Histochemical and surface microstructural analyses of floral cuticles provide evidence for differential behaviors in scent volatiles emission in two tuberoses (*Agave amica* (Medik.) Theide and Govaerts) cultivars from Asparagaceae

Raktim BHATTACHARYA [ID], Adinpunya MITRA* [ID]
Natural Product Biotechnology Group, Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur, India

Abstract: Two popular cultivars of tuberose (*Agave amica* (Medik.) Theide and Govaerts syn. *Polianthes tuberosa* L.) from the family Asparagaceae, widely cultivated for floricultural purposes are 'Calcutta Single' (CS) and 'Calcutta Double' (CD). It was evidenced that CS flowers emit intense scent volatiles as compared to CD flowers, while CD flowers retained higher contents of internal pool of scent volatiles than CS flowers. Such contrasting observations between the flowers of two cultivars suggest the role of possible barriers such as cuticles that prevent the emission of scent volatiles. The cuticular structure of both cultivars was studied using different histochemical approaches, including light and epi-fluorescence microscopies. Scanning electron microscopy (SEM) was used to understand the cuticular deposition in the cultivars. Our observations confirmed relatively higher cuticular thickness in CD flowers than in CS. SEM results showed the accumulation of wax crystalloids in CS flower and the formation of cuticular nanoridges in CD flowers over the surfaces of the tepals. In addition, accumulation of lipid droplets was also noticed on the tepal peels of the cultivars showing a denser deposition in CD. Our observations suggest that the presence of wax crystalloids in CS flowers enhances the emission of volatiles, while in CD flowers, the presence of thick cuticular nanoridges possibly deters the scent emission and accumulated as an internal pool in higher amounts. The stomatal behavior also indicates a proportional relationship between cuticular deposition and scent emission; low cuticular deposition corresponds to higher emission of floral volatiles emission and vice versa as observed in CS and CD flowers, respectively.

Key words: *Agave amica*, cuticular nanoridges, epicuticular wax crystals, lipid droplets, SEM

1. Introduction

Cuticle is the first line of defense against pathogen attacks in plants (Yeats and Rose, 2013). Besides acting as a defensive barrier, the cuticle also acts as a transpiration barrier to safeguard the plants from excessive water loss (Kunst and Samuels, 2009). The development of cuticles can vary differently depending on the plant species. Though the basic mode of development is similar the cuticular nature varies extensively among different species and in some cases among different cultivars (Yamamura and Naito, 1983). Another very important but less exploratory property of a cuticle is its self-cleaning nature (Shepherd and Griffiths, 2006). The self-cleaning nature of the cuticle is largely due to the hydrophobicity of the constituent chemicals. Cuticular compounds are mostly waxy in nature, mainly composed of fatty acids, and thus strongly hydrophobic (Samuels et al., 2008). Anatomically a cuticle is mainly composed of three basic parts, an external cuticle layer, and an internal cuticle layer; sandwiched between these two layers, sometimes

a middle layer can be found (Yeats and Rose, 2013). The anatomical features can be varied among different species but the external cuticle layer is a common occurrence throughout the plant kingdom.

Although plant cuticles from vegetative organs were studied for the last several decades, floral cuticle, however, attained attention in the past few years as it is considered as the final barrier to overcome for emission of volatiles in the atmosphere. Few studies have recently been undertaken to understand the nature of floral cuticle and its relation to volatile emissions. In *Petunia*, it has been found that floral cuticle plays an integral part in the scent volatiles network and actually initiates the emission of volatiles (Liao et al., 2021). A recent study reported a thin layer of epicuticular deposition in the floral cuticle, but the characteristic triple layered structure of cuticle is not a common occurrence in flowers (Livingston and Samuels, 2021).

Tuberose [*Agave amica* (Medik.) Theide and Govaerts syn. *Polianthes tuberosa* L.] from the family Asparagaceae are widely cultivated in the subtropics for harnessing flowers

* Correspondence: adinpunya@gmail.com

to be used for ornamentation and cosmetic application (Maiti et al., 2014). Several studies had been undertaken earlier on tuberose to understand the mechanism of scent volatile emission (Maiti and Mitra, 2017) including the characterization of scent volatiles in different commercially available cultivars of tuberose (Kutty and Mitra, 2019). Among the different cultivars of tuberose, two of them are very well-known for their ornamental and fragrant flowers. These are 'Calcutta Single' (CS) containing a single whorl of tepals and 'Calcutta Double' (CD) containing two whorls of tepals, commercially cultivated throughout India (Kutty and Mitra, 2019). From the previous studies conducted by Kutty and Mitra (2019), it was found that in CS flowers the amount of emitted volatile is higher than that of CD flowers, whereas the internal pool of volatiles retained in much higher amounts in flowers of the latter cultivar (Figure 1). Upon observing the floral surface, we conceived that the presence of a thick waxy cuticle in the tepal surface of the CD cultivar plausibly hinders the scent volatiles emission (Kutty and Mitra, 2019). However, no work has been done on the microscopic characterization of tuberose floral cuticle and its correlation with scent volatile

emission in different cultivars. In this study, we undertook tepal histochemistry along with surface microstructural analysis by light and scanning electron microscopy (SEM) to gain an insight into the roles of cuticles and epidermal cells for differential behaviors in scent volatiles emission from tuberose flowers of CS and CD cultivars.

2. Materials and methods

The tubers of the selected cultivars of tuberose were collected from Bidhan Chandra Krishi Viswavidyalaya (http://bckv.edu.in), Mohanpur, Kalyani, West Bengal, India. The collected tubers were planted in the experimental garden of Natural Product Biotechnology Group, Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India (22.3460° N, 87.2320°E) and maintained as described before (Maiti et al., 2014).

For histochemical analysis semithin transverse cryomicrotome sections (30 μm) were prepared. Flower tepals were collected at 6 p.m. in the evening (the time of anthesis) and immediately put in formalin-acetic acid-alcohol (FAA) for fixation (5% formalin i.e. 37% of aqueous
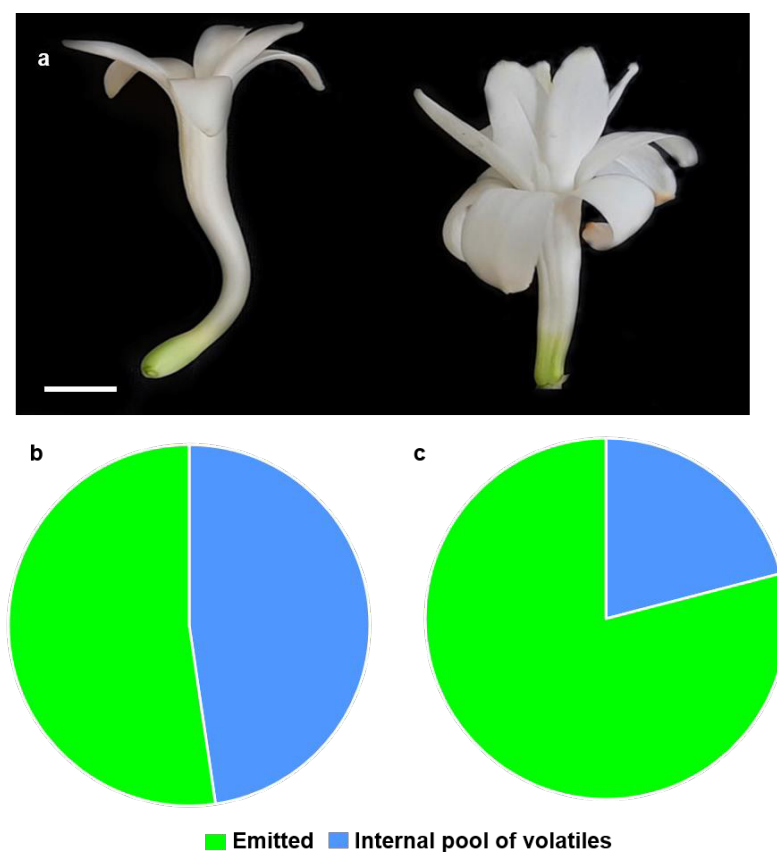
Figure 1. Tuberose cultivars and their volatile content. (a) 'Calcutta Single' (CS) and 'Calcutta Double' (CD) from left to right (scale bar 1 cm); (b–c) emitted and internal pool of volatile content of CS (b) and CD (c) (modified after Kutty and Mitra, 2019).

formaldehyde, 5% glacial acetic acid, 45% ethanol, and 45% distilled water) at a 1:10 volume ratio of tissue to fixative (Buda et al., 2009). Samples were then vacuum infiltrated with 10% sucrose followed by 20% sucrose for cryoprotection and finally, samples were embedded in M-FREEZE™ Cryoembedding media (Sigma-Aldrich, Germany) for sectioning. Cryosectioning was carried out at –25 °C using a Medimeas Manual Cryostat Microtome MCM-MT (Medimeas Instruments, India).

Calcofluor white/Auramine O was used to identify regions containing acidic and unsaturated waxes as well as cutin precursors (Buda et al., 2009). Sections were stained with calcofluor white (Sigma-Aldrich, Germany) at a concentration of 0.1% w/v in distilled water for 2 min and rinsed with distilled water for 1 min, followed by staining with 0.1% w/v auramine O (Sigma-Aldrich, Germany) in 0.05 M Tris-HCl (pH 7.2) for 15 min. Finally, the slides were rinsed thoroughly with distilled water for 5 min and mounted in a polyvinyl alcohol mounting medium with DABCO (Sigma-Aldrich, Germany), and viewed under blue light excitation (460 nm).

Nile blue A (sulphate) differentiates between the neutral lipids and the acidic lipids including free fatty acids and phospholipids by staining them red and blue, respectively (Gahan, 1984). Nile blue A (sulphate) (Sigma-Aldrich, Germany) was prepared at a concentration of 1% w/v in distilled water. Sections were stained for 30 s followed by immediate treatment with 1% aqueous glacial acetic acid for 30 s, this was followed by rinsing with distilled water and finally, slides were mounted in DABCO and viewed under a bright field.

Oil red O indicated the presence of lipids (Kromer et al., 2016). Oil red O (Sigma-Aldrich, Germany) was prepared at a concentration of 0.2% w/v in 60% aqueous 2-propanol. Sections were stained for 15 min and washed thoroughly in 40% (w/w) 2-propanol before mounting with DABCO and viewed under a bright field.

Sudan black B was used to identify the presence of phospholipids (Gahan, 1984). A saturated solution of Sudan black B (Sigma-Aldrich, Germany) was prepared in 70% aqueous ethanol to stain the floral sections for 15 min. After completion of staining, the sections were briefly rinsed with 50% aqueous ethanol and mounted in DABCO, and viewed under a bright field.

Sudan III was used to detect total lipids (Gahan, 1984). A saturated solution of Sudan III was prepared in 70% ethanol; it was then filtered before being used as a stain. Samples were kept in the staining solution for 15 min, after that they were washed with 50% aqueous ethanol to remove the excess stain and finally mounted in DABCO and viewed under a bright field.

Both bright field and fluorescence microscopic examinations were carried out in a Leica™ DM 2500 LED microscope (Leica™, Wetzler, Germany) coupled with a CoolLED pE-300[White] fluorescence illumination system (CoolLED Ltd., Andover, UK). Images after being viewed under objectives of 20X and 63X (Leica™, Wetzler, Germany) were digitally acquired using a Leica DFC7000 T camera and processed using Leica LAS X software (Leica™) using Windows 7 Professional platform.

Scanning electron microscopy (SEM) samples were prepared according to a previously published protocol using freeze-drying method (Trivedi et al., 2019). After collection, samples were immediately put for drying in a vacuum freeze drier (Indian Instruments and Chemicals, Calcutta, India) under a constant vacuum of 10^{-3} Torr at a temperature of minus (–) 40 °C. After drying, samples were fixed on the aluminium stubs. This was followed by sputter-coating with a 30 nm layer of gold (Quorum Q150RES, USA) and viewing under ZEISS EVO 60 scanning electron microscope (Carl Zeiss, Germany) at an accelerating voltage of 20 kV. The microscope's control program (Zeiss Smart SEM User Interface, Carl Zeiss, Germany) was used to generate digital images.

One-way ANOVA followed by Tukey's post-hoc analysis was performed using R software ($p < 0.05$) to find the significant differences in lipid droplet density among CS, CD inner whorl, and CD outer whorl. All the experiments were performed in triplicates.

3. Results

Calcofluor white/Auramine O is used to identify regions containing acidic and unsaturated waxes as well as cutin precursors (Buda et al., 2009). In Calcofluor white/Auramine O staining the cuticle in CS was found to be thin, and only the external cuticle layer was formed (Figure 2a). A similar type of external cuticle layer was also noticed in the inner whorl of the CD (Figure 2b). However, in the outer whorl of CD, a thick cuticular deposition was observed suggesting the formation of major cuticular layers (Figure 2c).

Nile blue A is a stain specific for fatty acid precursor molecules (Gahan, 1984). Cuticular membranes are dominated by the deposition of fatty acids. These fatty acid deposition can depict the details of cuticular polymerization. The external cuticles of both cultivars were found to be saturated with fatty acid deposition (Figures 2d–2f). The differences in cuticular thickness were found to be more prominent. As compared to the cuticular region of CS flower (Figure 2d) and the inner whorl of CD flower (Figure 2e), the outer whorl of CD flower showed a much thicker cuticle (Figure 2f).

Oil red O is a very specific stain for cuticular lipids (Kromer et al., 2016). In CS flower, the external cuticular region was shown to be lightly stained by oil red O (Figure 2g). The lipid droplets present in the epidermal region were brightly stained (Figures 2g–2i). A nonspecific staining of inner cellular region was observed. Also, in the inner whorl of CD feeble staining in the external cuticular region

was noticed (Figure 2h), suggesting a low accumulation of lipids on the external cuticle layer. Similar to CS, lipid droplet accumulation was noticed in the tepals of the inner cuticular region of CD inner whorl. A completely different pattern of staining was noticed in the case of the outer whorl of CD (Figure 2i). In this case, the external cuticle layer was stained along with the internal cuticle layer (Figure 2i), suggesting a thorough accumulation of lipids throughout the cuticular region.

Sudan black B known for staining acidic lipids (Gahan, 1984), showed a different kind of depositional pattern (Figures 2j–2l). In both the cultivars only the external cuticular region and the adjacent epidermal cells were stained with Sudan black B. In CS, the external cuticular region showed faint staining (Figure 2j) as compared to CD. In CD flower, the inner and outer whorls showed apparently similar staining pattern; careful examination, however, revealed that as compared to the inner whorl
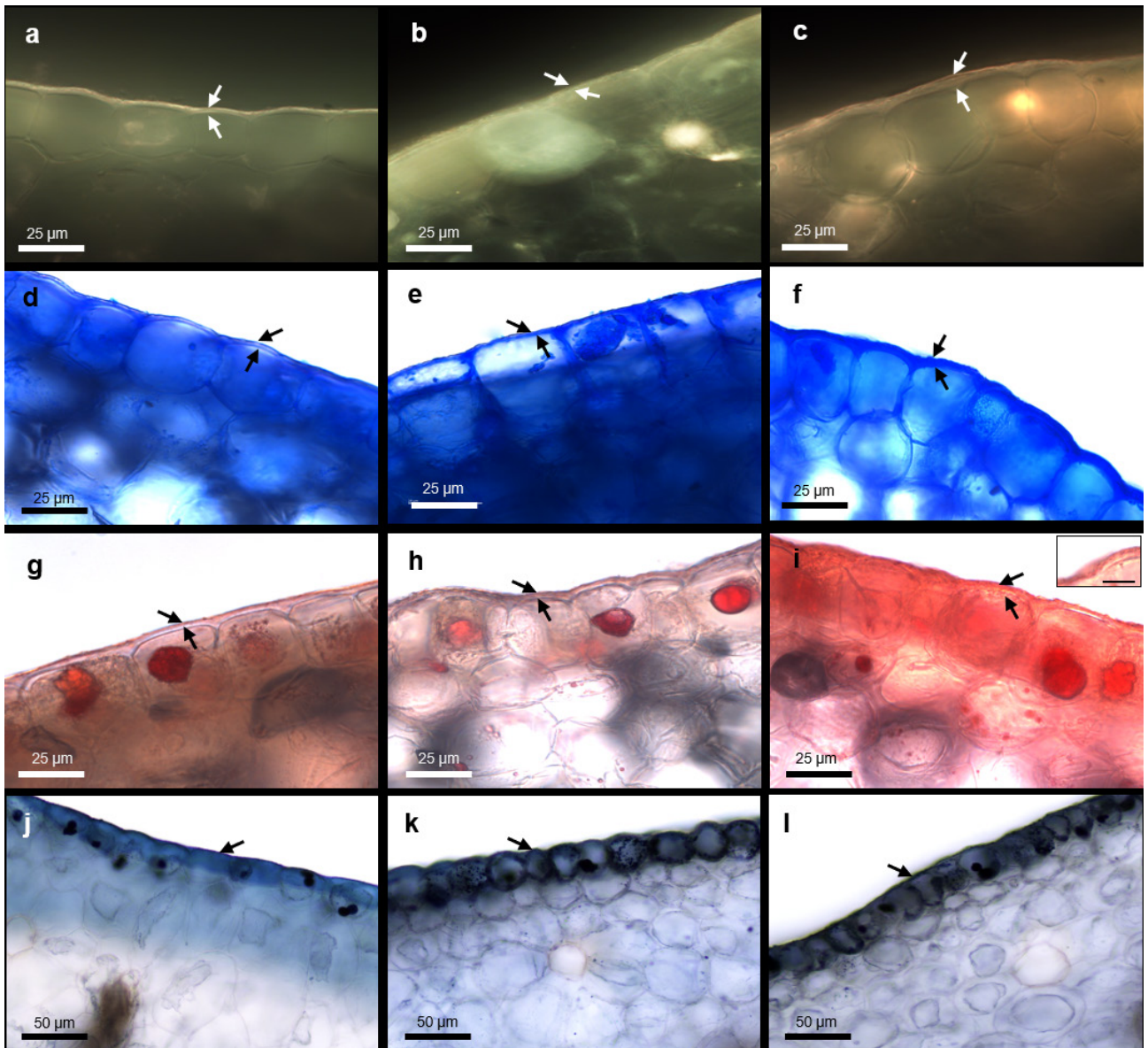
Figure 2. Histochemical characterization of CS, CD inner whorl, and CD outer whorl cuticles (from left to right). (a–c) Calcofluor white/Auramine O staining; (d–f) Nile blue A (sulphate) staining; (g–i) Oil red O staining; (i) Inset showing a magnified region of CD outer whorl showing intense staining of cuticle with Oil red O; (j–l) Sudan black B staining (a–i, scale bars 50 μm; j–l, scale bars 25 μm). Cuticular regions are marked with arrows.

(Figure 2k), the outer whorl (Figure 2l) stained relatively more intense.

The presence of lipid droplets has been observed in the tepal epidermal cells of both cultivars of tuberose (Figures 3a–3c). In CS flowers, we observed less number of lipid droplets accumulation as compared to CD (Figures 3a–3c). Assembly of the lipid droplets in the epidermal cells of the CD flower outer whorl was also observed (Figures 3d–3h).

Sudan III was used to stain whole tepals to detect lipid droplets (Gahan, 1984). Significant variations in lipid droplet density were also noticed among the tepals of CS, CD inner, and outer whorls. In CS flower, the lipid droplet density was observed to be quite low in tepal cells (Figure 4a); the droplets started getting denser in the tepal cells of the inner whorl in CD flower (Figure 4b). This was followed by highly densified lipid droplets that accumulate in the outer whorl tepal cells of CD flower (Figure 4c).

Floral Stomata were found to be stained with Sudan III as viewed under higher magnification (63×). We observed that stomata on the tepal surface of CS flower were feebly stained (Figure 5a). The stomatal aperture was colored light orange-red indicating less deposition of lipid bodies in the aperture region. Besides, the stomatal aperture in CS was found to be in an open position (Figure 5a), suggesting its role in scent emission (Maiti and Mitra, 2017). In the case of the CD inner whorl stomata, the aperture was brightly stained with Sudan III indicating a good deposition of lipids in the stomatal region. However,

it was found to be in partially open condition (Figure 5b). In the outer whorl of CD flower, the stomatal aperture was brightly stained but found to be in almost closed condition (Figure 5c).

The stomatal behaviors observed upon staining with Sudan III were further confirmed with SEM. An SEM image revealed that in CS flower stoma was found to be in a completely open condition (Figure 5d). When an inner whorl stoma of CD flower was observed under SEM, it was found to be partially opened (Figure 5e) whereas the outer whorl stoma of the same flower remained in a closed condition (Figure 5f). The stomatal behaviors, as observed under SEM completely correlates with those observed in bright field microscopy. Upon surface microstructural analysis under SEM, a difference in epicuticular wax deposition patterns in tepals was observed between the two cultivars (Figures 5g–5i). SEM analysis of tepal surfaces showed the presence of cuticular folds and nanoridges. In CS flowers, wax deposition in crystalloid form was noticed (Figure 5g), whereas, in CD, instead of crystalloid deposition, cuticular folds, and nanoridges were observed (Figures 5h–5i).

4. Discussion

Based on Calcofluor white/Auramine O staining it may be possible to estimate the extent of cuticle formation in both the studied cultivars. In both the inner and outer whorls of CD and in the single whorl of CS, the moderately fluorescent region of the internal cuticular region and the
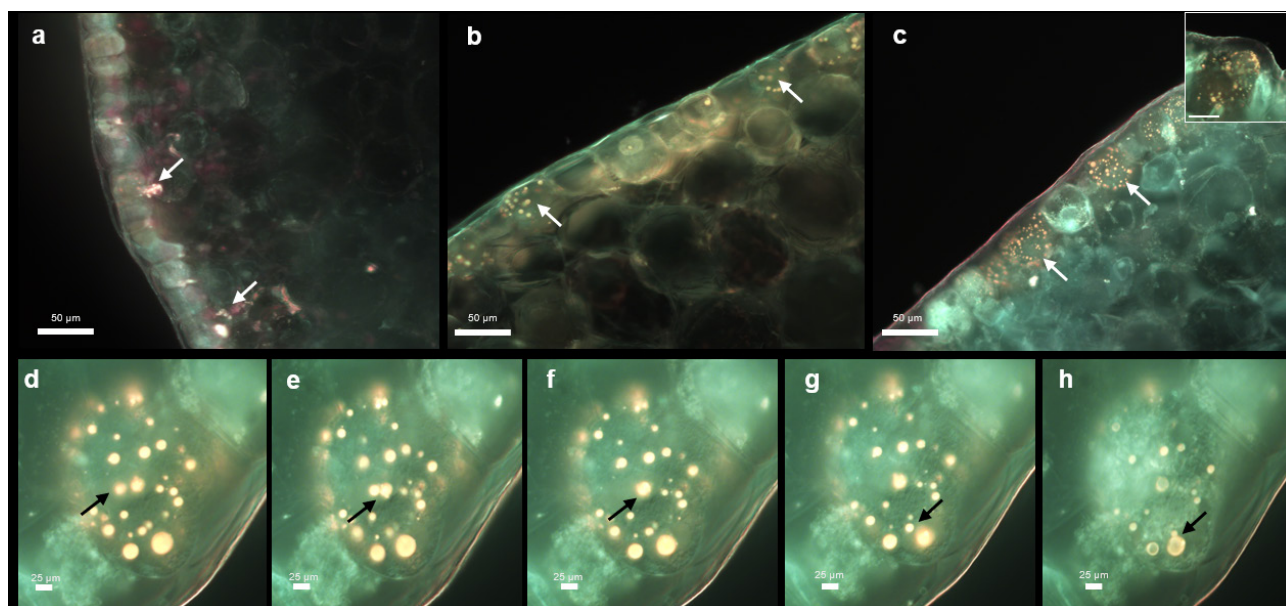
Figure 3. Lipid droplets in the epidermal cells of tuberose tepals. (a–c) Epidermal cells of CS, CD inner whorl, and CD outer whorl (from left to right) showing the presence of lipid droplets (indicated by arrows) (scale bars 50 μm); (c) Inset showing a protuberance containing lipid droplets in the CD outer whorl epidermal region; (d–h) From left to right, movements of lipid droplets (in 30 s interval) in the epidermal cell of outer whorl tepal in CD are indicated by arrows (scale bars 25 μm).
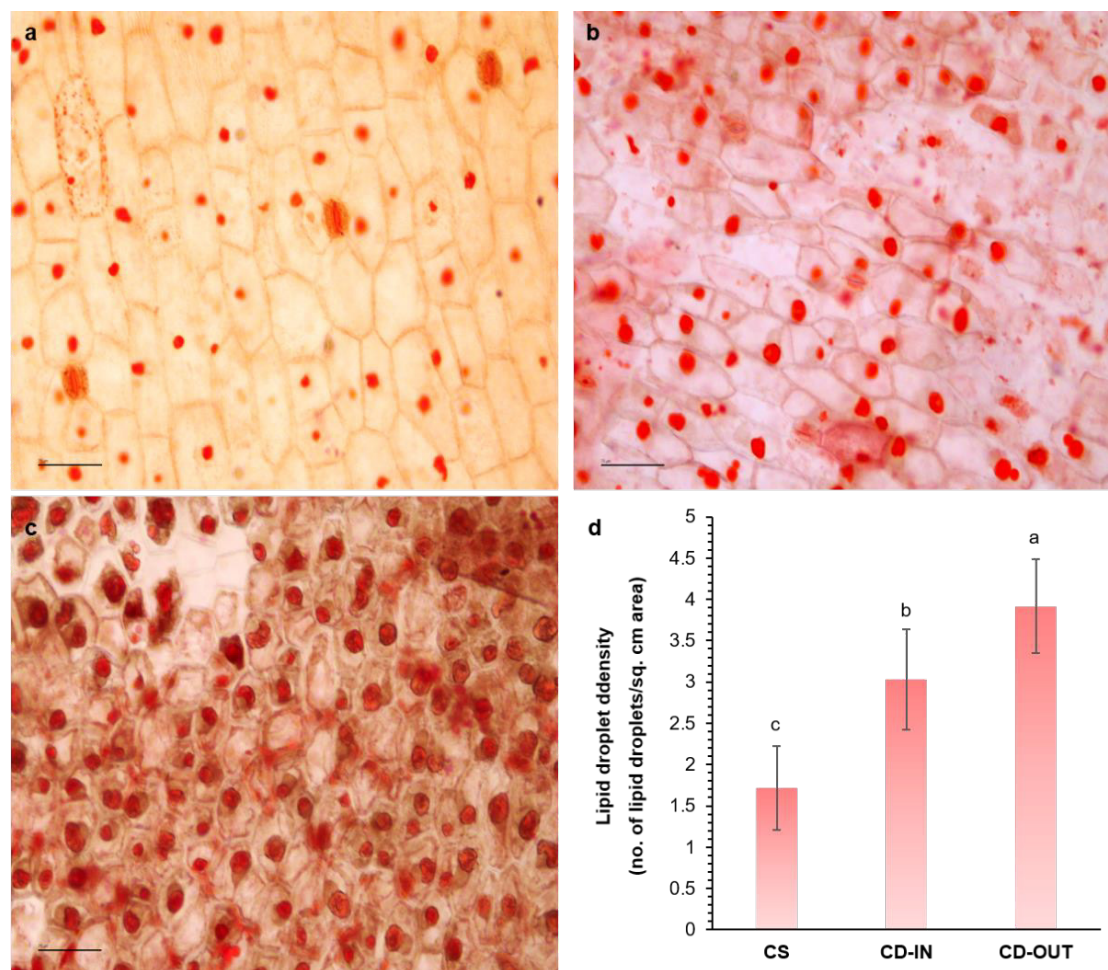
Figure 4. Lipid droplets as seen under bright field after staining with Sudan III. (a) Lipid droplets from CS; (b) Lipid droplets from CD inner whorl; (c) Lipid droplets from CD outer whorl; (d) Graphical representation of lipid droplet density in CS, CD inner, and CD outer whorl, statistically significant differences are indicated by different alphabets (Tukey's HSD, $p < 0.05$) (scale bars 75 μm).

epidermal region depicts the localization of most of the cutin precursor molecules (Figures 2a–2c). These cutin precursor molecules are the key elements of cutin, the building block of the cuticle (Bargel et al., 2006). In our study, we observed an accumulation of lipid droplets in the epidermal cells of the tuberose flower. The movement of lipid droplets as depicted in our study was earlier observed in in vitro experiments. Heredia-Guerrero et al. (2008) showed that cutin monomers can self-assemble in vitro forming the cutin polyester suggesting a plausible explanation of in vivo cutin synthesis. Recently, a similar type of lipid droplet movement was observed in *Arabidopsis thaliana* under a confocal microscope after staining with Nile Red (Krawczyk et al., 2022), where lipid droplets were shown to assemble near the plasma membrane with the help of lipid droplet tethering adaptor protein (Krawczyk et al., 2022).

Nile blue A staining patterns of tepal cuticles suggest that in the case of CS flowers the fatty acid deposition spans throughout the cuticular region (both external and internal cuticular regions). The intensely stained epidermal region in CS also suggests their possible role in fatty acid deposition. In the inner whorl of CD flower, fatty acids are mainly deposited in the internal cuticle (Figure 2e). The less coloration of the external cuticle suggests a low accumulation of fatty acid. The outer whorl of CD flower shows a completely opposite nature of the inner whorl (Figure 2f). Here, the fatty acids are deposited mainly in the external cuticular region. In the case of CS flower and CD flower inner whorl, the higher concentrations of fatty acids in the internal cuticular region and the epidermal cells indicate that the fatty acids were either synthesized here or synthesized elsewhere and finally deposited in the
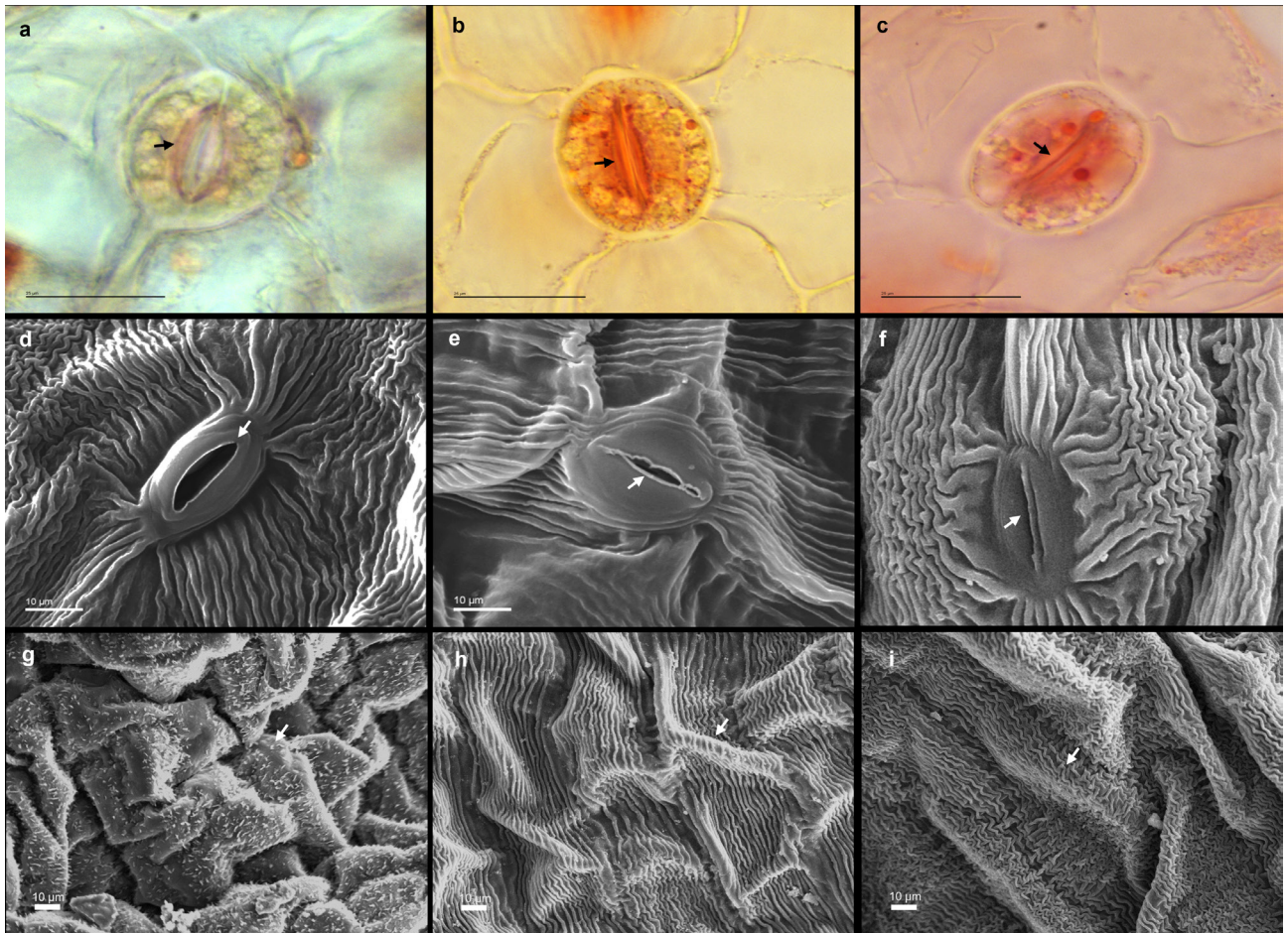
Figure 5. Floral stomatal behaviors and scanning electron micrograph of tepal surface in CS, CD inner whorl, and CD outer whorl (from left to right). (a–c) Stomata under bright field after staining with Sudan III (scale bars 25 **μ**m); (d–f) Stomata from the mature flowers under SEM (scale bars 10 **μ**m). Stomatal apertures are marked by arrows; (g–i) epicuticular wax deposition in CS, CD inner whorl, and CD outer whorl, respectively (scale bars 10 **μ**m); Cuticular depositional patterns are marked by arrows.

internal cuticular region so that these could be transported to the external cuticular region based on the requirement (Samuels et al., 2008). But in CD flower outer whorl, the feeble accumulation of fatty acids in the internal cuticular region suggests that fatty acid synthesis occurred rapidly, and immediately after synthesis moved towards the external cuticular layer.

Both in the tepals of CS and CD inner whorl, lipid droplets were mainly found to be localized in the internal cuticular region and the epidermal layer (Figures 2g–2i; Figures 3a–3h). This result suggests that the cuticular fatty acid synthesis may be happening within the cuticular region itself (both external and internal). In several cases, it was suggested that fatty acids could be synthesized in the epidermal region and transported to the external cuticle via the internal cuticle through possible channel formation between them (Pasacreta and Hasenstein, 1999; Samuels et al., 2008). In the past, it was also suggested that

the overall fatty acid synthesis might occur in the external cuticular region itself, and all the required precursors of fatty acid, cutin, and lipid perhaps transported through some transporter molecules to the external cuticle for the final synthesis (Pighin et al., 2004).

The differential staining pattern in Sudan black B is suggestive of the acidic lipid deposition in different regions of the cuticle of both cultivars. In CS flower, the less staining intensity suggests that the acidic lipids are deposited less in the cuticular region, whereas in CD inner and outer whorls, the intense coloration suggests more accumulation of acidic lipids. The intense lipid deposition in the stomatal aperture region of CD flower (Figures 4a–4d) can possibly explain the thicker cuticular region than CS. The deposition of lipid droplets in the stomatal aperture region suggests that the stomatal cuticle possibly acts as a physical linkage between the internal and external cuticular regions (Pesacreta and Hasenstein, 1999).

In an earlier study, floral stomata of tuberose CS cultivar were found to be actively involved in the scent emission process (Maiti and Mitra, 2017). The partially opened and closed stomata found in CD's inner and outer whorl respectively, provide an explanation for the less emission in CD than CS (Figures 5a–5i). The stomatal behavior can partially suggest that transpiration-mediated epi-cuticular wax deposition might occur in tuberose, especially in the stomatal region (Neinhuis et al., 2001). Such crystalloid deposition pattern of cuticular wax was also observed earlier in *Convallaria majalis* (Barthlott et al., 1998). However, in CD flowers, both the whorls showed characteristic cuticular folds of *Bellis*-type as observed earlier in *Bellis perennis* and *Grindelia robusta* (Koch et al., 2013). The presence of nanoridges on the tepal surface of CD suggests more deposition of cutin polyester materials (Li-Beisson et al., 2009). Cutin is the structural unit of the cuticle, and perhaps due to an excessive amount of cutin deposition, the cuticle of the CD flower was found to be thicker than the CS flower. In a recent study using *Petunia hybrida* flower as model, volatile diffusion was found to be increased up to 30-fold in petals with amorphous wax deposition (Ray et al., 2022). In tepals of CS, wax deposition was found to occur in crystalloid form, which provides an explanation for the higher emission of scent volatiles in CS flowers, whereas lower emission was reported in CD flowers that were devoid of crystalloid wax.

A previous study on metabolite profiling showed higher retention of volatiles internal pool in CD flowers than the CS cultivar, indicating possible interruption in scent volatiles emission (Kutty and Mitra, 2019). The microscopic evidence obtained from the present study can possibly explain the reason for this apparent interruption; the presence of a thick cuticle-like deposition on the tepal surface of the CD flower might be one of the responsible factors for the differential behaviors in scent volatiles emission in two tuberose cultivars. The relation between the volatile emission and cuticle formation is complex, nevertheless from the present study and based on our understanding from a few recent studies (Ray et al., 2022), we advocate that the presence of epicuticular waxes in crystalloid and nanoridges forms on tepal surfaces of CS and CD flowers, respectively, promote enhanced emission of scent volatiles in the former (CS) and hinder scent emission in the latter (CD). However, it is also necessary to characterize subcellular structures of floral epidermal cells and cuticle involved in the emission of volatiles, which requires analyses of ultrathin sections of the floral epidermis using high-resolution transmission electron microscopy (TEM). As the authors do not have a biological TEM facility at their disposal, such work in the future will be undertaken in collaboration mode to resolve the issue.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bargel H, Koch K, Cerman Z, Neinhuis C (2006). Structure–function relationships of the plant cuticle and cuticular waxes—a smart material? Functional Plant Biology 33:893-910.

Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I et al. (1998). Classification and terminology of plant epicuticular waxes. Botanical Journal of Linnean Society 126:237-260.

Buda GJ, Isaacson T, Matas AJ, Paolillo DJ et al. (2009). Three-dimensional imaging of plant cuticle architecture using confocal scanning laser microscopy. The Plant Journal 60:378-385.

Gahan PB (1984). Plant Histochemistry and Cytochemistry. Academic Press.

Heredia-Guerrero JA, Benítez JJ, Heredia A (2008). Self-assembled polyhydroxy fatty acids vesicles: a mechanism for plant cutin synthesis. BioEssays 30:273-277.

Kromer K, Kreitschitz A, Kleinteich T, Gorb SN, Szumny A (2016). Oil secretory system in vegetative organs of three Arnica taxa: essential oil synthesis, distribution and accumulation. Plant & Cell Physiology 57:1020-1037.

Kunst L, Samuels L (2009). Plant cuticles shine: advances in wax biosynthesis and export. Current Opinion in Plant Biology 12:721-727.

Kutty NN, Mitra A (2019). Profiling of volatile and non-volatile metabolites in Polianthes tuberosa L. flowers reveals intraspecific variation among cultivars. Phytochemistry 162:10-20.

Liao P, Ray S, Boachon B, Lynch JH, Deshpande A et al. (2021). Cuticle thickness affects dynamics of volatile emission from petunia flowers. Nature Chemical Biology 17:138-145.

Li-Beisson Y, Pollard M, Sauveplane V, Pinot F, Ohlrogge J et al. (2009). Nanoridges that characterize the surface morphology of flowers require the synthesis of cutin polyester. Proceedings of the National Academy of Sciences 106:22008-22013.

Livingston SJ, Samuels AL (2021). To protect and emit beauty. Nature Chemical Biology 17:124-125.

Maiti S, Moon UR, Bera P, Samanta T, Mitra A (2014). The in vitro antioxidant capacities of Polianthes tuberosa L. flower extracts. Acta Physiologiae Plantarum 36:2597-605.

Maiti S, Mitra A (2017). Morphological, physiological and ultrastructural changes in flowers explain the spatio-temporal emission of scent volatiles in Polianthes tuberosa L. Plant & Cell Physiology 58:2095-2111.

Neinhuis C, Koch K, Barthlott W (2001). Movement and regeneration of epicuticular waxes through plant cuticles. Planta 213:427-434.

Pesacreta TC, Hasenstein KH (1999). The internal cuticle of Cirsium horridulum (Asteraceae) leaves. American Journal of Botany 86:923-928.

Pighin JA, Zheng H, Balakshin LJ, Goodman IP, Western TL et al. (2004). Plant cuticular lipid export requires an ABC transporter. Science 306:702-704.

Ray S, Savoie BM, Dudareva N, Morgan JA (2022). Diffusion of volatile organics and water in the epicuticular waxes of Petunia petal epidermal cells. The Plant Journal 110:658-672.

Samuels L, Kunst L, Jetter R (2008). Sealing plant surfaces: cuticular wax formation by epidermal cells. Annual Review of Plant Biology 59:683-707.

Shepherd T, Wynne Griffiths D (2006). The effects of stress on plant cuticular waxes. New Phytologist 171:469-499.

Trivedi P, Karppinen K, Klavins L, Kviesis J, Sundqvist P et al. (2019). Compositional and morphological analyses of wax in northern wild berry species. Food Chemistry 295:441-448.

Yamamura H, Naito R (1983). The surface wax of several grapes in Japan. The Horticulture Journal 52:266-272.

Yeats TH, Rose JK (2013). The formation and function of plant cuticles. Plant Physiology 163:5-20.