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Plant biometric characterization and leaf micromorphometry of *Talinum triangulare* (Jacq.) Willd cultivated under shade¹

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ABSTRACT

Talinum triangulare (Jacq.) Willd, known as Ceylon spinach, Surinam purslane, or waterleaf, is cultivated for medicinal and food purposes. Among environmental factors, light is an important regulator of primary production, contributing to plant growth. However, the success of each plant species depends on its morphoanatomical responses to light. The aim of this study was to determine whether *T. triangulare* leaf micromorphometry is affected when grown in full sun and under shade, and if so, which plant biometric variables are affected. *T. triangulare* cultivation was tested in beds arranged in blocks under shade levels of 0, 18, 30, and 50%. Each bed was considered a basic experimental unit, totaling four replications per treatment. Plant height, stem diameter, leaf area, leaf fresh weight, stem fresh weight, specific leaf area, leaf area ratio, abaxial epidermis, and mesophyll thickness were higher as shade levels increased. However, there was a decrease in the number of leaves, root to shoot ratio, stomatal density on abaxial and adaxial surfaces, and palisade parenchyma thickness. Nevertheless, root length, leaf and stem dry weights, leaf weight ratio, adaxial stomatal index, adaxial epidermis, and spongy parenchyma thickness remained constant. Plants responded differently to shade, with morphological and anatomical changes, suggesting a phenotypic plasticity and raising the possibility of being grown both in shaded environments and in full sunlight.

Keywords: growth; anatomy; plasticity; luminosity.

RESUMO

Caracterização biométrica da planta e micromorfometria foliar de *Talinum triangulare* (Jacq.) Willd cultivada sob sombreamento

Talinum triangulare (Jacq.) Willd, conhecida como espinafre do Ceilão, beldroega graúda e folha d'água é cultivada com fins medicinais e alimentares. Entre os fatores ambientais, a luz é um importante regulador da produção primária, contribuindo para o crescimento das plantas. No entanto, o sucesso de cada espécie de planta depende das respostas morfoanatômicas à luz. O objetivo deste estudo foi determinar se a micromorfometria da folha de *T. triangulare* e qual das variáveis biométricas da planta são afetadas quando cultivada em pleno sol e sob sombreamento. O cultivo de *T. triangulare* foi testado em canteiros dispostos em blocos com níveis de sombreamento de 0, 18, 30 e 50%. Cada canteiro foi considerado uma unidade experimental básica, totalizando quatro repetições por tratamento. A altura da planta, o diâmetro do caule, a área foliar, a massa fresca da folha, a massa fresca do caule, a área foliar específica, a razão de área foliar, a espessura da epiderme abaxial e do mesófilo foram maiores à medida que aumentaram os níveis de sombreamento. No entanto, houve diminuição no número de folhas, relação raiz: parte aérea, densidade estomática das superfícies abaxial e adaxial e de espessura do parênquima paliçádico. O comprimento da raiz, as massas secas das folhas e do caule,

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a razão de peso foliar, o índice estomático da face adaxial, a espessura da epiderme adaxial e do parênquima esponjoso, por sua vez, permaneceram constantes. As plantas responderam de forma diferente ao sombreamento, com mudanças morfológicas e anatômicas, sugerindo uma plasticidade fenotípica e aumentando a possibilidade de serem cultivadas tanto em ambientes sombreados quanto em pleno sol.

Palavras-chave: crescimento; anatomia; plasticidade; luminosidade.

INTRODUCTION

Talinum triangulare (Jacq.) Willd belongs to the family Talinaceae, previously Portulacaceae (Brilhaus *et al.*, 2016). Its common names include Surinam purslane, Ceylon spinach, sweetheart, cariru, Florida spinach, potherb fame flower, lagos bologi, and waterleaf (Fasuyi, 2007; Aja *et al.*, 2010; Liao *et al.*, 2015). This species has pantropical distribution and adapts well to low fertility soils (Fasuyi, 2007; Nyffeler & Eggli, 2010). Native to tropical Africa, it is cultivated for medicinal (Agbonon *et al.*, 2010) and food purposes in South America, Asia, and Africa, especially in Nigeria (Ifon & Bassir, 1979, 1980; Fasuyi, 2007).

In Brazil, leaves and stems of *T. triangulare* are sold as unconventional greens, mainly in the North, Northeast, and Midwest regions, by smallholders as a source of extra income (Brasileiro, 2010; Alexandre, 2016). Brazil's agricultural agency, Empresa Brasileira de Pesquisa Agropecuária (Embrapa), has conducted research on this species, to promote its use as an unconventional leafy vegetable (Brasil, 2010). The species has agronomic and economic potential, mainly by smallholders in need of alternative income sources.

Since climatic and genetic factors influence the performance of plants, it is necessary to conduct research with genotypes under various climatic conditions. Among physical environmental factors, light levels regulate the primary production and influence plant growth (Dalmolin *et al.*, 2015). In this sense, shade screens have been used alone or in combination in protected environments, making the resulting microclimatic conditions appropriate for crop growth. Thus, the harmful effects of high incidence of solar radiation are reduced, also protecting against extreme temperatures (Moraes *et al.*, 2011).

It is crucial to understand the physiology of plant development, as well as the impacts of abiotic and biotic factors, so that management practices can be adopted to improve plant productivity (Goenaga, 1995). Several studies have reported the benefit of using shade for the cultivation of different plant species, such as leaf cactus (Queiroz *et al.*, 2015), taro (Oliveira *et al.*, 2011), rocket salad (Grangeiro *et al.*, 2011), coriander (Grangeiro *et al.*,

2011), and watercress (Hirata & Hirata, 2015), among others.

The intensity of photosynthetically active radiation plays an important role in plant acclimation to UV radiation. Morphological and physiological characteristics influenced by high irradiance are also affected by blue light (Hoffmann *et al.*, 2015). In pepper plants, these authors found that a high amount of blue light triggers specific biochemical and physiological processes, resulting in better acclimation and recovery of plants from stress produced by UV radiation. Among cultivated species, the ability to use light for biological production is variable, and morphoanatomical responses are responsible for the success of each species (Aragão *et al.*, 2014).

Plant biometrics and anatomical measurements help understand differences in growth of plants cultivated in different environments. Therefore, evaluating the productive and anatomical characteristics of *T. triangulare* grown under different shade levels and in full sun is extremely important when aiming to develop a base of reliable information to guide agronomic practices necessary for its economic exploitation. The purpose of this study was to determine whether *T. triangulare* leaf micromorphometry is affected when cultivated in different shade levels, and if so, which plant biometric variables are affected.

MATERIAL AND METHODS

The experiment was carried out from October 2014 to March 2015 in Rio Verde, Goiás, Brazil (geographical coordinates: 17°48'55" S and 50°56'28" W, altitude of 753 m). According to the Köppen and Geiger classification, the local climate is tropical (Aw). Annual precipitation varies from 1500 to 1800 mm and occurs mainly from October to May, with the dry season from June to September. Average annual temperature ranges from 20 to 35 °C.

Seeds for seedling production were obtained from plants in Rio Verde, in an area of natural occurrence. A voucher herbarium specimen (HRV 468) was deposited with the herbarium of the Federal Institute of Goiás in Rio Verde. After collection, seeds were germinated and developed

during 21 days in polypropylene trays containing commercial substrate, in which thinning was also carried out. Subsequently, seedlings were transplanted to beds.

The soil in the experimental area was classified as a clayey Oxisol with the following physicochemical characteristics, at a depth of 0-20 cm: pH (CaCl₂), 5.7; 0.13, 4.07, 1.21, 0.01, 2.89, 5.43, and 8.32 cmol_c dm⁻³ of K⁺, Ca²⁺, Mg²⁺, Al, H+Al, SB, and CEC, respectively; 27.74, 0.36, 5.0, 5.75, 18.55, 44.56, and 21.34 mg dm⁻³ of P, B, Na, Cu, Fe, Mn, and Zn, respectively; V% of 65.3%, and particle sizes of 590, 100, and 310 g kg⁻¹ of sand, silt, and clay, respectively.

The greenhouse used in the trial was built with an arched metal structure, with an ordinary ceiling height of 1.4 m and a high point of 3.0 m, length of 30 m, and width of 5.2 m, in north-south direction. The tunnel had galvanized iron structure with top and side closure with black shade screens for each shaded environment, representing 18, 30, and 50% radiation attenuation and full sun (100% of incident radiation). For each shaded environment, beds were made by using a rotary tiller, incorporating an organic compound at a dose of 1 kg m⁻². The beds had dimensions of 5 × 1 m (length × width). The spacing used was 0.25 m between rows and 0.25 m between plants. Each bed was considered a basic experimental unit, with four replications per treatment.

No phytosanitary products were applied during plant cultivation and weed control was manual. After harvest, at 118 days after transplanting, plant height, stem diameter, number of leaves, leaf area, length and volume of root system, and fresh and dry weight of leaves, stems, and roots of *T. triangulare* were assessed as a function of treatments. To measure plant height and root length, a ruler was used and for stem diameter, a caliper. To obtain root volume, roots were washed in tap water over a sieve until the complete removal of soil residues. Subsequently, the root system volume was measured by displacement of the water column in a graduated cylinder. Fresh weights of leaves, stems, and roots were obtained by weighing on an analytical balance. To determine dry weight, these materials were placed in paper bags and kept in a convection oven at 65 °C until reaching constant weight, which was expressed in g per plant. Leaf area was determined in cm² by integrating the digital image of leaves using the software ImageJ (Image Processing and Analysis in Java), version 1.47.

The micromorphometric analyses of the leaves regarding stomatal density index of the adaxial and abaxial surfaces and thickness of the adaxial and abaxial epidermis and palisade and spongy parenchyma were performed with the ANATI QUANTI® software (Aguiar *et al.*, 2007). To obtain the stomatal density and index, the epidermis-imprinting technique with cyanoacrylate was used, as described by Alves *et al.* (2014).

The effects of different environments were assessed on the anatomical structure of *T. triangulare* leaves using staining with toluidine blue. Leaf tissue thickening was measured with 0.5 cm² samples removed from the middle third of leaves of each plant from the experimental treatment (in full sun and 18, 30, and 50% shade) using a disposable razor. These samples were then fixed in Karnovsky's solution (1965) for 24 h, dehydrated in ascending ethanol series, and pre-infiltrated and infiltrated in historesin (Leica kit) according to manufacturer's recommendations.

Subsequently, the material was cut into 5 µm-thick sections with a rotary microtome (Ciencor Scientific Ltda, model 1508R). The sections were stained with toluidine blue at 0.05% in 0.1 M phosphate buffer at pH 6.8 (O'Brien *et al.*, 1964) for structural and micromorphometric analysis. Subsequently, images were obtained using an Olympus microscope, model BX61, with camera DP-72, described, and quantitatively estimated by using the software ImageJ (Image Processing and Analysis in Java), version 1.47.

The results were subjected to analysis of variance and, when significant, a regression test at 5% probability was carried out using the Sisvar® software.

RESULTS AND DISCUSSION

Depending on the environments to which plants of *T. triangulare* were exposed, a variation in growth pattern was observed in plant height, number of leaves, stem diameter, and leaf area (Figures 1A–D). *T. triangulare* showed an increasing linear behavior for plant height as shade intensity increased, with an increment of 0.31 cm for each unit increase of the shade level within the tested range (Figure 1A). The increase in heights represented 10.43, 14.39, and 37.74% for 18, 30, and 50% shade, respectively, as compared with the plant height grown in full sun (Figure 1A). These results are in line with those reported by Oliveira *et al.* (2009), who studied *Artemisia vulgaris* L. under red and blue Chromatinet-colored shade (50%) and full sun. They found that plants exposed to full sun presented lower height (36.68 cm) when compared with plants exposed to shade with blue (122.12 cm) and red (125.87 cm) screens, which did not differ from each other.

Conversely, the number of leaves linearly declined by 1.16 per unit of increase in shade levels, representing decreases of 5.9, 14.48, and 15.53% for 18, 30, and 50% shade, respectively, as compared with the number of leaves in plants grown in full sun (Figure 1B). For *Curatella americana* L. under full sun or 76% red screen shade, the larger leaf area growth counterbalanced the number of leaves. At 240 days, plants in full sun had a higher number of leaves and lower leaf area compared with plants grown under shade (Dalmolin *et al.*, 2015), in agreement with our results for both number of leaves and leaf area.

For some species, stem diameter is a highly plastic trait, and this is desirable because it ensures a greater support to shoots (Lee *et al.*, 1997). With increased shade, stem diameter linearly increased by 0.0369 mm per shade unit, representing gains of 7.12, 6.22, and 17.78% for plants under shade levels of 18, 30, and 50%, respectively, compared with stem growth of plants in full sunlight (Figure 1C). Nevertheless, Meira *et al.* (2012) observed that environments with full light and 25, 50, and 75% shade did not affect stem diameter of *Melissa officinalis* L., which presented values of 0.43, 0.68, 0.49, and 0.69 mm, respectively, not corroborating the behavior observed in this study.

Similarly, with increased shade, leaf area presented a linear increase of 70.84 cm² per shade unit, representing increases of 37.60, 30.42, and 94.81% for 18, 30, and 50% shade, respectively, compared with the leaf area in plants grown in full sun (Figure 1D). According to Lambers *et al.* (2008), plants under environments with low irradiance invest a higher amount of photoassimilates and other resources, aiming to increase leaf area and decrease weight

density to enlarge the capture of incident light and increase photosynthetic efficiency.

No significant effects were observed for root length of *T. triangulare* plants in function of the shade levels (Figure 2A). However, the variables root fresh weight, dry weight, and volume were affected with increased shade, having quadratic behavior with maximum points at 16.30, 16.87, and 11.19% shade, with values of 58.63 g, 9.62 g, and 59.45 mL per plant, respectively (Figures 2B–D). In *Aloysia gratissima* (Gill et Hook) Tronc., a significant effect was observed in root dry matter of plants grown in full sun and shaded by 40 and 80% with Aluminet® screens, obtaining values of 32.8, 43.8, and 15.8 g, respectively (Pinto *et al.*, 2007). According to Gonçalves *et al.* (2012), plants that grow in full sun present higher transpiration flows and require larger and more efficient root systems, with a better developed vascular system.

In relation to leaf and stem fresh weight, significant effects were observed, with linear increase in production with increasing shade levels (Figure 3A and B). For leaf

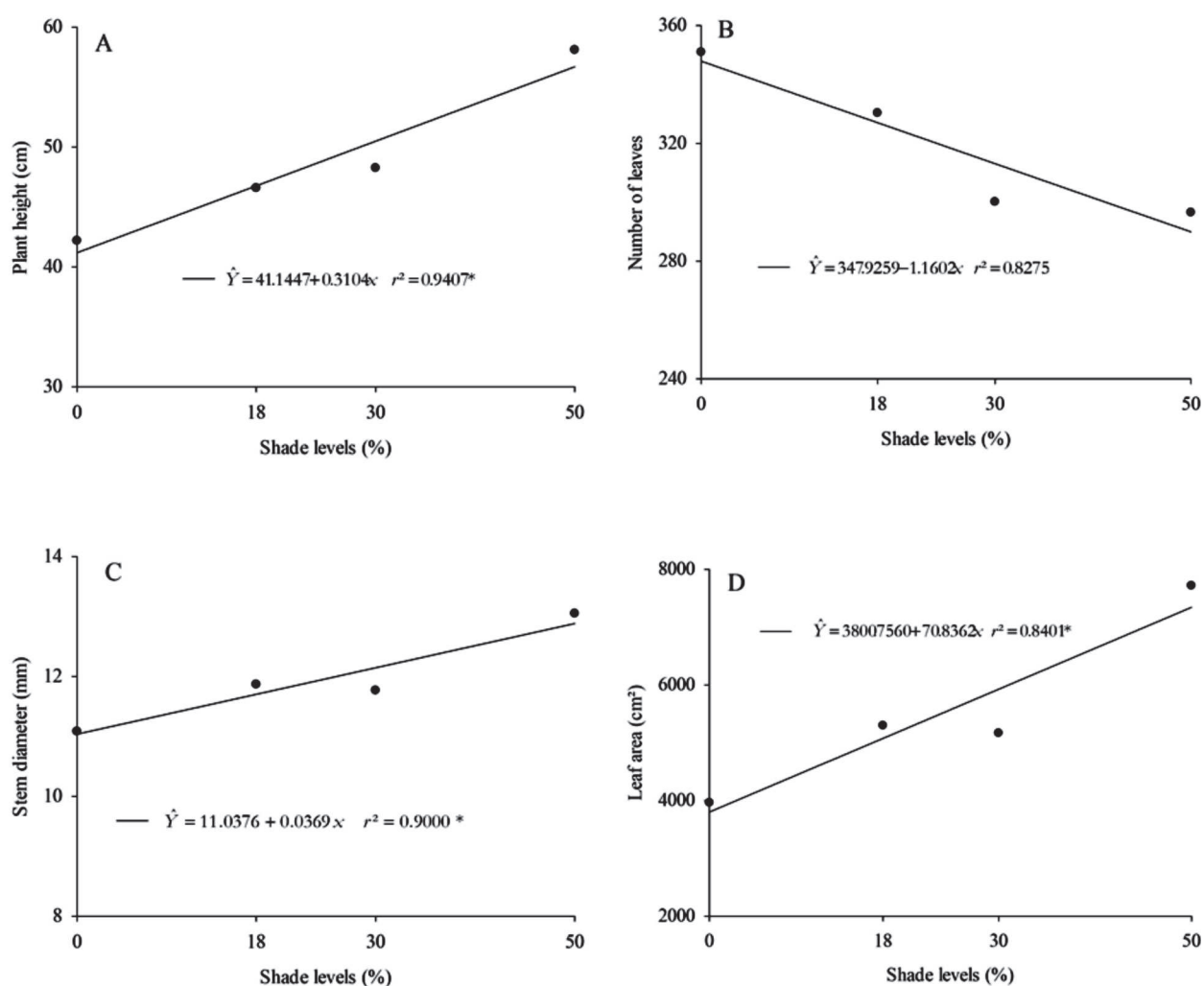


Figure 1: Plant height (A), number of leaves (B), stem diameter (C), and leaf area (D) of *Talinum triangulare* as a function of shade levels.

fresh weight, the increment for each shade unit was 0.6165 g per plant, which represented increases of 20.79, 14.52, and 36.09% under 18, 30, and 50% shade, respectively, compared with plants grown in full sun. Similarly, for stem fresh weight, the increment for each shade unit was 0.889 g per plant, which represented increases of 13.11, 16.15, and 29% under 18, 30, and 50% shade, respectively, compared with plants grown in full sun. However, no significant effects were observed for leaf and stem dry weight of *T. triangulare* plants in function of the shade levels (Figure 3C and D).

As shade levels increased, increases were observed in specific leaf area (Figure 4A) and leaf area ratio (Figure 4B). Leaf weight ratio of plants grown under shade did not change (Figure 4C), but their root-to-shoot ratio showed lower values (Figure 4D). For specific leaf area, increases of 11.64, 40.56, and 81.09% under 18, 30, and 50% shade, respectively, were observed compared with plants grown in full sun. Likewise, for specific leaf area, values represented gains of 11.39, 25.15, and 98.62% under 18, 30, and 50% shade, respectively, compared with plants grown in full sun. Shaded plants usually present low leaf area

ratio in full sun because of their ability to adapt to different lighting conditions. In *Mentha arvensis* L. plants, Chagas *et al.*, (2010) observed that shade levels (0, 30, 50, and 70%) imposed by Aluminet® and black screens led to linear increases in specific leaf area, leaf area ratio, leaf weight ratio, and specific leaf weight. According to the authors, for every 10% increase in shade, increases of 0.219 dm² g⁻¹, 0.087 dm² g⁻¹, 0.00747 g g⁻¹, and 0.0497 g dm⁻², respectively, were estimated.

Linear decreases of 16.21, 18.91, and 54.05% were observed for root-to-shoot ratio of plants under 18, 30, and 50% shade, respectively, compared with plants grown in full sun. According to Oliveira *et al.* (2009), root-to-shoot ratio can be changed by the interference of reallocating and distributing photoassimilates inside the plant due to environmental changes. In addition, plants of *Artemisia vulgaris* that were in full sun showed higher root-to-shoot ratio, suggesting priority allocation of dry weight to the root system as a consequence of light intensity.

Distribution of the dry weight of leaves, stems and roots of *T. triangulare* for 18, 30, and 50% shade levels

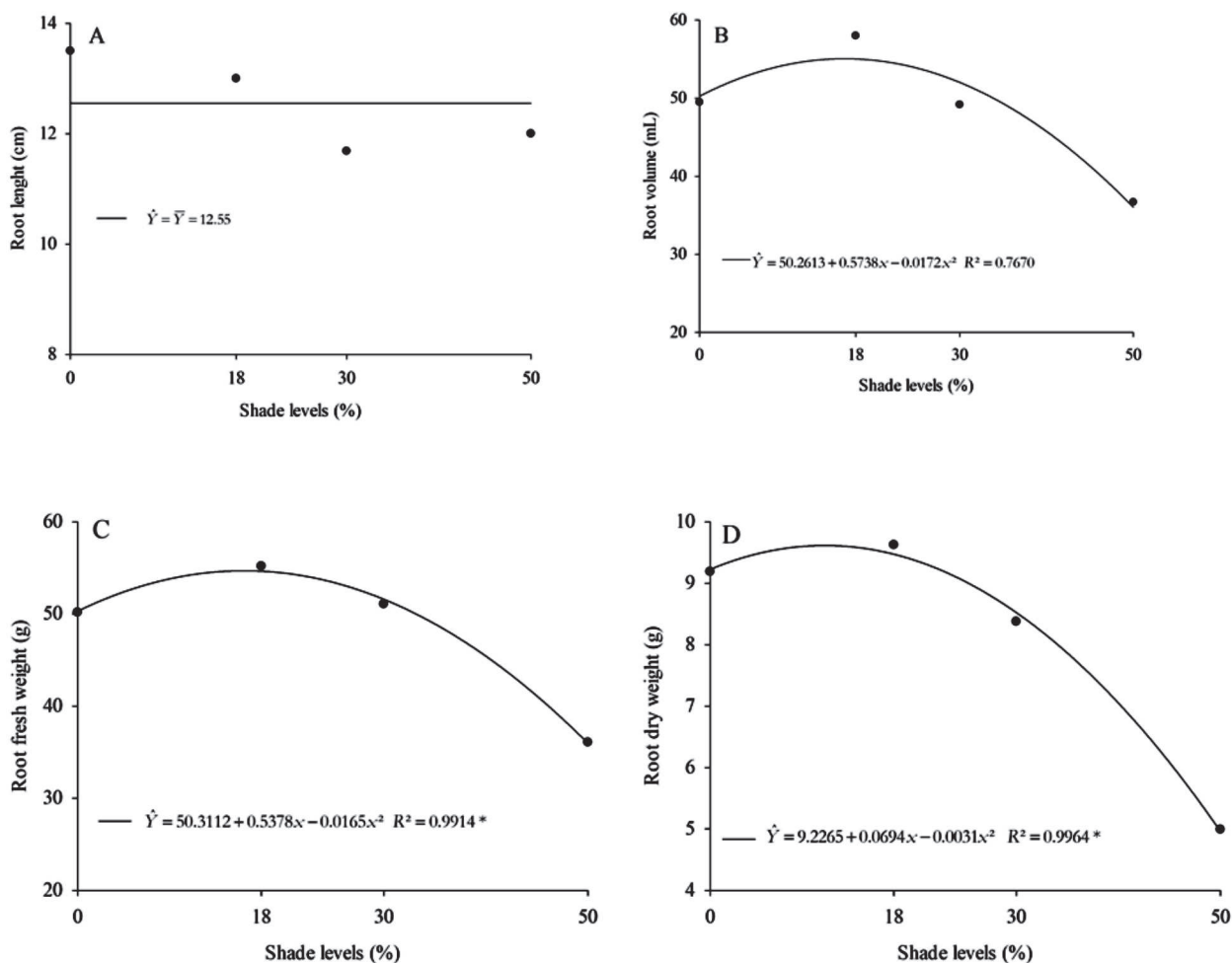


Figure 2: Root length (A), volume (B), fresh weight (C), and dry weight (D) of *Talinum triangulare* as a function of shade levels.

were 23.15, 55.55, and 21.3%, respectively (Figure 5). Plants grown under 50% shade showed the highest dry weight of leaves (24.4%) and stems (61.1%). Plants grown in full sun had the highest root dry weight (25.2%).

Leaves of *T. triangulare* are amphistomatic. The stomatal index of abaxial surface epidermis showed decreasing behavior (Figure 6A) compared with the adaxial surface epidermis, whose stomatal index remained constant regardless of shade levels (Figure 6C). Stomatal index of abaxial surface reached the highest value (13.58%) under 5.4% shade, and lower values were observed as shade levels increased, representing reductions of 0.22, 4.80, and 14.93% at levels of 18, 30, and 50%, respectively (Figure 6A). There are specific advantages of using differentiated screens that influence the microclimate, changing the composition and intensity of the light that reaches plants, to optimize productivity. In addition, this light interferes with leaf anatomy at both the early and adult stages of development due to the plasticity of the leaf internal structures to adapt to ambient light (Oliveira *et al.*, 2009).

In environments where plants were exposed to higher radiation intensities, greater stomatal density was observed, especially on the abaxial epidermis (Figure 6B), which presented values more than twice the stomatal density on the adaxial surface (Figure 6D). For both epidermises, a linear decrease in the stomatal density was observed as shade levels increased. On the abaxial epidermis, this reduction was more significant, with a value of 1.16 stomata per mm² of leaf, whereas on the adaxial surface this reduction was 0.2017 stomata per mm² of leaf for each increase in shade unit (Figures 6C and 6D).

The increase of stomatal presence in leaves exposed to high irradiance may be an important adaptation mechanism of species that tolerate arid environments. In *Curatella americana*, plants under shade showed amphihypostomatic characteristics, with a reduction in stomatal density on the adaxial epidermis of leaves. These characteristics are observed in plants growing in mesophytic environments, evidencing leaf plasticity (Dalmolin *et al.*, 2015).

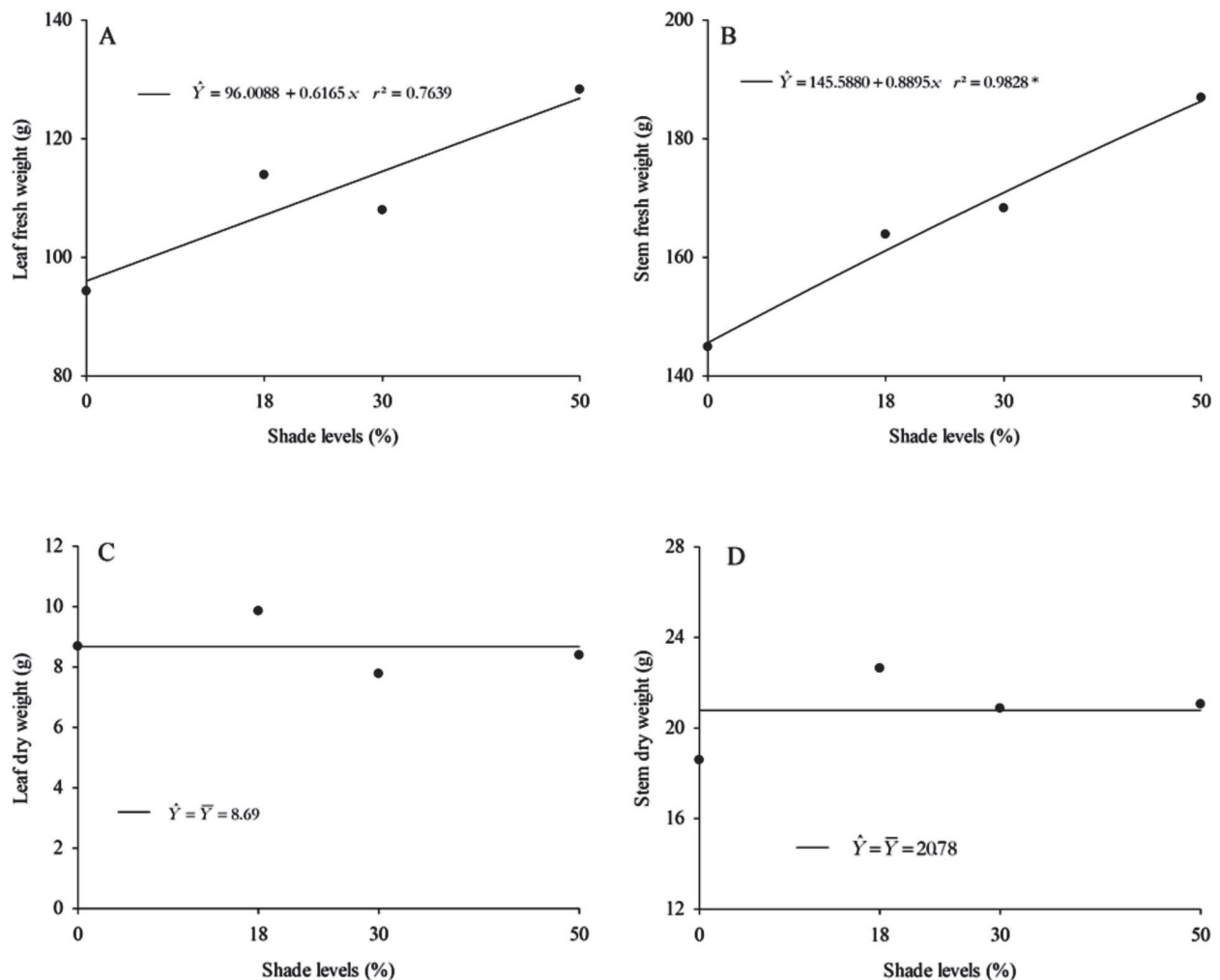


Figure 3: Leaf fresh weight (A), stem fresh weight (B), leaf dry weight (C), and stem dry weight (D) of *Talinum triangulare* as a function of shade levels.

The abaxial epidermis thickness (Figure 7) of the plants was influenced by shade, showing an increasing linear behavior with an increment of $0.196 \mu\text{m}$ per shade unit (Figure 7A) and an increase of 55.9% for this variable in plants grown under 50% shade compared with plants grown in full sun. In contrast, shade levels did not affect either the thickness of the adaxial epidermis (Figure 7B) or the spongy parenchyma thickness (Figure 7C). Full sun, as well as 40 and 80% shade levels, significantly influenced the spongy parenchyma of *Aloysia gratissima*, which presented values of 59.1 , 50.7 , and $44.9 \mu\text{m}$, respectively (Pinto *et al.*, 2007).

In leaves of *Aloysia gratissima*, Pinto *et al.* (2007) observed that the abaxial epidermis thickness was influenced by environments, with values of $9.5 \mu\text{m}$ for full sun, $8.1 \mu\text{m}$ for 40% shade, and $11.2 \mu\text{m}$ for 80% shade. According to the authors, those effects were also significant on the thickness of the adaxial surface epidermis of leaves, being higher in plants cultivated in full sun ($40.3 \mu\text{m}$) compared with 40% ($34.8 \mu\text{m}$) and 80% ($31.1 \mu\text{m}$) shade.

Palisade parenchyma thickness was influenced by treatments: higher thickness values were observed when *T. triangulare* was grown in full sun and decreased linearly as shade levels increased (Figure 7D). According to Lambers *et al.* (2008), spongy parenchyma thickness is identical when leaves from shade and full sun are compared, whereas palisade parenchyma thickness is commonly lower in leaves under shade, which was also observed for *T. triangulare*. Pinto *et al.* (2007) also observed significantly lower palisade parenchyma thickness of plants grown in full sun and under 40 and 80% shade, with values of 154.4 , 102.9 , and $75.7 \mu\text{m}$, respectively. Likewise, in plants of peanut forage grown in full sun and 50 and 80% shade, the palisade parenchyma thickness decreased linearly in order of 82.6 , 73.9 , and $65.6 \mu\text{m}$, respectively (Gobbi *et al.*, 2011). In *Artemisia vulgaris* grown in full sun, a thicker palisade parenchyma ($62.70 \mu\text{m}$) was observed compared with plants under red ($54.15 \mu\text{m}$) and blue ($54.57 \mu\text{m}$) Chromatinet-colored shade screens (50%) (Oliveira *et al.*, 2009).

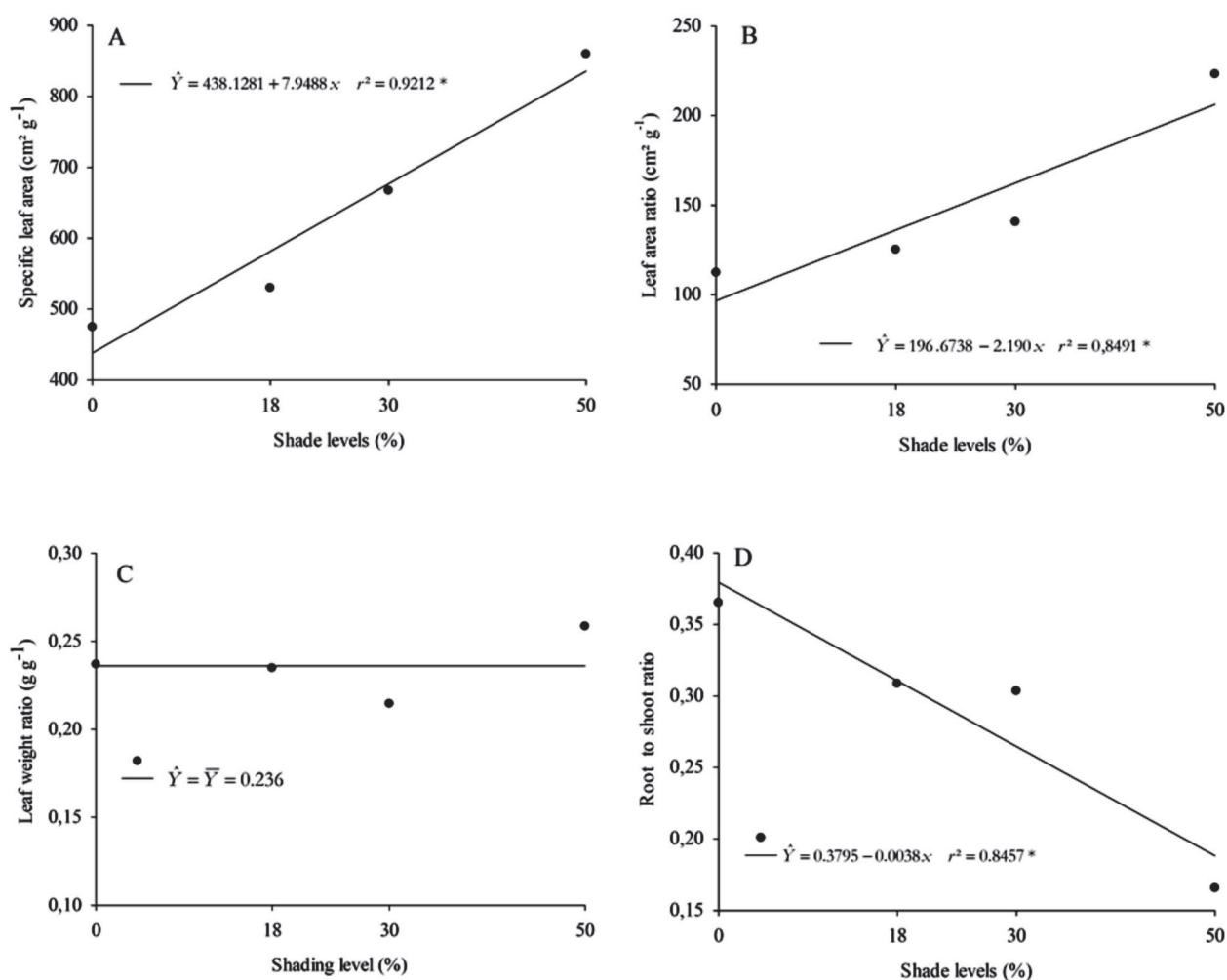


Figure 4: Specific leaf area (A), leaf area ratio (B), leaf weight ratio (C), and root to shoot ratio (D) of *Talinum triangulare* as a function of shade levels.

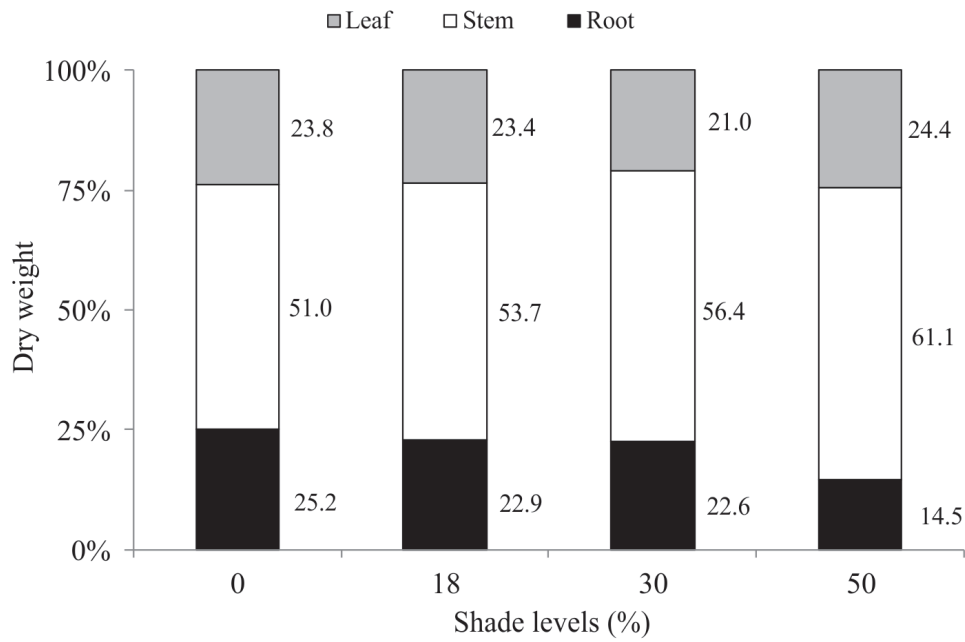


Figure 5: Dry weight distribution of leaves, stems, and roots of *Talinum triangulare* as a function of shade levels.

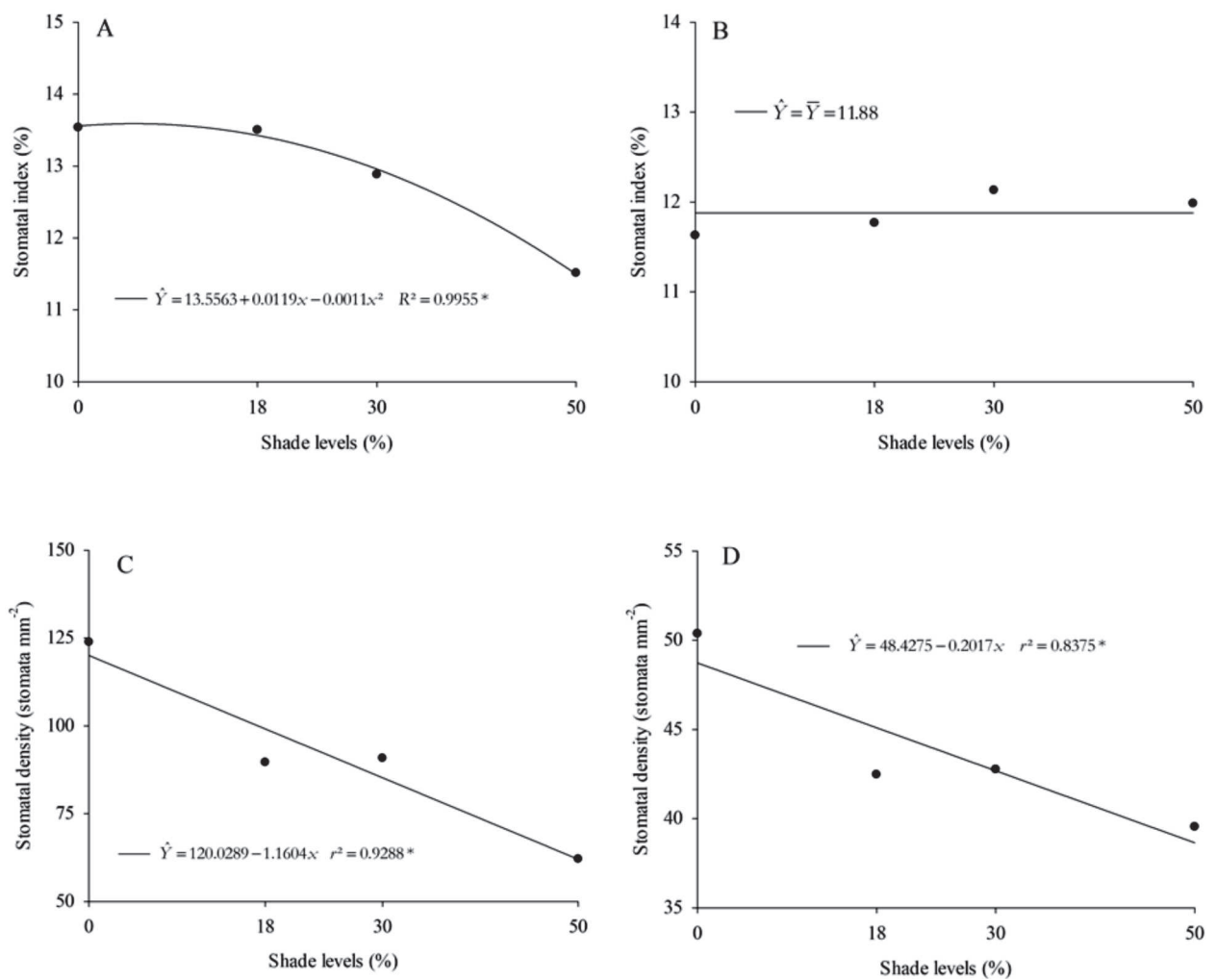


Figure 6: Abaxial surface stomatal index (A) and stomatal density (B) and adaxial surface stomatal index (C) and stomatal density (D) of *Talinum triangulare* leaves as a function of shade levels.

Mesophyll thickness was reduced with increasing shade levels, showing quadratic behavior with a minimum point at 34.66% shade with value of 500.83 μm (Figure 7E). Structural modifications found among leaves subjected to different radiation levels can be attributed to concentrations of distinct phytohormones, particularly auxin. Auxin enables greater distension of epidermal cells in leaves of shaded plants since it is concentrated in less illuminated regions of leaves. In contrast, more illuminated leaves

present higher levels of auxin in the mesophyll. In shaded leaves, auxin is located in the entire leaf, even the epidermis (Morais *et al.*, 2004).

The presence of reduced number of stomata and other cells, but larger in size, for all shade levels characterized in the epidermal imprinting (Figure 8) was observed on the leaf adaxial surface. On the other hand, on the abaxial surface, an increased number of stomata and other cells, but smaller in size (Figure 8), was observed.

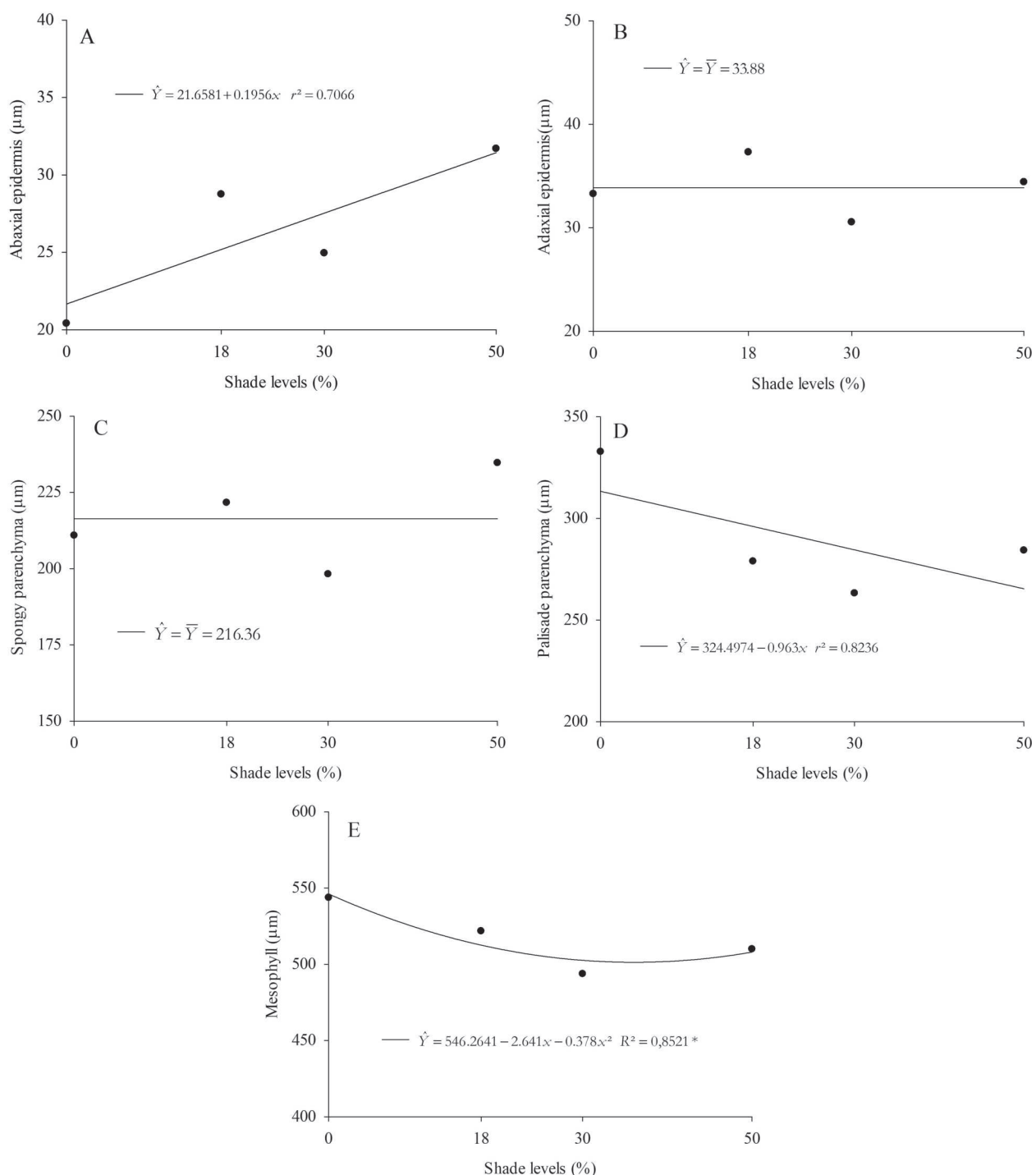
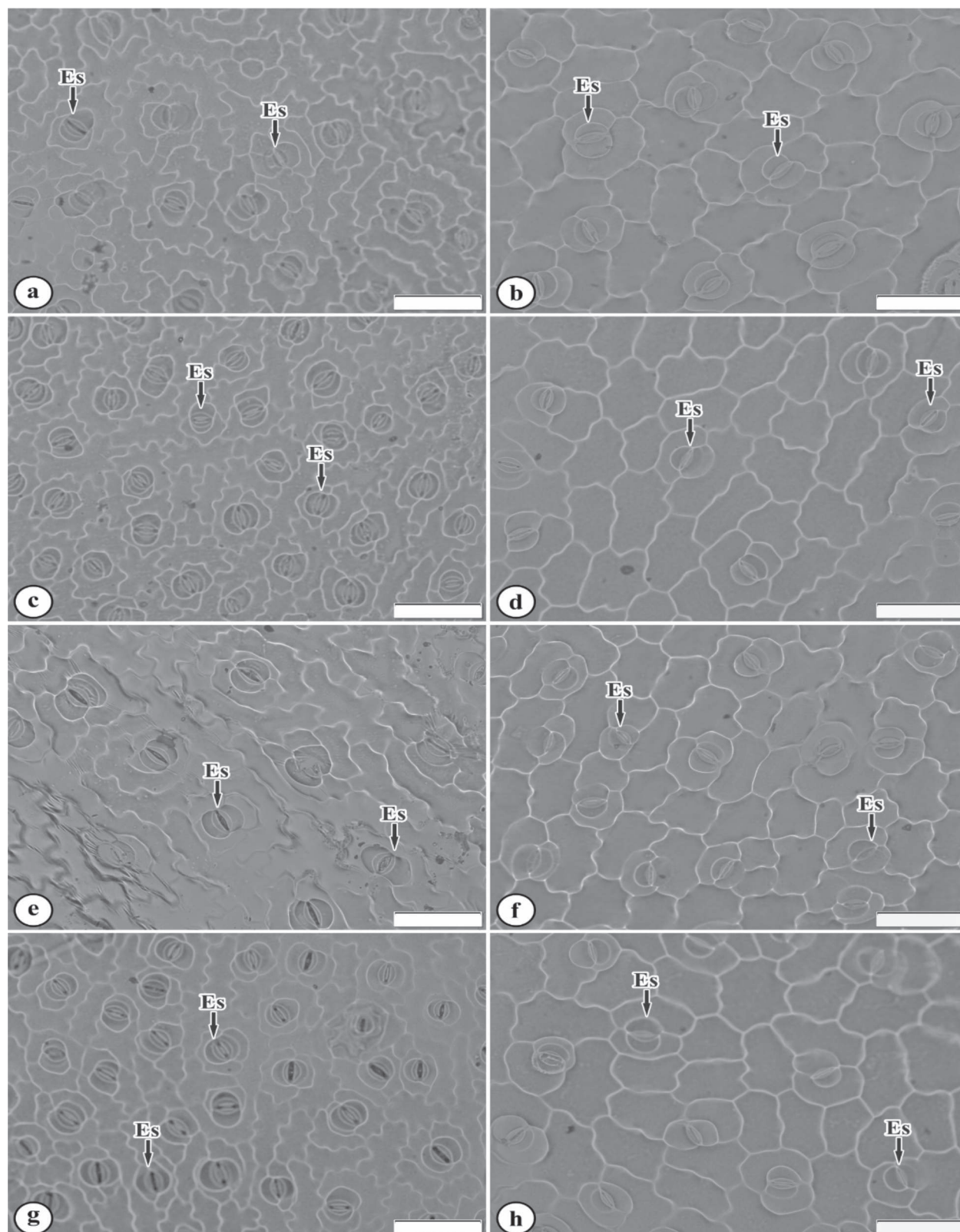


Figure 7: *Talinum triangulare* abaxial leaf (A) and adaxial leaf (B), epidermis spongy parenchyma (C), palisade parenchyma (D), and mesophyll thickness (E) as a function of shade levels.



Es = Stomata.

A-B: 18% shade; C-D: 30% shade; E-F: 50% shade; and G-H: full sunlight.

Photos on the left correspond to the abaxial surface and photos on the right correspond to the adaxial surface.

Bars: 100 μ m.

Figure 8: Epidermal imprinting of both leaf surfaces of plants of *Talinum triangulare* (Jacq.) Willd as a function of different shade levels.

CONCLUSIONS

Increasing shade levels increased height, stem diameter, leaf area, leaf and stem fresh weight, specific leaf area, leaf area ratio, and abaxial epidermis thickness.

Increasing shade levels decreased the number of leaves, root-to-shoot ratio, stomatal density of abaxial and adaxial epidermis, palisade parenchyma thickness, and mesophyll thickness.

Root length, leaf and stem dry weight, leaf weight ratio, stomatal index of the adaxial surface, adaxial epidermis thickness, and spongy parenchyma thickness of plants remained constant in full sun and 18, 30, and 50% shade levels. *T. triangulare* plants subjected to full sun and 18, 30, and 50% shade levels responded differently, showing morphological and anatomical changes, suggesting phenotypic plasticity and the possibility of being grown both in shaded environments and in full sun.

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