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Anatomical Structure of Young Oil Palm (*Elaeis guineensis* Jacq.) Leaves for Callogenesis Initiation

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Abstract

Plant anatomy, a key area within biology, examines the structure and organization of plant organs and tissues. It underpins various disciplines such as physiology, ecology, taxonomy, and evolutionary biology. Anatomical data are typically obtained using the paraffin embedding, which facilitates detailed microscopic observations. In oil palm (*Elaies guineensis* Jacq.) tissue culture, young leaves are commonly used as explants in callogenesis inducing undifferentiated cell growth. The study aimed to describe anatomical characteristics of young oil palm leaves, analyze the correlation between anatomical traits across different leaf positions, and examine anatomical changes during callogenesis. Leaf samples were collected from positions -4, -5, -6, -7 and -8, processed using paraffin embedding technique, and analyzed microscopically. The percentages of callogenesis were also calculated for each leaf position. Data were analyzed using ANOVA, followed by Duncan's Multiple Range Test (DMRT), and correlation analysis was performed in R Studio. The anatomical features included the adaxial and abaxial epidermis, cuticle, hypodermis, mesophyll (palisade and spongy parenchyma), vascular bundles (phloem and xylem), stomata, and sclerencyma. The correlations between mesophyll and leaf thickness were very strong ($r= 0.97$, $p>0.05$), indicating that as mesophyll tissue thickness increases, the overall leaf thickness also increases. Notably, leaves at position -8 exhibited the highest rate callogenesis, reaching 204%.

Keywords: anatomy; callogenesis; oil palm; paraffin; tissue culture.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) belongs to the family Arecaceae (formerly Palmae), which is part of the Monocotyledonae order and classified as a monocotyledonous plant (Cronquist, 1981). As with other monocots, the leaf venation in oil palm is either parallel or curved (Tjitrosoepomo, 2009). Oil palm leaves are characterized by large, pinnate structures, a single columnar trunk with short internodes, and irregular leaf vein patterns (Corley & Tinker, 2016). Plant anatomy, is a key subdicipline of biology, focusing on the structural organization of plant organs and tissues from multiple perspectives. It is crucial in deepening our understanding of plant biology— from molecular-level functions to broader ecological interactions. A thorough understanding of plant anatomy, grounded in its scientific principles and practical applications, contributes significantly to physiology, ecology, taxonomy, and evolutionary biology (Machado & Oliveira, 2024). Anatomical information can be effectively obtained using the paraffin embedding method, which has long been utilized in botany to prepare thin sections of plant tissue for microscopic analysis. This process involves replacing the water with paraffin, allowing for the production of smooth, thin ribbon sections suitable for detailed observation. Although widely used, the paraffin embedding technique is time consuming and requires careful execution of multiple steps, including fixation, dehydration, infiltration, de-alcoholization, staining, mounting to produce high-quality and permanent slide preparation (de Cássia Andreota & Sajo, 2015).

Previous research on the anatomical structure of oil palm leaves by Gomes et al. (2017) which utilized explants from young leaves as initial material for somatic embryogenesis, highlighted several key anatomical features of these tissues. The mesophyll cells of young oil palm leaves exhibit dorsiventral characteristics with two and three layers of palisade parenchyma are composed of elongated, rod-shaped cells arranged in an order beneath the adaxial hypodermis. Additionally, two to three layers of spongy parenchyma, consisting of more rounded cells with small intercellular spaces, were observed in the same region. The young leaf explants also possess a uniseriate (single-layered) epidermis composed of tightly packed rectangular cells. Stomata are present exclusively on the abaxial surface, indicating

hypostomatic leaf type. Beneath the epidermis on both leaf surface lies a double-layered hypodermis, consisting of cells larger than those of the epidermis (Gomes et al., 2017). In comparison, research on the anatomical characteristics of leaves in *Acrocomia* species within the Arecaceae family by Vianna et al. (2017) revealed several key structural features. These include an epidermis covered by a cuticle, hypodermis present on both leaf surface, non-vascular fiber bundles, and both primary and secondary vascular bundles. Similarly, Adzkia et al. (2020) reported that the anatomical characteristics of the leaf sheath in royal palms comprises vascular bundles, parenchyma tissue, metaxylem, protoxylem, and fiber bundles. These vascular bundles and parenchymatous tissues are essential for defining functional and structural properties of oil palm species. Furthermore, Samiyarsih, (2019) emphasized that several leaf anatomical traits, such as cuticle thickness, epidermal thickness, mesophyll thickness, stomatal size and density, and presence of trichomes can be effectively observed using light microscopy (Samiyarsih, 2019).

The propagation of oil palm through tissue culture has been widely practiced, particularly for the multiplication of elite Tenera clones. Various explant sources have been utilized in tissue culture, including mature embryos (Rabechaul et al., 1970), flowers (Smith & Thomas, 1973), roots (Jones, 1974), seeds (Ong, 1977), and young leaves (Schwendiman, 1988). Among these, young leaves especially spear leaves are considered highly suitable due to their totipotency and responsiveness to in vitro conditions. Propagation using young leaf explants on basal media supplemented with NAA (1-napthaleneacetic acid) as a plant growth regulator has effectively stimulated callogenesis development. Young leaf cells from spear leaves exhibit totipotency, highly receptive to signals that trigger the callogenesis pathway. Auxin in younger explants promotes callus induction (Constantin et al., 2015). This study focuses on the anatomical structures of young oil palm leaves as an initial indicator of their capacity. The objectives are to analyze the anatomical structure characteristics of young oil palm leaves (*E. guineensis* Jacq.), examine the correlation between the anatomical traits across individual leaf positions, and evaluate the anatomical structure involved in callogenesis based on leaf position.

MATERIALS AND METHODS

Materials

Spear leaf of young oil palm with La Mé genetic background, pure solid paraffin, fixative solution (FAA) composed of 70% ethanol; formalin; glacial acetic acid in a 90:5:5 ratio (v/v). A graded ethanol series for dehydration: 70%, 80%, 95%, and 100% ethanol. Clearing was performed using pure xylene, ethanol:xylene mixtures 3:1, 1:1, 1:3 (v/v). Additional reagents included distilled water, 1% safranin stain in 70% ethanol, xylene:paraffin mixture (1:9 v/v), liquid pure paraffin, glycerol, glycerin-albumin mixture (1:1), and canada balsam.

Sample collection and preparation of leaf anatomy

Young Oil Palm (*E. guineensis*) explants were obtained from the spear leaves at positions -4, -5, -6, -7, and -8. The leaves were collected for the Tissue Culture Laboratory of the Indonesia Oil Palm Research Institute (IOPRI), North Sumatera, Indonesia. Leaves lamina section was cut into small squares measuring 0.5 x 0.5 cm^2, with three replications performed (Maryani, 2009). Anatomical slide preparation was carried out using a modified paraffin embedding technique based on the protocols by Sutikno (2018). The embedding process began with fixation of the leaf samples in a solution of formalin, glacial acetic acid, and 70% ethanol in a 5:5:90 (v/v) ratio for 24 hours. The samples were then stained with 0.5% safranin in 70% ethanol. The clearing process involved sequential ethanol series of 70%, 80%, 96%, and 100% ethanol followed by ethanol:xylene mixtures at 1:3, 1:1, and 3:1 (v/v), each for 30 min. Infiltration was conducted by immersing samples in xylene:paraffin mixture (1:9) and placing them in an oven at 57°C for 24 h. The mixture was then replaced with pure paraffin, and the samples were maintained at 57°C for an additional 24 h. The leaf samples were placed in petri dishes and embedded in paraffin blocks. These 6-12 µm sections were obtained using a rotary microtome (HY-3615).

Microscopic Observations

Prepared slides were observed under a binocular microscope and documented using a camera (Olympus DP 23, Japan). The anatomical parameters of the young leaves that were observed and measured included: leaf thickness (µm), length of the upper epidermis (µm), length of the lower epidermis (µm), width of the upper epidermis (µm), width of the lower epidermis (µm), length of xylem (µm), length of phloem (µm), width of xylem (µm), width of phloem (µm), length of the palisade tissue (µm), length of the mesophyll (µm). The observations were conducted using magnifications 10x10 and 20x10.

Callogenesis Percentage (%)

Explants from 11 Marihat Clone were cultured in four replicates on media and incubated for 6–12 months. The number of primary calluses that developed on each leaf position (-4,-5,-6,-7,and-8) was recorded (n). The percentage of callogenesis was calculated using the following formula:

$$\%Callogenesis = \frac{Number\ of\ primary\ calluses\ (n)}{Total\ number\ of\ explants\ cultured} \times 100$$

This calculation was performed for each leaf position to determine the callus formation success rate at different developmental stages. The results were then analysed and presented in percentage form.

Data analysis

Qualitative data were analyzed descriptively and presented in the form of images. Quantitative data, derived from anatomical measurements, were statistically analyzed using SPSS software v. 25.0 (IBM, USA). The significance of the leaf anatomical characteristics was assessed through Analysis of Variance (ANOVA) with a 95% confidence level, followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. Correlation analysis was performed to evaluate the strength of the relationship between anatomical variables using R Studio software. The correlation coefficient (r) is calculated using the following equation:

$$r_{xy} = \frac{N\sum XY - (\sum X)(\sum Y)}{\sqrt{[N\sum X^2 - (\sum X)^2][N\sum Y^2 - (\sum Y)^2]}}$$

Where:

r : the correlation coefficient.
N : the number of pairs of data.
$\sum X$: Number of independent variables
$\sum Y$: Number of dependent variables

The interpretation of the sample correlation coefficient between variables is classified according to Table 1 (Usman & Purnomo, 2000).

Table 1. The correlation coefficient and its interpretation.

Coorrelation value	Interpretations
0,00 – 0.09	Negligible correlation
0.10 – 0.29	Low correlation
0.30 – 0.49	Moderate correlation
0.50 – 0.70	Strong correlation
> 0.70	Very strong correlation

RESULTS AND DISCUSSION

Young leaves are morphologically characterized by a smooth surface, flexible texture, and low chlorophyll content. Microscopic observations of their anatomical structure reveal several key components in cross-section. These include the upper epidermis (adaxial), lower epidermis (abaxial), hypodermis, palisade parenchyma (consisting of two to three cells), mesophyll, vascular bundles (phloem and xylem), stomata, cuticle, sclerenchyma, and spongy parenchyma (Figure 1).

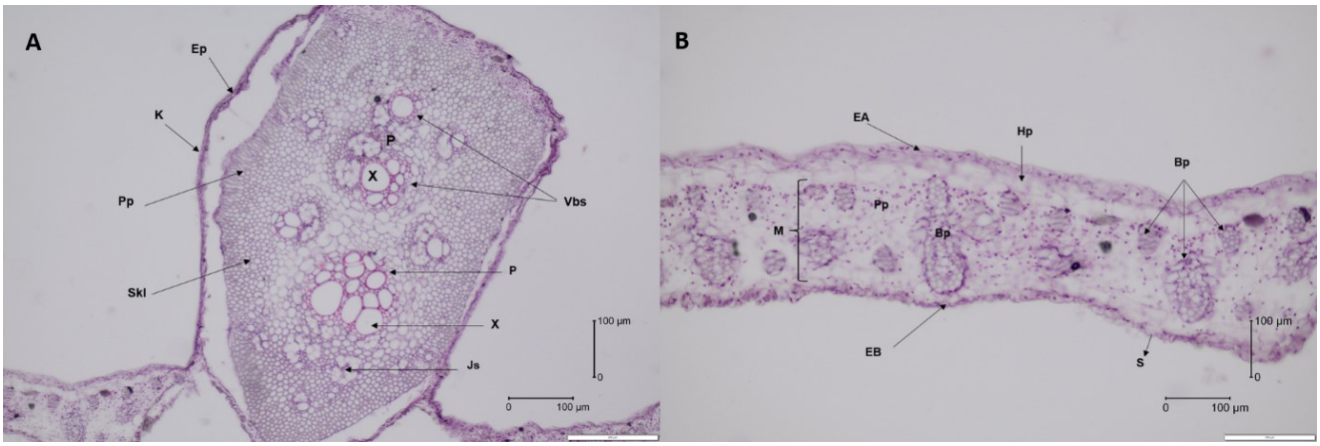

Figure 1. Representative anatomical structure of young oil palm (*E. guineensis)* leaves at 100X magnification. A) Anatomical structure of the leaf vein, B) Anatomical structure of the leaf. **Abbreviation:** EA: Upper epidermis, Hp: Hypodermis, Pp: Palisade parenchyma, M: Mesophyll, Bp: Vascular bundle (VBs), S: Stomata, EB: Lower epidermis, K: Cuticle, Ep: Epidermis, Skl: Sclerenchyma, X: Xylem, P: Phloem, and Js: Spongy tissue. Bar = 100 µm

The midrib of the oil palm leaf (Figure 1A) exhibits larger ones associated with smaller vascular bundles. This observation is consistent with the findings of Luis et al. (2010) who reported that the oil palm leaf anatomy includes a midrib containing prominent vascular bundles connected to smaller vascular elements. Stomata are observed exclusively on the leaf's abaxial (lower) epidermis.

According to Azmin et al. (2021), oil leaf anatomy comprises three major components: the epidermis, mesophyll (including palisade and spongy tissues), and vascular tissue. The epidermis comprises the adaxial (upper) and abaxial (lower) layers. The adaxial epidermis is located beneath the cuticle and above the hypodermal tissue, while the abaxial epidermis lies below the spongy parenchyma. Beneath both epidermal layers is a two-layered hypodermis composed of larger cells than those of the epidermis (Gomes *et al*., 2017). The epidermal tissue exhibits distinct characteristics: (1) rectangular cell shape, (2) tightly packed arrangement without intercellular spaces, (3) absence of chlorophyll, and (4) potential to differentiate into other epidermal cell types (Muttaqin, 2023). Both epidermal layers are composed of a single layer of compactly arranged cells, each covered

by a cuticle (Surya & Hari, 2017). The cuticle, a waxy or fatty layer, is a protective barrier, minimizing water loss through evaporation and shielding against pathogens and physical damage. Two or three layers of palisade parenchyma were observed beneath the adaxial hypodermis. These elongated, rod-shape cells are arranged vertically in rows. Beneath the palisade layers, two to three layers of spongy parenchyma are present, consisting of rounder cells with small intercellular spaces. Scattered throughout the mesophyll are clusters of underdevelop vascular bundles (Gomes et al., 2017). Quantitative measurements of the anatomical features of young leaves are summarized in Table 2.

Table 2. Range Of Average Values for Anatomical Parameters of Young Oil Palm (*E. Guineensis*) Leaves.

Anatomical parameter	Leaf sample				
	-4	-5	-6	-7	-8
Leaf thickness (µm)	203.20 ± 5.67	171.24 ± 13.94	66.68 ± 11.38	195.20 ± 15.00	207.65 ± 13.24*
length of the upper epidermis (µm)	10.40 ± 1.83	9.60 ± 1.27	6.20 ± 0.54	17.20 ± 4.85*	13.68 ± 0.59
length of the lower epidermis (µm)	8.00 ± 4.21	10.08 ± 4.27	6.53 ± 2.80	10.48 ± 3.95	10.65 ± 2.27
width of the upper epidermis (µm)	11.60 ± 1.39	12.80 ± 1.00	15.84 ±4.63	14.40 ± 2.08	9.17 ± 1.11
Width of the lower epidermis (µm)	18.00 ± 1.20	15.52 ± 6.45	8.64 ± 0.87	11.20 ± 2.27	15.16 ± 5.43
length of xylem (µm)	71.40 ±15.19	53.60 ± 9.97	93.52 ± 20.40	64.24 ± 12.16	75.00 ± 14.30
Length of phloem (µm)	13.80 ± 3.37	18.24 ± 1.92	23.44 ± 3.10	24.24 ± 3.86	17.16 ± 2.19
Width of xylem (µm)	87.56 ± 2.70	49.28 ± 15.38	89.56 ± 8.77	57.48 ± 15.67	54.10 ± 12.72
Width of phloem (µm)	13.96 ± 2.90	21.12 ± 3.84	17.56 ± 0.73	25.76 ± 5.49	19.96 ± 1.20
length of the palisade tissue (µm)	12.56 ± 2.22	15.16 ± 1.23	11.72 ± 0.54	30.00 ± 3.17*	9.00 ± 0.62
length of the mesophyll (µm)	120.80 ± 5.41	91.32 ± 7.62	66.68 ± 11.38	114.0 ± 12.98	127.20 ± 17.32*

Note *: significant at ($p \leq 0{,}05$).

The observations revealed that several anatomical parameters were statistically significant ($p \leq 0.05$) including leaf thickness (207.65 ± 13.24), mesophyll thickness (127.20 ± 17.32), upper epidermis length (17.20 ± 4.85), and palisade tissue length (30.00 ± 3.17). Leaf thickness is closely associated with cuticle thickness. The cuticle is relatively thick in oil palm leaves, contributing to an enhanced defense mechanism. Plants with thicker cuticles generally exhibit greater biotic stress resistance than those with thinner cuticles. This is attributed to the structural composition of the cuticle, which includes cutin, cellulose, oligosaccharides, and waxes. It compounds that act as physical and chemical barriers by inhibiting spore attachment, germination, and pathogen penetration (Pradana et al., 2017). Furthermore, as Farassati et al. (2021) noted, a well-developed cuticle may also facilitate the diffusion-based absorption of micronuteients, growth regulators, and oligo-elements, thereby supporting improved physiological responses during in vitro culture.

The mesophyll tissue of *E. guineensis* leaves consists of palisade parenchyma in the upper region and spongy parenchyma in the lower region. Vascular bundles are distributed throughout the mesophyll. According to Surya & Hari (2017) the mesophyll is anatomically divided into two distinct layers: the palisade tissue, located just below the upper epidermis, and the spongy tissue, located above the lower epidermis. Although these two tissues have different structures, both play essential roles in photosynthesis. Despite their structural differences, both tissues play vital roles in photosynthesis. The palisade tissue is particularly important due to its high chloroplast concentration, which enhances light absorption and carbon fixation (Tobing et al., 2021). Tihurua et al. (2020) stated that a higher ratio of palisade to spongy tissue indicates the effectiveness of the tissue in the photosynthesis process for capturing light and CO_2. In most plants, the products of photosynthesis in older leaves are transported to other parts, including younger leaves. According to Azmin et al. (2021) the photosynthetic products are allocated to all parts involved in growth and development. Juwarno et al. (2018) reported that increased thickness of the lower epidermis reduces water loss, supporting the plant adaptation to environmental stress.

This study examined the relationship between several anatomical characteristics of young oil palm (*E. guineensis*) leaves to assess potential correlations influencing the callogenesis process. The analysis focused on five leaf sections (positions -4, -5, -6, -7, and -8). The results revealed both positive (+) and negative (−) correlations among the anatomical traits of the leaves, as presented in Figure 2.

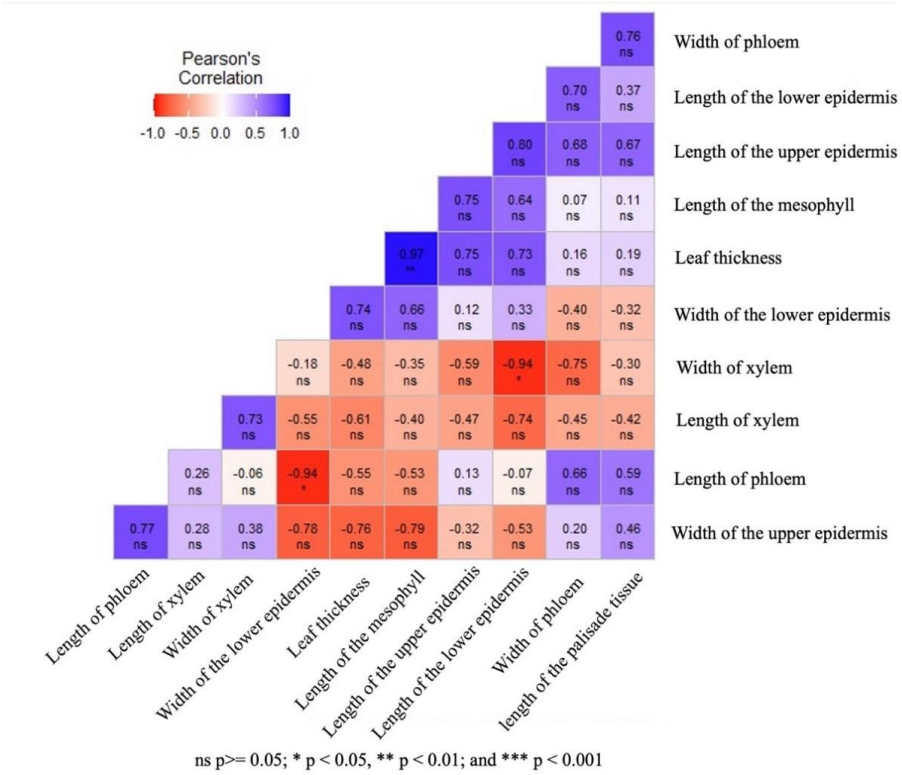

Figure 2. Heatmap of the correlation between several anatomical characteristics of young oil palm leaves (*E. guineensis*).

Based on Figure 2, a very strong correlation was observed between mesophyll tissue thickness and overall leaf thickness (r = 0.97, p > 0.05), indicating that leaf thickness also increases as mesophyll tissue becomes thicker. According to Cambaba et al. (2016), the mesophyll constitutes the primary component of the leaf blade; thus, any variation in mesophyll thickness has a significantly impacts on total leaf thickness. Variation in leaf thickness among plant species may be attributed to genetic factors that regulate plant anatomical characteristics (Coneva & Chitwood, 2018). Utami (2017) further noted that sunlight exposure influences leaf thickness, with increased light intensity promoting the development of a thicker palisade layer. In addition to light, leaf thickness is also affected by factors such as water content in plant tissues (Essaghi et al., 2016). Other environmental variables, including nutrient availability and light intensity, can also contribute to variations in leaf anatomical structure (Ningsih & Daningsih, 2022). Young oil palm leaves (*E. guineensis*) are frequently utilized as a source of explants for regeneration in tissue culture processes. According to Nurchayati et al. (2018) leaf explants are preferred due to their relatively thin morphology, which facilitates enhanced contact between the tissue surface and the culture medium, enhancing regeneration. One of the primary regeneration pathways for oil palm leaves is somatic embryogenesis. Leaves from positions -4, -5, -6, -7, and -8, used in the tissue culture process, exhibit varying levels of calogenesis, which are influenced by the anatomical characteristics of each leaf (Figure 3).

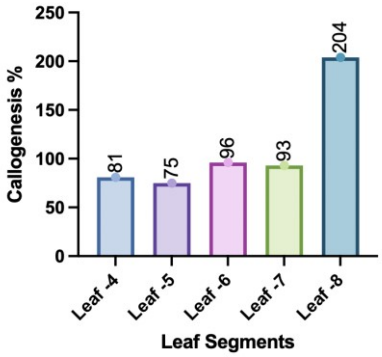

Figure 3. *The percentage of calogenesis derived from various responsive sections of young oil palm leaves (E. guineensis).*

Based on Figure 3, the highest percentage of calogenesis in young oil palm leaves (*E. guineensis*) was observed in leaf position -8, reaching 204%. This is associated with the positive correlation between leaf thickness and mesophyll thickness, which shows a positive correlation (+). A greater mesophyll cell thickness can enhance photosynthetic efficiency per unit area and improve the translocation of photoassimilates required by plant organs (Torres-Pio et al., 2021). Mesophyll cells can regenerate and differentiate into various types of tissues, including roots and shoots, when cultured in a tissue culture medium. According to Gomes et al. (2017), the early stages of calogenesis are closely linked to the central part of the leaf, particularly around vascular bundles (phloem and xylem). The cut edges of

young leaves, near these vascular bundles, have greater contact with the nutrient-rich culture media. This facilitates cell division near the vascular bundles, forming callus, which contains smaller cells, dense cytoplasm, and distinct nuclei. Callus development through calogenesis involves periclinal cell division, resulting in organized cell development and increased leaf thickness. Palisade parenchyma cells near the cell mass show minimal shape changes due to compression by the epidermal cells on the adaxial surface, leading to an increase in width and a decrease in height. In contrast, spongy parenchyma and abaxial epidermal cells near the developing propagules are often damaged or disrupted during callus formation.

CONCLUSIONS

The anatomical characteristics of young oil palm leaves (*E. guineensis*) include the upper epidermis (adaxial), lower epidermis (abaxial), hypodermis, mesophyll (palisade parenchyma and spongy parenchyma), vascular bundles (phloem and xylem), stomata, cuticle, and sclerenchyma. The correlation between the anatomical characteristics of the mesophyll tissue and leaf thickness shows a very strong correlation between mesophyll thickness and overall leaf thickness ($r = 0.97$, $p > 0.05$), indicating that an increase in mesophyll thickness contributes to greater leaf thickness. This increase may enhance the callogenesis process by promoting cellular activity within the tissue. Among the leaf positions examined, position -8 exhibited the highest callogenesis percentage, reaching 204%.

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Competing Interests: The authors declare that there are no competing interests.

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