Initial growth of Umbuzeiro seedlings (*Spondias tuberosa* arruda câmara) subjected to different water regimes: morphoanatomical characteristics

Crescimento inicial de sementes Umbuzeiro (*Spondias tuberosa* arruda câmara) sujeitos a regimes diferentes de água: características morfoanatômicas

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ABSTRACT
The present study aimed to evaluate the initial growth and leaflet and xylopod anatomical responses in seedlings when subjected to different hydraulic regimes. Seedlings after 150 days of germination were used, evaluating three hydraulic regimes after transplantation: T0= daily watering (control); T1= watering every three days and T2= watering every five days. After 90 days, growth parameters and qualitative and quantitative characterization of seedling leaflets and xylopods were evaluated. There were statistical differences only for xylopod diameter and main root length, where the highest average for xylopod diameter was verified with daily watering hydraulic regime (control treatment), 15.37 cm^{-1}. While for main root length, the highest average, 33,8 cm^{-1}, was verified with watering every five days. With higher biomass allocation percentage, the xylopod was the main drain, regardless of the hydraulic regime adopted. Higher averages for density, polar and equatorial diameter of the stomata were observed in seedling leaflets subjected to the hydraulic regime with watering every 5 days. It was observed that only palisade and sponge parenchymas presented difference, where the hydraulic regime with watering every three and five days presented higher density, respectively.

Keywords: Semiarid, hydraulic deficit, Caatinga.

RESUMO
Objetivou avaliar o crescimento inicial e respostas anatômicas foliolares e do xilopódio em plântulas quando submetidas à diferentes regimes hídricos. Foram utilizadas plântulas após 150 dias de germinação, avaliando-se três regimes hídricos após transplantio: T0= rega diária (controle); T1= rega a cada três dias e T2= rega a cada cinco dias. Após 90 dias, foram avaliados parâmetros de crescimento e caracterização qualitativa e quantitativa de folíolos e xilopódios das plântulas. Houve diferenças estatísticas apenas para diâmetro do xilopódio e comprimento da raiz principal, onde maior média do diâmetro do xilopódio foi verificada com regime hídrico de rega diária (tratamento controle) 15,37 cm-1, já para comprimento da raiz principal a maior média 33,8 cm-1 foi com rega a cada 5 dias. Com uma maior porcentagem de alocação de biomassa, o xilopódio foi o dreno principal, independente do regime hídrico adotado. Maiores médias para densidade, diâmetro polar e equatorial dos estômatos em folíolos de plântulas submetidas ao regime hídrico a cada cinco dias. Observou-se que apenas os parênquimas paliçádicos e esponjosos apresentaram diferença. Onde o regime hídrico com rega a cada três e cinco dias apresentaram maiores espessuras respectivamente.

Palavras-chave: semiárido, deficit hídrico, Caatinga.

1 INTRODUCTION

The Caatinga is the only genuinely Brazilian phytogeographic domain, encompassing approximately 70% of the Northest region and 11% of the national territory, rich in biodiversity and with high degree of endemism, presenting around 950 endemic vegetable species (REFLORA, 2020).

Despite the most densely populated semi-arid region in the world, it suffers with the anthropogenic pressure exercised on its natural resources, which is, most times, the only income possibility for the rural population, which provides the permanence and survival of this people in the Caatinga rural areas. Due to the low level of environmental awareness, the biome has been suffering a progressive destruction of its biodiversity, resulting in extinction events of economically important species and species that have not even been described (Siqueira-Filho, 2012).

In this context, vegetable species of the Anacardiaceae family deserve emphasis, as its trees are represented in almost all of the Caating territory, presenting high regional environmental and social-economic importance. Among the species of the family, the *Spondias tuberosa* Arruda Câmara, commonly known as imbuzeiro, umbuzeiro, cajá do sertão, among others, is highlighted. It is an important fruit tree, native to the Caatinga (Cavalcanti et al., 2006), with great social-economic application, due to it being used as food by humans, rich in vitamin C (Ribeiro et al., 2019 e Santos et al., 2006). The fruits are consumed and commercialized *in natura*, and also processed and industrialized as sweets, pulp, jams, juices and ice creams (Ferraz et al., 2005).

One of the factors that hinder the large-scale propagation of the umbuzeiro is the dormancy of its seeds, which causes a slow and uneven development of seedlings, as reported by some authors (Cavalcanti et al., 2006 and Lopes et al., 2009). In addition, the plants propagated this way have a long juvenile period (Aidar et al., 2013).

However, germination becomes important when used to obtain rootstock, grafting being the most recommended propagation method for the species, performed by forking at the top, in a full slit. According to Fonseca (2010), this method is viable for producing selected seedlings with several umbuzeiro accesses.

Throughout the evolution process, plants developed several strategies in order to overcome adverse environmental conditions, such as hydraulic deficit. The umbuzeiro, for example, presents seasonal leaf senescence, preventing dehydration during the drier periods of the year (Lima Filho, 2001). Another characteristic is the subterranean system, specialized and adapted to hydraulic deficit conditions, formed by long roots and with structures called xylopods that are able to store water (Gonçalves et al., 2006).

Few studies describe the importance of hydraulic availability for the initial growth of umbuzeiro seedlings, for example, the morpho-anatomical responses under these conditions. Although having good representation, anatomical variations in plants of this species are not very studied and basic information regarding physiological aspects and knowledge about external and internal structures related to such variations, specially in hydraulic stress conditions, and the implications in its characteristics, are scarce, despite its structure being adapted and associated to such environment.

Therefore, the objective of the study was to evaluate the initial growth responses as well as characterizing the leaflet and xylopod anatomy in umbuzeiro seedlings subjected to different hydraulic regimes.

2 MATERIAL AND METHODS

The fruits were collected in a 1.2 ha area of Caatinga, located in Juá, in the city of Paulo Afonso-BA (09°26’48,8" S and 38°25’53,1" W Gr., altitude of 428m – Datum WGS 84), within the Raso da Cararina Ecoregion. After collection, the fruits were taken to the Vegetable Physiology and Anatomy Laboratory at the University of the State of Bahia, UNEB Campus VIII, Paulo Afonso-BA. They were processed by manually removing the pulp and washed in running water, then dried at room temperature in the shade, for 48h, conditioned in brown paper bags and stored in a fridge for later use in the experiments.

2.1 HYDRAULIC REGIME IN THE INITIAL GROWTH OF UMBUZEIRO SEEDLINGS

The experiment was carried out in the greenhouse, which presents light retention coverage, obtained with 50% sombrite screen, from the University of the State of Bahia – Campus VIII, whose temperatures and luminosity during the experiment period are presented in Figure 1. The seedlings were obtained from seeds germinated after 150 days, being selected and transported to 700 mL^{-1} vases, drilled at the base, containing sand and vegetable soil substrate (1:1), using 300 $mg.L^{-1}$. The substrate’s water retention capacity (WRC) was determined according to the methodology described by Prado et al. (2006).

After transplantation, the seedlings were subjected to three irrigation regimes: T0 (control): daily watering; T1: watering every 3 days; and T2: watering every 5 days. The amount of water used in irrigation was calculated according to the WRC of the used substrate, administering 200 mL^{-1} per vase at each irrigation.

The experimental outline was completely randomized, with three treatments, with three parcels per treatment and three repetitions per parcel, making a total of 27 plants.

At 90 days, it was evaluated: number of leaves, aerial part height, stem diameter, leaflet area, leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and xylopod dry mass (XDM).

The values obtained for dry mass were used to calculate biomass allocation (%) for the leaves (LBA), root (RBA), stem (SBA) and xylopod (XBA) through the equations LBA= LDM/TDMx100; SBA= SDM/TDMx100, RBA= RDM/TDMx100 and XBA= XDM/TDMx100. The total dry mass (TDM) being determined by the sum of all organs dry mass.

The number of leaves, aerial part height and stem diameter were also evaluated on the first day of experiment implementation and every 30 days, until completing 90 days.

2.2 LEAFLET AND XYLOPOD ANATOMY OF UMBUZEIRO SEEDLINGS

Three individuals were collected randomly, resulting from the initial growth in different hydraulic regimes, per umbu seedling treatment after the 90-day period.

The completely expanded leaflets were quickly fixated in FAA 70% solution, according to the methodology described by Johansen (1940), staying in this solution for 72 hours, then transferred to an alcohol 70% solution (v/v), where they were stored until the anatomic cuts procedure.

2.2.1 Leaflet anatomy

The paradermic cuts of the leaflets were performed manually, according to the methodology described by Laboriau et al. (1961), the cuts were stained using saframin dye 1%.

For the transversal cuts, the methodology described by Kraus and Arduim (1997) was followed, the double staining being performed with safrablau (astra blue and safranin at 1%). For mounting the material's semi-permanent blades, glycerine-water 50% (v/v) was used, then sealed with colorless nail polish.

The blades with paradermic and transversal cuts were photographed with the AxioCam ERc5s coupled to Zeiss Primo Star optical microscope. The tissues thickness: abaxial and adaxial epidermis, palisade and sponge parenchyma, mesophyll and limbo, as well as stomatal density (SD), polar diameter (PD), equatorial diameter (ED) and PD/ED ratio were performed using the ImageToo® software.

The experimental outline was completely randomized, evaluating three blades in five different fields in each blade per treatment, making a total of 15 repetitions.

2.2.2 Xyplopod anatomy

The xylopod transversal cuts were performed in fresh material, from the same seedlings used for leaflet anatomy, before the material was fixed. The methodology determined by Kraus and Arduim (1997) was used, with safrablau staining (astra blue and saframin at 1%) and the semi-permanent blades mounted in glycerin0water 50% (v/v) and sealed with colorless nail polish.

For analyzing the presence of starch, the same methodology was used, however, the staining was performed with histochemical dye Lugol at 1%. The blades were photographed with the AxioCam ERc5s coupled to Zeiss Primo Star optical microscope. The images were used to performed the analysis and description of the structures.

The anatomical classifications followed Solereder (1908), Metcalf and Chalk (1950), Fahn & Cutler (1990) and Appezzato da Glória and Carmello Guerreiro (2012).

2.3 STATISTICAL ANALYSIS

The evaluated data were subjected to variance analysis, comparing the averages by Tukey test at 5% probability, using statistical software Sisvar 5.6 (FERREIRA, 2019).

3 RESULTS AND DISCUSSION

The initial growth of seedlings subjected to different hydraulic regimes, when compared to the evaluation period every 30 days, was significant for the three analyzed variables (Table 1), observing a growing increase of the variables throughout the days. Where the aerial part height and number of leaves presented higher averages, 18.16 cm^{-1} and 11.4 leaves, respectively, for the hydraulic regime every three days, while stem diameter presented higher average, 3.92 cm^{-1}, with watering every five days, all at 90 days of culture.

Regarding the variables analyzed at the end of the experiment, statistical difference was only verified for xylopod diameter and main root length (Figrue 2). For all the other growth variables, there was no difference after the 90-day period of exposure to the hydraulic regimes.

The highest xylopod diameter was observed with daily watering regime (control treatment), 15.37 cm^{-1}, while for main root length, the highest average, 33.8 cm^{-1}, was observed in the regime with watering every five days.

All the other growth variables did not present statistical difference, however, for aerial part height, number of leaves, leaf area and leaf, stem and xylopod dry masses, the highest averages were observed with watering every three days. For root dry mass, daily watering was more efficient.

Regarding biomass allocation percentage, when comparing hydraulic regime factors and different allocation organs, higher allocation percentage was observed in the xylopod, being highly significant, regardless of the adopted hydraulic regime (Figure 3).

Allocation in plants has been reported through the preferential distribution of biomass and nutrients among its several organs. This distribution depends on several factors, such as age, nutrition, competition, hydraulic relations and growth habit (Taiz & Zeiger, 2015). In perennial forage species, a balance that allows it persistence is preferable. A lower aerial part/roots relation can mean a more extensive root system and, possibly, more efficient, as observed in this study.

The presented results indicate a lower aerial part/roots relation, showing a strategy of the species in storing water and photosynthates in response to different hydraulic regimes to which it was subjected in the subterranean systems.

The umbuzeiro is well-adapted to the Caating low rainfall due to the presence of xylopods in the roots, this organ is composed of parenchymal tissue and stores water, mucilages, glucose, tannin, starch, acid, etc. These reserves nourish the plant during the dry periods (Cavalcanti & Resende, 2006).

Few studies report biomass allocation in umbuzeiro subterranean organs when related to hydraulic factors. Oliveira et al. (2019) evaluating the division of photoassimilates in umbuzeiro and amburana (*Amburana cearenses*) in different periods, observed that, for both species, the preferred drains are the tuberous roots, followed by branches, leaves and lateral roots. Specially during the initial growth stage, the tuberous root works as a high-activity drain, corroborating the results of this study, where the xylopod obtained higher biomass allocation percentage. Ramos (2004), evaluating *Amburana cearensis* plants under different shading levels, obtained results similar to the present study, the author observed that the plants allocated great part of the photoassimilates produced in the tuberous root, classified by the author as xylopod.

The results can be compared to studies on stomatal behavior, suggesting that, under hydraulic deficiency conditions, the umbuzeiro maintains a relatively stable hydraulic balance during the day, due to presenting low leaf density and exercising strict stomatal control. The interaction of this factors causes a drastic decrease in transpiration of the water stored in the subterranean organs, allowing higher water maintenance in these organs (LIMA FILHO, 2001).

The paradermic anatomical characteristics of the leaves of umbu seedlings are hypostomatic, with stomatas present only on the abaxial surface, of the anomocytic type, and with the presence of multicellular glandular trichomes, epidermic cells with little sinuous contours (Figures 5 E and G). In the study by Nascimento Silva & Paiva (2007), the authors observed anomocytic and tetracytic stomatas on the abaxial surface and the presence of simple tector trichomes on both epidermic surfaces, differing from the paradermic characteristics observed in this study. It is worth highlighting that the mentioned authors worked with adult umbuzeiro plants, which evidences the malleability of this species.

Statistical differences were observed for all studied paradermic variables, except for polar and equatorial diameter ratio (PD/ED ratio), where the highest density, stomatal polar and equatorial diameter in leaf of seedlings were verified in the individuals subjected to the hydraulic regime of watering every five days. Even not being significant, higher PD/ED ratio was also observed with watering every five days (Figure 4). Silva et al. (2008), working with hydraulic and saline stress in different adult umbuzeiro genotypes, observed a higher stomatal density in leaves of seedlings subjected to hydraulic stress, corroborating the results found in this study.

The increase in the number of stomatas in response to hydraulic stress shows the umbuzeiro's malleability to these conditions, which will provide more transpiration control in relation to the soil hydraulic potential (Silva et al., 2008). These data might be directly related to the PD/ED ratio results, which, even if not presenting differences, was higher in the seedlings subjected to hydraulic deficit.

Some authors state that the size and frequency of stomatas can vary depending on leaf position and environmental conditions (Appezzato – da - Gloria; Carmello – Guerreiro, 2012; Faria et al., 2000). Therefore, the high stomatal density is a characteristic of xeromorphic plants and, the higher the light intensity and water scarcity, the stomatal density also tends to be higher, since it makes gas exchanges more efficient and, thus, avoid dehydration (Nascimento-Filho & Paiva 2007).

Another stomatal characteristic observed in the present study was the stomatas elliptic shape. According to Khan et al. (2003), the elliptic shape is a characteristic of functional stomatas and the round shape is associated with stomatas that do not present normal functionality. The authors state that the higher the PD/ED ratio, the more ellipsoid the stomata shape will be, therefore, the higher its functionality will be.

In this study, it was not observed differences in the anatomical characteristics of leaflet blade in seedlings subjected to different hydraulic regimes. A blade can be observed with adaxial epidermis, dorsiventral mesophyll, with uniseriate palisade and sponge parenchyma with three to four cell layers, and leaf border with secretory duct (Figure 5 A and D). The central nerve presented subepidermal angular collenchyma, collateral vascular system, absence of sclerenchymatic fibers and presence of secretory ducts oriented to the adaxial and abaxial surfaces (Figures 5 B, C and F). The data presented for the species corroborate anatomical characteristics for the Anacardiaceae family mentioned by Anna-Santos et al. (2006) and Nascimento-Silva et al. (2008). Among them, the main characteristic is the presence of secretory structures in the main nerve.

Regarding the statistical differences of the leaflet blade, it was observed that only the palisade and sponge parenchymas presented difference. The hydraulic regime with watering every three and five days presented higher thickness of these tissues, respectively (Figure 6 B and C).

The increase in photosynthetic tissues also shows the plant’s adaptation to hydraulic stress conditions. For Silva et al. (2008), these differences in the tissues characterize an adaptation for higher photosynthetic efficiency.

For the other tissues that compose the leaflet blade, no statistical differences were observed in their thickness, however, the highest averages were observed with seedlings subjected to watering every five days, evidencing the malleability and adaptation to hydraulic stress conditions (Figures 6 A, D, E and F).

The xylopod is a subterranean system with storage capacity and, when it presents buds, it can also generate re-sprouting of aerial parts. This structure is of mixed origin, both stem and root. It is originated from the hypocotyl-root thickening, the xylopod can persist as a clearly morphologically distinct unit in the adult vegetable, or have its growth mistaken with the main root growth, making it indistinct from it (Figure 7 A) (Appezzato – da - Gloria & Carmello – Guerreiro, 2013).

The umbuzeiro subterranean system can be classified as a tuberous root, as the classification by Santos (2015). The author studied the anatomy of the umbuzeiro subterranean system in young plants, not observing lignification of the tissues, using this characteristic to classify it as tuberous root. However, there are no anatomical studies on subterranean systems of this species with adult individuals, requiring ontogeny studies to determine its origin ans structural nature.

Several authors classify the subterranean system of adult and young umbuzeiro plants as xylopod, based on morphological characteristic (Lima-Filho, 2001; Ramos, 2004; Melo et al., 2005; Andrade et al., 2013; Pinto et al., 2015).

Anatomically, the xylopod adult structure is similar to wood. The spatial orientation of the conductor elements can be diffuse, presenting lateral interconnections with roots and aerial shoots. Regarding the primary structure, it can present both as stem, due to the presence of marrow, and as root, according to the cut level (Appezzato – da - Gloria & Carmello – Guerreiro, 2013).

In the present study, no xylopod anatomical differences were observed when subjected to the different hydraulic regimes.

The coating system is the only tissue in the seedlings xylopod that presents the beginning of secondary growth, evidencing a developing periderm, it was also observed the presence of lenticels in the periderm (Figures 7 B and F). The fundamental system is constituted by cortical parenchyma, presenting secretory ducts and radial parenchyma cells. The vascular system is closed, showing the absence of medullary parenchyma, characteristic of dicotyledonae roots, highlighting the presence of primary xylemic tissue of centripetal growth (Figure 7 B, C, D and E).

The histochemical test with lugol reagent detected the presence of starch in the cortical tissue (Figures 8 A – C).

Starch presents other functions besides energy source or reserve carbon source. It would also be related to the tolerance of some species subjected to environmental stress, specially in the Caatinga, where there can be prolonged dry periods and vegetation fires, therefore, being an adaptation strategy of the plants to adverse environmental conditions (Vilhalva & Apezzato -da- Glória, 2006).

There are no studies that report the quantification and/or characterization of starch in the xylopod, however, countless studies quantified this polysaccharide in fruit, due to its great importance for *in natura* consumption or processing of food inputs. For example, Lopes (2007), who quantified starch in umbuzeiro fruits in different maturation stages, Santos et al. (2010), who characterized the physical-chemical composition of pulp and, in a more recent study, Ribeiro et al. (2019) quantified the nutrients and bio-active compounds of umbu pulp, peel and seeds.

The umbuzeiro, despite its social-economic and environmental importance, lacks complementary studies, specially about its subterranean systems, to better classify it and quantify the substances stored in these systems.

4 CONCLUSIONS

For the initial growth of umbuzeiro seedlings, it is possible to use a regime with watering every five days.

Higher biomass allocation percentage was observed in the xylopods, regardless of the adopted hydraulic regime, characterizing the organ as a high activity drain and great part of the photosynthesis liquid carbon gain.

The use of watering every five days provided higher thickness of the leaflet tissues, the increase in the number of stomatas in this regime was the main characteristic of adaptation to the hydraulic deficit.

For the qualitative characterization of the xylopod, no anatomical differences were observed in relation to the different hydraulic regimes, however, the presence of starch was detected.

Due to divergences in relation to the umbuzeiro subterranean system, ontogeny studies of the organ are recommended for better elucidation.

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ANEXOS

Table 1: Length of aerial part, number of leaves and stem diameter of umbuzeiro submitted to different water regimes and different evaluation periods.

Water Regimes	Evaluation period (days)			
	Length of Aerial Part (cm^{-1})			
	0	**30**	**60**	**90**
Daily	12,2 aA*	13,7 aA	12 aA	15,14 aA
3 days	13,58 aB	14,6 aAB	15 aAB	18,16 aA
5 days	12,2 aA	14,7 aA	15,4 aA	15,2 aB
	Leafs Number			
	0	**30**	**60**	**90**
Daily	5,2 aB	6,2 aAB	5,6 bAB	8,8 abA
3 days	5,2 aC	7,4 aBC	9,4 aAB	11,4 aA
5 days	4,8 aA	6,4 aA	7,2 abA	7,2 bA
	Stem Diameter (cm^{-1})			
	0	**30**	**60**	**90**
Daily	1,66 aB	2,94 aA	2,71 aA	3,49 aA
3 dias	2,34 aB	2,83 aB	2,42 aAB	3,51 aA
5 dias	2,23 aC	2,59 aBC	3,29 aAB	3,92 aA

* Averages followed by the same lowercase letter in the column and uppercase in the row, do not differ by Tukey's test at 5% probability.

Figure 1: Temperature and luminosity evaluated in the nursery during the period of the experiment

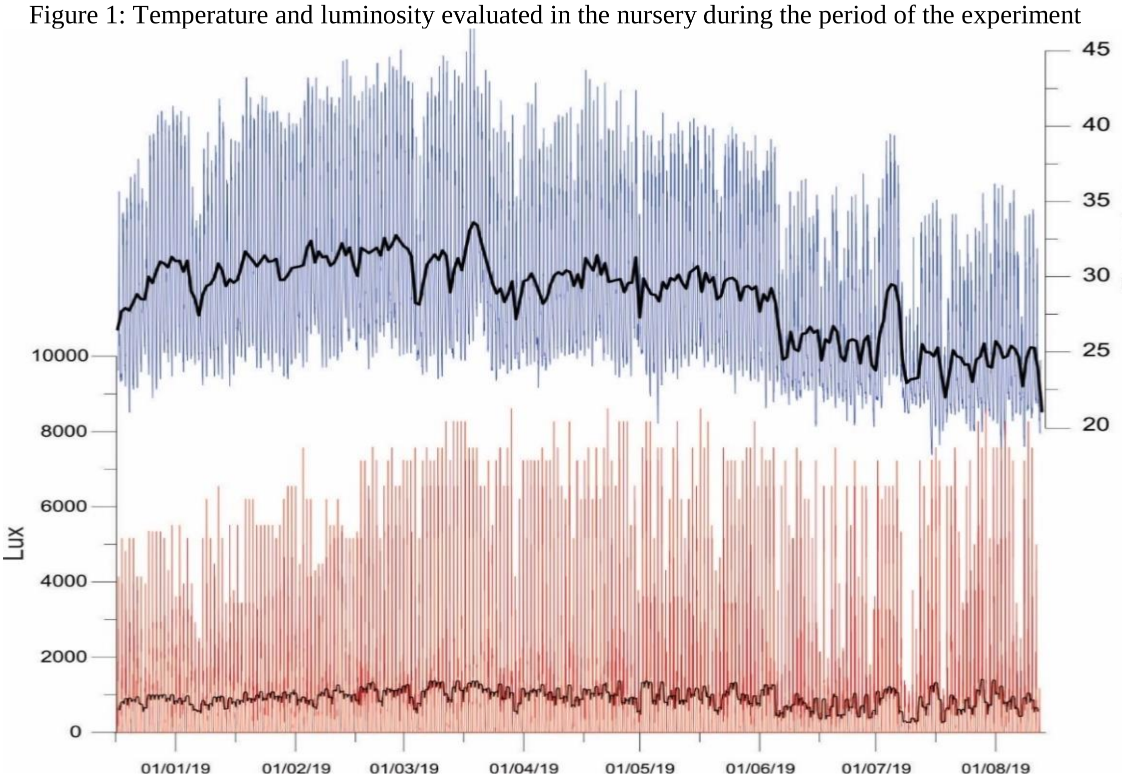

Figure 2. Diameter of the xylopodium (A) and length of the main root (LMR) (B) of umbuzeiro seedlings submitted to different water regimes.

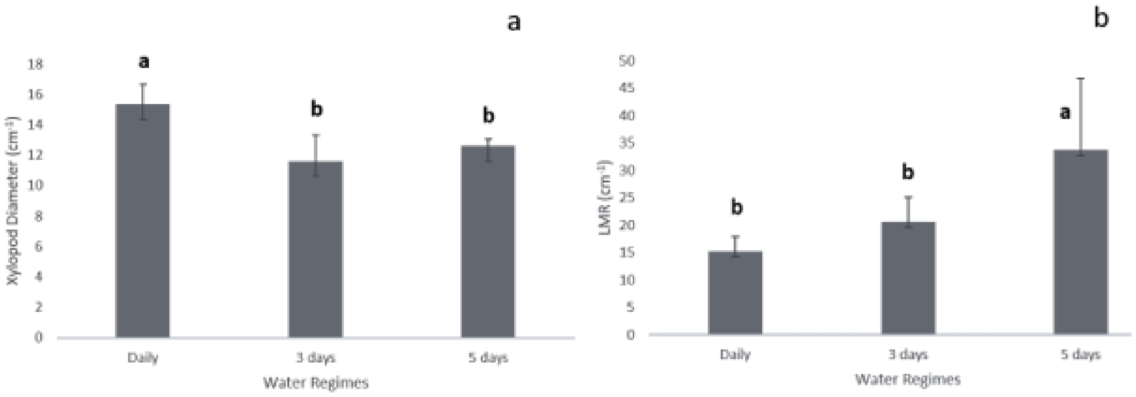

Figure 3. Percentage of Biomass Allocation in the different organs submitted to different water regimes.

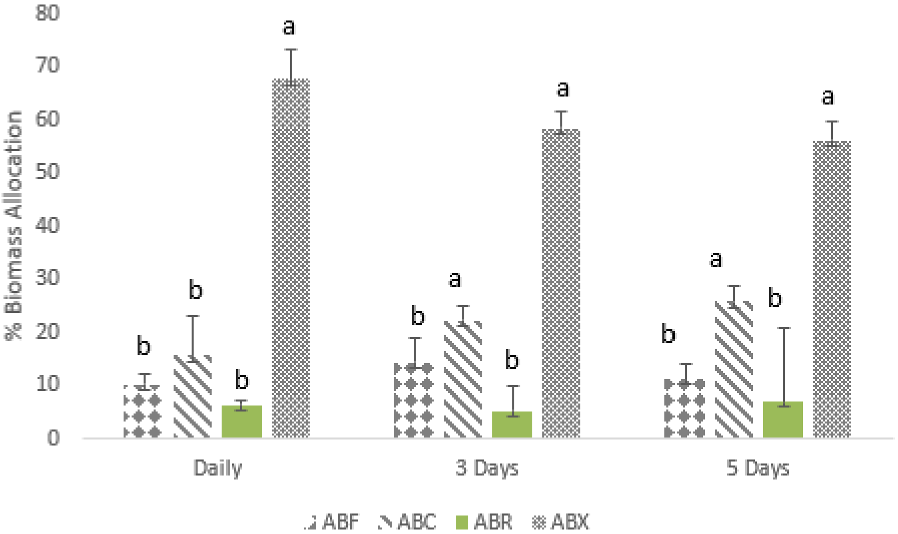

Figure 4. Stomatal density (A), polar diameter (B), equatorial diameter (C) and polar and equatorial diameter ratio (D) in umbuzeiro seedlings submitted to different water regimes. * Averages with the same letter do not differ by Tukey's test at 5% probability.

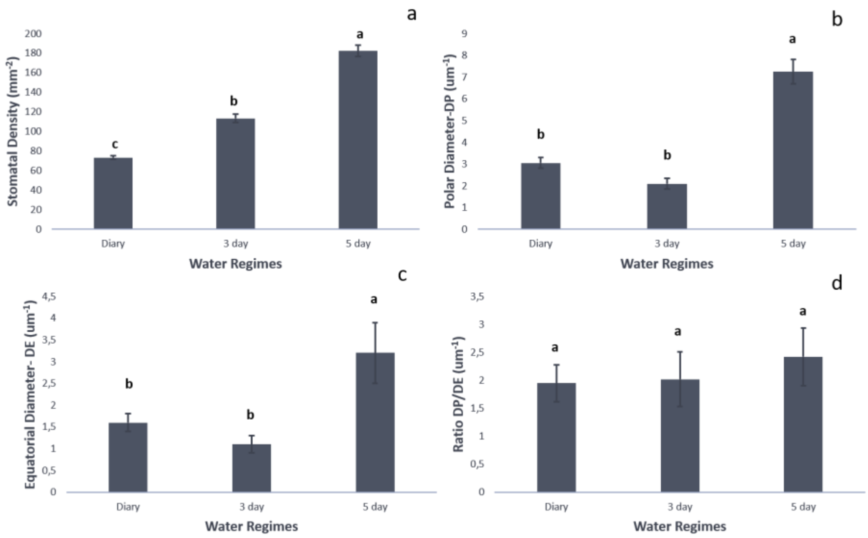

Figure 5. Pariolemic and leaf sections of Spondias tuberosa Arr. Cam. A- Cross-sectional view of the median region of the leaflet showing adaxial epidermis (EpAd) and abaxial epidermis (EpAb), palisade parenchyma (PP) spongy parenchyma (PE), vascular bundle (FVS); B- central rib (NC); C-xylem (XL), phloem (FL) and secretory duct (DS); D- detail of the secretory duct (DS) at the foliolar border; Glandular type (TG) trichoma; F- angular collenchymatic tissue (CoA); G - Stomach of the anomocytic type. Bars: A = 45um; B = 20um; C = 55um; D = 20um; E = 55um; F = 20um and G = 4um.

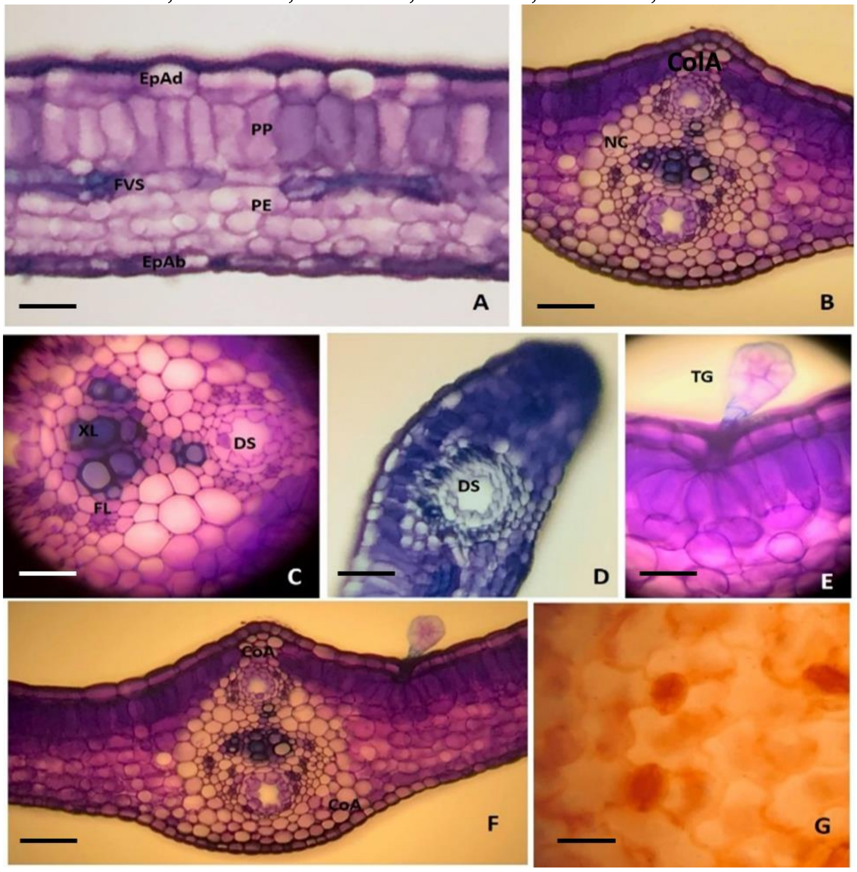

Figure 6. Adaxial epidermis (A), palisade parenchyma (B), spongy parenchyma (C), abaxial epidermis (D), mesophyll (E) and leaflet (F) of umbuzeiro seedlings submitted to different water regimes. * Means followed by the same letter do not differ by Tukey's test at 5% probability.

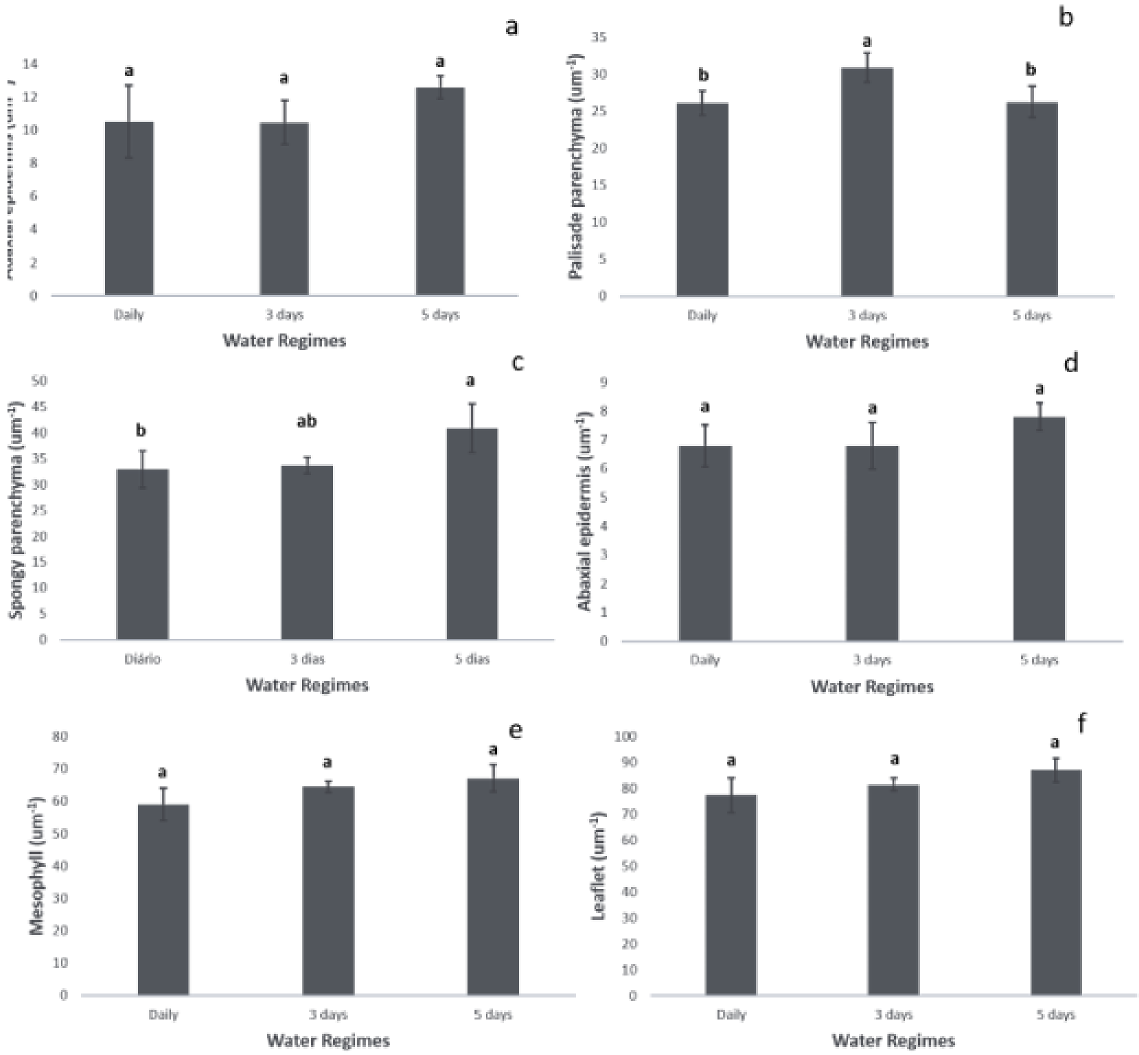

Figure 7. Cross sections of de Spondias tuberosa Arr. Cam. A- Morphological aspect of xylopodium of young seedlings; B- lining fabric with forming periderm; C- fundamental system with cortical parenchyma, detail of the secretory duct (DS); D-xylem; E- radial parenchyma (arrow); F- lenticels in the periderm. Bars: B, C, E and F = 45um and D = 60um.

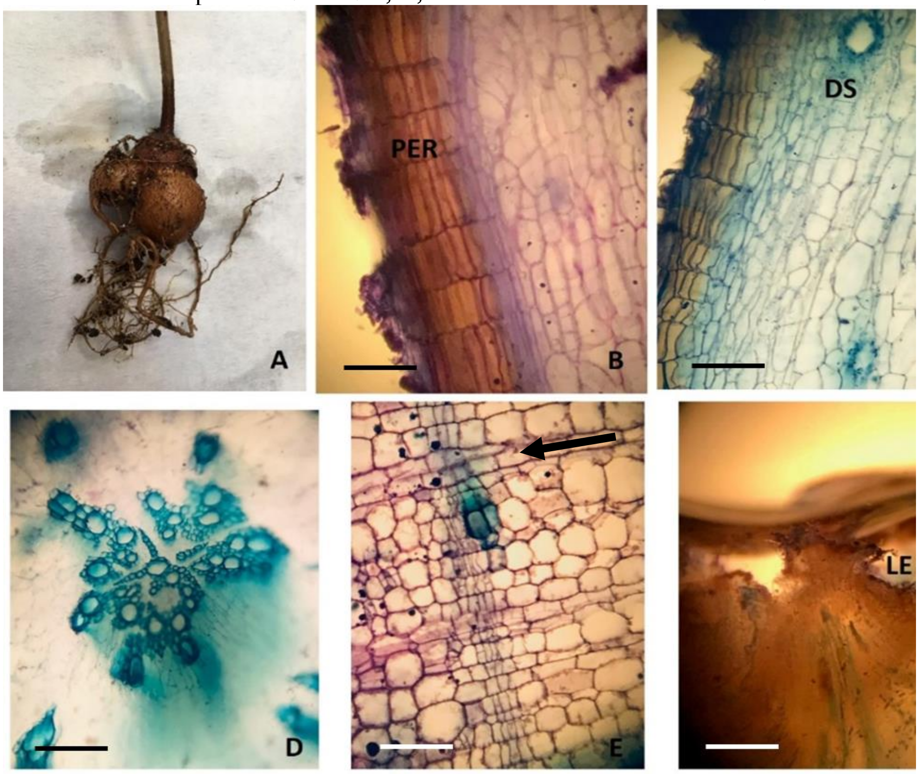

Figure 8. Cortical parenchyma with the presence of starch. Bars: A = 45um; B = 20um and C = 5um.