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ORIGINAL ARTICLE

Glandular trichomes and laticifers in leaves of *Ipomoea pes-caprae* and *I. imperati* (Convolvulaceae) from coastal Restinga formation: structure and histochemistry

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Abstract Glandular trichomes and laticifers occur in *I. pes-caprae* (L.) Stweet (Convolvulaceae) and *I. imperati* (Vahl) Griseb. However, the importance of their secretion for the species survival in "Restinga" environments had not yet been investigated. This study aimed to anatomically and histochemically characterize such secretory structures in the two species, indicating which type of laticifers they have and whether or not the trichomes secret saline solution. Moreover, knowing the composition of secretion can help to clarify the species strategies of survival under the stressful conditions in halophilous ecosystems. Leaf samples were used in light microscopy analyses. Both species have multicellular glandular trichomes on the leaf blade, and laticifers on the mesophyll and midrib. Trichome secretion is mucilaginous, but sodium was not detected, and therefore, such trichomes are not salt glands. Laticifers are typical and classified as articulated non-anastomosing, and are not covered by an epithelium, as reported in some studies. Mucilage secretion by the glandular trichomes can aid in the species survival in the "Restinga". The species latex contains terpenoids and rubber, which may constitute important defenses against herbivores.

Keywords Articulated laticifers · Mucilage · Secretory structures · Terpenoids

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Introduction

Ipomoea pes-caprae (L.) Stweet and *I. imperati* (Vahl) Griseb. (Convolvulaceae) are two creeping herbaceous species that colonize sandy and saline soils in the "Restinga", a coastal ecosystem. In Brazil, their occurrence has been reported to the phytophysiognomies nearest to the sea, in the creeping halophilous–psammophilous plant formation (Thomaz and Monteiro 1992). In this formation, plants are exposed to a broad range of stress factors, such as excess light, high temperatures, increased salinity, and water shortage (Crawford 2008). These factors may have driven the development of a set of morphological, anatomical, and physiological adaptations on local plants (Dickison 2000). Under stressful conditions such as high irradiation and salinity, secretory structures like glands and laticifers may play an important role in plant survival (Fahn 1979).

In *Ipomoea* species, some secretory structures have been described, such as glandular trichomes (Silva and Azevedo 2007; Arruda et al. 2009; Martins et al. 2012), laticiferous canals, secretory idioblasts (Metcalfe and Chalk 1957; Martins et al. 2012), floral nectaries on sepals (Keeler and Kaul 1984), and extrafloral nectaries on the petiole (Beckmann and Stucky 1981; Keeler 1977, 1980; Keeler and Kaul 1979, 1984; Martins et al. 2012). All these structures are present in both *I. pes-caprae* and *I. imperati* (Keeler and Kaul 1984; Arruda et al. 2009), except for nectaries in the latter. Only a few studies have described the chemical nature of the secretion of these structures and have sought to understand their role in plant survival in the "Restinga" environment. Pongprayoon et al. (1991, 1992) identified an acyclic diterpene (E-phytol) and a sesquiterpene (damascenone) in leaves of *I. pes-caprae*, but the authors did not correlate their occurrence with

environmental factors. These compounds were reported to have spasmolytic activity and to be effective against the dermatitis caused by jellyfish venom.

Salt glands are commonly reported in "Restinga" plants, as well as to others halophytes. These structures secrete solutions of excess mineral salts or organic compounds, and have been described in species from several families, like Plumbaginaceae, Frankeniaceae, Tamaricaceae, and Convolvulaceae (Fahn 1979). They have also been reported in mangrove plants, such as *Avicennia* (Acanthaceae) (Evert 2006) and *Laguncularia* (Combretaceae) (Fahn 1979), and in plants from sand-dune environments, like *Spartina* (Poaceae) (Levering and Thomson 1971). Silva and Azevedo (2007) described glandular trichomes in *Ipomoea* species that occur on the seashore. Their results have led us to question whether such trichomes can secrete saline solution, and thus be classified as salt glands.

Laticifers are frequently reported in Convolvulaceae (Metcalfe and Chalk 1957), but they are named "laticiferous canals" in some genera, like *Calystegia*, *Convulvulus*, *Ipomoea,* and *Dichondra* (Metcalfe and Chalk 1957), due to the presence of an epithelium (Arruda et al. 2009). Laticifers are mainly related to the defense against herbivores and microorganisms (Fahn 1979). According to Pickard (2008), laticifers produce secondary metabolites that form up the latex, which is stored inside the living cell(s) that produce(s) them. The latex is a fluid with variable chemical composition and may contain precipitates or suspended colloids, and a variety of solutes (Mahlberg 1993).

Thus, we aimed to characterize the structure and the secretion of glandular trichomes and laticifers in *I. pes-caprae* and *I. imperati*, in order to understand their role in the species survival in a stressful environment. For this purpose, we addressed the following questions: (1) are the glandular trichomes salt glands? and (2) which laticifer types are present in the species?

Materials and methods

Study area

Both *I. pes-caprae* (L.) Stweet and *I. imperati* (Vahl) Griseb. (Convolvulaceae) occur in the creeping halophilous–psammophilous formation of the Paulo César Vinha State Park. The park is located in Guarapari city, southern coast of Espírito Santo state, Brazil (23°33′–20°38′S and 40°26′–40°26′W), and comprises a coastal plain of approximately 1500 ha composed mainly of Restinga vegetation.

Collection of plant material

Flowering branches of *I. pes-caprae* and *I. imperati* were collected, and voucher specimens were deposited in the VIC herbarium at Universidade Federal de Viçosa (UFV), with numbers 32,586 and 32,585, respectively; and in the VIES herbarium at Universidade Federal do Espírito Santo (UFES), with numbers 18,723 and 18,721, respectively.

Vegetative branches were collected for the anatomical analyses. The characterization of secretory structures was made in ten leaves sampled between the first and fourth nodes from five individuals. Leaf samples were fixed in FAA (formalin, acetic acid, 50 % ethanol, 1:1:18 v/v/v) and stored in 70 % ethanol (Johansen 1940).

Anatomical characterization

After a minimum 24-h storage in ethanol, leaf samples were embedded in histological paraffin with dimethyl sulfoxide (DMSO) (Histosec, Merck, Germany) or in 2-hydroxyethyl methacrylate (Historesin, Leica Instruments, Germany). Longitudinal and transverse sections (5–8 μm) were obtained in a rotary microtome (RM2155 Leica, Deerfield, USA). Paraffin sections were stained with astra blue and safranin (Gerlach 1969), while historesin sections were stained with toluidine blue at pH 4.0 (O'Brien and Mccully 1981).

Sections were photographed in a photomicroscope (AX-70TRF, Olympus Optical, Tokyo, Japan) equipped with an U-photo system (Spot Insightcolour 3.2.0, Diagnostic Instruments Inc., New York, USA). Under epifluorescence, sections were visualized using an HBO50 W mercury vapor lamp and a UV light filter.

Histochemical tests

Histochemical tests for the contents of glandular trichomes and laticifers were performed using fresh, recently collected samples, which were sectioned using a LPC table microtome (Rolemberg & Bhering Trade and Import LTDA, Belo Horizonte, Brazil). The performed tests for lipids, terpenoids, phenolic compounds, alkaloids, polysaccharides, proteins, and sodium are listed in Table 1. For each investigated metabolite group (Table 1), a negative-control test was conducted in parallel, as recommended in the reference of the respective histochemical test. Glass slides were mounted either with the reagent itself or with glycerin jelly.

Results

Glandular trichomes

Ipomoea pes-caprae and *I. imperati* have multicellular glandular trichomes on both sides of the leaf blade (Figs. 1–3, 10) and in the petiole. The structures are

Table 1 Metabolite groups investigated and respective methodologies adopted in the histochemical study of leaf secretory structures of *Ipomoea pes-caprae* and *Ipomoea imperati*

Metabolite groups		Stain/reagent	References
Alkaloids		Wagner's reagent	Furr and Mahlberg (1981)
		Dittmar's reagent	
		Ellram's reagent	
General		Autofluorescence	–
Inorganic compounds	Sodium	Uranyl zinc acetate	Jensen (1962) and Harvey (1987)
Lipids	Total lipids	Sudan black B	Brundrett et al. (1991)
	Fatty acids	Copper acetate and rubeanic acid	Ganter and Jollés (1969)
Phenolic compounds	General phenolic compounds	Ferric chloride	Johansen (1940)
		Potassium dichromate	Gabe (1968)
	Tannins	Vanillin–hydrochloric acid	Mace and Bell (1974)
	Lignin	Phloroglucinol	Johansen (1940)
	Flavonoids	Wilson's reagent	Charrière-Ladreix (1976)
		Aluminum chloride	Charrière-ladreix (1976)
Polysaccharides	Neutral polysaccharides	Periodic Acid–Schiffs reagent (PAS)	Mace and Howell (1974) and McManus (1948)
	Starch	Lugol	Jensen (1962)
	Pectins	Ruthenium red	Johansen (1940)
	Acid mucopolysaccharides	Alcian blue	Pearse (1980)
	Callose	Aniline blue	Smith and McCully (1978)
Proteins	Total proteins	Xylidine ponceau	O'Brien and McCully (1981)
Terpenoids	Essential oils and resin-oils	Nadi reagent	David and Carde (1964)
	Terpenoids with carbonyl group	2,4-dinitrophenylhydrazine	Ganter and Jollés (1969)
	Steroids	Antimony trichloride	Hardman and Sofowora (1972) and Mace et al. (1974)
	Sesquiterpene lactones	Sulfuric acid	Geissman and Griffin (1971)
	Rubber	Oil red O	Jayabalan and Shah 1986)

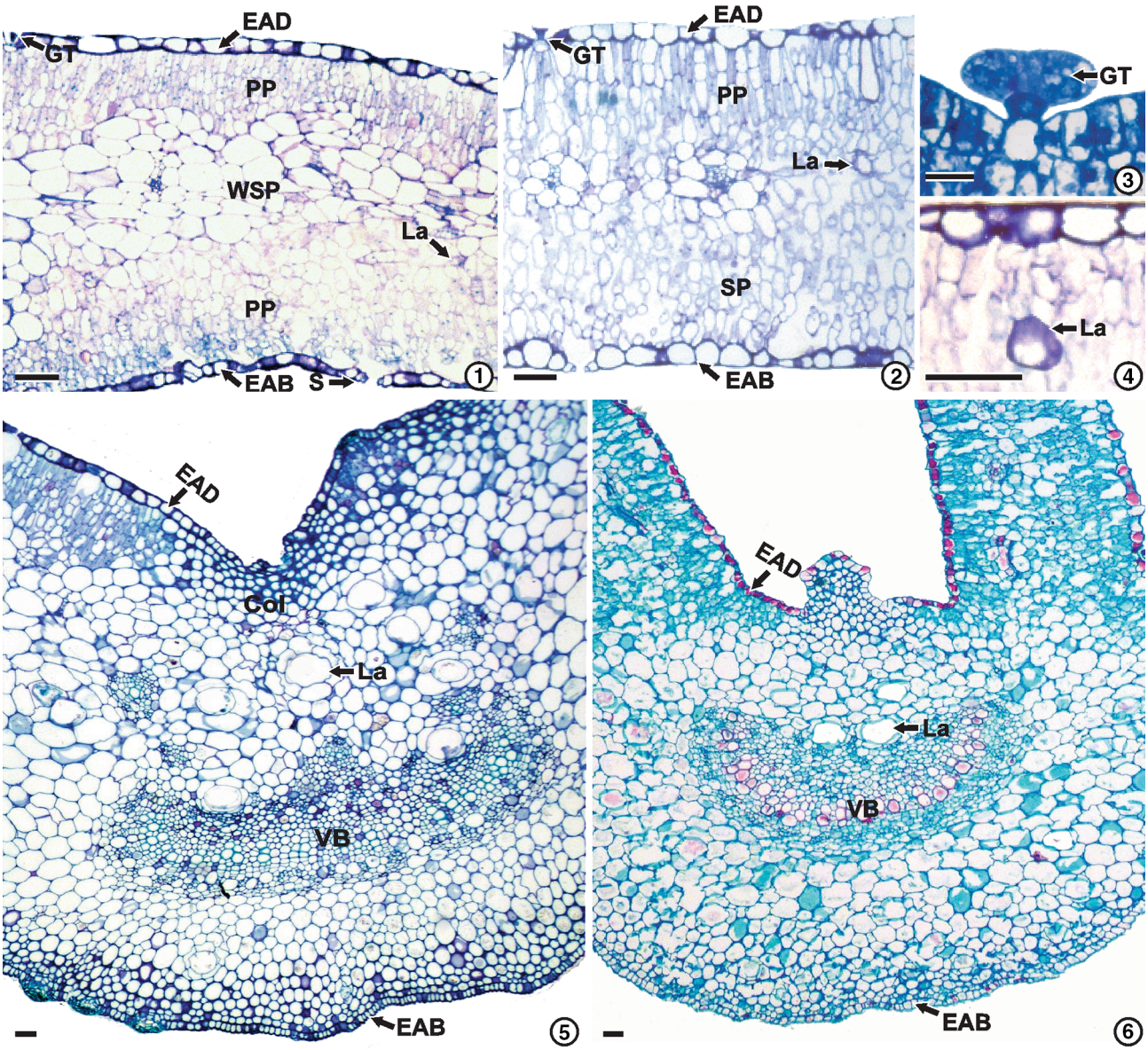
morphologically similar in the two species, having three regions: secretory head, stalk, and basal cell (Figs. 7, 9). The mature secretory head is composed of 12 radially arranged cells (Fig. 8) with thin primary walls (Figs. 7, 15). The stalk is short, being composed of a single rectangular cell with hyaline cytoplasm. The basal cell is thin-walled, isodiametric and vacuolated (Figs. 7, 9), and its conspicuous nucleus is located in the cell periphery (Fig. 9). This cell is located in a small depression on the leaf surface, slightly below the level of the other epidermal cells.

Histochemical tests showed similar results for both species. The negative control showed dense cytoplasm and a yellow-brownish content in the secretory head (Fig. 14). The stalk can be differentiated from the other trichome cells by its anticlinal wall, which has higher thickness than in the other parts of the trichome, as observed through autofluorescence (Figs. 15, 17) and with the NADI test (Fig. 16). The cuticle that covers the secretory head is thinner than in the other epidermal regions (Fig. 16).

Reactions on trichome contents were positive for polysaccharides (Figs. 18, 20), specifically pectins (Fig. 19; Table 2), and negative for all the other compounds, including sodium. This mucilaginous secretion promotes the adherence of the two lobes of the adaxial leaf surface of young leaves (Fig. 10), from the first to fourth nodes, only the abaxial surface remaining exposed to the external environment. This secretion has pectin substances and other polysaccharides, similarly to the observed in glandular trichomes (Fig. 20; Table 2).

Laticifers

Laticifers are distributed throughout the mesophyll (Figs. 1, 2, 4) and midrib (Figs. 5, 6). The structures are morphologically similar in both *I pes-caprae* and *I.*



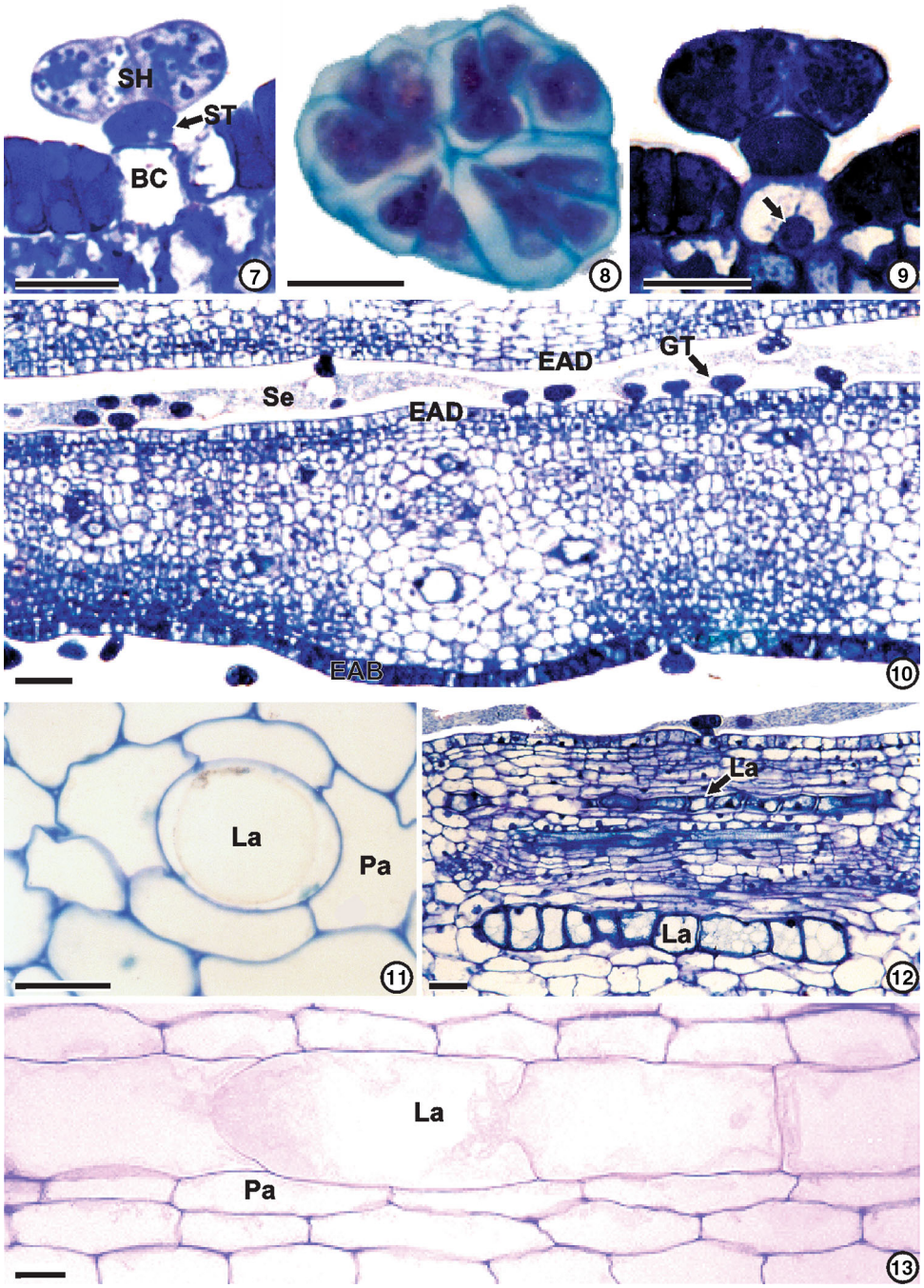
Figs. 1–6 Leaf secretory structures of *I. pes-caprae* (**1**, **4**, **5**) and *I. imperati* (**2**, **3**, **6**) in cross section. **1–4** Leaf blade, **5**, **6** midrib, **3** detail of secretory trichome, **4** detail of laticifer. *Col* collenchyma, *EAB* epidermis of the leaf abaxial surface, *EAD* epidermis of the leaf adaxial surface, *GT* glandular trichome, *La* laticifer, *PP* palisade parenchyma, *S* stomata, *SP* spongy parenchyma, *VB* vascular bundle, *WSP* water-storage parenchyma. *Bars* 100 µm

imperati. On the midrib, laticifers have a large lumen and are clearly delimited by flat cells (Figs. 5, 6 11). On the leaf blade, laticifers occur between cells of the palisade and spongy parenchymas (Figs. 1, 2, 4), but flat cells cannot be visualized adjacent to them, which render them difficult to be identified. These laticifers are classified as articulated and are composed of a single row of cells (Figs. 12, 13), which have large peripheral nuclei (Fig. 12) and lipid-impregnated walls (Fig. 21; Table 2).

Histochemical tests identified the following compounds in the latex of the two *Ipomoea* species: lipids (Fig. 21), terpenoids (Fig. 22), rubber (Figs. 23, 24), and polysaccharides (pectins) (Fig. 25; Table 2). There was neither production nor accumulation of any investigated chemical compound in the cells that delimit midrib laticifers.

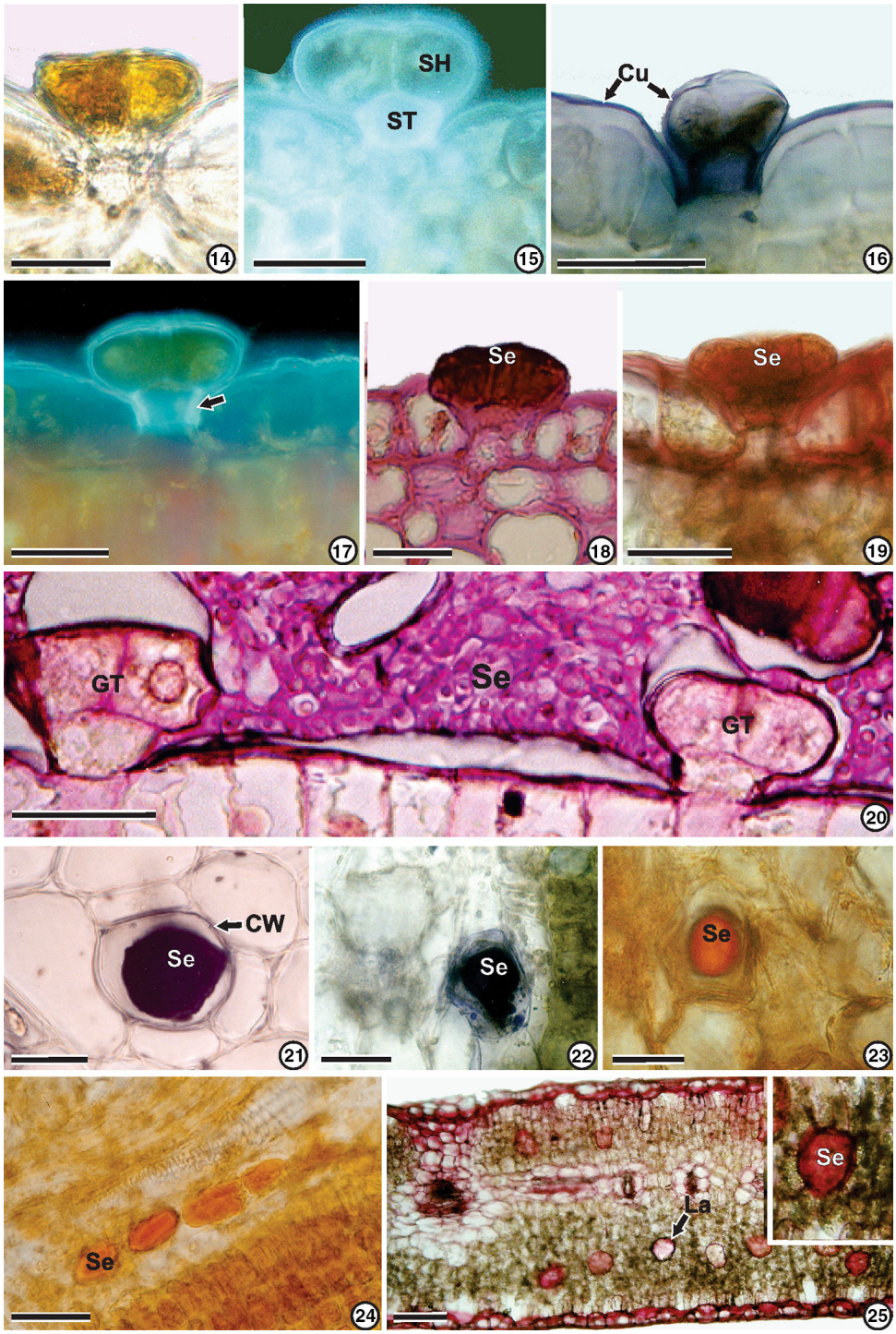
Discussion

Ipomoea pes-caprae and *I. imperati* occur in coastal environments, where salt tends to be highly concentrated (Suguio and Martin 1993; Scarano 2002; Crawford 2008). For this reason, their multicellular secretory trichomes



Figs. 7–13 Glandular trichomes and laticifers in leaves of *I. pes-caprae* and *I. imperati*. **7** Overview of an immature trichome, **8** front view of a mature trichome, **9** overview of a mature trichome. Notice the large nucleus (*arrow*) of the basal cell, **10** young leaf in longitudinal section. Notice the mucilaginous secretion (Se) produced by the glandular trichomes (GT) on the leaf adaxial surface, **11** laticifer on the midrib in cross section, **12** young laticifers on the leaf blade in longitudinal section, **13** laticifer on the midrib in longitudinal section. *BC* basal cell, *EAB* epidermis of the leaf abaxial surface, *EAD* epidermis of the leaf adaxial surface, *GT* glandular trichome, *La* laticifer, *Pa* parenchyma, *Se* secretion, *SH* secretory head, *ST* stalk. *Bars* 30 μm

were initially thought to be salt glands, representing an adaptive strategy that would help in removing the excess salt from the plants. However, no sodium was histochemically detected in these trichomes. Only polysaccharides (neutral ones and pectins) were detected, which demonstrates the mucilaginous nature of the secretion. Martins et al. (2012) observed a mucilaginous composition in the content of glandular trichomes of *Ipomoea asarifolia* (Desr.) Roem. & Schult. An analysis for sodium detection, however, was not performed in their study.



Figs. 14–25 Histochemical characterization of glandular trichomes (**14–20**) and laticifers (**21–25**) in leaves of *I. pes-caprae* and *I. imperati*. **14** Nonstained leaf section; **15**, **17** autofluorescence. Notice both secretory head (SH) and stalk (ST) cells, **16** NADI test showing the cuticle covering the trichome, in comparison with the thicker cuticle covering the adjacent epidermal cells; **17** thickening of stalk cell anticlinal wall (*arrow*); **18**, **20**, **25** positive reactions for pectins; **19** positive reaction for neutral polysaccharides; **20** detail of secretion from glandular trichomes; **21** positive reactions for lipid compounds, both in the wall of laticifer cells (CW) and in laticifer content; **22** positive reaction for terpenoids; **23**, **24** positive reactions for rubber in transverse (**23**) and longitudinal (**24**) sections. *Cu*- cuticle, *GT* glandular trichome, *Se* secretion, *SH* secretory head, *ST* Stalk. *Bars* 30 μm

Table 2 Histochemical positive results on leaf secretory structures of *Ipomoea pes-caprae* and *Ipomoea imperati*

Metabolite groups		Stain/reagent	Secretory structures	
			Glandular trichome	Laticifer
Lipids	Total lipids	Sudan black B		+
Polysaccharides	Pectins	Ruthenium red	+	+
	Neutral polysaccharides	Periodic Acid–Schiffs reagent (PAS)	+	
Terpenoids	Rubber	Oil red O		+
	Essential oils and resin-oils	Nadi reagent		+

+ Positive reaction

Mucilage may play an important role in wound responses (Fisher et al. 2009), host-pathogen interactions (Pérez-de-Luque et al. 2006), and water transport (Zimmermann et al. 1994; Czarnes et al. 2000; Zimmermann et al. 2007). The mucilaginous secretion in the trichomes of plants from the coastal "Restinga" formation is believed to contribute to the species adaptation to high levels of light irradiation, high temperatures, and low water availability due to low field capacity and/or high soil salinity (Ghanem et al. 2010). The secretion may also provide protection against herbivores (Mafokoane et al. 2007; Rocha et al. 2011; Woodward et al. 2012) and help decreasing transpiration rates (Fahn 1979). Mucilage secretion on the young leaves of both plant species promotes the adherence of the two leaf blade lobes, and therefore reduces the leaf area in direct contact with the external environment. Such reduction may diminish water loss through transpiration, and it could therefore represent a water-saving adaptive strategy.

Laticifers are universally present in the Convolvulaceae (Solereder 1908; Metcalfe and Chalk 1950). The laticifers of *Ipomoea* are classified as articulated non-anastomosing, as already described by Solereder (1908), Metcalfe (1967), Fahn (1979), and Evert (2006). This same type has also been reported in *Convolvulus* and *Dichondra* (Metcalfe and Chalk 1950; Metcalfe 1967; Fahn 1979). In addition, the presence of lipids in laticifers of *I. pes-caprae* and *I. imperati* corroborates the report of lipid deposition in the walls of laticifer cells in other Convolvulaceae species (Fineran et al. 1988).

Metcalfe and Chalk (1957) and Arruda et al. (2009) used the term "laticiferous canals" to describe the laticifers in some *Ipomoea* species. In fact, *I. pes-caprae* and *I. imperati* have both been reported to possess an epithelium contouring their laticifers (Arruda et al. 2009). However, our histochemical analyses showed that there is neither production nor accumulation of latex or its components in this group of cells. Furthermore, the occurrence of two types of laticifers (i.e., with an epithelium, on the midrib and petiole; and without an epithelium, on the leaf blade) in the same plant species seems to be rather morphologically improbable. Thus, they are typical laticifers, just with small ordinary parenchymatous cells that delimit the ones occurring on the midrib.

Laticifers can produce chemically complex and diverse types of latex (Van Die 1955; Yoder and Mahlberg 1976; Endress and Bruyns 2000), which may contain sugars, tannins, alkaloids, and/or protein crystals (Heinrich 1967; Fahn 1979). Laticifers of *I. pes-caprae* and *I. imperati* produce rubber particles, pectins, lipids, and terpenoids, all of which have already been reported to other Convolvulaceae species, like *Calystegia silvatica* (Kit.) Griseb. (Condon and Fineran 1989). Terpenoids, which had already been detected on pharmacological studies (Pongprayoon et al. 1992), were found to be restricted to the laticifers in the studied species. Laticifers have been mentioned to perform multiple functions, including regulation of water balance, mediation of oxygen transport, wound healing, and protection against herbivores and pathogens, as well as to compose the plant excretory system (Fahn 1979, 1990; Farrell et al. 1991; Evert 2006).

The secretory structures found in *I. pes-caprae* and *I. imperati* contribute to the development of different adaptive strategies that allowed their successful establishment in the coastal sand-dune environment. The secretion of glandular trichomes is purely mucilaginous, and they, therefore, cannot be considered salt glands. The mucilage enables the species to overcome stress by high irradiation, high temperatures, and low water availability. Moreover, the laticifers are typical and can be classified as articulated non-anastomosing, with no production or accumulation of metabolites in the surrounding cells, thus rendering inadequate the use of the term "epithelium" to describe their structure.

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