

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

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SEXUAL DIMORPHISM OF GALLS INDUCED BY *LOPESIA MATAYBAE*
(DIPTERA: CECIDOMYIIDAE) ON *MATAYBA GUIANENSIS*
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Trabalho de Conclusão de Curso ao Instituto de Biologia, da
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Orientador: Prof. Dr. Denis Coelho de Oliveira

Co-orientadora: MSc. Uiara Costa Rezende

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Sexual dimorphism of galls induced by *Lopesia mataybae* (Diptera: Cecidomyiidae) on *Matayba guianensis* (Sapindaceae)

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ABSTRACT

- Usually one species of galling insect can induce just one gall morphotype on the host plant. The galling insect *Lopesia mataybae* (Diptera: Cecidomyiidae) induces two similar gall morphotypes on the leaflets of *Matayba guianensis* (Sapindaceae). However, these galls differ in thickness and size, a possible evidence of sexual dimorphism occurring in galls induced by a cecidomyiid.
- In the present study, the morphological, anatomical and histochemical attributes of male and female galls were examined and compared.

- Both gall morphotypes presented the galling insect in larva or pupa stages, and the emerged insect from different galls were sexually distinct. Galls induced by females were significantly bigger (height average = 4,67mm, S=0,43/ width average = 4,59mm, S=0,70) than male galls (height average = 2,95 mm, S=0,46/ width average = 2,34 mm, S=0,45), and the quantity and size of cell layers in female induced galls were higher. There were no differences in histochemical compounds detected in both male and female galls.
- The differences between the female and male galls found in this study may be due to differences in the life cycle of the insects, where female's stimuli on the host plant lasts more than males, causing plant responses to be different and thus promote bigger galls for females.

Key words: plant-interaction, plant anatomy, dimorphism, galling insects

INTRODUCTION

Galls are new plant organs developed by chemical and/or mechanical stimulus of specialized organisms, especially insects (Mani 1964, Shorthouse *et al.* 2005). The host plant interaction with these specialized herbivores, leading to gall formation, is one of the most efficient strategy of herbivory (Roskam 1992; Stone & Schönrogge 2003). The galling insects are truly phenotype manipulators changing the host tissues into its own benefit and, in general, induce cell hypertrophy and tissue hyperplasia during the gall development (Oliveira *et al.* 2016). Despite of the site of gall induction and development (e.g. leaf, stem, flower bud), the gall structure provide a favorable microclimate to the galling insect larvae, protection against

natural enemies and provide nutritional resources, especially with the formation of the nutritive tissues. (Bronner 1992; Hawkins *et al.* 1997; Hartley 1998; Shorthouse *et al.* 2005; Oliveira 2011; Ferreira *et al.* 2017a). The development of gall tissues depends on continuous chemical and/or mechanical stimuli of the galling insect during its feeding activity, which act like a biotic modulator of plant tissues metabolism, but it is limited by host plant morphogenetic constraints (Mani 1964; Meyer & Maresquelle 1983; Rohfritsch 1992; Oliveira & Isaias 2010a; Bartlett & Connor 2014; Oliveira *et al.* 2016; Amorim *et al.* 2017; Rezende *et al.* 2019).

Some plant species can host different gall morphotypes, induced by different species of galling organisms, the superhosts (e.g. Hayward & Stone 2005; Oliveira 2008; Costa *et al.* 2010; Oliveira *et al.* 2013). In a general way, each insect species induces just a single gall morphotype (Rohfritsch & Shorthouse 1982; Price 2005). Sometimes, insects from the same species can induce galls in distinct host plant species (Malenovský *et al.* 2015). Therefore, in some cases, one insect species can induce two, even three gall morphotypes at the same or different host plant organs, as well as at different periods of its life cycle (e.g. Gonçalves *et al.* 2005; Dorchin *et al.* 2009; Kurzfeld-Zexer *et al.* 2015; Pfeffer *et al.* 2018; Silva *et al.* 2019). Induction of more than one gall morphotype indirectly increases plant resource variability that galling insects is able to access. This peculiarity can be related to the sexual dimorphism of the galling organism (Rhombert 1980; Wool & Burstein 1991; Miller 1998; Dorchin *et al.* 2009; Gonçalves *et al.* 2005). Sexual dimorphism is common in some taxa of galling insects as the Eriococcidae gall systems (Gullan *et al.* 2005; Gonçalves *et al.* 2005; Magalhães *et al.* 2015) but no reports have been shown for Diptera: Cecidomyiidae.

The galling *Lopesia mataybae* (Diptera - Cecidomyiidae), induces two gall morphotypes on leaflets of *Matayba guianensis* (Sapindaceae). We believe that females and males occupy distinct galls, although these two gall morphotypes are quite similar, they have considerably differences in size and thickness. Cecidomyiidae females usually emerges later, are bigger and have a longer life cycle than males (Condrashoff 1962; Gagné 1994; Dorchin *et al.* 2007, Tabadkani *et al.* 2012), and considering that gall tissue develop in response to insect feeding stimuli (e.g. Ferreira *et al.* 2017b, Rezende *et al.* 2019), cecidomyiid female feed more frequently and induce bigger galls. In addition, the female galls should present different distribution of nutritional compounds in the tissues compared with male galls.

MATERIALS AND METHODS

Study area and sampling

The study was carried out at Estação Ecológica do Panga (19°10=S, 48°24=W), Uberlândia city, Minas Gerais State, Brazil. We collected 124 galls from different sizes induced by *L. mataybae* on leaflets of *M. guianensis* from July 2019 to October 2019 for sexual morphological, anatomical and histochemical characterization.

To determine the possible sexual dimorphism, i.e. the presence of *L. mataybae* females or males, the collected galls were put separately on plastics microtubes until adult emergence. The emerged adults were analyzed in a stereomicroscope, to distinguish males from females according to Garcia and Urso-Guimarães (2018) insect descriptions. Female and male galls were measured using a digital caliper (Digimess®). Moreover, galls from both morphotypes were opened to determine the stages of development of the insect.

For anatomical analysis, samples were fixed in FAA 50 (formalin, acetic acid, 50 % ethanol, 1:1:18 v/v/v) for 48 hours (Johansen, 1940), dehydrated in an ethanol series, embedded in 2-hydroxyethyl methacrylate (Historesin[®], Leica[®] Instruments, Germany), and sectioned using a rotary microtome (YD-315 model, China) at a thickness of 5µm. The sections were stained with 1 % toluidine blue at pH 4.0 (O'Brien et al. 1965) and mounted with Entellan[®].

To determine the possible sexual dimorphism, i.e. the presence of *L. mataybae* females or males, the collected galls were put separately on plastics microtubes until adult emergence. The emerged adults were analyzed in a stereomicroscope, to distinguish males from females according to Garcia and Urso-Guimarães (2018) insect descriptions. Female and male galls were measured using a digital caliper (Digimess[®]). Moreover, galls from both morphotypes were opened to determine the stages of development of the insect.

RESULTS

Female-induced galls are larger and thicker (height average = 4,67mm, S=0,43/ width average = 4,59mm, S=0,70) than those occupied by males (height average = 2,95 mm, S=0,46/ width average = 2,34 mm, S=0,45) (Fig.1 A). In these galls of different sizes were found both larva and pupa stages of the galling insects. The insects emerged from both morphotypes were sexually different according to Garcia and Urso-Guimarães (2018) insects' descriptions, males emerged from small galls and females emerged from bigger galls. However, some characteristics remains similar, both galls are induced on the abaxial leaflet surface, with globoid morphotype (Isaias et al. 2014a) and covered by projections. These

galls are composed by two parts, one outer vessel-like structure that surrounds another innermost cocoon-like structure, that is made up of harder tissues (Fig. 1 B, C). In the tip of the cocoon-like structure there is nutritive cells, where the galling insects can feed (Fig. 2 A, D). The insect emerge of the gall pushing out this cocoon-like structure and the wrapping part remains fixed to the leaf base

The galls develop initially from cell hypertrophy and tissue hyperplasia of the abaxial epidermis and spongy parenchyma of the leaflet, neoformed vascular bundles are noted in the base of the gall (Fig.2 C, D). In the point of insertion, female and male galls maintain only parts of the spongy parenchyma (Fig. 2 E, F). Toward gall tissue, it continues to expand by tissue hyperplasia with aleatory cell divisions. The gall is covered by the epidermis that continue from the abaxial leaf epidermis. In addition, galls have projections which are composed of homogeneous parenchyma with elongated cells.

In both female and male galls, the cocoon-like structure goes through walls hardening due the intense lignification (Fig. 2 A, B). The differences between the galls are in the wrapper that remains active until these structures' senescence. This wrap on female-induced galls are smaller at the base and expands in thickness in the region surrounding the cocoon-like structure, with more than 25 lateral cell layers approximately. In male-induced galls, the base is the bigger part, with greater cellular hyperplasia and hypertrophy comparing with the walls surrounding the cocoon-like structure with approximately 15 lateral cell layers.

Based in histochemical tests, there is no difference between female-induced galls and male-induced galls. Both accumulate starch in the base and lateral regions (Fig.3 A). The presence of reducing sugars was detected on both galls, mainly next to the "cocoon-like

structure” region, probably a true nutritive tissue (Fig.3 B). The tests for proteins e lipids were negative for both female and male galls.

DISCUSSION

Some morphological features are clear indicative of sexual dimorphism in galls induced by *Lopesia mataybae* on leaflets of *Matayba guianensis*. The female galls are bigger, and the vessel-like structure showed more hypertrophied cells compared with male induced galls. However, most of anatomical and histochemical traits are similar in galls induced by both female and male galling insects. Presumably, the morphology and life-cycle differences among *L. mataybae* females and males (Garcia & Urso-Guimarães 2018) are responsible for the morphological differences in galls structure, determining the sexual dimorphism.

In addition to gall induced by *L. mataybae*, *M. guianensis* host galls induced by the parthenogenetic galling insect, the *Bystracoccus mataybae* (Eriococcidae) (Hodgson *et al.* 2013). *B. mataybae* produces two different morphotypes of galls on *Matayba guianensis*, depending on the developmental stage of the insect and the site of oviposition. The first instar nymph induces simple stem galls and the second instar nymph induce more complex leaflet galls (Pfeffer *et al.* 2018; Silva *et al.* 2019). Although induced in the same host plant species, these galls induced by *B. mataybae* have different morphology, anatomy and histochemistry than those induced by *L. mataybae*. These findings indicate that the galling species, their development stage and the sex has a direct influence on the gall morphology. For example, *B. mataybae* galls are glabrous (Pfeffer *et al.* 2018), while *L. mataybae* galls have trichomes. The mature leaves of *M. guianensis* are smooth, but the leaves in early development stages

have trichomes. Therefore, the galls induced by *L. mataybae* not only maintain this characteristic of young leaves but increase its expression. The lignified tissue in the cocoon-like structure can protect the galling insects from environment stress and natural enemies, as discussed by Stone & Schönrogge (2003).

The gall shape determination depends on the galling feeding behavior, that stimulates differently the process of gall tissues development (Rohfritsch & Anthony 1992). So, the gall tissues differentiation and growth depend on the constant specific galling feeding stimulus (Rezende *et al.* 2019). Cecidomyiidae male insects usually emerge earlier from the galls, thus has shorter life cycle than females (Dorchin *et al.* 2007; Gagné 1994; Yukawa *et al.* 2013). The differences between life cycles of the sexes can be a possible explanation for the occurrence of sexually dimorphic galls (Gullan *et al.* 2005). Females staying longer in galls than males promote more stimuli in the plant that can result in more tissues hyperplasia and cell hypertrophy and consequently developing sexually dimorphic galls. The gall systems *Pseudotectococcus rollinae* (Eriococcidae) - *Rollinia laurifolia* (Annonaceae) (Gonçalves *et al.* 2005) and *Trichilogaster acaciaelongifoliae* (Pteromalidae) - *Acacia longifolia* (Fabaceae) (Dorchin *et al.* 2009) are examples of female-induced galls being larger than male-induced ones. In both systems the galls are similar in general patterns, however, differ in size according with the galling sex. Corroborating the hypothesis that morphological dimorphism of the inducers reflects a difference in responses from the plant, resulting in sexually morphological dimorphic galls.

Galls are true sinks of photoassimilates from different plant parts (Jankiewicz *et al.* 1970; Larson & Whitham 1991; Castro *et al.* 2012). These resources are used to support gall development and metabolism, as well as the galling insect diet (Hartley 1998; Oliveira *et al.*

2006; Isaias *et al.* 2014b). Usually, Cecidomyiidae galls develop a typical nutritive tissue with high sugar metabolism, and a storage tissue with starch, as detected in galls induced by *Eugeniomyia dispar* in *Eugenia uniflora* (Rezende *et al.* 2018) and by a Cecidomyiidae in *Copaifera langsdorfii* (Oliveria *et al.* 2010). In both galls of *L. mataybae*, starch was detected in the storage tissue, and reducing sugar detected in the typical nutritive tissue. However, there was no detection of protein in the tissues of both galls, which is unusual for Cecidomyiidae galls.

The gall induction usually increases the number of cell layers and reduces the intercellular spaces, this developmental feature guarantees parenchyma homogenization, formation of the gall cortex and the neoformation of vascular bundles with cytological distinctiveness (Arduin & Kraus 1995; Isaias *et al.* 2011; Magalhães *et al.* 2014; Fleury *et al.* 2015; Rezende *et al.* 2018). The development of different cell layers' quantities between the sexes of *L. mataybae* can be a reflect of the different insects feeding stimuli and consequently different plant cell responses. The vascular connections derived of the cells redifferentiation, found between host organs and galls, are also important for the establishment and maintenance of the gall structure, allowing the sink status of galls (Oliveira & Isaias 2010b; Isaias *et al.* 2011; Fleury *et al.* 2015; Amorim *et al.* 2017). In a co-generic species of *Lopesia* sp. inducing galls on leaves of *Mimosa gemmulata*, the neoformation of vascular bundles indicates the changing fates of vascular parenchymatic cells towards the re-differentiation of procambium, followed by the differentiation of xylem and phloem elements. Such alterations in cell functions is an essential process for the maintenance and development of the gall structure (Costa *et al.* 2018; Ullrich & Aloni 2000). In galls induced by both sexes of *Lopesia mataybae*, the presence of many hypertrophied vascular bundles

demonstrates that despite the lack of differences in production and storage of primary metabolites between both gall morphotypes, these galls still have a high sink status in consequence of its development.

In our study, we have shown that galls induced by different sexes of *Lopesia mataybae* result in sexually dimorphic galls, with significant differences in morphology (size and thickness) and anatomy (quantity and size of cell layers), but histochemically equals in production of some primary metabolites such as starch and reducing sugars. This indicates that, these differences between the two gall morphotypes, may be due to differences in the life cycle of the insects, where female's stimuli on the host plant lasts more than males, causing plant responses to be different and thus promote bigger galls for females.

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Figure Captions

Figure 1: Galls from both morphotypes. Galls induced by *Lopesia mataybae* in leaves of *Matayba guianensis* are divergent in size and thickness, even when occurring in the same leaf (A). These differences are reflexes of the galling insect's sexual dimorphisms. The galls occupied by females (B) are bigger and thicker laterally than those occupied by males (C). (PP) Pupae, (CCL) “Coccon-like” Structure, (VL) “Vessel-like” Structure. Scale bar: 2mm.

Figure 2: Anatomical structure of both galls induced by *Lopesia mataybae*. (A) (B) lignification region above the “coccon” like larval chamber, giving a harder aspect for both galls. (C, E) Female gall; (C) Female's base smaller, but expanding in thickness around the larval chamber; (E) Cortex cells resulted from hypertrophy and hyperplasia, with the presence of vascular bundles; (D, F) Male gall; (D) Male's base is the bigger part, containing more cell layers, the regions around the larval chamber is thinner; (F) Cortex cells with presence of vascular bundles. (VB) Vascular Bubbles, (SP) Spongy Parenchyma, (TNC) Typical Nutritive Tissue, (ST) Storage Tissue, (LC) Larval Chamber, (SC) Sclerenchyma, (AEP) Adaxial Epidermis.

Figure 3: Histochemical analysis of Starch and reducing sugars. (A) Presence of starch in the storage tissue; (B) Presence of reducing sugar in larval chamber cells and in some regions of the base. (BLR) Base's Lateral Region, (TNC) Typical nutritive tissue.

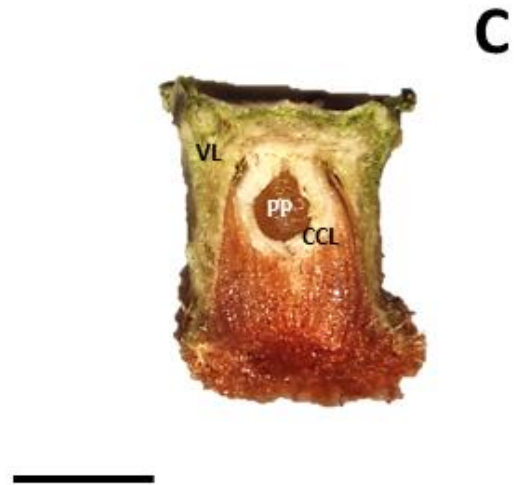
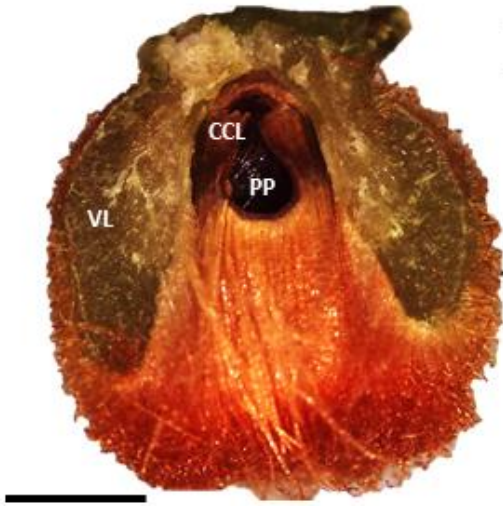
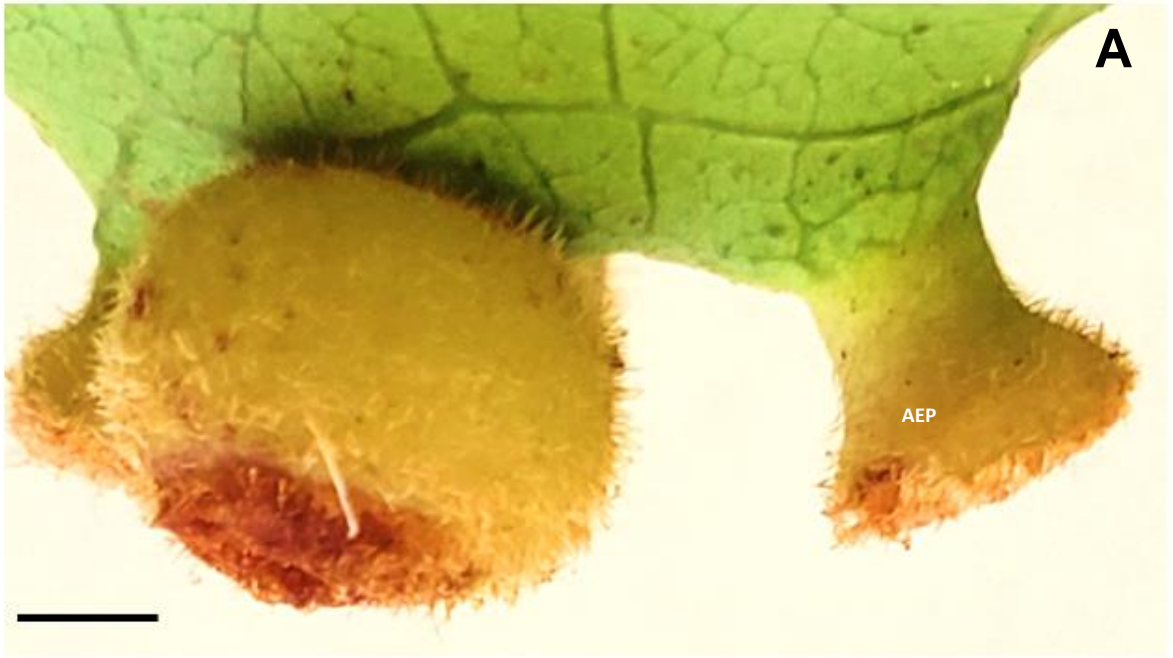


Figure 1

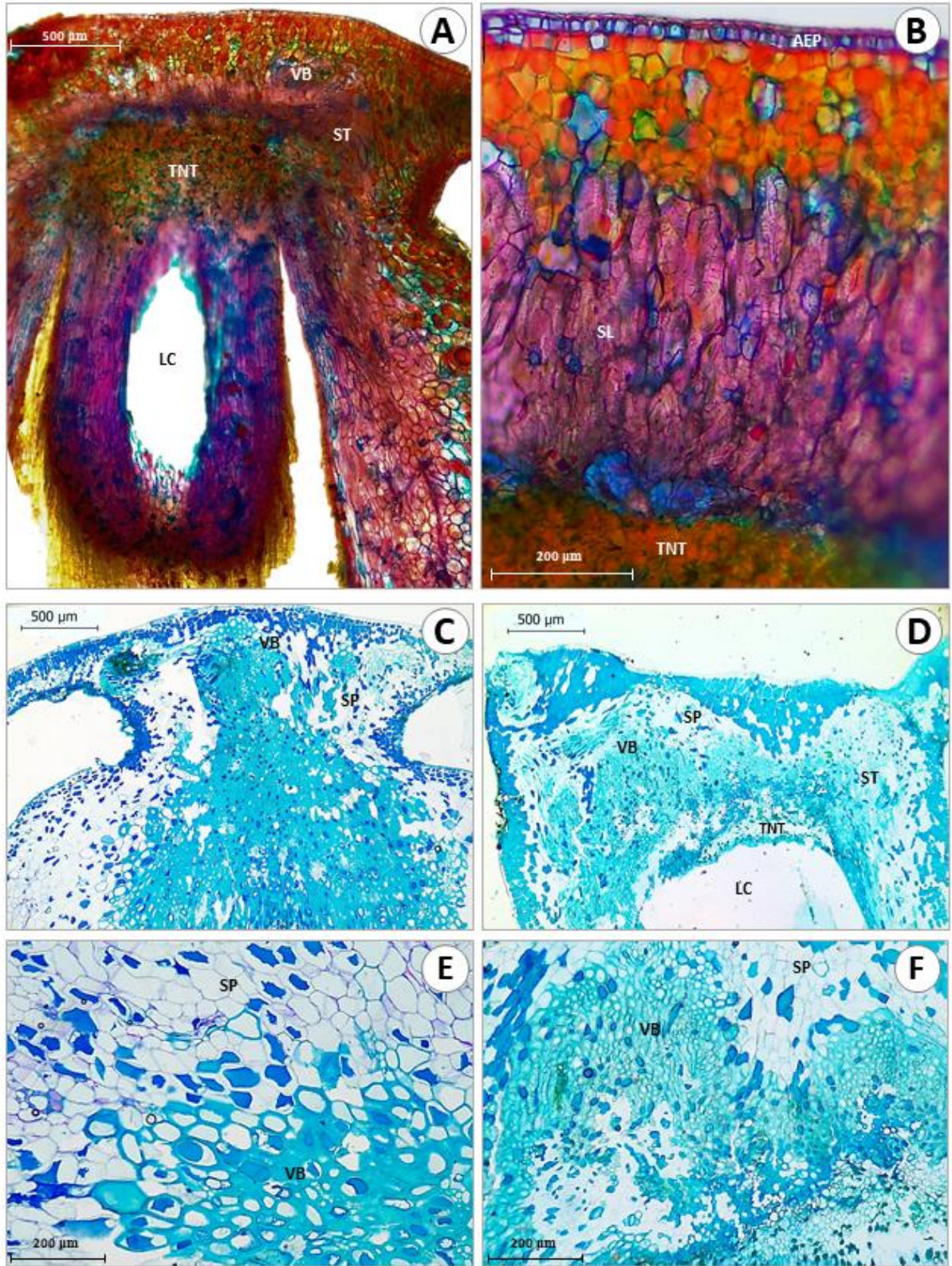


Figure 2

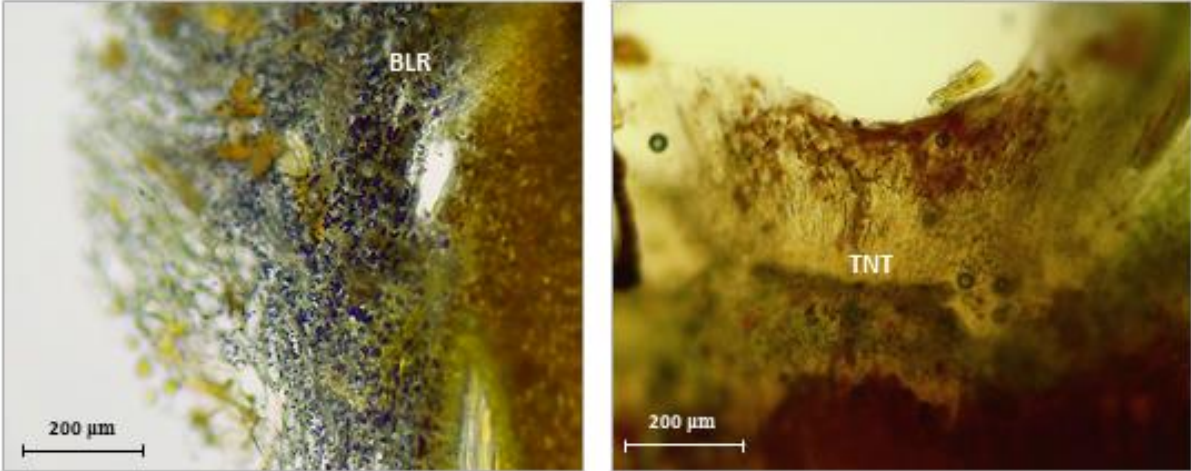


Figure 3

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REQUIREMENTS