



RESEARCH ARTICLE

Micromorphological variations of trichomes in the genus *Ocimum* L.

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ABSTRACT

Ocimum L. is an attractive fragrant ornamental plant with medicinal aromatic compounds. The study using digital microscopy revealed the distinct epidermal trichome morphology in five species of *Ocimum* including *O. americanum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum* Gurke and *O. tenuiflorum* L. A distinguished variation in size, shape, type and abundance of trichomes on leaves and stem of all species are described and photographed. Both glandular and non-glandular trichomes scrutinized in all the species in which glandular type comprised capitate and peltate trichomes. On the stem, the longest glandular capitate trichome (GCT) and non-glandular trichome (NGT) were observed in *O. tenuiflorum* while *O. basilicum* possessed longest glandular peltate trichome (GPT). In case of foliar trichomes, the longest GCT observed on leaves of *O. americanum*; and *O. basilicum* showed the longest GPT and NGT. In the species, the septate or aseptate NGT possessed uni, bi or multicellular base with acute or broad apex. A distinguished variation in the number of cells in stalk and head of GT was observed in the study. Presence of silica bodies in peltate trichome of *O. kilimandscharicum* was noticed as the characteristic feature. Among the five species, the trichomes were abundant in *O. kilimandscharicum*; while *O. basilicum* showed sparse distribution. Trichomes with disrupted cells were also noted in *O. americanum*. Due to the presence of specific trichome characteristics, the efficient taxonomic key is prepared to identify the taxa at species level.

Introduction

Lamiaceae includes around 250 genera and 7000 species that particularly well represented in tropical and temperate areas (1). While some species are characteristic of semi-arid conditions and many others are adapted to wet habitats in seasonally flooded areas or along river banks in forest. The family divided into two main groups based on number of aperture and nuclei in shed pollen (2). The first and second group comprises the subfamilies Lamioideae and Nepetoideae respectively (3). Nepetoideae has often strongly aromatic species with volatile terpenoids, rosmarinic acid and hexacolpate pollens.

Most of the members of Lamiaceae display both glandular and non-glandular trichomes on different plant parts (4, 5). The xeromorphic species have well-developed trichomes to ensure the preservation of entire plant from xeromorphic changes (6). Three main types of trichomes like peltate, capitate and non-glandular were observed on the stem, inflorescence axis, leaf and calyx surfaces of *Salvia chrysophylla* Stapf, *Thymus quinquecostatus* Celak and *Lamium truncatum* Boiss that possess the secretions contained

essential oil, polyphenols, flavonoids and acid polysaccharides (7-11). In many of the species, the essential oils produced glandular trichomes spread over the aerial vegetative and reproductive parts. Moreover, the non-glandular trichomes protect the plants from different abiotic stresses like high temperature and light intensity, UV rays, low humidity etc. Recent studies indicate the common occurrence of glandular trichomes in members of Lamiaceae, Bignoniaceae, Asteraceae and Brassicaceae which release the proteins for defensive mechanism or valuable secondary metabolites that can be utilized for the production of pharmaceuticals (4, 12-16).

The genus *Ocimum* L. belongs to the sub-family Nepetoideae of the tribe Ocimeae that included about 65 species of herbs and shrubs (17). Plants of this genus are collectively called 'basil' which play as the source of essential oils and aromatic compounds (18). The valuable medicinal plant, *Ocimum* sp., thrives well in a variety of soils and climatic conditions. Soils suitable for cultivation are rich loam to poor laterite, and saline and alkaline to moderately acidic. Well-drained soil helps to encourage improved vegetative growth. It flourishes well under fairly to high rainfall

and humid conditions, and long days and high temperature have been found to be favourable for plant growth and higher oil production (19). It is native in Asia, Africa, South and Central America, but widely distributed around the world (17). The aromatic and medicinal plants used fresh or dried as a drug in traditional medicine and as a flavoring agent for foods, confectionary products and beverages (20). The plants are used to prepare many herbal medicines due to the antimicrobial properties (21). Plant trichome induction is depending to the level of phytohormones and other primary metabolites produced in the plants during different stages of life cycle that including gibberellins (GA), salicylic acid (SA), jasmonic acid (JA) and cytokinin (CTK). The differential plant metabolism in turn causes variation in trichome morphology and distribution (22). While the information on trichome morphology in different species of *Ocimum* is scanty; so the present study focused on scrutinization of trichome diversity among six species of *Ocimum* in Kerala.

Materials and Methods

Plant collection

For the study, five species of *Ocimum* including *O. americanum*, *O. basilicum*, *O. gratissimum*, *O.*

variations of trichomes. The size, position, type, abundance and structure of base, stalk and apical cell of trichomes were studied using Motic B1-Series Digital Microscope. The morphological trichome variations among the species were photographed by the attached camera of microscope. The size of trichomes measured using Motic Image Plus 2.0 software.

Results and Discussion

Recent plant taxonomy considers trichome structure as the key character for identification of taxon. The present investigation focused on plant morphology and trichome analysis in five major species of *Ocimum* in Kerala, provides a better taxonomic key to identify the species (Table 1). The study revealed the presence of both glandular and non-glandular trichomes in all five species; beyond, the glandular trichomes included capitate and peltate types. The trichomes of the genus categorized into six major types based on their micromorphology (Table 2). As part of the plant systematics, Abdulrahman and Oladele studied the glandular and non-glandular trichomes in six species of the genus *Ocimum* (23). Similarly, Observations are made on the glandular trichomes on the vegetative organs of *Ocimum selloi* Benth (24). In *Ocimum*, the peltate and sessile capitate glandular trichomes were distinct on the

Table 1. Morphological characters of *Ocimum* L. spp.

Species	Morphological characters
<i>O. tenuiflorum</i> L.	Subshrub. Leaves elliptic-oblongeolate, obtuse at both ends, margin coarsely serrate, puberulous; petiole covered with reddish-purple hairs. Raceme simple, occasionally branched at base. Bracts suborbicular. Calyx purplish, campanulate; lobes 5, upper lip ovate, acute, lower lip hispid. Corolla reddish-pink, tubular-campanulate; tube with sessile oil glands; lobes of upper lip oblong-orbicular; lower lip ovate-oblong. Stamens 4. Nutlet sub-trigonous, dark brown.
<i>O. americanum</i> L.	Woody herb. Leaves simple, opposite, elliptic, acute to acuminate, base truncate or obtuse, subentire, sparsely hairy or glabrous. Raceme densely pubescent; flowers 5-7 in each whorl. Calyx upper lip orbicular, densely hairy. Corolla white, glabrous. Staminal filaments glabrous. Nutlet sub-trigonous, dark brown.
<i>O. basilicum</i> L.	Erect annual herb; stem obtusely 4-angular. Leaves ovate-elliptic, base cuneate, margin entire to distantly crenulate, apex acute, puberulous above, pubescent with sessile oil glands beneath. Calyx tube c. 3 mm long, sparsely strigose; upper lip broadly ovate; lower lip hairy within. Corolla cream; tube inflated below; lobes 5. Stamens 4. Nutlet ellipsoid, black, mucilaginous.
<i>O. gratissimum</i> L.	Shrub. Leaves simple, opposite, elliptic to obovate, acuminate at apex, base attenuate serrate. Racemes terminal, panicles; bracts oblanceolate; flowers 4-6 at each node. Calyx glabrous; upper lip ovate, obtuse, lobes of lower lip acuminate, glandular. Corolla white, lobes obtuse. Staminal filaments glabrous or villous at base; anthers sagittate at base. Nutlet brown.
<i>O. kilimandscharicum</i> Guerke.	Perennial herb. Leaf ovate-oblong, pubescent with white hairs. Inflorescence greenish-greyish. Flowers white. Calyx greenish-greyish, densely hairy. Seed black, mucilaginous.

kilimandscharicum and *O. tenuiflorum* were collected from the 'Herbal Garden' of Arya Vaidya Sala, Kottakkal, Malappuram, Kerala and identified by Dr. A. K. Predeep, Department of Botany, University of Calicut.

Trichome study

Both preserved and fresh materials were used for the study. The plants preserved in 10% formaldehyde and prepared herbarium specimens by conventional dry method. For micromorphological study, the thin hand cross-sections made from the live or preserved materials using commercial razor blade. The sections stained with safranin and mounted in glycerine. About 3-5 specimen slides of leaf and stem were prepared in order to study the morphological

stem and leaves of the species. Obviously, the presence of glandular trichomes can be considered as the key character of the present genus.

Trichome diversity on stem

The distinct morphological structural variations in trichomes are visible on leaves and stem of all species. Both environmental and genetic factors determine the unique features of trichomes in each taxon. Sometimes, the different species grown in same environmental conditions showed dissimilar trichome morphology due to genetic reason (25). In *O. americanum*, *O. kilimandscharicum* and *O. tenuiflorum*, stem possesses three types of trichomes including capitate, peltate and non-glandular; while capitate and peltate types were observed in *O.*

Table 2. Different types of trichomes observed in selected species of *Ocimum* L.

Type	Micromorphology
1	Non glandular, base unicellular
	A. Short, aseptate with acute apex
	B. Short, septate with acute apex
2	Non glandular, base bicellular
	A. Septate with acute apex
3	Non glandular, base multicellular
	A. Septate with acute apex
4	Glandular, stalk unicellular
	A. Unicellular stalk with unicellular head
5	Glandular, stalk bicellular
	A. Bicellular stalk with unicellular head
6	Glandular, stalk multicellular.

basilicum and *O. gratissimum*. Among the 5 species studied, *O. tenuiflorum* showed the longest capitate trichome; whereas *O. basilicum* displayed the shortest one on stem. In case of peltate type, *O. basilicum* had longest as well as shortest trichomes. At the same time, *O. tenuiflorum* showed the longest non-glandular trichomes and *O. americanum* exhibited the shortest (Table 3). The considerable variation in density of trichomes also observed between species. The higher trichome density scrutinized on stem of *O. gratissimum*, *O. kilimandscharicum* and *O. tenuiflorum* and these three species had better abundance of different types of trichomes that indicate the formation of unique trichome types in each species (Table 4).

The effect of environmental stimuli such as temperature, altitude and season, drought stress, light intensity and toxicity of minerals on glandular trichome formation has been found in different medicinal plants (15). This multifactorial character is clear from the investigation describing the specific

in the study. In *Begonia dregei*, the deeply incised leaves had larger numbers of longer trichomes at the sinuses; beyond, higher numbers of trichomes observed on the abaxial surface (26).

Among the *Ocimum* spp., *O. basilicum* possessed glandular trichomes only. In this, the capitate type was with unicellular stalk and head, whereas head of peltate type had unicellular or multicellular nature (Fig. 2. M-O). The species is considered as the main site of production of essential oils due to the presence of glandular trichomes (27). The major phytoconstituents of essential oil in *Ocimum* species are phenylpropanoids and terpenoids. Production of these secondary metabolites is correlated with specific genes. This trichome related genes identified from the *Ocimum* species; however, these genes are highly expressed in *O. basilicum* (28).

In *O. gratissimum*, the presence of capitate, peltate and non-glandular types was observed on the stem. Peltate type had basal epidermal cell, unicellular stalk and multicellular head. In contrast, capitate type developed from single epidermal cell. Oftenly, uniseriate 4-celled non-glandular trichomes with multicellular base intermingled with glandular types (Fig. 1. A-C). Similarly, microscopic examination on stem of *O. kilimandscharicum* described the presence of capitate, peltate and non-glandular trichomes in varying density. Capitate trichomes showed unicellular or multicellular base and unicellular head, while the multicellular head observed in peltate type. The long uniseriate non-glandular trichomes had unicellular or multicellular base and hooked or acute apex with flagelliform shape (Fig. 1. H-K). In *O. tenuiflorum*, capitate type possessed unicellular or multicellular stalk and secretory unicellular or multicellular head. In addition to this, peltate type exhibited short and unicellular stalk with broad round multicellular secretory head composed of 4-12 cells. Whilst, the

Table 3. Types and length of trichomes on leaves and stem in different species of *Ocimum* L.

Species	Foliar trichomes (µm)			Stem trichomes (µm)		
	GCT	GPT	NGT	GCT	GPT	NGT
<i>O. americanum</i>	26–79	34–75	50–140	40–60	c. 32.7	81–389
<i>O. basilicum</i>	–	41–83	c. 390	30–32	25–80	–
<i>O. gratissimum</i>	31–44	–	68–322	31–44	33–42	–
<i>O. kilimandscharicum</i>	c. 39	40–80	26–256	40–67	48–56	232–526
<i>O. tenuiflorum</i>	31–48	–	34–145	31–112	43–65	157–846.6

(GCT-Glandular Capitate Trichome, GPT-Glandular Peltate Trichome, NGT-Non-Glandular Trichome)

characteristic features of base, stalk and head in trichomes of *Ocimum* species. In *O. americanum*, capitate trichomes were with multicellular or unicellular base and single celled head; while peltate trichomes possessed unicellular base, stalk and head. A distinguished structural variation observed in non-glandular trichomes; these were uniseriate and 2-5 celled with unicellular base. Moreover, the trichomes carrying disrupted cells were also observed on the surface of stem (Fig. 2. G-J). A significant correlation between the shapes of leaves and the presence, number and size of trichomes among taxa was clear

uniseriate unbranched non-glandular type showed broad or curved apex and unicellular or multicellular base (Fig. 1. P-R).

In most plants more than one type of trichomes was observed; while, the presence of non-glandular trichomes was the most dominant feature of *Vitex negundo* L., *Isodon rugosus* (Wall. ex Benth.) Codd, *Colebrookea oppositifolia* Sm. and *Marrubium vulgare* L. (29). In members of some families, the glandular trichomes were only detected that may or may not be bearing secondary metabolites. The stalked glandular trichomes of *Pelargonium* sp., *Solanum* sp.,

Table 4. Occurrence of different types of trichomes in *Ocimum* spp.

Trichome types	<i>O. americanum</i>		<i>O. basilicum</i>		<i>O. gratissimum</i>		<i>O. kilimandscharicum</i>		<i>O. tenuiflorum</i>	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Type 1-A	+b	-	-	+b	+b	-	-	-	-	-
Type 1-B	+a	-	+b	-	-	-	-	+a	-	-
Type 1-C	-	+b	-	-	+a	-	+a	+b	+a	+a
Type 2-A	+b	-	-	-	-	-	-	-	+b	-
Type 2-B	+b	-	-	-	-	-	+b	-	-	-
Type 3-A	+a	-	-	-	-	-	-	-	+a	+a
Type 3-B	-	+b	-	-	-	+b	-	-	+a	+b
Type 4-A	+b	-	-	-	+b	+a	+b	+a	+b	+a
Type 4-B	-	-	-	-	-	+b	-	+b	-	-
Type 5-A	-	-	-	-	-	-	+b	+b	-	+b
Type 5-B	-	-	-	-	-	-	-	-	+b	+b
Type 6	-	-	-	-	-	-	-	-	+b	-

+ = Present; - = Absent; a = Abundant; b = Occasional

Lycopersicon sp., *Cucurbita* sp., and *Abutilon* sp. consisted ω5-fatty acids, precursor of anacardic acid which can be separated from tissues by isolating the glandular trichomes. The anacardic acid is using in chemical industry to prepare resins, coatings, frictional materials etc. (30).

Foliar trichome morphology

The leaves display trichomes on both upper and lower epidermis having specific functions like protection from environmental stresses. Each species shows distinct diversities in trichome distribution in turn helps to identify the taxa at taxonomic level. However, the young leaves are densely covered with trichomes and the density of trichomes decreases progressively with leaf maturity. Hence, the trichomes are established early in leaf differentiation and their density decreases with leaf development and age (31). The occurrence of capitate and peltate glandular trichomes in leaves of *Ocimum selloi* has been documented (32, 33). It is characteristic feature of the genus *Ocimum* that produce oil drops by the activity of plastids present in the head cells of trichomes (34). Present study confirms the occurrence of glandular and non-glandular trichomes on the abaxial and adaxial sides of *Ocimum* leaves. Leaves showed the occurrence of numerous peltate trichomes compared to the stem. Among the species, *O. basilicum*, *O. americanum* and *O. kilimandscharicum* had glandular peltate trichomes of which *O. basilicum* displayed the longest and *O. americanum* possessed the shortest. Interestingly, all species except *O. basilicum* exhibited capitate trichomes in which *O. americanum* showed the longest one. Always, the length of non-glandular trichome is higher than glandular trichomes that vary from 26-390 μm. From the study, it is clear that *O. basilicum* had longest non-glandular trichomes whereas *O. kilimandscharicum* showed the shortest (Table 3).

There are reports on the occurrence of 8 different types of non-glandular and glandular trichomes on the leaf surfaces in eight species of *Kalanchoe* Adans. (Crassulaceae DC.), which is considered as an important adaptation to the conditions of the natural environment (35).

Likewise, a wide diversity in trichome structure and occurrence is evident on leaves of *Ocimum* species. In *O. americanum*, capitate trichomes were with unicellular base and head; while unicellular or multicellular head observed in peltate type. The uniseriate non-glandular trichomes had acute apex and unicellular or multicellular base (Fig. 2. A-F). But, *O. basilicum* showed peltate and non-glandular foliar trichomes in which peltate type was with multi-cellular head. The non-glandular trichomes had uniseriate and segmented nature with acute apex (Fig. 2. K-L).

Peltate trichomes arise as epidermal protuberances that possess basal cell, stalk and apical head cell. Commonly, peltate glandular trichomes are localized on the adaxial and abaxial leaf surfaces in Lamiaceae. Eventhough, variation in size is highly noticeable among the taxa; they can act as a well-generated excretory system (36). In *O. gratissimum*, capitate and non-glandular trichomes were examined on adaxial side of leaves. Capitate type showed unicellular base, stalk and head; while, the non-glandular trichomes are long, unicellular or multicellular with acute apex (Fig. 2. P-R). Trichomes with homogenous morphology are described in *O. gratissimum* and in other Lamiaceae spp. (13, 37). Similar to *O. americanum*, the trichomes in *O. kilimandscharicum* were glandular and non-glandular type in which capitate trichomes had unicellular base and multicellular head. Interestingly, peltate type showed silica deposition in the multicellular head. The non-glandular type was segmented uniseriate broad with hooked or acute apex (Fig. 1. D-G). Silica deposition in plant tissues is one of the important characteristics of the families Poaceae, Asteraceae, Cucurbitaceae etc. (38-40). In *Pleioblastus chino* (Franch. & Sav.) Makino, the silica deposition observed in epidermal prickly hairs of leaves (40). Similarly, silica bodies were also detected on both abaxial and adaxial epidermis of *Koeleria macrantha* leaves, although their sizes, density and distribution patterns were different.

Usually, the light intensity and trichome development is positively correlated in plants. But there was no effect of light treatments on the glandular trichome density and essential oil

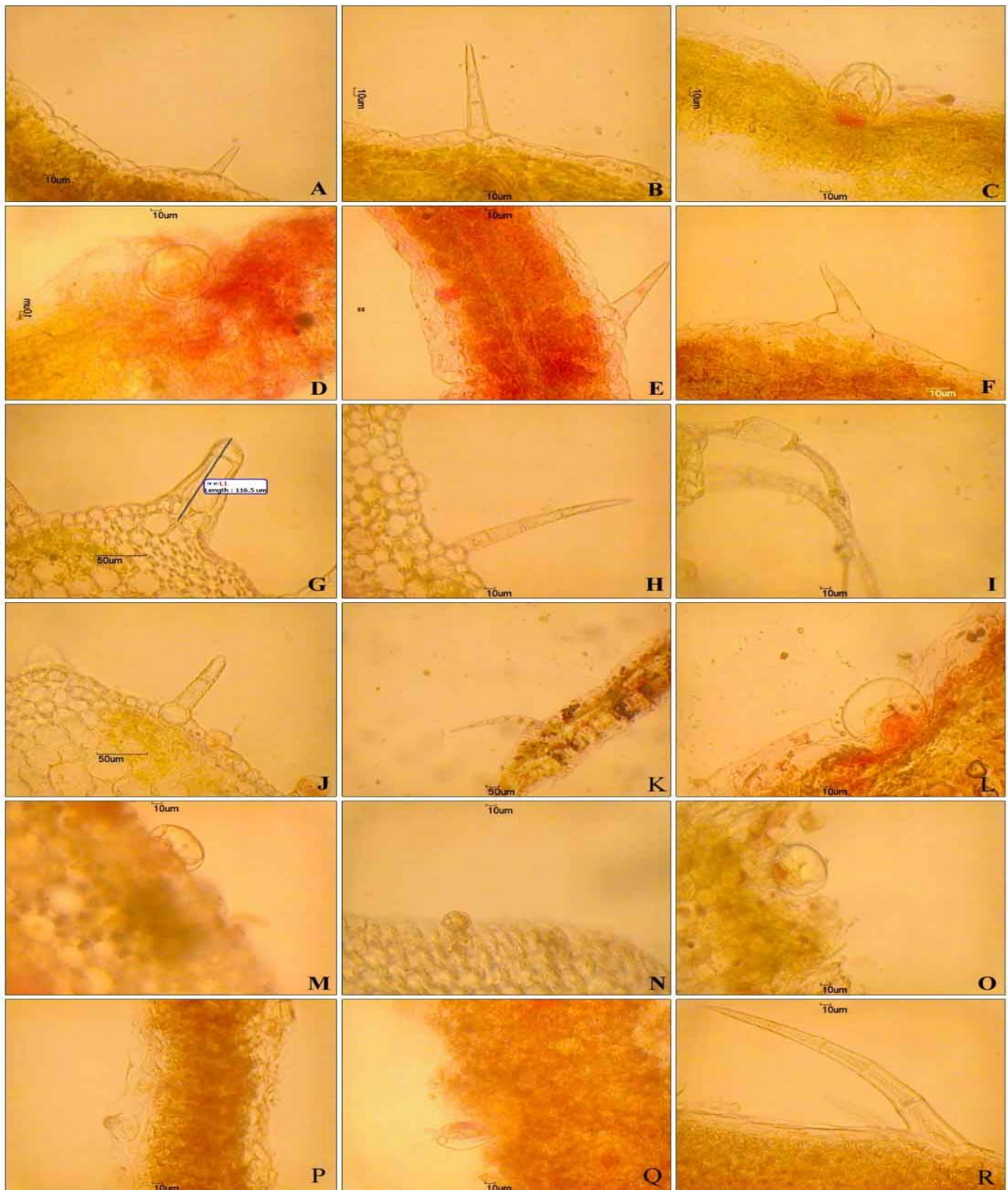


Fig. 2. Trichomes; A-F. *O. americanum* – Leaf; G-J. *O. americanum* – Stem; K-L. *O. basilicum* – Leaf; M-O. *O. basilicum* – Stem; P-R. *O. gratissimum* – Leaf.

content (11). *O. obovatum* had two types of glandular trichomes on the leaves: large peltate and small capitate. In this species, the secretions of peltate and capitate heads are gradually transported from protoplast to the subcuticular space via fracture or pore at the connecting walls of the head cells (27). The rupture of the cuticle on the head cells seems to be a common feature of glandular trichomes in Lamiaceae spp. (41). The essential oil produced on the leaves of *O. selloi* has

repellent and antimicrobial activities to protect the plant from herbivores and pathogens (42). Presence of capitate and non-glandular trichomes in *O. tenuiflorum* makes the species unique from others. Here, capitate type possessed single celled stalk with secretory uni or multicellular head. The non-glandular trichomes were long, segmented, uniseriate with multicellular or unicellular base and broad or acute apex (Fig. 1. L-O). The pronounced expression of animal metallothioneins

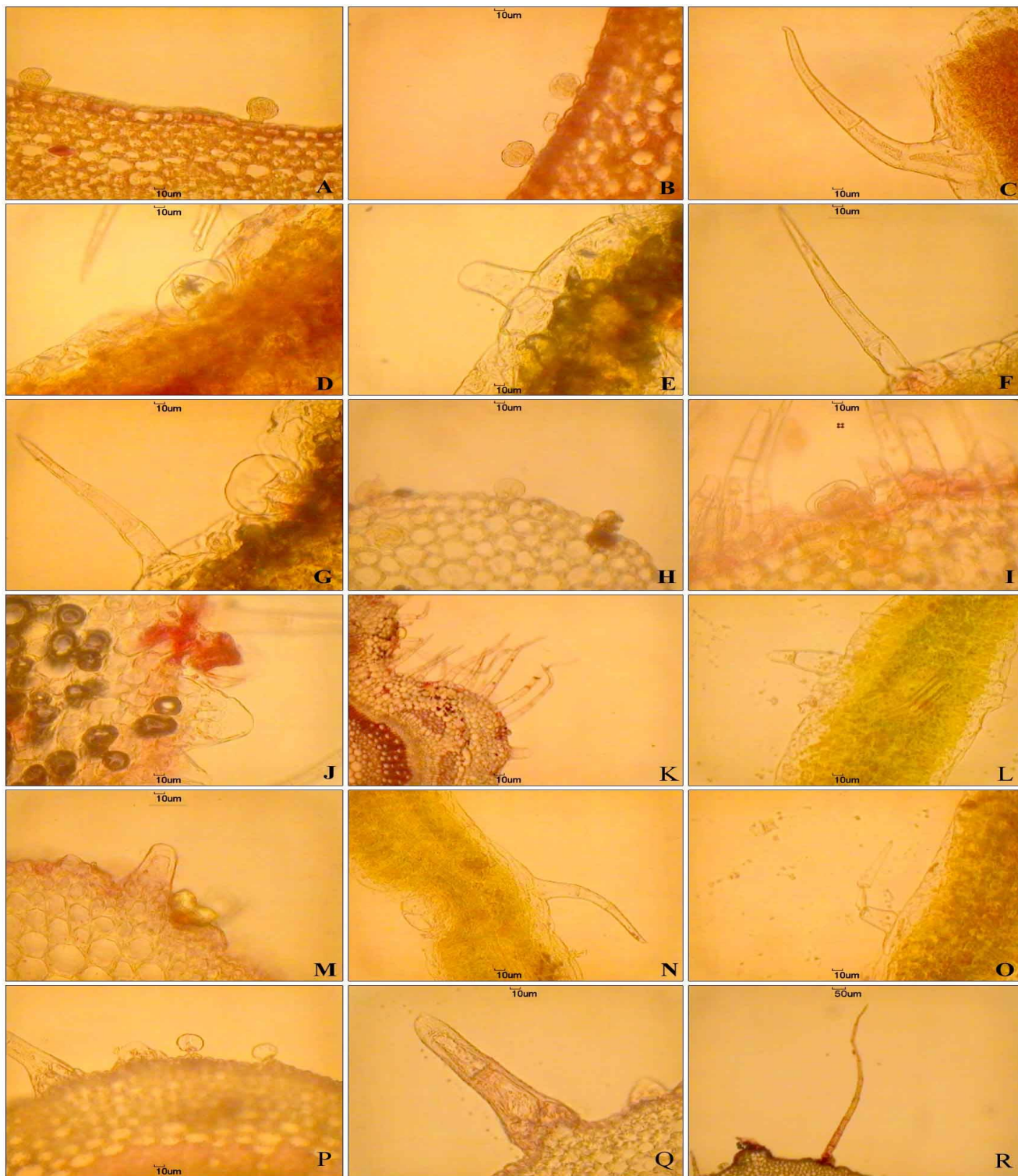


Fig. 1. Trichomes: A-C. *O. gratissimum* – Stem; D-G. *O. kilimandscharicum* – Leaf; H-K. *O. kilimandscharicum* – Stem; L-O. *O. tenuiflorum* – Leaf; P-R. *O. tenuiflorum* – Stem.

(MT) in leaf trichomes are discussed in detail (43). The MTs are cysteine-rich, low-molecular-weight proteins that bind to heavy metals and play a role in plant metabolism and detoxification. It indicates the role of trichomes in detoxification of accumulated heavy metals and corresponding enhancement in plant metabolism.

Key to the species

- 1a. Stem trichomes only glandular type..... (2)
- 1b. Stem trichomes both glandular and non-glandular types..... (3)

- 2a. Foliar glandular trichomes only capitate type *O. gratissimum*
- 2b. Foliar glandular trichomes only peltate type *O. basilicum*
- 3a. Stem or foliar trichomes having internodal thickening (4)
- 3b. Stem or foliar trichomes lack internodal thickening *O. americanum*
- 4a. Foliar trichomes both capitate and peltate type *O. kilimandscharicum*
- 4b. Foliar trichomes only capitate type..... *O. tenuiflorum*

Conclusion

Due to the presence of phytochemical compounds, *Ocimum* spp. are economically important. The glandular trichomes play significant role in the production of these compounds. In this aspect, the study on type and abundance of trichomes on leaf and stem of *Ocimum* spp. are very relevant. Eventhough they are collected from same environmental conditions, the selected species showed prominent micromorphological diversity. In the study, the species have unique trichome features in case of abundance, length and types that are very useful to identify the taxon at genus and species level. Both GTs and NGTs observed in all the species in which *O. kilimandscharicum* possessed abundant trichomes and *O. basilicum* was with less density of trichomes. GTs were peltate or capitate in nature in which the production of useful secondary metabolites were easily visible. The number of cells in head and stalk of GTs were varied in species and some of them possessed silica depositions. Septate or non-septate NGTs observed on leaves and stem, of which disrupted cells included in NGTs of *O. americanum*. The study promises the occurrence of phytochemical compounds in the head of GTs that may enlighten a new path to future studies.

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Authors' contributions

SE conceived, designed and performed the present work. DP critically analyzed the data and wrote the manuscript.

Conflict of interests

The authors have declared that no competing interests exist.

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