Anatomical changes in leaves of *Piper aduncum* L. under different irradiances

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ABSTRACT
The aim of the study was to evaluate the effect of different light conditions on the internal structure of *Piper aduncum* L. leaves. *P.aduncum* seedlings were grown for 4 months under different light conditions achieved with shading nets (50, 70, and 100% of luminosity) and with photoconversion nets in red (RN) and blue (BN) colors. Two fully expanded leaves located at the third node were collected from five plants per treatment for paradermal and transverse sections. In the paradermal sections, stomatal density, polar and equatorial diameters, the ratio between polar and equatorial diameters, and trichome density on the abaxial and adaxialsurfaces of the epidermis were evaluated. In the transverse sections, the thickness of the adaxial and abaxial epidermis, cuticle of the adaxial surface, palisade and spongy parenchyma, and leaf blade were assessed. The vessel elements parameters were also evaluated and Carlquist vulnerability index were measured. The results show that *P. aduncum* changes the internal structure of its leaf when grown under different radiation conditions. It presented greater epidermis thickness, greater stomatal density and smaller diameters of vessel elements, anatomical characteristics favorable to its growth under high irradiances.
Keywords: Monkey pepper, Ecological anatomy, Luminosity, mesophyll.

INTRODUCTION

Light, as a primary source of energy, is one of the most important environmental factors for plant growth and development and changes in irradiance, whether in intensity or spectrum, influence the anatomy, physiology, and morphology of leaves (Brodribb et al. 2020). In general, adaptations to different irradiance conditions are associated with the adjustment of the photosynthetic apparatus to optimize the capture of incident light (Brestic et al. 2023). Besides the modifications that occur in the photosynthetic tissues, studies have shown significant changes in stomatal density, stomatal functionality, trichome number, alterations in vascular bundles, and epidermal thickness (Silva et al. 2020; Haworth et al. 2023).

Zhang et al. (2022) observed changes in the morphoanatomy of *Heptacodium miconioides* Rehder (Caprifoliaceae) leaves when exposed to three levels of irradiance, including an increase in palisade parenchyma, stomatal density, and leaf thickness. Similar responses were observed by Gomes et al. (2023) for *Lippia origanoides* Kunth (Lamiaceae) regarding different light intensities. Frade et al. (2023) also observed that the anatomical responses to the spectrum are not limited to leaves but also influence root growth and stem elongation in *in vitro*-cultivated *Eucalyptus* (Myrtaceae).

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Piper aduncum L. (Piperaceae), popularly known as “monkey pepper” or “long pepper,” is a pioneer shrub native to the Americas (Rocha et al. 2008). It has a wide geographical distribution in Central America, the Antilles, and South America. In Brazil, it canbefound in the Acre, Pará, Mato Grosso, Ceará, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, and Paraná states (Guimarães et al. 2024). Although not yet commercially cultivated, the species has high potential due to its antimicrobial properties (Nor et al. 2020; Morais et al. 2023), insecticidal properties (Durofil et al. 2021; Morais et al. 2023), and essential oil with low toxicity (Sousa et al. 2008).

Species belonging to the Piperaceae are of interest in anatomical studies due to their medicinal importance. Most studies focus on the anatomical description of species or the identification of chemical components present in their essential oil-producing structures (Gogosz et al. 2012; Arroyo et al. 2022). Therefore, little attention has been given to the influence of environmental conditions on these characteristics.

However, it is precisely these aspects that largely determine the plasticity of plants in response to the environment. The morphological characteristics of the leaf surface determine the amount of light absorbed or reflected the degree of organ hydrophobicity, the vapor pressure of the air in contact with the leaves, the organ’s efficiency in defending against parasites and pathogens, the amount of absorbed pollutants or pesticides, the magnitude of transpiration, and growth (Yang et al. 2023).

In this context, the aim of this study was to evaluate the characteristics of the mesophyll and vascular bundles of *P. aduncum* leaves cultivated under different irradiances in a greenhouse.

MATERIAL AND METHODS

Seedlings of *P.aduncum* were produced in the Departamento de Fisiologia Vegetal at the Universidade Federal deLavras - UFLA, from seeds collected from selected parent plants located on the university campus. The seeds were pre-germinated on petri dishes, placed on three filter paper sheets, and kept in a Mangesdorf-type germination chamber at 25 °C under a 12-h photoperiod for 30 days. The plant was identified by DSc.Valéria Evangelhista Gomes and the exsiccates of the species are listed as a taxonomic document in the Herbarium of the Federal University of Lavras, under registration number 20275.

After this period, the seedlings were transferred to expanded polypropylene trays with 72 cells, each containing 55 g of commercial substrate Tropstrato HA® per cell. They were then kept in a nursery with 50% shading until they reached a height of 2.5 cm. The plants intended for full-sun cultivation (100% radiation) were previously acclimated for seven days at 70% irradiance and then for seven days under full sun before being transplanted into the final substrate.

After acclimation, the seedlings were transplanted into 6-l plastic pots filled with a substrate composed of subsoil, sand, and bovine manure in a ratio of 2:1:1, according to the different irradiance treatments. The physicochemical characteristics of the soil were analyzed by the Soil Analysis Laboratory of the Departamento de Ciências do Solo at UFLA, and the following parameters were obtained: pH: 5.4; P: 4.13 mg/dm^3; K: 73.32 mg/dm^3; Ca: 2.30 cmolc/dm^3; Mg: 0.30 cmolc/dm^3; Al: 0.10 cmolc/dm^3; H+Al: 2.90 cmolc/dm^3; V: 49.00%; organic matter: 2.10 dag/kg; clay: 70.00 dag/kg; silt: 16.00 dag/kg; and sand: 14.00 dag/kg.

For anatomical analyses, two fully expanded leaves located at the third node of five plants per treatment were collected 150 days after transplanting into the pots. These leaves were fixed in a solution of formaldehyde, acetic acid, and 70% ethanol (F.A.A.70) for 72 hours, and then stored in vials containing 70% ethanol (Arik and Altindişli 2021). Paradermal sections were obtained by chemical tissue dissociation in Jeffrey’s solution for 7 days, followed by clarification with 50% sodium hypochlorite. The sections were then washed in distilled water three times for 10 min, stained with 1% safranin solution, and mounted on a slide and cover slip with 50% glycerin (Kraus and Arduin 1997). The Jeffrey’s solution was prepared using equal parts of chromic acid (10%) and nitric acid (10%) (Johansen 1940). Transverse sections were made from two-centimeter leaf fragments taken from the middle region of the leaf containing the central vein, using a table microtome model LPC.

The sections were clarified in 50% sodium hypochlorite, washed in distilled water three times for 10 min, stained with Safrablau solution (1% safranin and 0.1% astral blue in a ratio of 7:3, v/v), and mounted on slides and cover slips with 50% glycerin. The slides were photographed using an Olympus® BX 60 microscope, coupled with a Canon A630 digital camera. The photomicrographs were analyzed using UTHSCSA Image Tool® software, measuring the quantitative characteristics of the tissues on four slides per replication, with five sections per slide and five fields per section.

From the transverse sections, the thickness of the adaxial epidermis (TAE) and abaxial epidermis (TAB), cuticle of the adaxial surface (CAS) of the leaf blade, palisade parenchyma (PP), spongy parenchyma (SP), and leaf mesophyll (LM) were evaluated.

In the paradermal sections, the polar diameter (PD), equatorial diameter (ED), the ratio between polar and equatorial diameter (PD/ED), stomatal density (SD), density of trichomes on the abaxial surface (TAB) and adaxial surface (TAD) of the epidermis were evaluated. Stomatal density (number of stomata per mm²) was measured on the abaxial surface of the epidermis (hypostomatous leaf), and trichome densities (number of trichomes per mm²) were obtained on the abaxial and adaxial surfaces of the epidermis.

The thickness of the phloem (TP), phloem proportion in relation to the total area of the vein (PR), xylem diameter (XD), number of xylem vessels (XN), proportion of xylem in relation to the total area of the vein (XR), and Carlquist's vulnerability index (CVI = xylem vessel diameter/number of xylem vessels) calculated according to Carlquist (1975) were also measured.

The experimental design was completely randomized, with five treatments and five replications. The data were subjected to analysis of variance and the Scott-Knott test at $p<0.05$ using the SAEG statistical software (SAEG 2007).

RESULTS AND DISCUSSION

The experiment was conducted at Gota de Esperança Farm, Departmento de Agricultura (DAG) of the UFLA, located at coordinates 21°14'07"S and 44°58'22"W, at an altitude of 879 m. The climatological averages observed during the experimental period were provided by the Climatological Station of the Departmento de Engenharia Agrícola at UFLA, with a maximum temperature of 25.06 °C and a minimum temperature of 13.54 °C, precipitation of 1.28 mm, and relative humidity of 72.7%.

The treatments consisted of cultivating the plants for 150 days under five irradiance spectra: two shade net (30 and 50% shading), two Chromatinet® netes in red (RN) and blue (BN) colors blocking 50% of the incident radiation, and a full-sun treatment (100% irradiance). Irrigation was performed daily, maintaining the substrate at field capacity.

Using a USB-650 RED TIDE portable spectroradiometer coupled with an electromagnetic radiation source DT-MINI (200 to 2000 nm) and an R400-7-VIS-NIR reflectance probe (US Bio Solutions Ocean Optics®), the reflectance spectrum of the different radiation environments was evaluated with a spectral resolution of 1 nm. The normalized irradiance for each environment showed higher values in terms of spectrum quantity and size for the 100% irradiance environment, followed by the RN environment, 70 and 50% irradiance environments, and the MA environment (Figure 1). It was also observed that the blue net provided a peak of irradiance between approximately 450-550 nm, while the red net exhibited a peak between 490-690 nm.

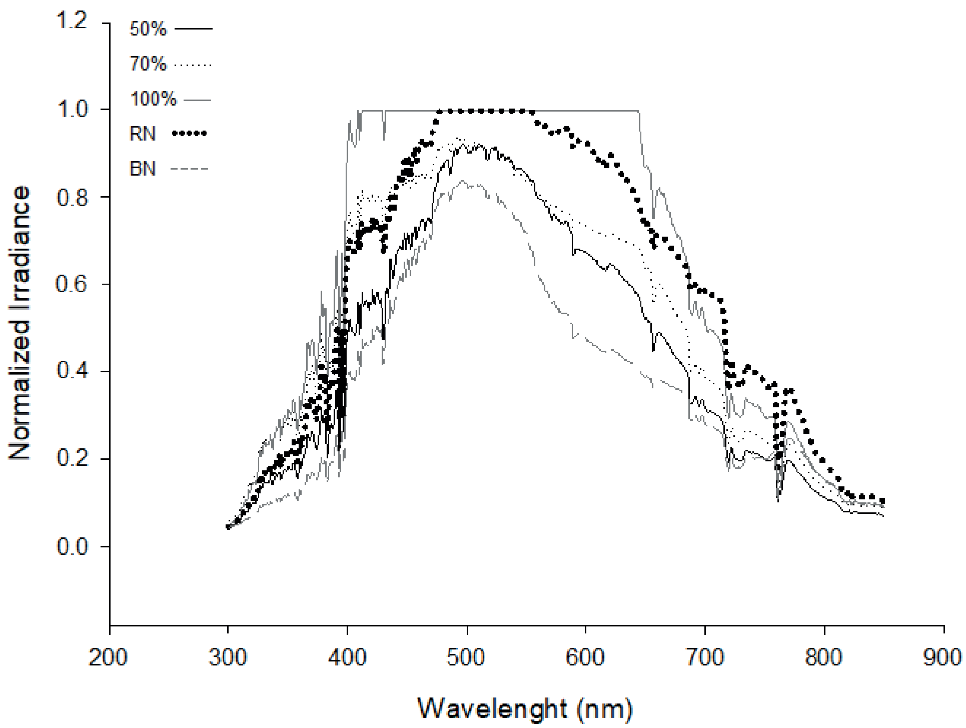

Figure 1. Normalized irradiance/wavelength in five radiation environments: 100% irradiance; 70% irradiance; 50% irradiance; RN (red net) and BN (blue net).

Significant changes in the leaf anatomy of *P. aduncum* were observed when cultivated under different irradiance levels ($p<0.05$). In the transverse sections of the leaf blade, it was observed that the thickness of the adaxial and abaxial epidermis, cuticle, palisade parenchyma, spongy parenchyma, and leaf lamina were greater in plants grown under 100% irradiance, with a reduction in treatments with lower irradiance levels (Table 1, Figure S1 - Supplementary Material).

The increase in leaf tissue thickness under high irradiance conditions may serve as a way to mitigate or prevent damage caused by excessive light to the photosynthetic apparatus, allowing for the maintenance of photosynthesis (Matos et al. 2009). Thicker epidermis and cuticle can enhance leaf reflectance and improve light interception efficiency (González Moreno et al. 2022). Additionally, the thickening of leaf tissues can be understood as a species' plasticity to this irradiance condition. Similar results were observed in *Nicotiana glauca* Graham (Solanaceae) plants, which exhibited increased cuticle thickness and palisade parenchyma thickness in higher irradiance environments (Negin et al. 2023).

Regarding the plants grown under colored net, an increase in the thickness of the abaxial epidermis, cuticle, palisade parenchyma, and leaf lamina was observed in plants cultivated under blue net (Table 1). A study conducted with cherry plants also observed thickening of the palisade parenchyma and epidermis when grown under blue monochromatic light, while a reduction in these tissues was observed when cultivated under red light (Sarropoulou et al. 2023). According to Momayyezi et al. (2022), an increase in the red band of light promotes an energy imbalance that interferes with the functioning of photosystem I and II.

Therefore, the reduced thickness of the palisade parenchyma and epidermis is a result of the interference in the distribution of radiation between the photosystems, which leads to changes in the development of these leaf tissues. Additionally, a higher amount of blue light can promote the development of leaves with characteristics similar to those developed under high irradiance conditions (Brestic et al. 2023).

The stomatal density of plants grown under 100% irradiance showed higher values compared to the other treatments (Table 2 and Figure S2 - Supplementary Material). Additionally, the stomata of plants cultivated under 100% irradiance exhibited a reduction in size. An increase in stomatal density may be related to the plant's plasticity in order to increase stomatal conductance and prevent limitations in photosynthesis (Revathi et al. 2020). In *Gerbera jamesonii* Bolus ex Hook.f (Asteraceae) grown in vitro under different light qualities, higher stomatal density was observed in plants illuminated with predominantly red spectra (Meng et al. 2019).

Regarding the PD/ED ratio of the stomata, it was found that plants grown under colored net had a higher value compared to those under 100% irradiance (Table 2 and Figure S2 - Supplementary Material). The blue and red light spectra are important for controlling stomatal opening by regulating phototropins and phytochromes that activate a signaling cascade controlling stomatal opening and closure (Al Murad et al. 2021). According to Khan et al. (2002), a higher DP/DE ratio indicates a more ellipsoidal shape of the stomata, which can result in greater functionality, as observed in plants grown under colored net. Changes in this ellipsoidal structure affect stomatal opening and closure control, resulting in high stomatal conductance and water loss (Brodribb et al. 2020).

Plants under 100% irradiance showed a lower PD/ED ratio and higher stomatal density, indicating that under high light conditions, plants

Table 1. Anatomical variables observed in cross-sectional sections of leaf tissues in *Piper aduncun* cultivated under different irradiance levels.

TRAT	TAE (µm)	TAB (µm)	CU (µm)	PP (µm)	PJ (µm)	LF (µm)
50%	50.8d	42.2a	5.60b	39.23c	46.67d	85.97d
70%	56.2b	37.2b	5.51b	40.24c	49.19c	83.47d
100%	63.7a	42.7a	8.88a	56.52a	71.80a	125.63a
RN	53.7c	43.2a	8.33a	45.60b	56.68b	98.53c
BN	56.6b	42.0a	8.76a	54.52a	50.49c	105.49b
CV(%)	2.17	3.24	6.79	4.34	2.98	1.70

*Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test ($p<0.05$). TAE = thickness of the adaxial epidermis; TAB = thickness of the abaxial epidermis; CU = cuticle thickness; PP = palisade parenchyma thickness; PJ = spongy parenchyma thickness; LF = leaf blade thickness.

Table 2. Anatomical variables observed in paradermal leaf sections of *Piper aduncun* cultivated under different irradiance levels.

TRAT	PD (µm)	ED (%)	PD/ED (µm)	SD. (stom./mm²)	TAB (trich./mm²)	TAD (trich/mm²)
50%	27.06^{ns}	18.58^{ns}	1.76b	177.25b	22.98c	28.93d
70%	28.66	18.64	1.54c	163.21c	60.71a	33.69c
100%	25.48	17.72	1.41d	217.54a	44.86b	53.54b
RN	28.34	15.22	1.84a	141.42d	25.70c	37.96c
BN	27.62	15.88	1.84a	150.97d	25.09c	61.29a
CV(%)	8.12	10.87	2.36	4.87	6.72	7.25

*Means followed by the same letter in the column do not differ significantly according to the Scott-Knott test (p<0.05). ns: not significant. PD: polar diameter; ED: equatorial diameter; PD/ED ratio; SD: stomatal density; TAB: trichome density on the abaxial surface of the epidermis; TAD: trichome density on the adaxial surface of the epidermis.

increase the number of stomata but with smaller dimensions, allowing for more accurate regulation of water and photosynthetic relationships under these conditions (Haworth et al. 2023).

The trichome density on the abaxial and adaxial epidermis showed significant results in relation to irradiance treatments (Table 2; Figure S3 and S4 - Supplementary Material). On the abaxial side of the epidermis, the treatment with 70% irradiance exhibited the highest trichome density, while on the adaxial side of the epidermis, higher trichome density was observed in plants cultivated under 100% irradiance and the blue net. This result suggests that in *P. aduncun*, both the spectral quality and intensity of irradiance influence the formation of these structures. Studies conducted on *Mentha canadensis* L. (Lamiaceae) also found a relationship between spectral quality, irradiance intensity, and trichome formation (Ueda et al. 2021).

Trichomes are a morphological adaptation that can act in plant protection by regulating leaf temperature through light reflection. Glandular trichomes can also secrete substances that protect leaves against parasites and predators (Escobar-Bavo et al. 2018). Additionally, it can be inferred that the higher trichome density, increased thickness of the palisade parenchyma and epidermis observed in treatments with 100% irradiance and the blue net is a response aimed at enhancing the efficiency of radiation utilization in the photochemical process (Brousseau et al. 2021).

There was a greater thickening of the phloem in plants cultivated under 70% irradiance and the blue net, with no changes in the proportion of this vascular tissue in relation to the total area of the vein (Table 3 and Figure S5 - Supplementary Material). The phloem distributes the photoassimilates produced by the leaves and, therefore, may undergo changes due to the light environment (Agustí et al. 2020). Thus, the increased thickness of its structure in the 70% irradiance environment and the blue net

Table 3. Anatomical variables observed in cross sections of the vascular bundles of *Piper aduncun* leaves grown at different irradiances.

TRAT	PT (µm)	PP (%)	XD (µm)	XVN (unid)	XVR (%)	CVI
50%	37.48b	0.064^{ns}	9.66a	66.04c	0.060b	0.15a
70%	44.18a	0.067	10.22a	113.02a	0.079a	0.09c
100%	38.50b	0.058	8.81b	59.13c	0.063b	0.15a
RN	36.38b	0.065	8.66b	56.03c	0.060b	0.16a
BN	46.89a	0.072	9.85a	80.08b	0.085a	0.12b
CV(%)	9.05	13.04	4.64	9.33	8.53	10.45

*Means followed by the same letter in the column do not differ significantly from each other according to the Scott-Knott test (p<0.05). ns. not significant. PT= phloem thickness; PP= phloem proportion; XD= xylem diameter; XVN= xylem vessel number; XVR= xylem vessel proportion; and CVI= Carlquist vulnerability index.

may be promoting a more balanced distribution of photoassimilates to different plant organs.

The number and diameter of xylem vessels in relation to the total area of the vein were higher in treatments with 70% irradiance and the blue net (Table 3, Figure S5 - Supplementary Material). Larger vessel elements (in diameter and proportion) are more efficient in conducting water compared to narrower and smaller elements, but they provide less hydraulic safety (Belyaeva and Butenkova 2021). Similarly, in *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), an increase in the size of vascular elements and vessels and higher photosynthetic rates were observed in environments enriched with blue spectrum (Silva et al. 2020). Plants cultivated under 50 and 100% irradiance and the red net reduced variables related to xylem in order to increase hydraulic safety, which may indicate that these conditions caused stress in the species. However, studies on the effect of light quality and intensity on the development of this tissue in medicinal plants are still limited.

The Carlquist Vulnerability Index (CVI) allows inferences about xylem vulnerability to embolism - the lower the CVI, the higher the hydraulic conductivity of the plant. Plants cultivated under 70% irradiance and the blue net exhibited the lowest CVI values (Table 3 and Figure S5). The increased phloem thickness associated with the low CVI observed in the 70% irradiance and blue net treatments indicates that the species has appropriate responses when cultivated under these conditions. Since the phloem is directly involved in the flow of photoassimilates from the leaves to different plant organs, these conditions may allow for greater growth and a more balanced distributionthe of plant. Thus, it can be concluded that *P. aduncum* alters its leaf anatomy according to different light conditions, which allows better adaptability in both full sun and shaded environments or with altered light spectra.

CONCLUSIONS

Based on the observed data, it can be noted that the species *P. aduncun* alters its leaf anatomy when cultivated under different irradiance levels. Additionally, it exhibits favorable leaf anatomical characteristics for its development under high irradiance conditions and under spectra enriched with blue light wavelengths.

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AUTHORS' CONTRIBUTION

Conceptualization: FVP, MFPL, and AAA; Methodology: FVP and MFPL; Formal analysis: ICAA; Investigation: FVP, ICAA, and ACRT; Resources: AAA; Data curation: FVP and ACRT; Writing - original draft: FVP and ICAA; Writing - review & editing: ICAA; Visualization: ICAA; Supervision: FVP and MFPL; Project administration: FVP and AAA; Funding acquisition: AAA. All authors read and approved the final manuscript

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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