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Glandular trichomes on the leaves of *Trigonella foenum-graecum*: Development, Structure and Histochemistry

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ABSTRACT

Light and scanning electron microscopy of Trigonella foenum-graecum leaves confirmed the presence of one type of non-glandular trichome and two types of capitates glandular trichomes (clavate and elongated). Capitate glands arise as protodermal protuberances that divide asymmetrically to produce a basal cell and an apical cell. Further divisions of the apical cell produce a capitates trichomes with one basal cell, one to three stalk cells and two to ten cells of the head arranged in one to several layer of cells, one to four cells in each. Alkaloids, polysaccharides, acidic lipids and amino acids were histochemical detected in all the glandular cells; phenolic substances were detected in the epidermal cells and in secretory head cells. There are no differences in gland development or secretion between the clavate and elongated capitate glandular trichomes.

Key words: *Trigonella foenum-graecum*, glandular trichomes, secreted material, ontogeny.

INTRODUCTION

The trichome appendages arise from the elongation or a series of divisions of epidermal cells to form specialized trichomes that function as glandular or non-glandular trichomes [1]. They can be unicellular or multicellular, uniseriate or multiseriate, and are variously shaped [2]. Glandular trichomes produce various substances, which are stored at the plant surface [3].By their physical properties and by the production of different chemical products they play a great role in the protection against herbivores and pathogens, in the pollination and in the other interactions between plants and environment, as well as secondary metabolites in the secreted products are used as food additives or pharmaceuticals [4].

Trigonella foenum-graecum is an aromatic species of the Fabaceae (subfamily; Faboideae) is commonly known as Fenugreek and is considered as one of the oldest multipurpose medicinal plant, as well as a fodder plant [5].It is grown as a spice crop in many parts of the world. The species is used in folk medicinal as a tonic, blood sugar lowering, treatment for weakness and edema of legs [6]. In modern medicinal fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects and also as a source for preparing raw materials of pharmaceutical industry, especially steroidal hormones [7].

The structure, development and histochemistry of the glandular trichomes of related families, such as Lamiaceae are well document [8]. In contrast, little information is available on the structural and histochemical aspects of trichomes of Fabaceae. The potential pharmacological interest of *T. foenum-graecum*, as well as, capacity of glandular trichomes for synthesize and store varied types of secondary metabolite led us to investigate the development, structure and secretion of different trichome types on leaves of this plant. In the present paper we present result obtained from studies carried out mainly with the aid of light, fluorescent and scanning electron microscopy and some histochemical tests.

MATERIALS AND METHODS

Plant material

Leaves at various developmental stage were collected from *Trigonella foenum-graecum* during the vegetative growth period prior to the flowering period from plant growing in the experimental field of Tarbiat Modares University, Tehran, Iran (35° 43′ N, 51° 22′ E, 1283.7 m), (pH, 7.4). Ten samples from each stage were selected for structural, chemical and developmental studies.

Scanning electron microscopy (SEM)

Small leaves were fixed in FAA (formalin, acetic acid, 70% alcohol) for 24 h, stored in 70% ethanol [9]. After dehydration in a graded ethanol series, the material was critical point dried with CO2, sputter-coated with a thin layer of gold and, finally, examined in a Philips XL30 scanning electron microscope at 20 kV.

Light microscopy (LM)

For structural studies, free-hand sections or peeled epidermis of emergent (very young) leaves (middle leaflet at first or second node) were prepared and mounted in water on glass slides.

For developmental studies, leaf primordium were fixed in absolute ethylalcohl and glacial acetic acid (AA - 3:1) for 48 h, then squashed in glycerin water. The slides examined with an Olympus BH2 light microscope.

Histochemical tests

Fresh hand sections of emergent leaves were stained with a series of histochemical regent: Periodic acid-Schiff's (PAS) for polysaccharides [10]. Hematoxylin for pectin substances [10].Fehling's solution these for reducing sugars [11]. Sudan red 7B for neutral lipids [12].Nile blue for neutral and acidic lipids [10].Coomassie brilliant blue for total proteins [13].Ninhydrin test for amino acids [14]. Dittmarre agents for alkaloids [15]. Nadi reagent for essential oils [16]. Potassium dichromate for phenolic compounds [17]. Neu's reagent for flavonoids under UV [18].Toluidine Blue for nuclear staining [19].Sudan III for Cutinized or suberized walls [9]. Phloroglucinol for Lignified walls [9].Iodine-Sulphur for cellulosic walls ([19].Standard control procedures were carried out simultaneously.

Table 1. Histochemistry of the glandular trichomes on the leaves of *T. foenum-graecum.*

Target compound	Reagent	Colour observed in secretory cells	Trichome type	
			Clavate	Elongated
Polysaccharides	Schiff (PAS)	Pink	+	+
Pectins	Hematoxylin	Violet	+	+
Neutral lipids	Sudan red 7B		-	-
Neutral and acidic lipids	Nile blue	Blue	+	+
Reducing sugars	Fehling's solution		-	-
Alkaloids	Dittmar	Brown-orange	+	+
Essential oils	NADI		-	-
Aminoacids	Ninhydrin	Violet	+	+
Proteins	Coomassie brilliant bl		-	-
Phenolic compounds	Potassium dichromat	Light brown	+	+
Flavonoids	Neu's reagent	Yellow	+	+

+, Positive; -, Negative.

RESULTS

Structure and distribution of the trichomes

The leaves of *T. foenum-graecum*bear non glandular and two types of glandular trichomes (Fig. 1).The trichomes are often falcate and leaning towards the leaf apex. Two types of glandular trichomes are capitate, which can be divided into two subtypes according to the morphology of the secretory head: 1) Clavate trichomes, with oval or ovoid head; 2) Elongated trichomes, with long and thin head (Fig. 1A). The glandular trichomes, although appearing to differ in morphology, are structurally similar. These glandular trichomes are made up of a prominent basal cell (Fig. 1B) with hexagonal surface (Fig. 1A, L), 1-3 short or long uniseriate stalk cells with thick lateral wall towards the base (Fig. 1B) and a 2-10 secretory cells, which are enclosed in a smooth cuticle (Fig. 1D, E). The heads of the gland are uni or multilayered, with one to four cells in each layer (Fig. 1C). Clavate trichomes are more frequent than elongated trichomes. The Non glandular trichomes are uniseriate and unbranched structures (Fig. 1F) that consisted of a broad

and protruding hexagonal basal cell (Fig. 1G, H, l),generally one but occasionally two or three stalk cells (Fig. 1I, J) and a long pointed apical cell with warty surface (Fig. 1K). Most of the glandular trichomes and the Non glandular trichomes of *T.foenum-graecum* are very long and scanning electron microscopy induced dehydration and shrinkage in apical cells (Fig. 1M).

Adaxial surface is glabrous (Fig. 2A). Trichomes are only present abaxially, which are mainly distributed on the midrib, leaf base and margin. Emergent leaves are covered with Non glandular trichomes which partially obscures the glandular trichomes (Fig. 2B).As the leaf expands, the density of both glandular and Non glandular trichomes decreases (Fig. 2C), so that few trichomes are only observed on the midrib of mature leaves (Fig. 2D).

Development of the trichomes

The development of trichomes begins at very early stages of leaf primordial appearance, even before the differentiation of stomata mother cells. The rate of trichome differentiation is rapid and start functioning very early before the leave reaches its full size. In emergent leaves fully developed and actively secreting trichomes are observed. Trichome cells do not develop synchronously across the leaf primordium, new trichomes initiate progressively in between developing trichomes (Fig. 3H).Thus, trichomes at various stages of differentiation were observed in primordial leaves (Fig. 3I). However, fully developed leaves bear generally mature trichomes but early ontogenetic stages of trichomes can occasionally be found.

The clavate and elongated glandular trichomes display similar developmental process. For both of them, three different stages of development can be recognized: presecretory, secretory and post secretory phase (Fig. 3A-M). The first sign of trichome initiation is expansion and differentiation of a protodermal cell from the other cells that appears as an enlarged spherical cell (initial cell) (Fig. 3A). After its initiation, trichome enlarges in the apical part, forming a tubular extension with asymmetrical cytoplasmic distribution (show polarization) (Fig. 3B). The cell then divides asymmetrically and periclinally to form a basal and an apical cell with dense cytoplasm (Fig. 3C). The basal cell increases substantially in volume during development, thus lead to protrude above the level of the epidermal cells. The top cell undergoes another periclinal division produces the stalk cell and the mother cell of the head (Fig. 3D). The final number of the stalk cells (1 to 3 cells) depends on the number of the stalk cell periclinal divisions. The head initial cell divides by periclinal divisions forming a row of cells (Fig. 3E), which after a period of enlargement, undergoes anticlinal divisions as necessary, depending on contains numerous head cells, to form a two to ten- celled glandular head. The first anticlinal division generally occurs in the cells of the upper layer (Fig. 3F), but may occur in the lower cells (Fig. 3G) or the divisions in these cells are synchronized. When the trichome has reached its full size and shape, the cuticle thicken, particularly on the lateral walls of the lowest stalk cell. All of the above described divisions are at presecretory phase and the cuticle is attached to the cellular surface (Fig. 3A-I).

Onset of the secretory phase is characterized by separation of the cuticle from the cell wall and formation a small subcuticular space across the apical surface of the gland. Cuticle detachment occurs only in the upper region of the glandular cells. As a result of the pressure promoted due to secretion accumulation, the subcuticular space gradually enlarged, becoming fully distended at the end of secretory process (Fig. 3J).

At the post secretory phase, the secreted material stored in the subcuticular space, is released to the trichome surface via cuticular micropores (Fig. 3K).In the absence of cuticular micropores, it is possible that the cuticle, with its narrow channels (Fig. 3M), can allow the release of secretion components and, or tip of the trichome breaks (Fig. 3L).As a consequence of releasing, the cytoplasm begin to degenerate, then other parts of the trichome lose their stability, followed by shrinkage, and finally ends development of the trichome.

Solubility of the released secretion in different solvents

Treatment of the secretion droplets of the glandular trichomes with different fluids demonstrated differences in droplet solubility. The droplets kept their volume and shape after treatment with aqueous fixative. Treatment of the trichomes with absolute ethanol and acetone resulted in total removal of the released secretion from the trichome tip.

Histochemistry

Secretory product of clavate and elongated trichomes was colorless, and located in the head region (Fig. 4A). The secretory droplets were also detectable within the subcuticular space that confirmed perfectly to the histochemical investigation. The histochemical result revealed that both capitate trichomes produced a heterogeneous substances (table 1): PAS reaction for polysaccharides gave positive result in the head cells, indicated by light pink color (Fig. 4B). Hematoxylin showed pectin substances within the secretory cells, which became violet (Fig. 4C). Treatment with Fehling's solution for reducing sugars proved negative in glandular trichomes (Fig. 4D), whereas in non-glandular trichome, reddish spots appeared on the outer surface of the cuticle (Fig. 4E) that finally became to form flower shape ornamentations (Fig. 4F).

Acid lipids were identified mainly in the distal part of the head area, by a blue color with nile blue, whereas basal and lowest stalk cells showed a pink staining (Fig. 4G).Treatment with Sudan Red 7B for neutral lipids gave negative result in secretory cells, although red color observed in basal and lower stalk cell (Fig. 4H).Amino acids within the head cells stained light violet with Ninhydrin (Fig. 4I). Dittmar reagent gave positive response for alkaloids, showing orange to brown coloration of the head cell and subcuticular space (Fig. 4L, M). Phenolic compounds were evidenced in the protoplast secretory cells by brown color with potassium dichromate (Fig. 4N). Neu' s reagent for flavonoids detection, induced an intense yellow florescence of the head cells, as well as epidermal cells of the leaves (Fig. 4O). For other histochemical tests, Nadi reagent and Coomassie brilliant blue reagent produced negative result, indicating the absence of essential oils (Fig. 4K) and proteins (Fig. 4J), respectively. Staining with toluidine Blue showed the presence of two to ten nuclei in the head region (Fig. 1D, E). The nuclei was also detectable in basal and stalk cells (Fig. 1B). The absence chlorophyll florescence revealed that chloroplasts were not present in the trichomes.

Cell wall histochemistry

In all trichome types, the composition of basal and lowest stalk cell walls changed during trichome development. In the young trichomes PAS reactions for polysaccharides gave positive result in the basal and stalk cell walls, whereas in mature trichomes, these walls became PAS-negative and Sudan III-positive, indicating that cutinization had occurred, specifically in the lateral walls of the lowest stalk cells. In addition, the outer layer of the head cells (cuticular sheath) were stained lightly with Sudan III (Fig. 5A-C). As well as, the silvery white auto fluorescence on the outer layer of basal, stalk and secretory cells indicated the presence of cutin substances in the trichomes structures (Fig. 5I). The stalk cell walls appears to be brittle because are fractured during tissue processing (Fig. 5D). Although the basal and stalk cell has thick cutinized cell wall, this cells are living and its protoplast has a nuclei (Fig. 5C).Staining with Iodine-Sulphur for cellulosic walls was positive in primary walls of glandular head cells (Fig. 5E) and apical cell of Non glandular trichomes (Fig. 5F), which became bright blue. Cutinized walls also stains brownish (Fig. 5E, F). Histochemical test with Phloroglucinol (which stains red for lignified walls) was negative in all types of trichomes (Fig. 5G, H).

DISCUSSION

As in plants of most Faboideae species, the leaf surface of *Trigonella foenum-graecum* showed one non glandular and two glandular trichomes type. Non glandular trichomes are uniseriate, that consist of protruding basal cell, 1-3 stalk cells and a long pointed apical cell with thick and warty wall. Both clavate (with oval head) and elongated (with long and thin head) gland are capitates. The basic structure of these glandular trichomes are similar. In both of the glandular trichomes, multicellular head (2-9 cells) supported by 1-3thick-walled stalk cells and a prominent basal cell. In contrast to non-glandular trichomes, the surface of glandular trichomes is smooth and lack a micro-ornamentation. All trichome types are only presented on abaxial surface.

Non glandular trichomes resembling in general features the type described here were reported earlier in some members of Faboideae like in *Trifolium lappaceum*, *Medicago lupolina* [20].

Capitate trichomes have been reported in *Trifolium* sp, *Medicago* sp, *Melilotus* sp and *Onions* sp. These trichomes are generally club-shaped (clavate) with uniseriate stalk. However, biseriate stalk in *Trifolium pretense* and multiseriate stalk in *Ononisnatrix* is also reported [20]. Willison and retelack (1988) [21] reported that in *Trifolium* sp are presents procumbent and erect capitates trichomes. The heads of the procumbent trichomes were long and thin, consisting of 4-8 layers of cells. By contrast, the heads of the erect trichomes were globose and not organized in layers, although they were multicellular. The structure of the clavate trichomes of *T. foenum-graecum* is also similar with that of *Theobroma* sp. (Malvaceae) [22].

The trichomes of *T. foenum-graecum* appear to develop rapidly on leaf primordial. The high number of fully developed trichomes are observed on emergent growing leaves. Trichomes are also found in mature leaves, but their distribution frequency is lower in comparison to the young leaves .In many plant species trichome density is very high in young leaves but decreases rapidly with leaf expansion ([23], [24]).The rapid differentiation and high density of trichomes in early leaf development has been suggested that, in emergent leaves lacking difference epidermis, trichomes and their exudates may serve as a functional analogue of the epidermis in mature leaves [25] since they play a similar protective role against biotic and abiotic factors such as water deficit, insect herbivores, phytopathogenic fungi, and UV-B radiation[1]. At later stages of leaf development, when the formation of the epidermis is completed, the functional role of the trichomes becomes less important, and they often senesce and shed. In some cases, however, trichomes remain viable and functional in mature leaves [2]. However, fully developed leaves of *T. foenum-graecum*bear generally mature trichomes but immature trichomes can occasionally be found. Therefore trichome production no ceases during leaf enlargement and epidermal cells of mature leaves can be also committed to trichome fate determination. In some plants, the final number of trichomes is established early

during leaf differentiation [26], while in others new trichomes are formed throughout all the stages of leaf development [23].

The ontogeny of *T. foenum-graecum* trichomes follows the pattern described for other Fabaceae species, such as *Trifolium alpinum*, *Medicago falcate* [20], Vicia faba [27] and *Glycine max* [28]. Both the glandular and non-glandular trichomes recorded originate from a single initial protodermal cell. This is a relatively common developmental pattern for many trichomes [29]; [20].Uphof (1962) [29] is of the opinion that the initial of glandular trichome is usually tipped while that of a non-glandular trichome is acute. But according to Gupta and Murty (1977) [20] both types of trichomes show only initial whit a round trip. The present study in agreement with the conclusions of Uphof.

The clavate and elongated trichomes display similar secretory behavior. Both glandular trichome accumulates their secretory product within the subcuticular space. Pressure exerted by the secretion caused the cuticle separate from the underlying cell wall, creating a subcuticular space in the gland apex. The formation of subcuticular space is a notable characteristic of the secretory cells, as in glandular trichomes of Lamiaceae [30], Scrophulariaceae [31], Asteraceae [26] and Bignoniaceae [32].The secreted material is usually released through cuticular micropores, in form of small droplets. Sometimes, after cuticular repressor formation of the channels, which open to the outside, secreted material is released. The release of secretory product following the micropore or repture of the cuticle is common feature of many glandular trichomes [33]; [34]. Secretion release, particularly by passing droplets through the channel on *T. foenum-graecum* leaves is noteworthy and has been reported in few species: *Fagonia* (Zygophyllaceae) [35], *Leonotis leonurus* (Lamiaceae) [36], *Caesalpinia crista* (Fabaceae) [37].

Glandular trichomes of *Cicer arietinum*, which very similar in appearance to the glandular trichomes described here, have been considered by Schnepf (1965) [38] to be trichome-hydathodes. In the present observations, our efforts in visualizing sol went-treated droplets indicate, unlike ethanol and acetone, secretion droplets on the trichome tip was not removed by aqueous treatments. These solubility characteristics suggest that the secretion is lipophilic. Thus, these trichomes does not seem to act as hydathodes in *T.foenum-graecum*. However, this hypotheses deserve further experimental investigation.

The present histochemical result indicate that the secreted material in both types of *T. foenum-graecum* capitates trichomes were of complex nature. Positive reaction to PAS, indicated that polysaccharides were present of in the protoplast of the head cells, which could act as an energy source [39]. Acid polysaccharides, as pectin was shown to hematoxylin within the secretory cells. The presence of these substance may have a lubricant role in facility leaf growth and expansion [40]. Fehling's test designed to detect the presence of reducing sugars in glandular trichomes gave negative result. Clavate trichomes in many species is often considered as a nectar-secreting [41]. Diaz-Castelazo et al. (2005) [37] reported the capitates trichomes of *Macroptilium atropurpureum* (Fabaceae) with Fehling's technique was colored red (indicating presence of reducing sugars). The lack of sugars in the secretion confirm that the glandular trichomes on the leaves of T. *foenum-graecum* are not involved with nectar production. As a consequence, existence of reducing sugars on the outer surface of Non glandular trichomes can be suggested that these sugars probably guide the path of the pollinators and attracted insects [42].

The reaction of Nile blue showed the head cells of the capitates trichomes contained acid lipids, which may have protective functions [43].Ninhydrin test indicated the presence of amino acids in the secretory cells. This class of compound can be present in cell protoplasts as a precursor of the secretory product. It can be also for of the presence of amino acid 4-hydroxyisoleucine, which is one of the most important compounds in *T. foenum-graecum*.

Alkaloids are widespread in Fabaceae. The present data showed that the capitates trichomes appear to be the site of synthesis and or storage of alkaloids. Alkaloids are efficiently used as defensive agents and they may be moved around the plant to those parts needing greater protection [44].Phenolic compounds were detected by potassium dichromate and Neu' s reagent in head cells of capitates trichomes and the epidermal cells. These result are consistent with the phytochemical data obtained from other analyses of the T. *foenum-graecum* [45].The phenolic substances could deter herbivory, as well as ameliorate the effect of the intense solar radiation and prevent other compounds from oxidation [44]. Essential oils and proteins were not detectable in the secretion that confirm the glandular trichomes on the leaves of *T. foenum-graecum* are not involved with either biosynthesis or secretion these metabolites.

The auto fluorescence and reaction of Sudan III showed a strong positive reaction to the existence of cutin or suberin in the basal and lowest stalk lateral walls of the fully developed glandular and Non glandular trichomes. Whereas the upper stalk cell walls showed faintly cutinization. Young stages featured no suberin or cutinization, either. PAS and I/KI reactions for cellulosic walls gave positive result in the periclinal walls of the stalk cells, the distal wall

bordering the secretory cells, and the lower wall bordering the basal cell. These characteristics, furthermore the presence of nuclei in the basal and stalk cell despite the cutinized walls suggests that symplastic transport was promoted and cutin barriers on the side walls of the basal and stalk cell were able to prevent apoplastic water flow in to the trichomes [30].Lignin was not clearly located in the all trichome types of *T. foenum-graecum.* Cell wall lignification in glandular trichomes were not typical and only certain trichomes synthesized lignin, e.g. trichomes of *Salvia aurea* (Lamiaceae) [46] and *Cucurbita pepo* (Cucurbitaceae) [47].

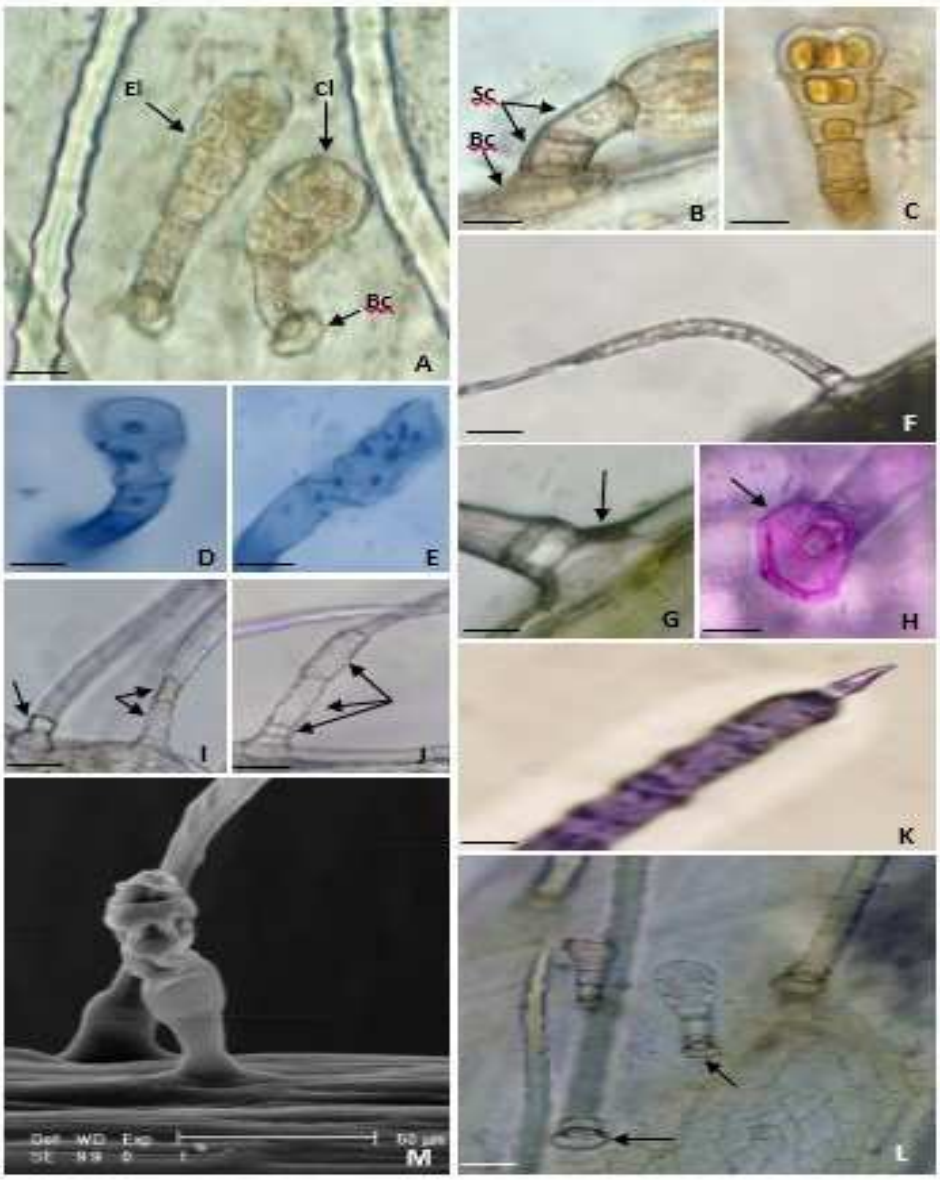

Fig. 1. (A-M) *T. foenum-graecum* leaf trichomes (A-E) capitates glandular trichomes: (A) Clavate and elongated glandular trichomes. (B) Protruding basal cell and 2-celled stalk, (C) Multilayered secretory head with 1-4 cells in each layer. (D) 2-celled head. (E) 10-celled head. (F-K) Non glandular trichomes: (F) uniseriate non glandular trichome; (G) broad and protruding basal cell. (H) Hexagonal Surface of basal cell, (I) Uni and bicelled stalk. (J) 3-celled stalk (K) pointed apical cell with warty surface. (L) Glandular and Non glandular trichomes with hexagonal basal cell (arrows), (M) SEM micrograph of collapsed glandular and non-glandular trichomes. Ec, elongated trichome. Cl, clavate trichome; Bc, basal cell; Sc, stalk cell. Bar = 7 µm.

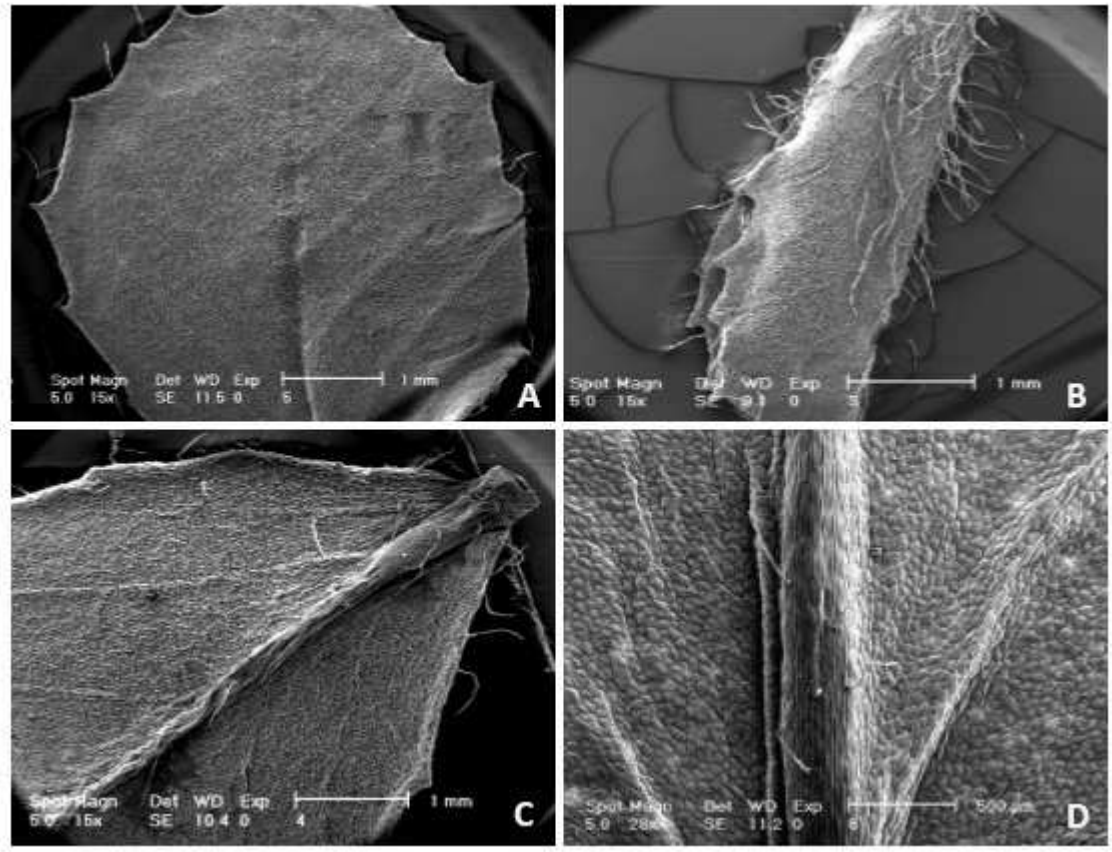

Fig. 2. SEM micrographs showing distribution and density of trichomes on leaves of T. foenum-graecum. (A) glabrous adaxial surface of leaf; (B) abaxial surface of emergent leaf with trichomes mainly distributed on the midrib, leaf base and margin; (C) low density of trichomes in abaxial surface of portion of young leaf; (C) a portion of mature leaf with trichomes restricted to the midrib.

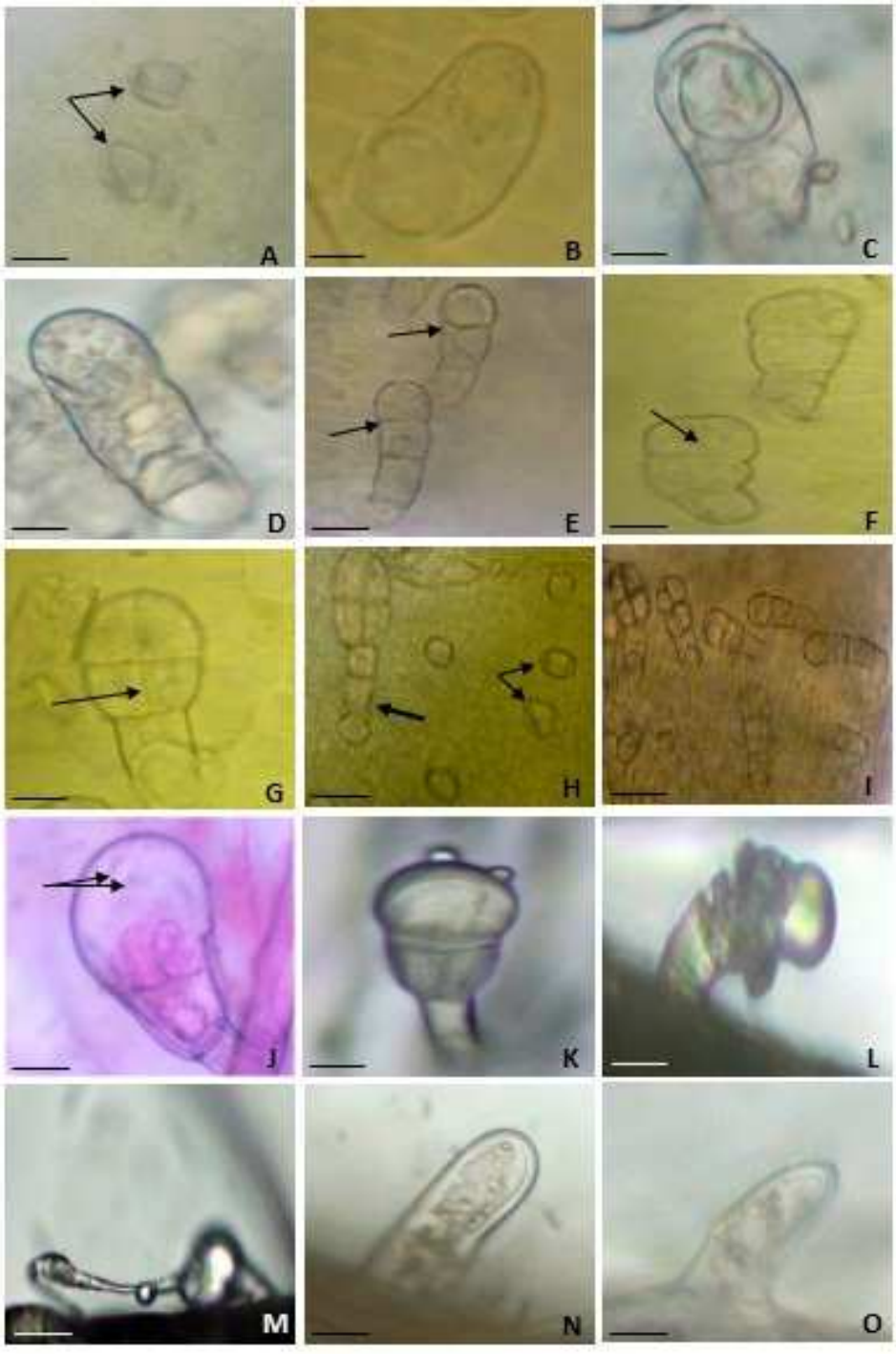

Fig. 3. Ontogenetic stages of *T.foenum-graecum* trichome. (A-I) Presecretory phase: (A) Trichome initial as a bulge on the leaf epidermis. (B) A tubular trichome initial (1-celled stage), (C) 2-celled stage with basal and upper cell, (D) 3-celled stage with a basal, stalk and head initial cell, (E) Periclinal divisions in head cell (arrows), (F) head cells and the first of anticlinal division in upper cell (arrow), (G) The second anticlinal division in lower cell of the head cell. (H) The mature glandular trichome (large arrow) and initial trichomes (narrow arrows) side by side. (I) Various stages of glandular trichome development, (J) Secretory phase with fully extended subcuticular space, secretory droplets can be observed within the subcuticular space (arrows), (K-M) Post secretory phase (releasing secretions) via (K) Micropores, (L) Repture, (M) Channel. (N, O) The trichomes in early ontogenetic stage with round tip for glandular (N) and acute for non-glandular (O) trichome, bar = 20 µm.

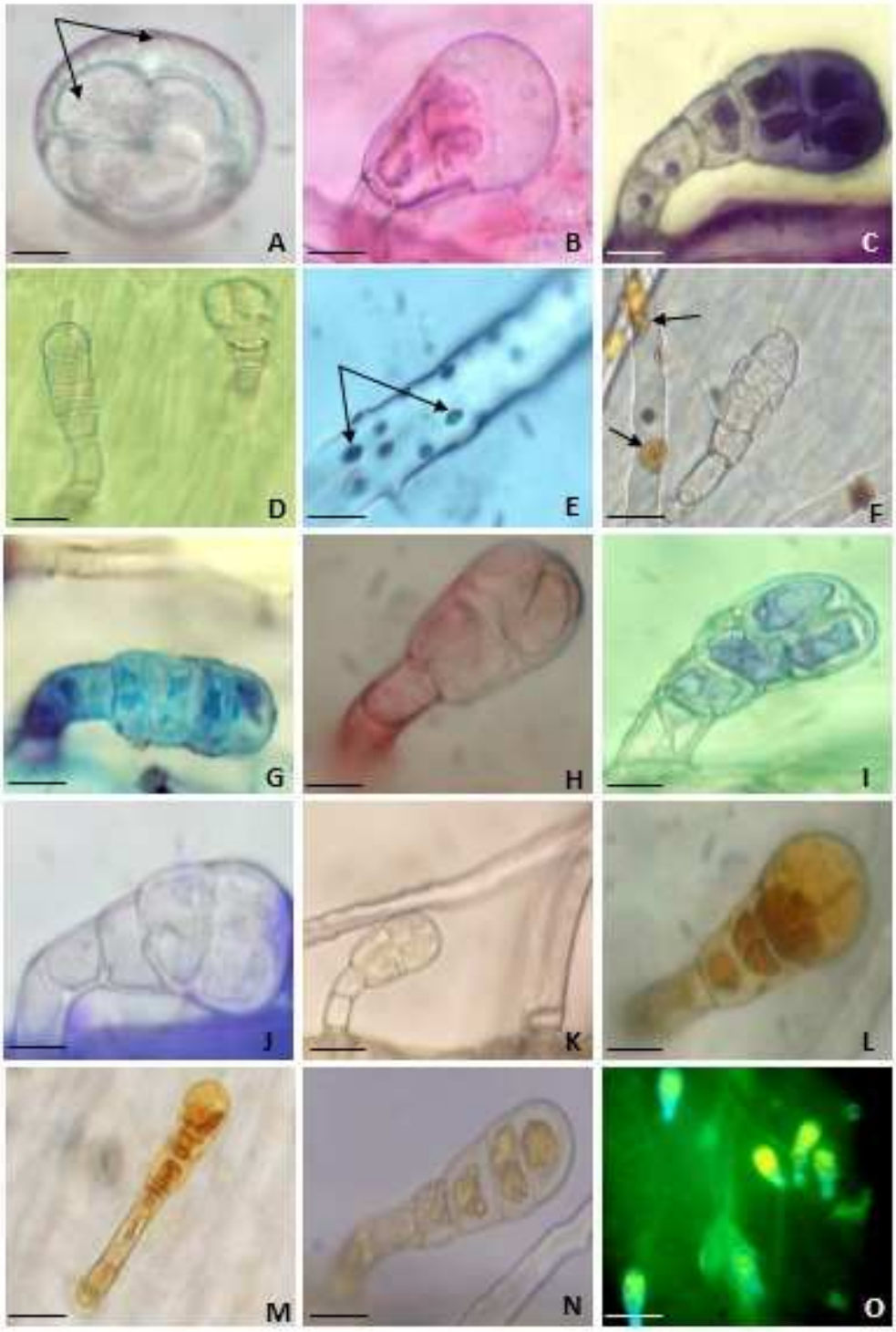

Fig. 4 Light (A-N) and florescence (O) micrographs showing the response of glandular trichomes of *T. foenum-graecum* leaves to histochemical tests. (A) glandular trichome showing colorless secretory material, (B) Positive reaction with PAS for polysaccharides, (C) Pectin stained violet with Hematoxylin, (D) Negative result with Fehling's solution, (E, F) Appearance of ornamentations, (F,G) Nile Blue test showing blue staining of acid lipids, (H) Basal and stalk cell stained red with Sudan red VII B, (I) Ninhydrin test showing violet staining of the amino acids, (j) Negative reaction with Coomassie brilliant blue for proteins, (K) Negative result for essential oils with Nadi reagent, (L, M) Alkaloids stained orange to brown, (L-N) Light brownish color of phenolic compounds with potassium dichromate, (O) Yellow florescence with Neu's reagent for flavonoids in the epidermal cells and head of glandular trichomes,(bar = 7 µm).

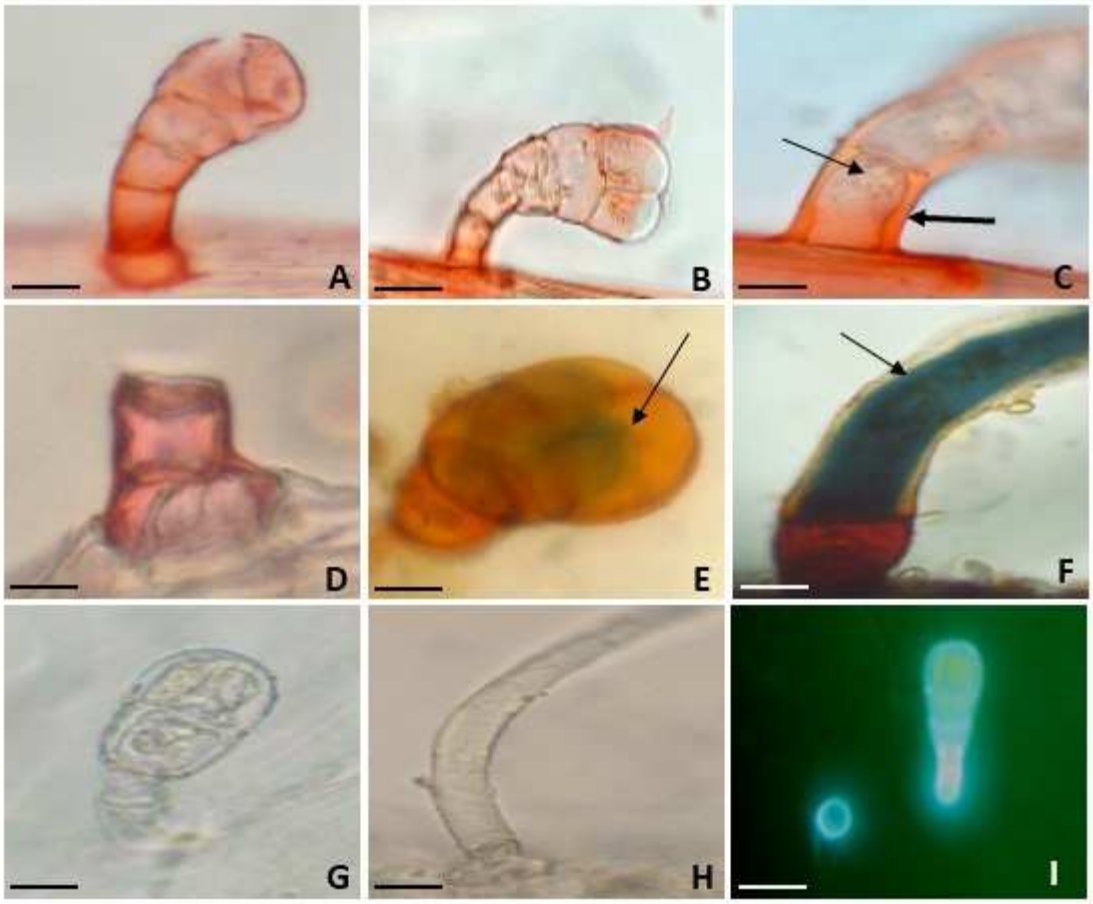

Fig. 5.Light (A-H) and florescence (I) Micrographs showing the cell wall histochemistry of trichomes on the leaves of *T.foenum-graecum*. (A, B) Mature trichome, showing cutinization in the cell walls of basal, stalk and cuticular sheath stained with Sudan III, (A-B). (C) The presence of nuclei in stalk cell, large arrow indicating a thick cutinized stalk cell lateral wall, bar = 7 µm. (D) Fractured stalk cell during tissue processing, (E, F) Primary walls of secretory cells (E) and apical cell of non-glandular trichome (F) was colored blue with Iodine-Sulphur for cellulosic walls, (G, H) Negative result for lignified walls with Phloroglucinol in glandular (G) and non-glandular trichome (H). (I) Silvery white fluorescence for cutinized walls, bar = 40 µm.

CONCLUSION

Glandular trichomes are common in vegetative and reproductive organs of Fabaceae and recent data [28] suggest that they can exert different functions. In this work, histochemical techniques was applied for the first time to the leaves of *T. foenum-graecum*. The present data showed that the secretory trichomes on the leaves cannot be considered as a hydathodes and or nectar, but have protective function as a first line of defence at the surface of the leaf. As well as, these trichomes secrete compounds that are medicinally important. The study of developmental stages indicate that the presence of secretory compounds was independent of the leaf development stage, but it is dependent on the trichome development stage. Since the trichomes density and their exudates decreases with leaf maturity, for optimal exploitation of this external storage compartment, it is recommended the use of emergent leaves. Furthermore, structural characteristics of the secretory trichomes and histochemical analysis would be useful in deciding the protocol required for isolation of their compounds.

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