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Morphological and Anatomical Studies of Floral and Extrafloral Nectaries in Some *Vicia* taxa (Fabaceae)

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Abstract: Morphology and anatomy of the floral and extrafloral nectaries were studied by light and Scanning Electron Microscope (SEM) in seven *Vicia* taxa. The floral nectaries are diverse into three types; half-ring with ligulate nectary projections arising from its center in four taxa, a big ring without nectary projections in *V. peregrina*, or half-ring without nectary projections in *V. ervilia* and *V. monantha*. They are covered with several large modified nectary stomata located only near their tips in the examined species without nectary projections, or located only near their nectary projections tips in the studied species with nectary projections. These nectaries are vascularized by phloem only derived from the closet vascular traces of the staminal column to them. Most of the studied taxa showed the presence of two prominent brown spots of extrafloral nectaries on the abaxial surface of each two adjacent stipules, while absent in *V. ervilia* and *V. monantha*. They present in the middle of the stipules in *V. faba* and *V. narbonensis*, or at its base in the other studied taxa. These spots are pear-shaped in *V. narbonensis*, or reniform in the other taxa consisting of clavate glandular hairs densely gathering together and long or short unicellular hairs. The glandular hairs formed of a secretory head (2-4, rarely 8 cells), one stalk cell and a basal cell. Only *V. peregrina* is characterized by the presence of short unicellular hairs aggregated around the shallow pits edges of the nectary spots. The secretory subepidermal parenchyma consists of one layer in *V. narbonensis* and *V. sativa* subsp. *cordata*, two layers in *V. faba* and *V. peregrina*, or three layers in *V. sativa* subsp. *nigra*.

Key words: Extrafloral nectarines, floral nectaries, nectary projection, secretory tissue, *Vicia*

INTRODUCTION

The genus *Vicia* L., belongs to tribe Vicieae (Faboideae-Fabaceae) and comprises 140 species of erect, climbing or scrambling herbs. It is mainly distributed in N temperate Europe, extending to S America, Hawaii and tropical E Africa (Kupicha, 1981; Mabberley, 1997). Täckholm (1974) recognized 12 species and Boulos (1999) enumerated 14 species of *Vicia* occurring in Egypt.

Flowers often bear specialized secretory structures, such as nectaries, which are clearly localized areas of tissue that regularly secrete nectar; a sugary substance for attracting and feeding pollinators (Daumann, 1970; Rudall, 2000). In Faboideae, the floral nectaries occur most frequently as a ring surrounding the base of the gynoecium. They are closely associated with the staminal column and usually vascularized by branches from the stamen traces (Fahn, 1953; Frei, 1955; Esau, 1965). Vascular tissue, when present, ranges from well developed bundles with both xylem and phloem to phloem strands only, which transports sugars to the secretory region (Frey-Wyssling, 1955; Rao *et al*., 1958; Rudall, 2000). Nectaries usually consist of secretory epidermal cells with dense cytoplasm and the nectar passes to the surface either through the ruptured cuticle or through the modified nectary stomatal pores (Fahn, 1979; Rudall, 2000). Modified nectary stomatal pores are found in Faboideae, such as *Trifolium pratense* (Picklum, 1954; Eriksson, 1977), *Medicago sativa* (Teuber *et al*., 1980), *Lotus corniculatus* (Murrell *et al*., 1982), *Phaseolus vulgaris* (Webster *et al*., 1982), *Vicia* species (Davis *et al*., 1988; Davis and Gunning 1991, 1992; Stpiczyñska, 1995), *Pisum sativum* (Razem and Davis, 1999), *Glycine max* (Horner *et al*., 2003). The nectary stomatal pores are considered to be modified because the guard cells of mature stomata rarely contract sufficiently to close their pores and they do not have a regulatory role in nectar secretion by flowers (Davis and Gunning, 1992, 1993).

Bhattacharyya and Maheshwari (1971) studied the extrafloral nectaries of the Leguminales and found that they are rather uncommon in the subfamily Faboideae

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unlike the other two subfamilies; Caesalpinioideae and Mimosoideae. They explained the ontogeny of the stipular secretory and nonsecretory trichomes of *Vicia faba*. The ultrastructure of secretory trichomes and their underlying epidermal and subepidermal cells of *Vicia faba* stipules has been studied in great detail by Wrischer (1962), Figier (1971), Tarkowska *et al.* (1981) and Davis *et al.* (1988). Kupicha (1976) classified the two subgenra of the genus *Vicia* on the basis of the inflorescence characters and stipules with nectariferous spots on abaxial surfaces in subgenus *Vicia* or without these spots in subgenus *Vicilla*. Wilkinson (1979) divided the extrafloral nectaries into two basic types; the epidermal nectaries and the hair nectaries which consist of local concentrations of glandular trichomes. Fahn (1990) mentioned the presence of nectar-secreting glandular trichomes on the stipules of *Vicia faba*. The distributional patterns of glandular hairs on the calyx-tooth were examined in 30 species of subgenus *Vicia* of genus *Vicia* by Endo and Ohashi (1998). They observed two types of glandular hairs; a few glandular hairs sparsely scattering on the whole surface, or many glandular hairs densely gathering at the center of the teeth. Stpiczyñska (2000) studied extrafloral nectaries located on the stipules in four *Vicia* taxa; *V. sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. He found that they consisted of secretory hairs building of four cells of head, one stalk and basal cell and 2-3 layers of subepidermal cells.

In the present study, a comprehensive investigation of the floral and extrafloral nectaries was found for their better understanding in the genus *Vicia*. The SEM examination and comparison of morphology and anatomy of the floral and extrafloral nectaries of seven *Vicia* taxa were done.

MATERIALS AND METHODS

Seven *Vicia* species were collected from their natural habitats in Egypt and the herbarium specimens were kept at Biological Sciences Department, Suez Faculty of Education and Agricultural Botany Department, Faculty of Agriculture, Suez Canal University. Taxa names and places of collection are given in Table 1. The stipules and Flowers were dissected to reveal the nectaries to scanning electron microscopy. The materials were fixed in FAA then 70% ethyl alcohol, dehydrated with an ethanol series and critical-point dried using Critical Point Drying Apparatus (FL 9496 Balzers). They were mounted onto stubs, sputter coated with gold and scanned with a Jeol SEM (JSM-T20) at 20 kV at central services lab., National Research Centre (Dokki, Cairo).

Table 1: Classification and places of collection of the *Vicia* species used in the study. The classification is based on Kupicha (1976) and Maxted (1993)

Subgenus	Section	Species	Place of collection	Date of collection
Vicia	Faba	*V. faba* L. cv. giza 2	Ismailia, Fanara	15.3.2004
	Narbonensis	*V. narbonensis* L.	Dessuk, Aboe Ghoneina	22.3.2004
	Peregrinae	*V. peregrina* L.	El-Sharkia, Menia El Kameh, El Saadiin	26.3.2004
	Vicia	*V. sativa* L. subsp. *cordata*	Ismailia, Fanara	15.3.2004
		V. sativa L. subsp. *nigra*	Ismailia, Fanara	15.3.2004
Vicilla	Ervilia	*V. ervilia* (L.) Willd.	South east El Arish, Wadi Hareidin	03.4.2004
	Cracca	*V. monantha* Retz	El-Arish, Airport protected area	20.3.2004

For anatomical studies, fresh flowers were fixed in FAA solution, then transferred to 70% ethanol and dehydrated through a graded ethanol series. Samples were then embedded in paraffin wax M.P. 56-58°C (Johansen, 1940). Using a rotary microtome, serial sections (12 μ) were obtained and double stained by safranin and light green (1% solution in 50% ethanol) according to Willey (1971). They were then dehydrated through an alcohol series and mounted in Canada balsam. These sections were photographed using a Zeiss photomicroscope at Agricultural Botany Department, Faculty of Agriculture, Suez Canal University. Where the same SEM and anatomical characters are shared by more than one species only one photograph is presented for either one.

RESULTS AND DISCUSSION

Floral nectaries

Morphological characters: Floral nectaries are located on the receptacle extending shortly up between the base of the staminal column and the ovary (Fig. 1a-f). They are surrounding the base of the ovaries opposite to the tenth free stamens differentiating in shape and size in the studied *Vicia* taxa as follows:

- Big nectaries of a half-ring with ligulate nectary projections arising from its center in *V. faba*, *V. narbonensis* and the two subsp. of *V. sativa* (Fig. 1a, b, c and Fig. 3b, e). These projections lie in the direction of the keel petals (Fig. 3a, f) and retain structural and functional trends for the nectaries. They are elongated, broad at the base and gradually tapering at the tip, whereas a short and wide in

V. faba and two subspecies of *V. sativa*, or narrow and more prolonged in *V. narbonensis*.

- A big ring without nectary projections in *V. peregrina* (Fig. 1d and Fig. 3d).
- Small nectaries of half-ring without any projections, in *V. ervilia* and *V. monantha* (Fig. 1e, f and Fig. 3c). The smallest ones appeared in very small flowers of *V. ervilia* (Fig. 1e).

In general, most of the studied taxa belonging to subgenus Vicia (*V. faba*, *V. narbonensis* and *V. sativa*) have long nectary projections, while the species of subgenus Vicilla (*V. ervilia* and *V. monantha*) and *V. peregrina* from subgenus Vicia are without these projections. The presence of floral nectaries with long projections in *Vicia faba* confirm the result of Davis *et al.* (1988) and do not confirm the result of Gulyás and Kincsek (1982), who classified the same species under species with the simple nectaries located around the base of the gynoecium in the inner side of the receptacle. The big ring floral nectaries in *V. peregrina* are similar to those of *V. sepium* and *V. grandiflora* (Stpiczyñska, 1995), while their nectaries with nectary projections.

The floral nectaries are covered with large modified nectary stomata only near their tips for releasing the nectar in the examined *Vicia* species without nectary projections (*V. peregrina*, *V. ervilia* and *V. monantha*). The surface of their epidermal cells does not contain pores or cracks and the only visible way for nectar secretion is these modified nectary stomata, which usually open, lack subsidiary cells and surrounded by two guard cells (Fig. 2a). This is in accordance to Picklum (1954), Waddle and Lersten (1973), Eriksson (1977), Teuber *et al.* (1980), Murrell *et al.* (1982) and Webster *et al.* (1982) who observed that the discoid floral nectaries of most of the species in Faboideae have modified nectary stomatal pores at its top. Whereas, in the studied *Vicia* species with nectary projections (*V. faba*, *V. narbonensis* and *V. sativa*), the nectar is released also through the modified nectary stomatal pores, located on these projections only, particularly their tips (Fig. 2b). These projections show several large nectary stomata, which sometimes differentiate adjacent to one another and the longer ones have more modified stomatal pores. These modified nectary stomata of the nectary projections appear similar to that recorded by Davis *et al.* (1988) for *Vicia faba*.

The nectaries consists mainly of turgid cuboidal and slightly convex epidermal cells (Fig. 2c) which are prolonged only near their tips. The surface of the nectary projections consists of narrow elongated or rectangular epidermal cells (Fig. 2d).

Anatomical characters: In cross sections, the floral nectaries consist of a conspicuous outer epithelial layer, which shows tightly packed and thin-walled cells with a densely stained cytoplasm and a large prominent nucleus and lightly stained inner parenchyma cells (Fig. 3a-f). This agrees with Fahn (1979) and Rudall (2000) who reported that the cells of the floral nectaries are smaller, more compact and more intensely stained than adjacent cells of the staminal column as is characteristic of all nectaries.

Vascular supply: The floral nectaries are vascularized by phloem only derived from the staminal traces. The column of sieve elements originates from the closet vascular traces of the staminal column to the nectaries (Fig. 3c). There were two openings leading to a nectar chamber which formed from the space between the free stamen and the staminal column (Fig. 3a). Frei (1955) and Frey-Wyssling (1955) noticed that the floral nectaries supplied by phloem produced a nectar with a high concentration of sugars. Where, those with bundles built of phloem and xylem, especially when xylem dominated, produced a lot of nectar with a low content of sugars.

Extrafloral nectaries spots

Morphological characters: A prominent brown spot of extrafloral nectary is present on the abaxial surface of each stipule only in the five studied taxa of subgenus *Vicia*, *V. faba*, *V. narbonensis*, *V. peregrina*, *V. sativa* subsp. *cordata* and subsp. *nigra* (Fig. 4a-e). The features of extra floral nectaries of these five taxa are compared in Table 2. The spots are noticed in the middle of the stipule in *V. faba* and *V. narbonensis* (Fig. 4a, b), or at the base in *V. peregrina*, *V. sativa* subsp. *cordata* and subsp. *nigra* (Fig. 4c-e). These spots are absent on the stipules in the other examined two species of subgenus Vicilla (*V. ervilia* and *V. monantha*). This is in agreement with Kupicha (1976) as well as Endo and Ohashi (1998) who differentiated between these two subgenra of the genus *Vicia* according to the pesence or absence of nectariferous spots on abaxial surfaces of stipules. However, Endo and Ohashi (1998) observed many glandular hairs densely gathering at the center of the two lateral calyx teeth in *V. narbonensis*, or at the center of the two lateral and lower calyx teeth in *V. sativa*, or a few glandular hairs sparsely scattering on the whole surface at the center of all calyx teeth in *V. peregrina*.

The shape of these extrafloral nectaries spots are pear-shaped in *V. narbonensis* (Fig. 5a) or reniform in the other taxa (Fig. 5b, c). They consist of clavate glandular hairs densely gathering without any spaces between them and unicellular hairs. These hairs aggregate

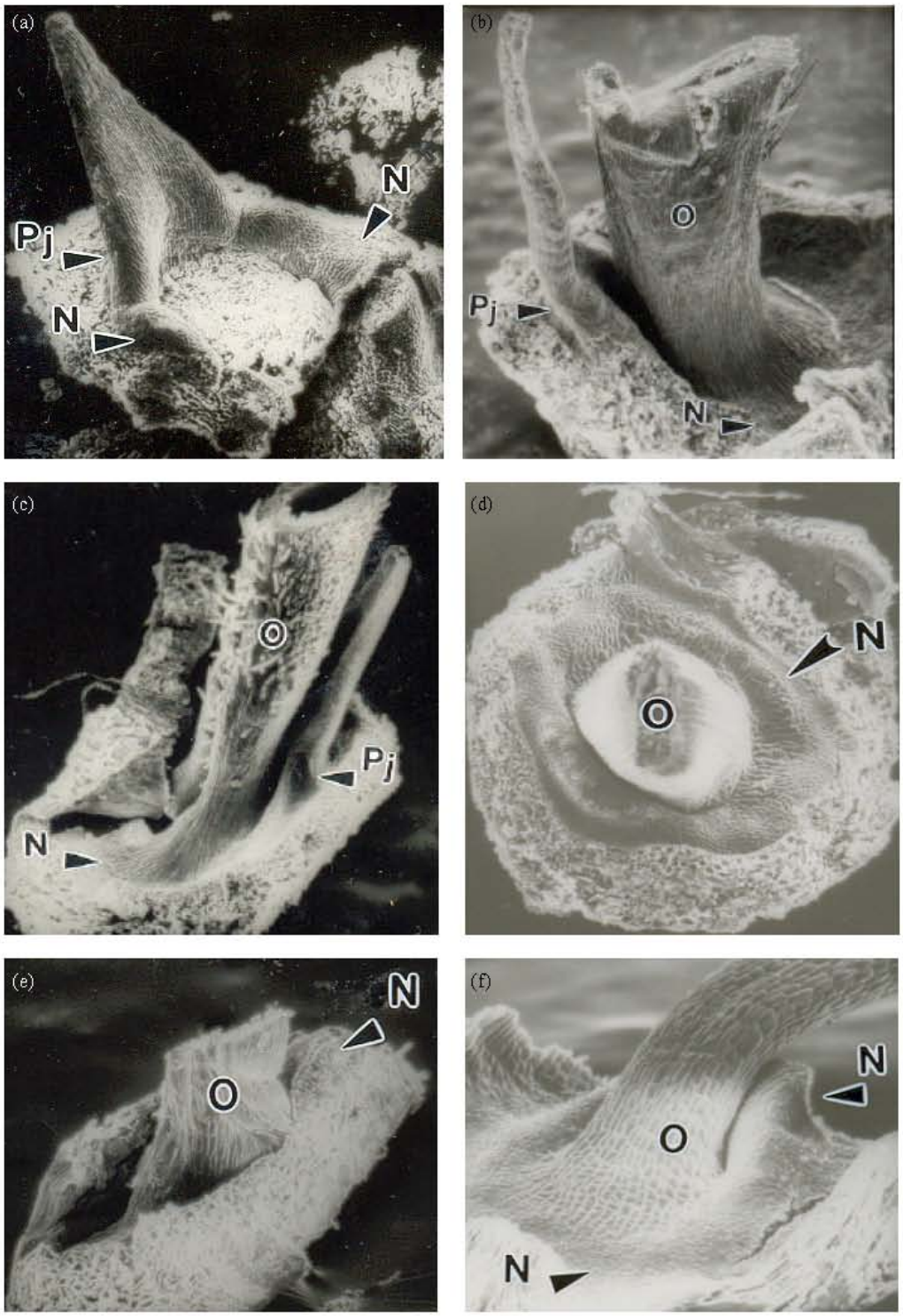

Fig. 1: SEM micrographs of the base of the dissected flowers showing nectaries surrounding the ovaries. (a) *V. faba*, (b) *V. narbonensis*, (c) *V. sativa* subsp. *cordata*, (d) *V. peregrina*, (e) *V. ervilia* and (f) *V. monantha*. (Magnification: x 50 in a-d; x 100 in e and f). Nectary (N), nectary projection (Pj), ovary (O)

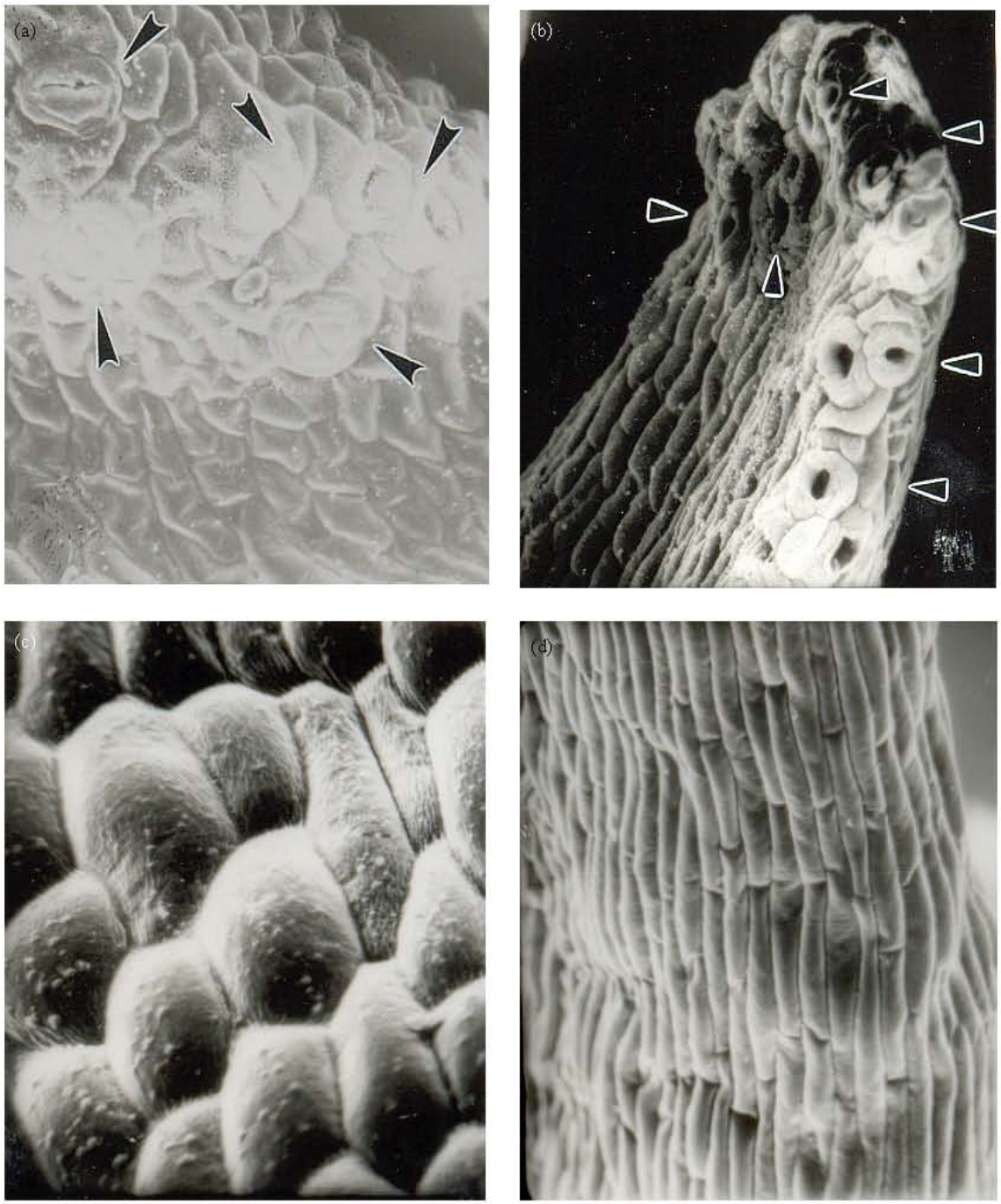

Fig. 2: (a) and (b) SEM micrographs of the surface of the tips of the nectary and nectary projection in *V. peregrina* and *V. sativa* subsp. *cordata* respectively, showing several large modified nectary stomata, sometimes adjacent to each other and usually open (arrowheads), (c) SEM micrograph of the surface of the nectaries in *V. monantha* and (d) SEM micrograph of the surface of the nectary projection in *V. narbonensis*. (Magnification: x 500 in a, b and d; x 1000 in c)

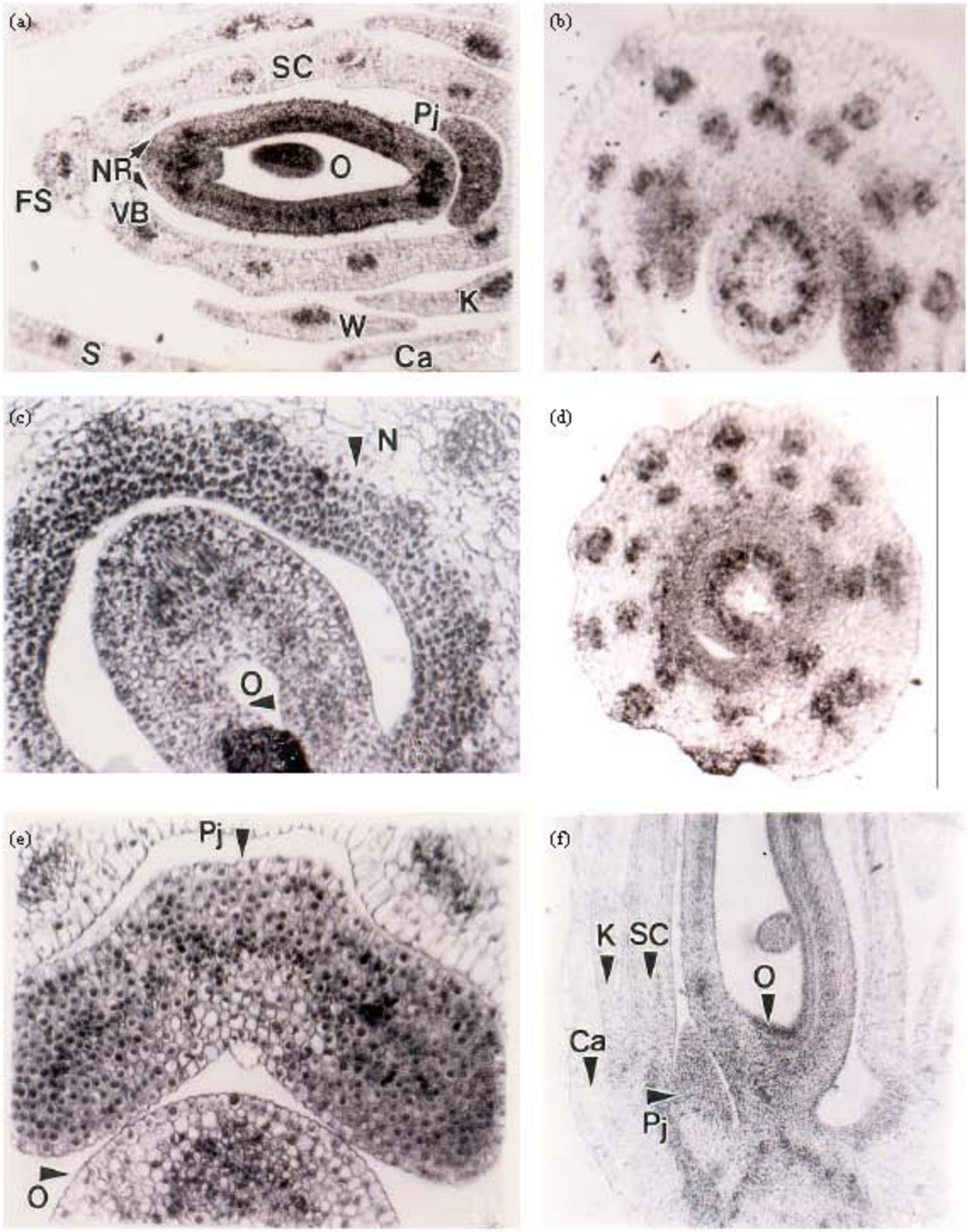

Fig. 3: Cross sections of nectaries (a-e) and longitudinal section (f). (a) and (f) *V. narbonensis*, (b) *V. faba*, (c) *V. ervilia*, (d) *V. peregrina* and (e) *V. sativa* subsp. *cordata*. (Magnification: x 16 in a, b, d and f, x 50 in c and (e). Calyx tube (Ca), free stamen (FS), keel petal (K), nectar reservoir (NR), nectary disc (D), nectary projection (Pj), ovary (O), standard petal (S), staminal column (SC), vascular bundle (VB) and wing petal (W)

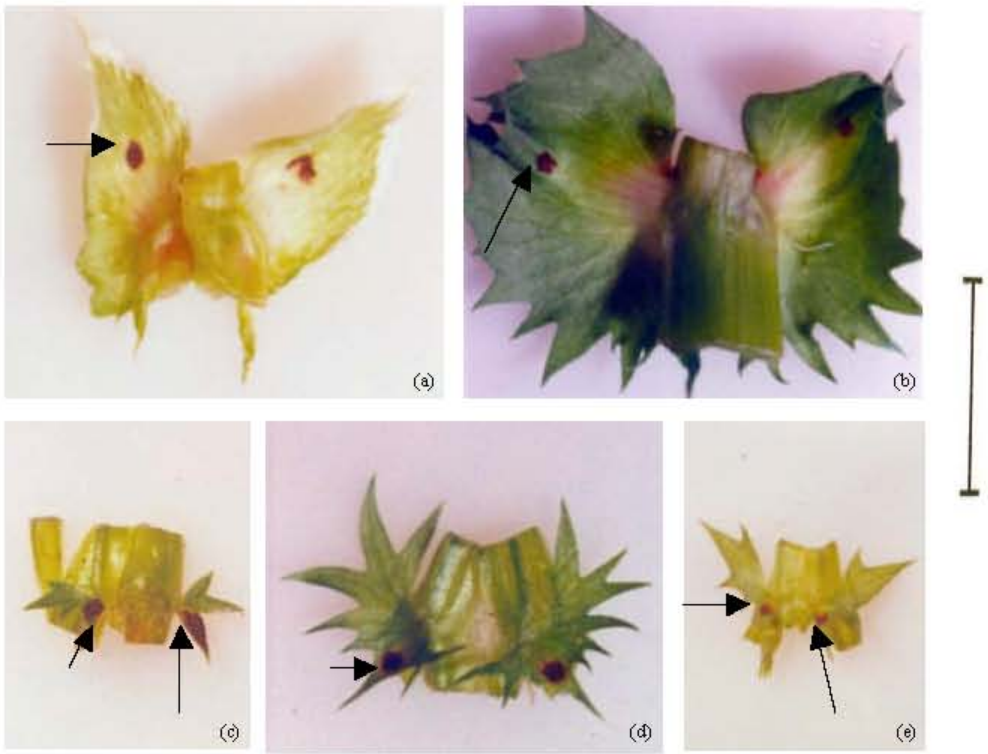

Fig. 4: Photographs of the third stipule showing extrafloral nectaries spots (arrowheads) of: (a) *V. faba*, (b) *V. narbonensis*, (c) *V. peregrina*, (d) *V. sativa* subsp. *cordata* and (e) *V. sativa* subsp. *nigra*. (scale bar = 4 cm)

Table 2: Characters of the extrafloral nectaries spots on the stipules of the five studied *Vicia* taxa

Characters	*V. faba*	*V. narbonensis*	*V. peregrina*	*V. sativa* subsp. *cordata*	*V. sativa* subsp. *nigra*
Position	In the middle part	In the middle	At the base	At the base	At the base
Average length × width (mm)	0.40×0.50	0.48×0.30	0.30×0.36	0.25×0.28	0.27×0.30
Spots shape	Reniform	Pear-shaped	Reniform	Reniform	Reniform
Glandular hairs	In shallow depressions	In shallow depressions	In shallow pits	In shallow depressions	In shallow depressions
Heads of Glandular hairs	Four (rarely eight)	Two (rarely three)	Two (rarely three)	Four (rarely two)	Four (rarely two)
Glandular stalk cell	Long	Long	Short	Short	Short
Unicellular hairs	Long	Short	Short aggregating around shallow pits	Long	Short
Secretory subepidermal layers	Two layers	One layer	Two layers	One layer	3 layers

in shallow depressions in *V. Faba*, *V. narbonensis* and two subspecies of *V. sativa* (Fig. 5a, c), or in shallow pits in *V. peregrina* with short unicellular hairs aggregated around them (Fig. 5b). In contrast, Wilkinson (1979) reported that the hair nectaries consist of local concentrations of glandular trichomes. The present result supports by Bhattacharyya and Maheshwari (1971), Wrischer (1962), Figier (1971), Tarkowska *et al.* (1981) and Davis *et al.* (1988) and Fahn (1990) who observed the presence of nectar-secreting glandular trichomes on the stipules of *Vicia faba*. As well as, Stpiczyñska (2000) noticed extrafloral nectaries on the stipules in *Vicia sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*.

Glandular hairs: The clavate glandular hairs are capitate trichomes consisting of a secretory head supported by one stalk cell and a basal cell. The stalk cell is radially elongated and long in *V. faba* and *V. narbonensis* (Fig. 5e), or short in the other taxa (Fig. 5d, f). These capitate trichomes divided into two types according to the number of cells in their heads as follows:

- Bicellular uniseriate secretory heads (rarely three), in *V. narbonensis* and *V. peregrina* (Fig. 5f and 6b, c, h, i).
- Multicellular biseriate secretory heads (four cells), in *V. Faba* (rarely eight cells), *V. sativa* subsp. *cordata* and subsp. *nigra* (rarely two) (Fig. 5d, e and 6a, d-g).

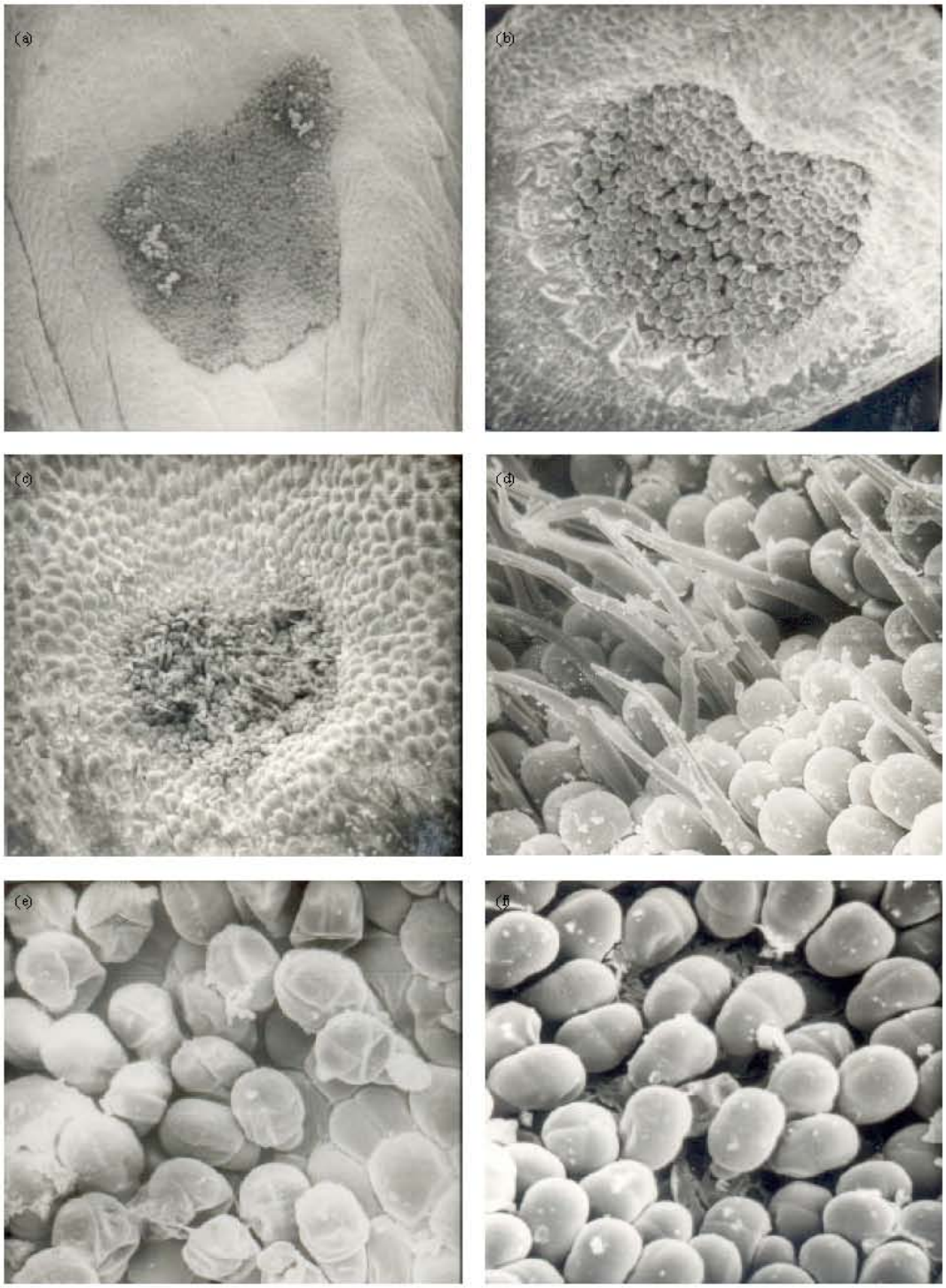

Fig. 5: (a-c) Scanning electron micrographs showing the shape of the stipular nectaries spots on the stipules of: (a) *V. narbonensis*, (b) *V. peregrina*, (c) *V. sativa* subsp. *cordata*. (d-f) Scanning electron micrographs appearing their structure of: (d) *V. sativa* subsp. *cordata*, (e) *V. faba* and (f) *V. peregrina*. (Magnification: x 50 in a; x 100 in b and c; x 500 in d-f)

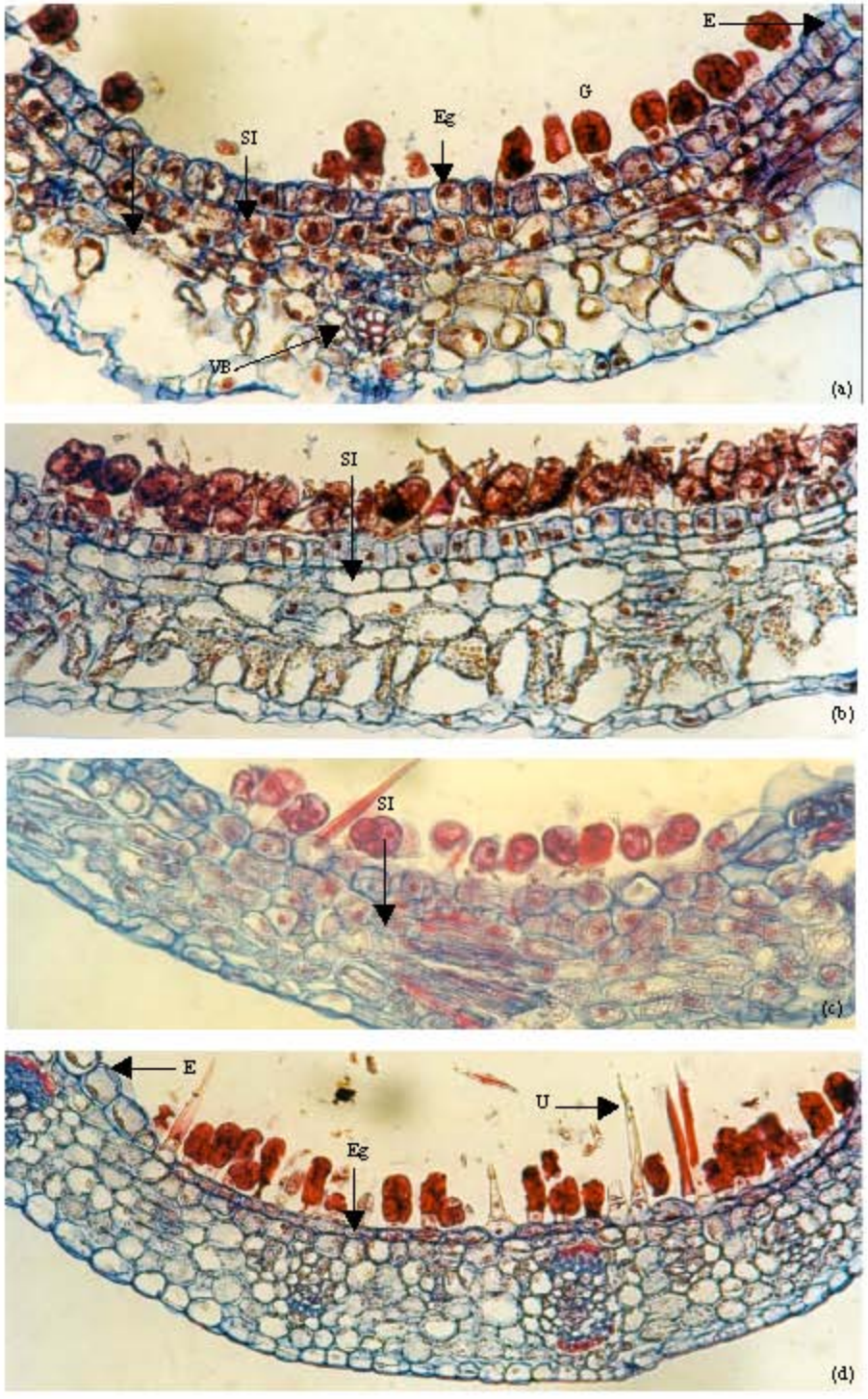

Fig. 6: (a-d) Cross sections of the stipules showing the nectary spots of: (a) *V. faba*, (b) *V. narbonensis*, (c) *V. peregrina* and (d) *V. sativa* subsp. *cordata*. (Magnification: x 60 in a-d). Epidermal cells (E), Epidermal cells under glandular hairs (Eg), Glandular hairs (G), Subepidermal secretory layer (Sl), Unicellular hair (U), Vascular bundle (VB)

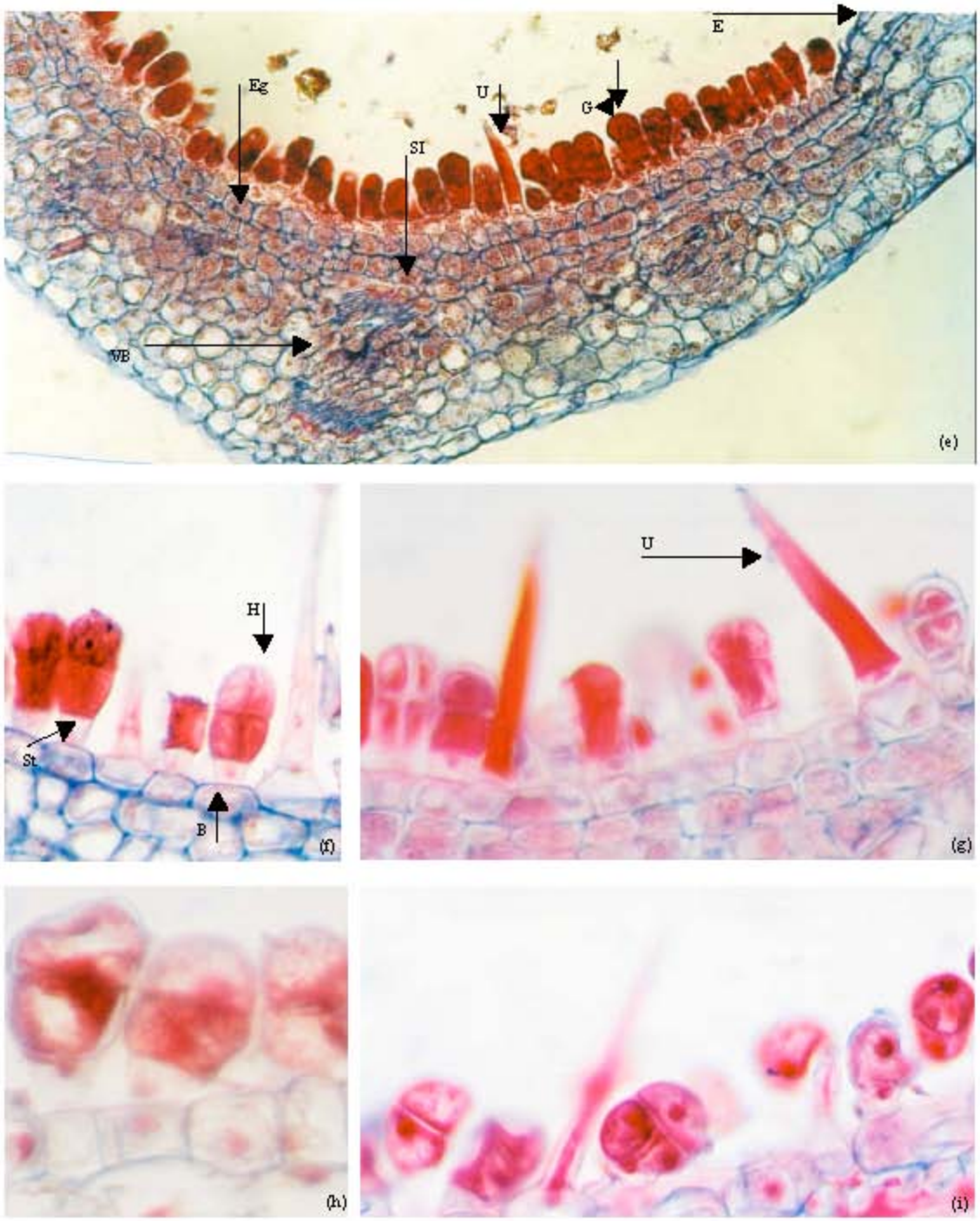

Fig. 6: (e) Cross sections of the stipules showing the nectary spots of *V. sativa* subsp. *nigra*. (f-i) Enlarged view of glandular and unicellular hairs in cross sections: (f) *V. sativa* subsp. *cordata*, (g) *V. sativa* subsp. *nigra*, (h) *V. narbonensis* and (i) *V. peregrina*. (Magnification: x 60 in a; x 132 in f-i). Basal cell (B), Epidermal cells (E), Epidermal cells under glandular hairs (Eg), Glandular hairs (G), Head of glandular hairs (H), Subepidermal secretory layer (Sl), Stalk cell (St), Unicellular hair (U), Vascular bundle (VB)

Stpiczyñska (2000) found that secretory hairs built of four cells head, one stalk and basal cell in stipular spot of *V. sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. As well as, Diaz-Castelazo *et al*. (2005) detected the capitate trichomes in *Macroptilium atropurpureum* (Fabaceae) with histochemical techniques, which are coloured red by Fehling's technique (indicating presence of reducing sugars).

Unicellular hairs: They are intersperse among the glandular hairs and extended above them, long in *V. faba* and *V. sativa* subsp. *cordata* (Fig. 5d and 6d), or short in *V. narbonensis* and *V. sativa* subsp. *nigra* (Fig. 5a and 6b, e). Only *V. peregrina* is characterized by the presence of short unicellular hairs aggregated around the shallow pits edges of the nectary spots (Fig. 5b). Stpiczyñska (2000) mentioned that these unicellular hairs are mechanical hairs in *V. sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. Recently, Diaz-Castelazo *et al*. (2005) proved for the first time the unicellular trichomes as nectar-secreting structures in *Crotalaria incana*. They found that these unicellular trichomes were hollow inside in the sections and when they were submitted to sugar-detecting histochemical techniques appearing red coloration indicating the presence of reducing sugars and revealing accumulation of nectar.

Anatomical characters: The cross section of stipular spot showed layer of glandular hairs and unicellular hairs following by secretory epidermal cells and subepidermal layers (Fig. 6a-e). The secretory epidermal cells and subepidermal layers are composed of tightly packed and thin-walled cells characterizing by a densely stained cytoplasm and a large prominent nucleus. The secretory subepidermal parenchyma consists of one to three layers; one layer in *V. narbonensis* and *V. sativa* subsp. *cordata* (Fig. 6b, d), two layers in *V. faba* and *V. peregrina* (Fig. 6a, c), or three layers in *V. sativa* subsp. *nigra* (Fig. 6e). The epidermal cells are thinner at the nectary spot than the other epidermal cells on the two sides of it, as well as subepidermal layers (Fig. 6a-e). These differences in cells thickness appearing the surface at the nectary spot is sunken forming the shallow depressions or pits which contain the glandular hairs representing the stipular nectaries. These results are in agreement with Stpiczyñska (2000) who recorded 2-3 subepidermal secretory layers in cross sections of stipular spot of *V. sativa* subsp. *angustifolia*, *V. sativa* subsp. *sativa*, *V. sepium* and *V. grandiflora*. However, Endo and Ohashi (1998) reported that the surface of the calyx-tooth is sunken at the nectary spot in *V. narbonensis*, or flat in *V. peregrina* and *V. sativa*.

Vascular supply: The extrafloral nectaries are not vascularized and a central portion of them were located directly above the main vein of the stipules reaching the periphery of secretory subepidermal layers (Fig. 6a-e). Both xylem and phloem were present in the main vein and usually in minor veins located two or three cell layers below the epidermis bearing the nectary hairs. This observation supports those of Figier (1971); Davis *et al*. (1988) but not the statement of Durkee (1983) who reported that only phloem was present.

It is concluded that the floral nectaries differentiate in the examined species into half-ring or complete ring with or without ligulate nectary projections, which are covered with several large modified nectary stomata located only near their tips and vascularized only by phloem. There are two prominent brown spot of extrafloral nectary on the abaxial surface of each two adjacent stipules in most of the studied taxa except in *V. ervilia* and *V. monantha*. They present in the middle or at the base of the stipules consisting of unicellular hairs and glandular hairs which formed of a secretory head (2-4-8 cells), one stalk cell and a basal cell. The secretory subepidermal parenchyma consists of one layer to three layers.

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