

The Role of Floral Morphology and Epidermal Outgrowths in *Etilingera elatior* (Jack) R. M. Smith (Zingiberaceae) True Flower

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ABSTRACT

The inflorescence of *Etilingera elatior* forms tubular, true flowers in an acropetal sequence that blooms ostentatiously. The aromatic plant also produces excellent flavour and fragrance afforded by the presence of secretory structures capable of accumulating, storing, and releasing volatile compounds. However, there is a lack of botanical description of the flowering morphology and the type of secretory structures involved in plant-pollinator interactions. This study aims to describe the floral morphology, characterise the diverse micromorphology of the epidermal outgrowths in the true flower, and analyse their histochemical contents by scanning electron and light microscopes. Labellum defines the anthesis stage of the true flower: unveiling by petals at anthesis and furling inwards that closes the top part of the flower at post-anthesis. In addition to the floral advertisement accessory function demonstrated by the labellum, the closure provides additional exaptation of post-pollination protection to the flower. Both glandular trichomes and osmophores are involved in biochemical functions that release heterogeneous substances (mucilage, terpenes, and phenolic compounds) to help secure anthesis. Non-glandular trichomes, on the other hand, are structurally involved in the floral development by providing physical

and mechanical protection to the flower by acting as glue to maintain the closed structure of the flower, connecting floral accessories, and forming a formidable barrier surrounding the ovary, the most important reproductive organ of the flower. Findings from the present study demonstrate that the presence of secretory structures coordinated with the flower's functional traits enhances the pollination mechanism. It is the maiden report for *E. elatior* on

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epidermal outgrowths and their participation in floral structure and development besides plant-pollinator interaction.

Keywords: Capitate glandular trichomes, corolla tube, epigynous glands, labellum, non-glandular trichomes, osmophores

INTRODUCTION

Flowers are sophisticated, primary organs demonstrating luxuriant display with specialised functions to ensure plant fitness (de Santiago-Hernández et al., 2019). The form and function of flowers often correlate to plant-pollinator interactions designed to maximise the chances of reproductive success (van der Kooi et al., 2021). Floral rewards and secretory structures such as nectary glands, osmophores, and trichomes are often enlisted as accessories to secure pollination. Their presence is associated with olfactory cues that, along with a visual display by the floral ornaments, aim to entice pollinators into visiting the flowers.

Trichomes are epidermal outgrowths on plant surfaces that act as the primary line of defence by providing chemical, mechanical, and physical protection against ecological adversities (Wang et al., 2021). Trichomes are composed of single-cell or multicellular structures. These structures are divided into two general categories: glandular (presence of glandular head) or non-glandular (absence of glandular head), depending on their morphology and secretion ability. Glandular trichomes are the metabolically active type of secretory structures capable of synthesising olfactory or gustatory-repellent compounds (Chalvin et al., 2020). The significance of

glandular trichomes is often highlighted as a natural factory of compounds with high biotechnological interest, such as artemisinin, the antimalarial drug produced in *Artemisia annua* (Huchelmann et al., 2017). The non-glandular type of trichomes, on the other hand, usually protect the plant against excessive light, water loss and extreme temperature by decreasing light absorbance and trapping layers of air that conserve heat and moisture (Karabourniotis et al., 2019; Lusa et al., 2015).

In flowers, trichomes are not only responsible for releasing volatiles with distinctive secondary metabolites to help secure anthesis by glistening the floral surface to appeal to pollinators and facilitate pollen capture and redistribution (Tissier et al., 2017), but they are also structurally important in maintaining floral shape (Tan et al., 2016). According to Tan et al. (2016), trichomes act as glue, especially in young petals, that ultimately help shape the floral bud of cotton (*Gossypium hirsutum*). Reports of trichomes participating in the connection of floral organs are not new; in fact, El Ottra et al. (2013) described the terms ‘coherent’ and ‘adherent’ for trichomes involved in joining the same whorl and different whorls of organs, respectively. Furthermore, the description of trichomes also provides characters for phylogenetic and comparative morphological studies.

Etlingera elatior is a perennial, herbaceous plant indigenous to Indonesia and Malaysia. The plant consists of a leafy and inflorescence shoot system emerging from the rhizome. The leafy shoot can

grow up to \approx 3-4 m in height, whereas the inflorescence shoot can reach up to 1.5 m (Choon & Ding, 2017a). The inflorescence of *E. elatior* is a raceme with 90 to 120 flowers (Choon & Ding, 2016) initiated in an acropetal sequence. *E. elatior* is one of the most popular and most studied species in the family of Zingiberaceae, especially in phytochemistry (Juwita et al., 2018; Sungthong & Srichaikul, 2018). Ornamentally, the perennial plant demonstrates distinct inflorescence borne on a peduncle that blooms ostentatiously like a torch, hence its common name, torch ginger (Choon et al., 2016). The aromatic plant also produces excellent flavouring and fragrance that has attracted scientific communities to investigate the chemical composition of its essential oil (Anzian et al., 2020; Bezerra-Silva et al., 2016; Sangthong et al., 2022). However, despite a great deal of attention on the chemical aspects of the plant, there is a lack of botanical characterisation of the floral morphology and their epidermal outgrowths. More importantly, the type of secretory structures responsible for the floral volatiles in torch ginger has yet to be reported, undermining the plant's potential as a source of pharmacological feedstock. Thus, this study was undertaken to provide data on the role of floral morphology and epidermal outgrowths in the true flower of *E. elatior*.

MATERIALS AND METHOD

Plant Materials

The true flowers of *Etilingera elatior* were collected randomly from Field 2, Faculty

of Agriculture, Universiti Putra Malaysia ($3^{\circ} 00'28''$ N, $101^{\circ} 42'10''$ E). True flowers at pre-, during, and post-anthesis were removed from inflorescence and dissected for examination using a digital colour camera Leica DFC310 FX (Leica Microsystems, Germany) equipped with Leica software application suite (LAS, version 3.8) (Leica Microsystems, 2013).

Surface Analysis

The true flowers were subjected to surface analysis using a scanning electron microscope (SEM). Samples were fixed in FAA (10% formaldehyde, 5% acetic acid, 50% ethanol, which were purchased from Sigma Aldrich[®], Germany) for 24 hr and placed in a vacuum pump to remove air from tissue (Choon & Ding, 2017b). Samples were rinsed thoroughly with distilled water and then post-fixed in 1% osmium tetroxide (Sigma Aldrich[®], Germany) for 24 hr. After a series of dehydration in graded ethanol (50, 60, 70, 80, 90, and 100%, Sigma Aldrich[®], Germany), samples were subjected to critical point drying (Leica EM CPD030, Germany) and then sputter coated (Baltec SC030, Switzerland) with gold. Observations were carried out using JEOL JSM-5610V SEM (JEOL Ltd., Japan) at an accelerating voltage of 15 kV.

Histochemistry Analysis

The chemical content in the secretory structures was investigated in fresh hand-section materials using the following histochemical tests: Nadi reagent (Sigma Aldrich[®], Germany) for essential oils

and terpenes (Caissard et al., 2004), ruthenium red (Sigma Aldrich®, Germany) for mucilage and pectin (Johansen, 1940), Sudan IV (Sigma Aldrich®, Germany) for lipids (Jensen, 1962), and toluidine blue O (Sigma Aldrich®, Germany) for phenolic compounds (Uzelac et al., 2015). Distilled water and lipid removal solution of methanol (Sigma Aldrich®, Germany), chloroform (Sigma Aldrich®, Germany), water, and chloride acid (Sigma Aldrich®, Germany) mixture (66:33:4:1) (Machado et al., 2006) were performed as blank and negative control procedure, respectively. All sections were mounted on a glass slide with a cover slip and then examined with a light microscope (Meiji Techno, Japan) equipped with a digital single-lens reflex (DSLR) camera (Olympus E-420, Japan).

Quantitative Measurements and Analyses

Digitally recorded micrographs were subjected to quantitative measurements and estimation using Image J software (National Institute of Health, Bethesda, USA). The morphological characteristics of the branched, non-glandular trichomes were measured according to Zhang et al. (2005). The secretory structure distribution was quantified in a 10 mm² area and then averaged from ten samples of each plant part and developmental stages. In addition, the dimensions of stalk length, basal cell, and head diameter; shortest, second, and longest branch length of trichomes were measured and expressed in mean ± standard deviation.

RESULTS

Floral Morphology

The true flowers of *E. elatior* are distended by floral bracts (Figure 1a). The floral symmetry of a true flower exhibits zygomorphic (Figure 1b)—a floral accessory comprised of a tubular bracteole with a deep slit on one side. The perianth consisted of a calyx of three-toothed dentate with the apex pubescent and three petals of tubular or oblong shaped with entire margin (Figures 1c–e). The anthesis stage of the *E. elatior* true flower is indicated by the emergence of the labellum, where the pink-coloured petals conceal the labellum at the pre-anthesis stage. During anthesis, deep red coloured with yellow margin labellum emerged and then wilted at post-anthesis by the end of the day, enclosing the top part of the flower (Figure 1f). The pistil is comprised of a long, pubescent style with the upper part wrapped by an anther crest, and the stigma is simple, capitate shaped and is positioned on top of the other (Figure 1g). The stamen of the true flower comprised an anther and a short, arching filament (Figure 1h). The anther is adnate, extrorse, and pubescent around the opening, with the tetrasporangiate pollen dehisced longitudinally prior to anthesis (Figure 1i)—the labellum and the short filament connate to an elongated tube that extends to median-superior of the ovary. The corolla tube of *E. elatior* true flower is reflexed. The lower part of the true flower is comprised of an epigynous gland wrapping the basal style and sits above the closure of the ovary (Figure 1j). The ovary placentation of *E. elatior*'s true flower is the trilocular axile.

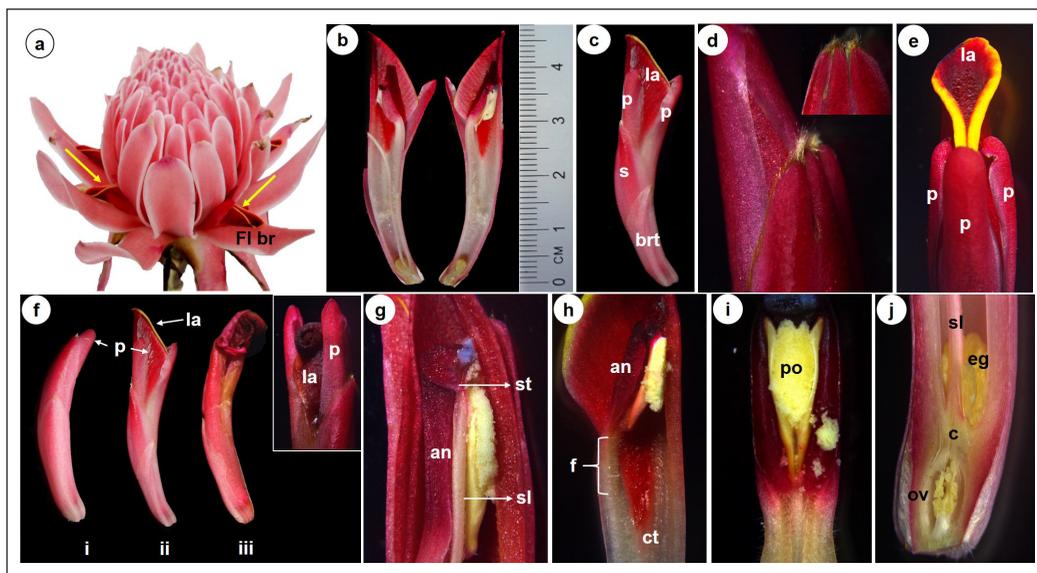


Figure 1. (a) Inflorescence of *Etlingera elatior* at full bloom stage indicated by the emergence of red-coloured true flower (arrow) distended by a floral bract (fl br); (b) Longitudinal cut of true flower showing the floral symmetry as zygomorphic; (c) Lateral view of the whitish pink colour of *E. elatior* true flower comprising a one-sided, deep slit bracteole (brt), sepal (s), and two of the three petals (p); (d) Three-toothed dentate and hairy apex of the sepal; (e) Ventral view of the folded, deep red, and yellow margin labellum (la) surrounded by three petals; (f) True flower at three anthesis stages indicated by the emergence of the labellum, where (i) at pre-anthesis, labellum is concealed by petal, (ii) petals unfurl and labellum emerged during anthesis, and (iii) at post-anthesis, labellum wilted, and enclosed the top part of flower (inset); (g) Lateral view of the upper part of the true flower. Stigma (st) positioned on top of anther (an) that embraces the long style (sl); (h) Arching stamen with short filament (f) and anther is adnate and extrorse. Labellum is connate to the basal filament (fi) that elongates to form corolla tube (ct); (i) Ventral view of the anther with tetrasporangiate dehisced longitudinally; (j) Lower part of the true flower. Epigynous gland (e.g.) surrounding the basal style that sits above the closure (c) of ovary (ov)

Epidermal Outgrowths

Trichomes and osmophores were the epidermal outgrowths of *E. elatior* true flower revealed in this study. There are three floral trichomes in the true flowers of *E. elatior*, i.e., capitate glandular, unbranched, and branched non-glandular.

Capitate Glandular Trichomes. The basal anther and filament of *E. elatior* true flower is covered by a dense capitate type of glandular trichomes (Figure 2a) constituted by basal cell, unicellular stalk,

and glandular head. Two morphotypes are observed as characterised by the stalk length: short and long, undulating stalk (Figure 2b). Short- and long-stalked capitate glandular stalk lengths were 43.1 and 104.0 μm , respectively (Table 1). Short-stalked capitate glandular basal cell and head diameters were 19.0 and 21.9 μm , respectively (Table 1). At the same time, the basal cell diameter and head diameter of long-stalked capitate glandular were 21.8 and 23.9 μm , respectively (Table 1). Three secretory stages are observed,

where, at pre-secretory, a copious number of secretory products accumulate and occupy the entire glandular head (Figure 2c). Secretions are released during the secretory stage, as seen on the glandular head, presumably by means of cuticular exudation since no cuticle ruptures are observed (Figures 2d-e). Following the release of secretion, an empty glandular head is observed at the post-secretory while maintaining its round/oval shape without shrinking (Figures 2f-g). The glandular head was stained positively by Nadi reagent and Sudan IV, indicating the presence of terpenes and lipophilic contents, respectively (Figures 2h-k).

Branched Non-glandular Trichomes. Three morphotypes of branched non-glandular trichomes were found specifically on the protruding region of *E. elatior* true flower corolla tube at exceptionally high density (Figures 3a-b). The morphology of branched non-glandular trichomes is characterised according to Zhang et al. (2005). Type A is characterised by two branches with distinctively unequal lengths (Figure 3c), where the stalk length, basal cell diameter, and shortest and longest branch length were 903.2, 41.3, 125.4, and 307.4 μm , respectively (Table 1). Type B is characterised by two branches, where the shortest branch seemingly appeared defective as if branch

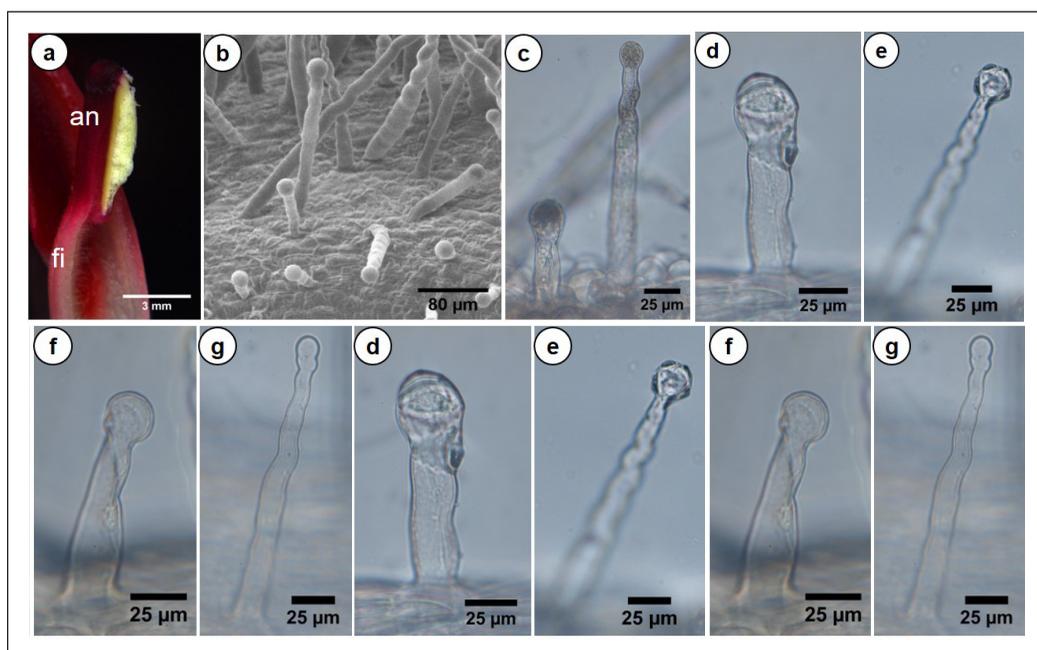


Figure 2. (a) Dense pubescence on basal anther (an) and filament (fi) of *Etilingera elatior* true flower; (b) Scanning electron microscope micrograph of short- and long-undulating stalk of capitulate glandular trichomes; (c) Capitulate trichomes at pre-secretory stage with secretion materials accumulated in the glandular head; (d–e) Trichomes at secretory stage with exudates secreted from the glandular head: (d) Short-stalked; (e) Long-stalked trichome; (f–g) Empty glandular head at post-secretory stage; (h–i) Positive histochemical tests by Nadi reagent indicating the presence of terpenes; (j–k) Secretion materials tested positive by Sudan IV indicating the presence of lipophilic materials

Table 1
Morphological characteristics of floral trichomes in true flowers of *Etilingera elatior*

Type of trichomes	Stalk length (µm)	Basal cell diameter (µm)	Head diameter (µm)	Shortest branch length (µm)	Second branch length (µm)	Longest branch length (µm)
Capitate glandular						
Short-stalked	43.1 ± 15.7	19.0 ± 4.4	21.9 ± 2.5	N. A.	N. A.	N. A.
Long-stalked	104.0 ± 43.9	21.8 ± 4.0	23.9 ± 4.2	N. A.	N. A.	N. A.
Branched, non-glandular						
Type A	903.2 ± 175.4	41.3 ± 15.5	N. A.	125.4 ± 41.2	N. A.	307.4 ± 106.4
Type B	513.7 ± 118.3	29.2 ± 12.6	N. A.	29.8 ± 9.4	N. A.	220.6 ± 66.2
Type C	489.7 ± 61.1	31.2 ± 7.6	N. A.	26.3 ± 8.9	181.3 ± 30.8	272.0 ± 56.8

Note. Data are mean ± standard deviations of $n = 10$; N. A. = Not applicable

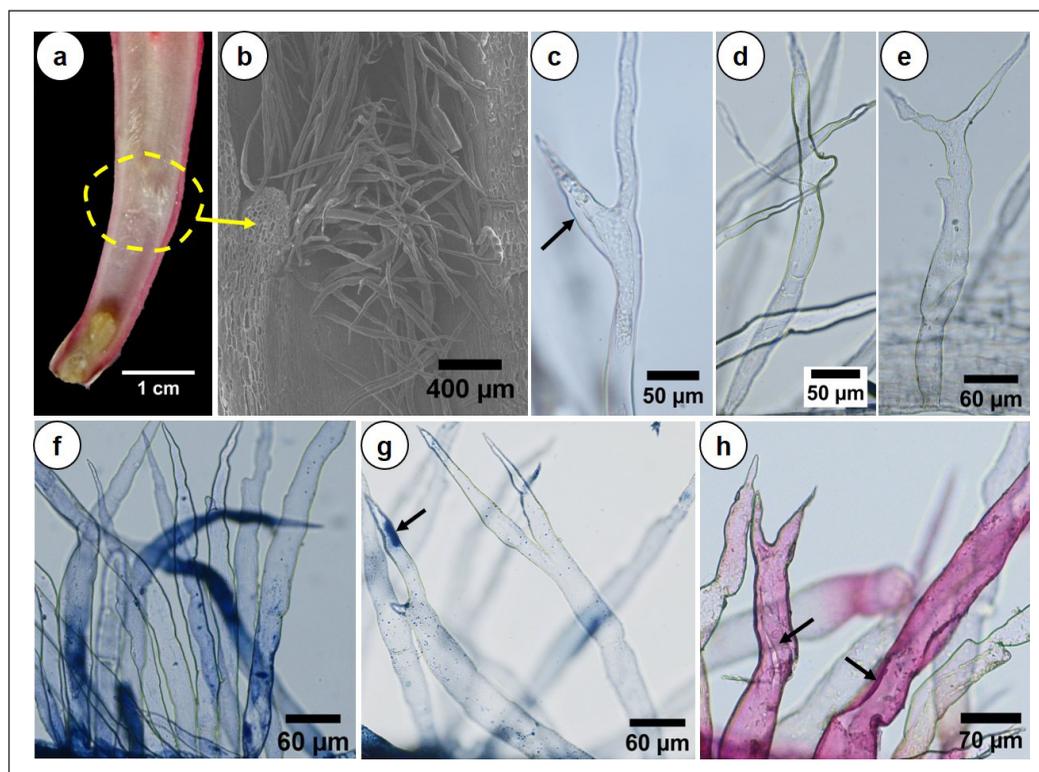


Figure 3. (a) Corolla tube of *Etilingera elatior* true flower. Protruding region (circle), where branched non-glandular trichomes occurred at high density; (b) Close-up view of the protruding region viewed under scanning electron microscope; (c–e) Branched non-glandular trichomes: (c) Type A with one of the branches shorter than the other. Note the thick cuticle of the trichomes indicated by arrow; (d) Type B with the shortest branch seemingly appeared as defect due to aberrant expansion of the branch; (e) Type C with three branches and the third, shortest branch on the lower stalk; (f–g) Nadi reagent staining of the trichomes indicating the presence of terpenes; (g–h) Arrows indicate the slit cuts resulted during the transverse hand sectioning of corolla tube that provided leeway for the staining of the internal content of the trichomes; (h) Staining by ruthenium red for mucilage content

elongation stopped abruptly during trichome morphogenesis (Figure 3d). The stalk length, basal cell diameter, and shortest and longest branch of *E. elatior* type B branched non-glandular trichomes were 513.7, 29.2, 29.8, and 220 μm , respectively (Table 1). Type C appeared as a morphed combination of types A and B, with three branches total (Figure 3e). Two branches are either similar in length or slightly shorter than the other one, whereas the third branch appeared as a defect. The stalk length, basal cell diameter, shortest, second, and longest branch of *E. elatior* type C branched non-glandular trichomes were 489.7, 31.2, 26.3, 181.3, and 272.0 μm , respectively (Table 1).

All three types of branched trichomes are constituted by thick cuticles, as evidenced by the histochemical tests. Staining was proven difficult to permeate the hydrophobic cuticle unless the cut trichomes (indicated by arrows in Figures 3g–h) resulted from the hand sectioning provided leeway for the reagents to react with the internal content of the trichomes. Nonetheless, positive staining by Nadi reagent and ruthenium red indicated the presence of terpenes and mucilage in the trichomes (Figures 3f–h).

Unbranched Non-glandular Trichomes.

The true flowers of *E. elatior* bear three morphotypes of non-glandular trichomes. The first type is undulating, unicellular with thinly cutinised lateral walls and pointed apex, mostly found on the filament (Figures 4a–b). The second type is uni-, bi-, or multicellular, with a thickly cutinised lateral wall and a rounded apex (Figures 4c–d).

These structures are found on the protruding regions of the corolla tube. The third type is a simple, unicellular structure with a thinly cutinised lateral wall and a pointed

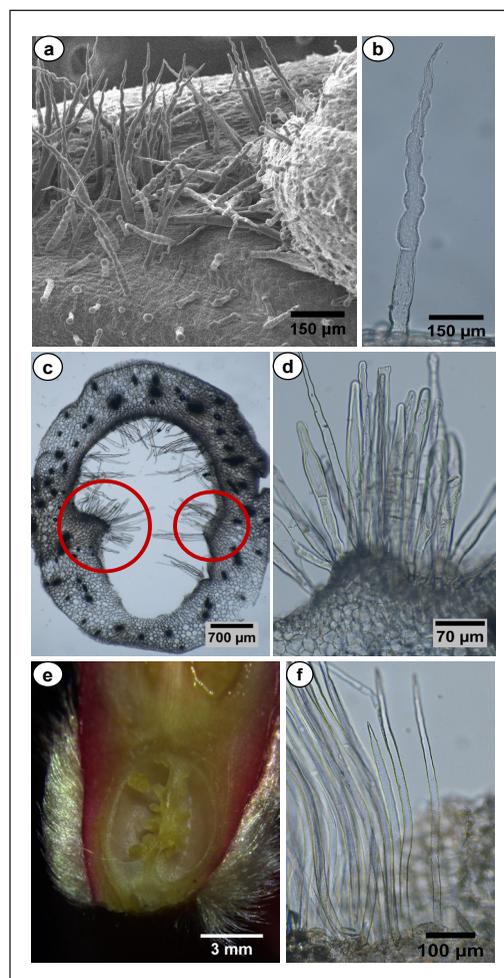


Figure 4. (a) Scanning electron microscope micrograph of dense non-glandular trichomes on the filament of *Etilingera elatior* true flower; (b) Undulating stalk with thinly cutinised lateral wall and pointed apex; (c) A transverse section of the true flower, viewed using a light micrograph, shows the protruding regions (red circled) of the corolla tube where non-glandular trichomes occur abundantly; (d) Uni- to multicellular non-glandular trichomes with thickly cutinised lateral wall and globose apex; (e) A thick layer of trichomes at the base of the true flower that surrounds the ovary; (f) Long, unicellular trichomes with pointed apex

apex (Figures 4e–f). These structures occur abundantly on the basal part of the true flower, creating a thick layer that surrounds the ovary.

Osmophores. In addition to trichomes, osmophores that consist of an epidermis of specialised secretory cells and/or secretory parenchyma (Tölke et al., 2018) were also observed in *E. elatior* true flower. Unicellular, rounded and sometimes irregular protuberances are observed on the basal anther (Figures 5a–b) and are distinct from all other neighbouring cells. Light micrographs of fresh, hand-cut sections revealed that the structures appeared as layers of translucent convex with smooth cuticles (Figure 5c). The epidermal cells exhibited polarity, composed of thick cuticles, and stained positively by ruthenium red for mucilaginous polysaccharides (Figures 5d–e). The presence of mucilage, terpenes, and phenolic compounds by Nadi reagent, ruthenium red, and toluidine blue O, respectively, indicated that the structures may be osmophores (Figures 5f–h) because *E. elatior* true flowers engaged in scent production (Lee, 2019). The floral fragrance of *Anacardium humile* and *Mangifera indica* have been related to the presence of osmophores (Tölke et al., 2018). However, further investigation using a transmission electron microscope is needed to confirm this structure.

DISCUSSION

Floral Traits and Structure

The floral characteristics of *E. elatior* reflect the general features of Zingiberaceae, being

pentacyclic and zygomorphic (Piñeyro-Nelson et al., 2017). Both labellum and epigynous glands are prominent features of the family, especially in terms of formation (Song et al., 2007). In this study, labellum

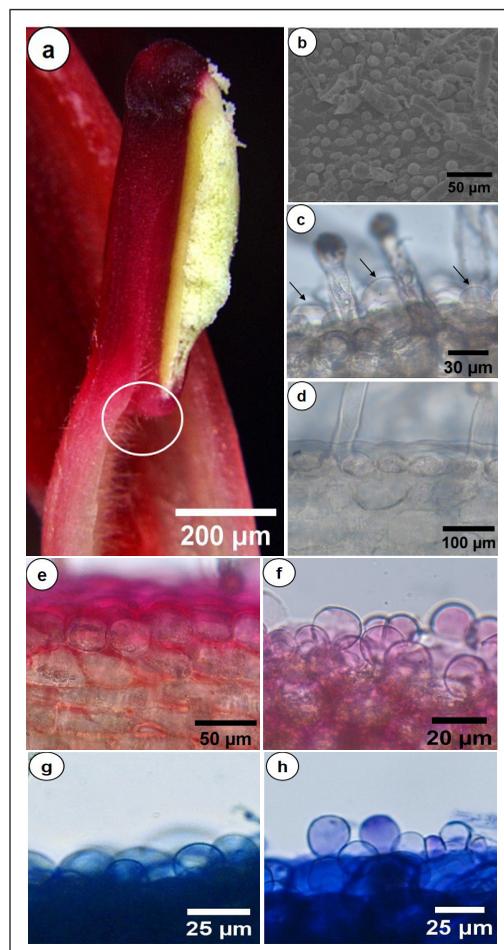


Figure 5. (a) Dense pubescence on basal anther (circle) of *E. elatior* true flower; (b) SEM micrograph of the osmophores appeared as globular trichomes; (c) Light micrograph of the osmophores as translucent convex (arrows) layers with smooth cuticle on the epidermal surface; (d) The epidermal cells comprised of single layer exhibiting polarity; (e) Positive staining by ruthenium red indicates the presence of mucilaginous polysaccharides on the epidermal surface; (f–h) Positive histochemical tests of the osmophores for ruthenium red (f), Nadi reagent (g), and toluidine blue O (h)

demonstrates an important function as an additional exaptation with the striking combination of red-coloured and yellow margins, serves primarily as a visual attraction (Armbruster, 1997). The classic floral advertisement system indicates an open invitation and an announcement that the pollen dehisced prior to the anthesis of *E. elatior* is ready for pollination, or rewards are available for collection. By the end of the day, the labellum wilted, and the top part of the flower was marked as post-anthesis. Given the acropetal architecture of the inflorescence, where flowers open sequentially by the ring, the labellum closure suggests protection, especially for successfully pollinated flowers and ensures limited contamination or disruption from constant visitation by pollinators.

Structurally, the labellum contained within the petals provides a tubular rigidity of the flower (Bull-Hereñu et al., 2022), presumably assumed by the folding of lateral lobes that enclose the stamen and pistil. The connotation of the labellum and the basal filament that elongates to the base of petals and extends to the median-superior of the ovary forms the corolla tube of *E. elatior*. According to Pedersen (2004), the corolla tube formation and the labellum furling inwards that encloses the top part of the flower at post-anthesis are automorphy characteristics of *Etilingera*.

The tubular flowers of *E. elatior* is associated with nectar-feeding birds such as hummingbirds, sunbirds, and spiderhunters as the main pollinators of *E. elatior* (Kittipanangkul & Ngamriabsakul,

2006; Sakai et al., 2013). Lesser banded hornets (*Vespa affinis*), spiders, and ants are also commonly observed visiting the flowers at the research plot. Accordingly, the long needle-like bills of the avian are adapted to nectar exploration long enough to prod through the corolla tube (Rico-Guevara et al., 2019; Riegert et al., 2011). The main source of rewards in *E. elatior* is epigynous glands located at the basal corolla tube surrounding the style (distance from stigma was 3.7 ± 0.7 cm). In Zingiberaceae, nectaries are transformed into epigynous glands that are also known as “supragynopleural” nectaries (Box & Rudall, 2006) or stylodes (Khaw, 2001). Closure of the locules that separate the epigynous nectary provides protection, especially the ovule, from damage inflicted by the rigorous movements of pollinators (Kirchoff, 1998).

Floral Trichomes and Osmophores as Secretory Structures

The true flowers of *E. elatior* produce sweet, scented fragrance as non-food rewards and nectar, protein-rich pollen, and oil as food rewards. Osmophores have been associated with producing heterogeneous substances such as mucilage, terpenes, and phenolic compounds (Tölke et al., 2018). On the other hand, capitate glandular trichomes secrete terpenes and lipophilic compounds (Guo et al., 2020). Generally, the chemicals produced by the secretory structures on the stamen indicate floral cues that attract pollinators and signalling pathways to locate primary rewards (Essenberg, 2021). The abundance of mucilaginous polysaccharides

suggests the significance of the secretion as glistening the epidermal surface of anther to attract pollinators and help pollen get glued on the bodies of visitors (Koptur et al., 2020), as well as a carbohydrate source for metabolic processes (Pansarin et al., 2014).

Terpenes and lipophilic compounds constitute floral volatiles that enable long-distance attraction of pollinators by perfuming their immediate environment (Guo et al., 2020). Essential oils analysis of the inflorescence head at full bloom stage revealed the presence of (*E*)-caryophyllene and (*E, E*)- α -farnesene (Lee, 2019), which are commonly emitted in floral organs (Chakira et al., 2022; Qiao et al., 2021; Yang et al., 2022). The secreted materials observed on the glandular heads suggest oil rewards that pollinators can collect, especially bees, although there is no direct evidence to support this. Alternatively, the exudates could play dual roles as repellents to herbivores or illegitimate, non-pollinating nectar collectors (Johnson et al., 2017; Mesquita-Neto et al., 2020).

The occurrence of osmophores and glandular trichomes indicates the strategic pollination mechanism exhibited by *E. elatior*. The olfactory cues that led visitors to the flower increased the possibility of pollen distribution from the anther to the body parts of pollinators by the sticky mucilage as they foraged deeper into the corolla tube for nectars. Subsequent pollen redistribution onto the stigma is possible as the visitors retreat from the corolla tube. On the other hand, the presence of phenolic compounds suggests deterrent chemicals

that make plants hostile to herbivores, especially predatory insects, from acquiring protein-rich pollen (Wang et al., 2021; War et al., 2018).

Floral Trichomes Participate in Floral Development

Trichomes are abundant in several locations of the *E. elatior* true flower: the apex of three-toothed dentate margins of sepal, filament and style, corolla tube, and basal flower surrounding the ovary. The apical trichomes on the sepal suggest mechanical protection, especially during the early ontogeny prior to the petal emergence and anthesis. The trichomes acted as a seal by interlocking the apex of the true flower, thereby maintaining a closed structure that would allow the development of internal floral organs with minimal interruption from external forces (Takeda et al., 2013) (Supplementary data Figure S1). This finding appeared to substantiate Zhao and Chen's (2016) on the function of trichomes in cotton (*Gossypium hirsutum*), which maintains the correct flower shape while protecting the young tissues.

Similarly, the non-glandular trichomes on the basal true flower of *E. elatior* enveloped the sepal act as 'Velcro' to the bracteole (Supplementary data Figure S2). In addition, the thick layers of trichomes surrounding the ovary create a formidable barrier that enhances greater protection of the most important reproductive organ by providing insulation from desiccation and, particularly, the seeds against predators during the later fruiting stage (Armbruster, 1997).

The clusters of trichomes on the protrusion regions of the *E. elatior* corolla tube coincided with the fusion of petals (Supplementary data Figure S3). Observation in the early ontogeny of the true flower showed that the clusters of trichomes are present during the simultaneous deepening of the corolla tube

and petal elongation, as well as separation of common primordia (Supplementary data Figure S4), indicating their presence as anchor and protection to the developing reproductive organ. In addition, the clusters also indicate another hurdle of deterrents for predatory microorganisms such as parasitoids from accessing the source

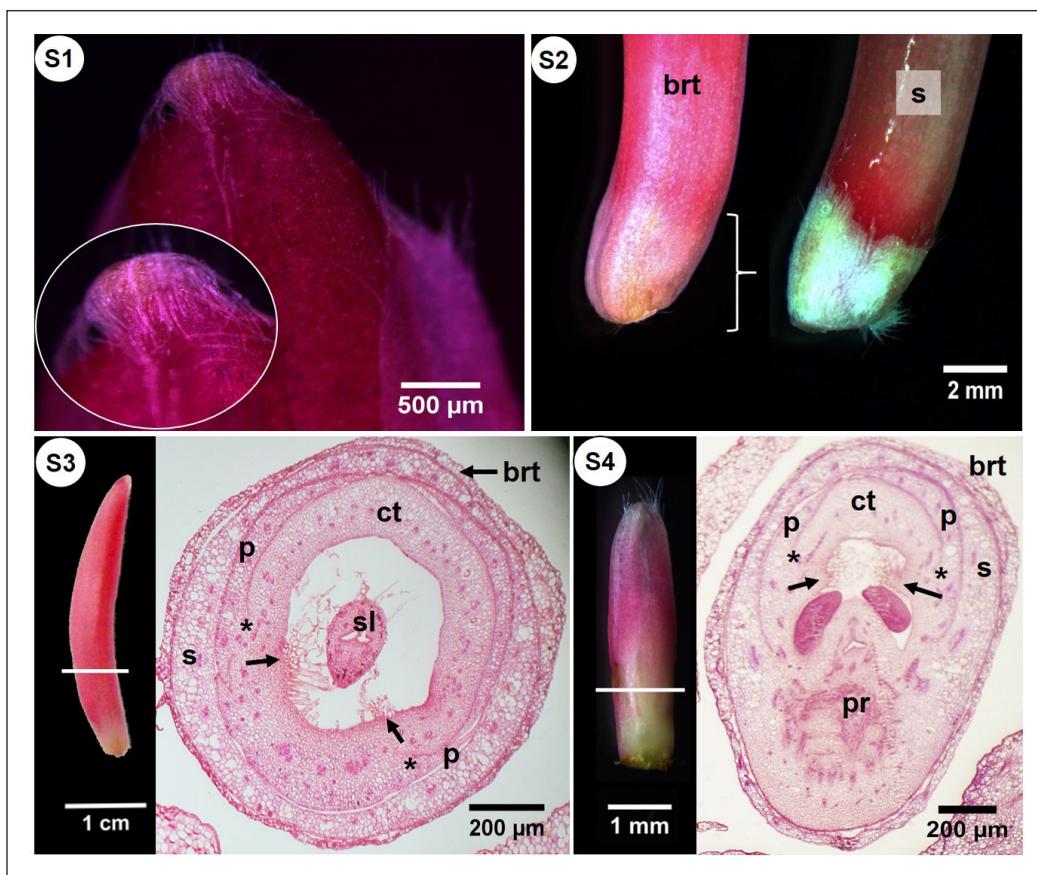


Figure S1. The apex of the sepal covered by interlocking non-glandular trichomes helps to maintain a closed structure of the developing true flower of *Etilingera elatior*; (S2) Basal true flower showing the pink-coloured bracteole with the curly brace indicates the non-glandular trichomes enveloping the red-coloured sepal and acting as ‘Velcro’ to the bracteole; (S3–S4) Transverse section of true flower stained in astra blue where the occurrence of branch non-glandular trichomes (arrows) coincided with the growth of petals on the corolla tube (asterisk). The solid line on the left figure of the true flower reflects the transverse cut of the anatomy: (S3) True flower development at stage completed sepal development; (S4) Early ontogeny of the true flower where petal primordia elongate, corolla tube deepens, and common primordia separates
Note. The clusters of trichomes (arrows) on the corolla tube present prior to the separation of common primordia; brt = Bracteole; ct = Corolla tube; p = Petal; pr = Primordia; s = Sepal; sl = Style

of rewards located at the basal flower, especially during the anthesis.

Interestingly, the morphology of branched trichomes in *E. elatior* also appeared similar to the mutational phenotype of *IRREGULAR TRICHOME BRANCH₁* (*ITB₁*), which controls trichome morphogenesis in *Arabidopsis*. According to Zhang et al. (2005), the *ITB₁* genes regulate anisotropic expansion of the stalk and branches that produce bloated and misshapen branched trichomes. The fact that both branch and unbranched non-glandular trichomes with diverse morphotypes occurred in two specific clusters suggests a complex interplay of cellular interactions during the expansion of the corolla tube and petal elongation. Trichome formation in the corolla tube is regulated by the *AmMYBML1* gene in *Antirrhinum majus* (Serna & Martin, 2006).

The developmental processes of flowering organs such as sepals, petals, stamens, and carpels involve specific control by floral organ identity genes that are able to repress trichome proliferation (Matías-Hernández et al., 2016). Given that the clusters of trichomes occurred at the fusion point of petals to the corolla tube, it is, therefore, curious if the branched trichomes in *E. elatior* are associated with any mutational consequences of cell expansion derived from the ontogeny of floral organs in the true flower. Genetic analysis of the trichomes will provide insights into the differential regulation of cell expansion within the floral tissues and characters for future taxonomic delimitation

and phylogenetic relationships currently lacking in Zingiberaceae.

CONCLUSION

The floral traits of *E. elatior*, mainly the tubular characteristic, demonstrate how nectar-feeding birds are associated as the main pollinators in the flower-pollinator relationships. The labellum proved to be more than just an advertisement accessory as it is commonly regarded; it was also an additional exaptation to protect the flower. The present study also demonstrates the presence of secretory structures coordinated with the functional traits of the flower to enhance the pollination mechanism. Both glandular trichomes and osmophores signified their importance in securing anthesis by releasing secretion materials as indicated by the histochemistry, providing a signalling pathway that attracts pollinators and deters herbivores from robbing the floral rewards. The diversity of trichome structures, possibly with different concoctions of chemical signals, contributes to the survival strategies of the plant in mediating the environment during the anthesis period. Specifically, the non-glandular trichomes continually provide physical and mechanical protection and are involved structurally throughout flower development. Although further studies are required to confirm the underlying mechanism of the occurrence of trichomes in clusters at the corolla tube, this is the maiden report for *E. elatior* on trichomes diversity and their participation in floral structure and development besides plant-pollinator interaction.

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