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Nota Científica / Short Communication: The formation of the stigmatic surface in *Passiflora elegans* (Passifloraceae)[1]

A formação da superfície estigmática em Passiflora elegans *(Passifloraceae)*

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Abstract

The stigma surface is a complex multicellular structure where the development of the pollen tube begins. This development is necessary for sucess in fertilization and depends on recognition processes that involve the anatomy of the stigma. *Passiflora* is an economically important genus because of its edible fruits. Many authors have described the stigma of *Passiflora* but nothing is known about the ontogenesis of this structure. This work aimed to describe the formation of the stigmatic surface of *Passiflora elegans*. Results showed that, in bud, the stigmatic surface of this species is flat with small cells. The cells in the subdermal layer have large vacuoles and the nucleus, near to the external periclinal walls. During its development the stigma surface becomes uneven due to the elongation of cells in the subdermal layer. Elongation results in an increase of external secretory surface area of the stigmas, and probably plays an important role in pollen recognition. The polysaccharide content found in the inner walls of these structures might be involved in the signal process for pollen tube growth during its early development. The morphological evidence presented here shows that, as the stigma of *Passiflora* is formed by dermal and subdermal cells, it should not be characterized as colleters or papillae and, therefore, it is defined here as stigma emergences.

Key-words: anatomy, stigma development, stigma emergence, pollination.

Resumo

A superfície estigmática é uma estrutura multicelular complexa, onde o tubo polínico inicia o seu desenvolvimento, necessária para a fecundação. Este desenvolvimento depende de condições favoráveis que envolvem a anatomia do estigma durante o processo de reconhecimento. *Passiflora* é um gênero economicamente importante devido aos seus frutos comestíveis. O estigma de *Passiflora* tem sido descrito por vários autores, mas o seu processo de formação é desconhecido. Esse trabalho tem por objetivo descrever o processo de formação da superfície estigmática de *Passiflora elegans*. Os resultados demonstram que durante a fase de botão jovem, a superfície estigmática é composta por pequenas células e apresenta superfície plana. As células da camada subdepidérmica apresentam grandes vacúolos e núcleo, próximo da parede periclinal externa. Durante o seu desenvolvimento, a superfície estigmática torna-se irregular devido ao alongamento de células da camada subdepidérmica. Essas modificações resultam em um acréscimo da superfície secretora externa do estigma, e provavelmente desempenham um importante papel no reconhecimento do pólen. Os conteúdos polissacarídicos encontrados na superfície interna dessas estruturas podem estar envolvidos com os processos de sinalização do tubo polínico durante seu desenvolvimento inicial. As evidências morfológicas observadas nesse trabalho demonstram que as estruturas presentes na superfície do estigma de *Passiflora* são constituídas por células de origem dérmica e subdérmica, e não devem ser caracterizadas como coléteres ou papilas, sendo assim, caracterizadas nesse trabalho como emergências estigmáticas.

Palavras-chave: anatomia, desenvolvimento do estifma, emergência estigmática, polinização.

Species of *Passiflora* L. are characterized by having a sporophytic and gametophytic self-incompatibility system (Rêgo *et al.* 1999, 2000; Suassuna *et al*. 2003), and the recognition reaction of pollen, as in other angiosperms, occurs mainly on the stigmatic surface (Rêgo *et al.* 2000). The stigma of

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Passifloraceae has been described in previous studies. Puri (1947) described the flower anatomy of this genus, and considered the stigma to be large, with massive structures; however, no further anatomical comments were provided. Raju (1956) classified these structures as projections that facilitate pollen grain retention during pollination events, and also as a site for the growth of pollen tubes during their passage towards the transmitting tissue. In one study, the stigma of Passifloraceae was classified as dry with unicellular papillae (Heslop-Harrison & Shivanna 1977), and in another study *Passiflora racemosa* Brot. and other *Passiflora* species and genera in the family were reported to have multicellular papillae (Bernhard 1999). The classification used by Bernhard (1999) was also used by Souza *et al.* (2006) for *P. edulis* f. *flavicarpa* Degener. These authors noted that the papillate structures had cells with large vacuoles and thin walls. However these characterizations of the pappilate stigmatic surface of *Passiflora* were based only on the final stages of stigma development. There is still no consensus on the origin of this structure, which is probably induced by the lack of specific ontogenetic studies. The goal of this work is to analyze the ontogenetic process of these structures at the stigmatic surface of *Passiflora elegans* Mast., an endemic species of southern Brazil.

Stigmas of 50 floral buds, measuring 0.3 to 2 cm, and 20 buds in pre-anthesis were collected from plants found on the Campus do Vale, at Rio Grande do Sul Federal University. A voucher specimen was deposited in the ICN Herbarium (ICN 52108).

The material was fixed in a 2% formaldehyde and 2.5% glutaraldehyde solution, in a 0.1 M sodium phosphate buffer, at 7.2 pH (Roland & Vian 1991). For the bright-field microscopy analysis, the material was washed in 0.1 M sodium phosphate buffer, at 7.2 pH, dehydrated in an ethanol series, and embedded in hydroxyethylmetacrylate (Gerrits & Smid 1983). Sections between 2 and 4 μm thick were made using a Zeiss Mikron rotation microtome and stained with 0.05% Toluidine Blue O, at 4.4 pH (Feder & O'Brien 1968). Histochemical tests were performed using fresh material, in combination with Ruthenium red to detect pectic acids (Johansen 1940) and Sudan III to test for lipids (Sass 1951). These tests were observed with a Leica DMR-HC microscope, and the images were obtained using a Leica DFC 500. For the scanning electron microscopy analysis, the material was post-fixed in 1% OsO_4, washed in 0.1 M sodium phosphate buffer at pH 7.2 (Weber 1992), dehydrated in acetone, critical point dried (Gersterberger & Leins 1978), sputter-coated with gold using a Balzers SCD 050, and examined using a Jeol 6060 SEM.

It was found, during the initial stages of development, that the apical portion of the stigma has a slightly sinuous surface (Fig. 1a), the dermal layer has cells with an evident nucleus and portions of condensed chromatin (Fig. 1b), and the subdermal layer has cells with large vacuoles and respective nucleus displaced near the external periclinal walls (Fig. 1b).

Subsequent to the initial developmental phase, the external surface of the stigma begins to form multiple dome-shaped projections, as a result of anticlinal divisions followed by anticlinal and radial elongation of the dermal and some of the subdermal cells (Fig. 1c). The height of each emergence on the stigmatic surface continues to increase, while the expanding subdermal cells divide transversally giving rise to projections that have an apical and a basal cell (Fig. 1d). The apical cells remain in direct contact with epidermal cells, in which the internal periclinal wall and the proximal portion of the anticlinal wall accumulate compounds of pectic nature (Fig. 1d). At the end of development, a specialized structure is formed on the stigma, comprised of cells from the dermal and subdermal layer (Fig. 1e). Once these structures have formed the stigma surface, it appears papillate, but in cross-section it can be seen that each projection has a multicellular organization around a central axis formed by the subdermal cell (Fig. 1f). Scanning electron microscopy revealed numerous multicellular projections on the stigmatic surface (Fig. 1g).

During pollination, the pollen germinates on this pappilate surface (Fig. 2a) and the pollen tube path follows the central region of the structure, which is rich in pectic compounds that have accumulated along the anticlinal and periclinal walls (Fig. 2b-c). Beyond the stigma, the pollen tubes grow into parenchyma (Fig. 2c-e) and the transmitting tissue that have cells with similar chemical properties.

The surface of the stigma is crucial during pollination, because pollen recognition depends on the lipids stored in the stigmatic cells and on the glycoproteins secreted from them onto the outer surface (Tilton *et al.* 1984). After hydration, the pollen grain germinates and the pollen tube emerges and grows over the stigma. During this time, specific enzymes loosen the cell wall of the papillae

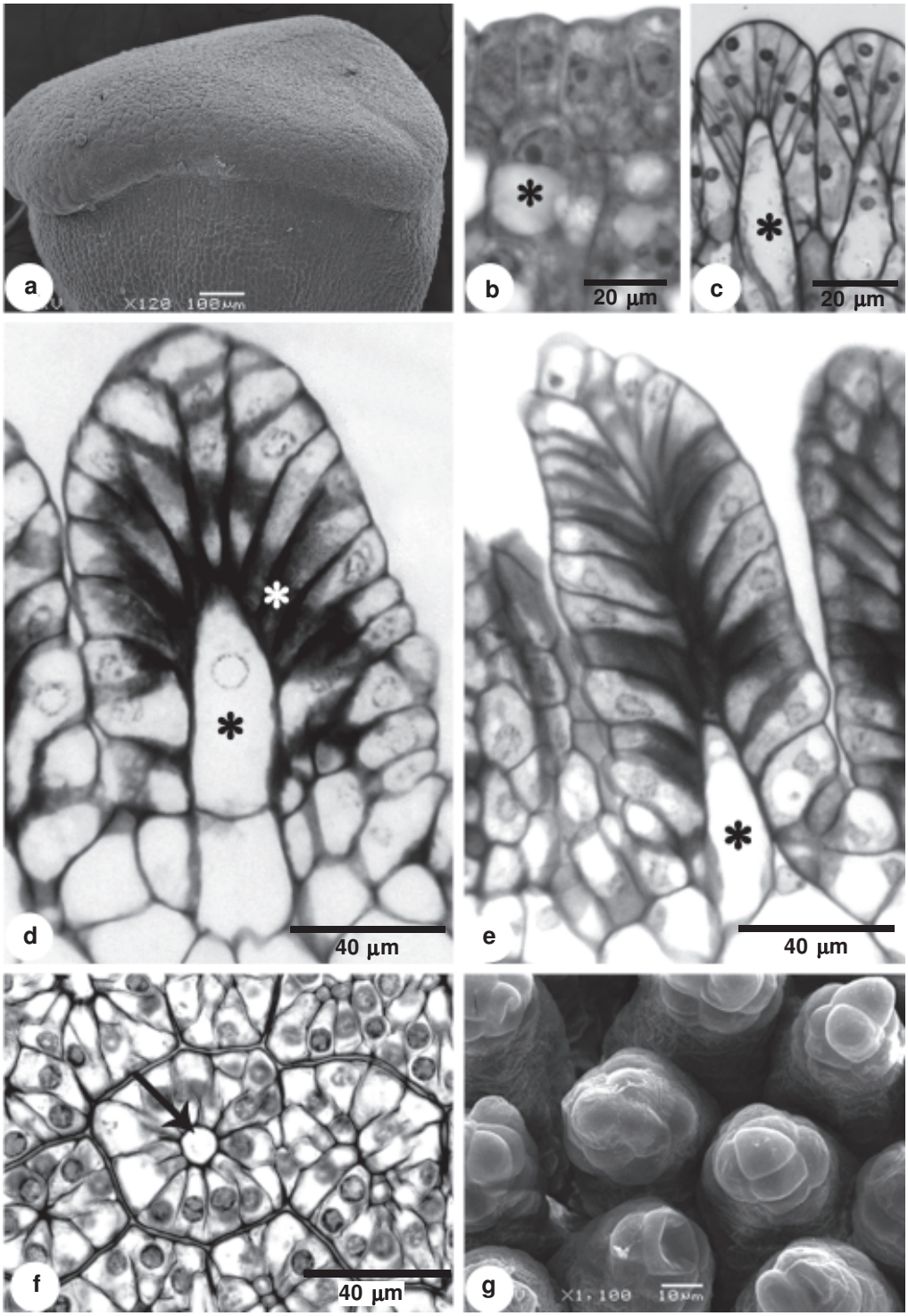

Figure 1 – Development of the *Passiflora elegans* stigma – a. young stigma under scanning electron microscopy; b. longitudinal section of the young stigma with the cells of the subdermal layer with large vacuoles (*); c. longitudinal section of the stigma showing epidermal cells pushed by cells from the subdermal layer (*); d. cross-sectional of the dermal cell with pectins walls (white asterisk) and division of subdermal cell (*); e. longitudinal section of the stigma emergence in the final phase of development; f. cross-section of the stigma emergence region in the final phase of development, showing the cell of the subdermal layer positioned in the central region; g. electromicrography of the stigma surface showing the stigma emergences.

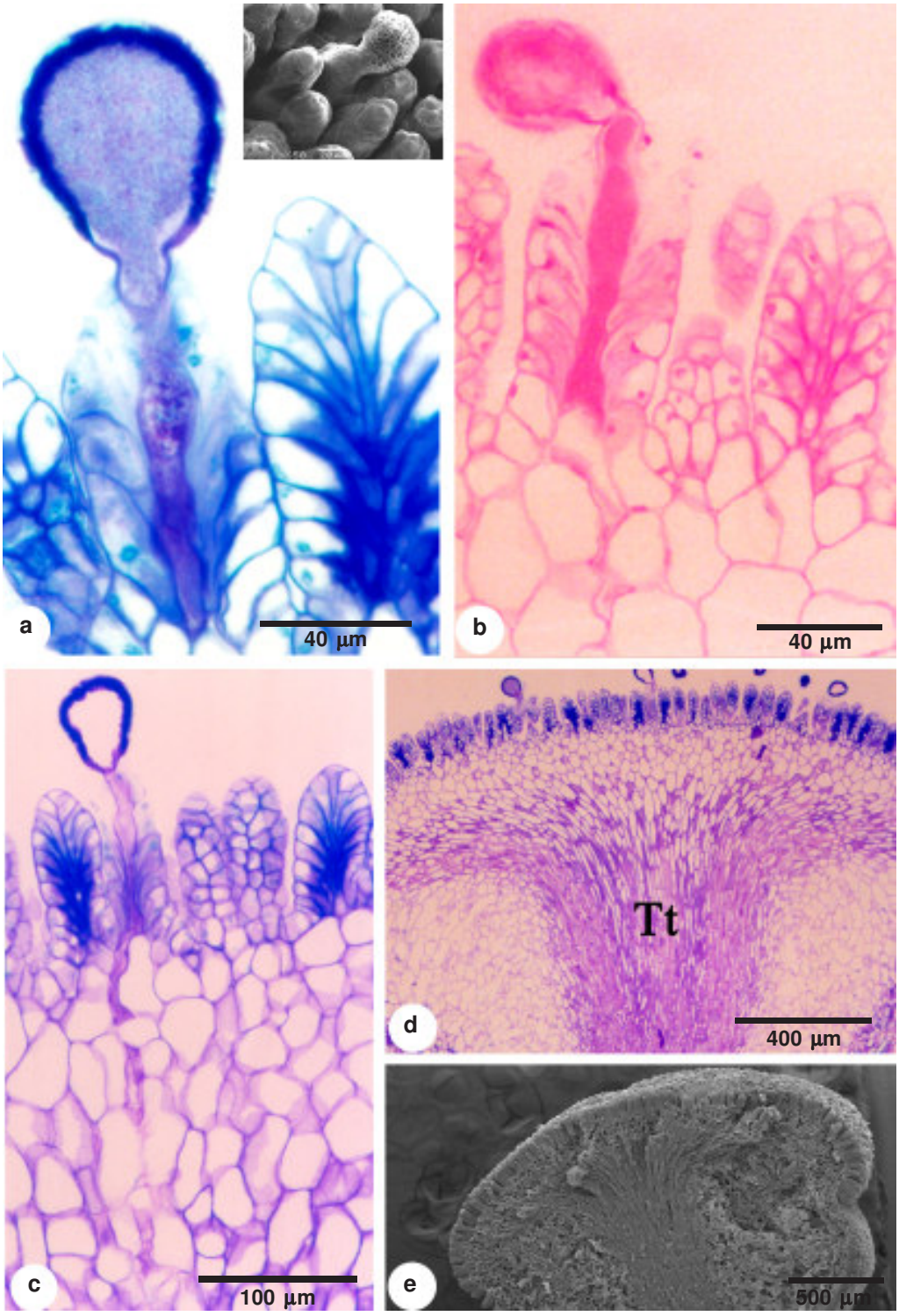

Figure 2 – Stigma and style of the *Passiflora elegans* flower – a. stigma surface with a pollen tube on the stigma emergence. Detail under scanning electron microscopy; b. histochemical test for the presence of pectins; c. longitudinal section of the stigma surface and the way of the pollen tube penetration through the stigma emergences and parenchymatous tissue; d. longitudinal section of the stigma and style showing the transmitting tissue at the apical center of the style (Tt); e. stigma and style under scanning electron microscopy.

preparing it for the penetration of the pollen tube (Micheli 2001). Calcium is probably a messenger during this process, inducing enzyme secretion and the consequential loosening of the cell wall (Elleman & Dickinson 1986; Hiscock *et al*. 2002).

In this study, a large amount of pectin was observed on the dermal and subdermal cell walls of the stigma, which coincides at the cellular level with the pollen tube path during its germination. Pectins probably stimulate the pollen tube growth of *P. elegans*, and calcium is made available to this structure along its course. Calcium (Ca^{2+}) is a key element in this process, regulating elongation and orientation of the pollen tube during its development (Malhó *et al*. 2006).

Pectins are synthesized in dictyosomes, in a methyl-sterified form. The methyl-sterification of carboxylic groups prevents Ca^{2+} binding, making the cell wall less rigid. As methyl-sterification increases, the fluidity of the pectin gel also increases, allowing the cell to expand while the integrity of its structure is maintained, due to the hydrophilic properties of pectins (Micheli 2001; Taylor & Hepler 1997).

Braum (2008) observed in style cells, adjacent to a growing pollen tube, the accumulation of pectic material in the vacuoles and, near the cell walls. These traits are important because they promote changes in the cell walls of the transmitting tissue, allowing for the passage of the pollen tube. In *P. edulis*, there are reports of the occurrence of pectic compounds, mainly along the inner periclinal walls of the cells that constitute the dermal layer structures (Souza *et al.* 2006). It is possible that the same mechanism described by Braum (2008) occurs in the stigma of *P. elegans*.

The stigmatic surface cells of *Passiflora* are structurally and ontogenetically similar to colleters (Paiva & Machado 2006), which are usually associated with the secretion of mucilaginous compounds. Thomas (1991) cites the occurrence of colleters in approximately 60 families of angiosperms, mainly on stipules and sepals. In Passifloraceae, these structures are known to occur on leaf surfaces (Solereder 1908), and are abundant on young plant parts, especially along the borders of foliar primordia and stipules (González 1998).

Colleters secrete a viscous material on the external surface (Thomas 1991; Klein *et al.* 2004; Barreiro & Machado 2007). This process differs from what was observed in this study, as the stigmatic emergences found on *P. elegans* have pectic compounds in their inner walls.

The morphological characteristics of the stigmatic projections revealed in this work do not agree with the previous descriptions of the literature, that used the term papilla to define these "projections of epidermal cells." In addition, previous studies did not classify these structures as colleters. For this reason, we conclude that "stigma emergence" is a better term to classify the structures found on the stigmas of *Passiflora*, as they are formed from the dermal and subdermal layers and they do not secrete mucilage.

Bernhard (1999) considered the characteristic stigma of Passifloraceae to be largely distributed among the genera of this family, but rare in the other families of angiosperms. From a taxonomic perspective, the stigmatic surface also appears to be an important trait that could be used to help describing Passifloraceae. Additional studies on other taxa in the family are needed to confirm this.

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