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Original Article

Influence of seed size on drought tolerance in *Eugenia uniflora* L. seedlings

Influência do tamanho da semente na tolerância à seca em plântulas de *Eugenia uniflora* L.

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Abstract

Drought events have become more intense and frequent in tropical ecosystems, which can limit plant growth and development, as well as the germination and recruitment of tree species. The variation in seed size may give plants advantages in the establishment in an environment and greater tolerance to environmental stress. *Eugenia uniflora* L. is a species found in different Brazilian biomes, such as Atlantic Forest and Pampa, and has high survival capacity in degraded areas and in various environmental conditions. To analyze the effect of seed size on the emergence capacity and drought response of *E. uniflora* seedlings, seeds were separated into two classes according to size and later analyzed for emergence, growth variables, soluble carbohydrate content, and anatomical changes of plants under drought settings. Analysis of the results showed that seed size and drought affected most growth parameters, in which drought caused a reduction in the number of leaves, fresh leaf mass, fresh stem mass and fresh root mass, and collar diameter. Drought also induces a larger diameter of the central cylinder in the root of plants originating from large seeds and a smaller thickness of the cortical parenchyma of the root of plants originating from small seeds. The content of soluble carbohydrates was reduced in the roots of plants under drought compared to the control, and there was also a blockage in sap transport in plants under drought. The results obtained show that water stress caused by drought reduced the growth and anatomical characteristics of root; however, plants from large seeds had greater leaf mass, fresh stem mass, root dry mass, and total dry mass, and higher shoot/root dry mass ratio. Thus, seed size influenced the performance of *E. uniflora* seedlings, both under drought and in the control setting.

Keywords: water stress, climate change, morphological responses, anatomy, soluble carbohydrates.

Resumo

Os eventos de seca tem se tornado mais intensos e frequentes em ecossistemas tropicais, o que pode limitar o crescimento e desenvolvimento das plantas, assim como a germinação e o recrutamento de espécies arbóreas. A variação do tamanho da semente pode conferir vantagem no estabelecimento das plantas no ambiente e maior tolerância ao estresse ambiental. *Eugenia uniflora* L. é uma espécie encontrada em diferentes biomas brasileiros, como Mata Atlântica e Pampa, e possui alta capacidade de sobrevivência em áreas perturbadas e em diversas condições ambientais. Com o objetivo de analisar o efeito do tamanho das sementes na capacidade de emergência e na resposta à seca das mudas de *E. uniflora,* sementes foram separadas em duas classes de acordo com o tamanho e posteriormente analisadas a emergência, as variáveis de crescimento, o teor de carboidratos solúveis e alterações anatômicas das plantas sob seca. A análise dos resultados mostrou que o tamanho da semente e o fator seca afetaram a maioria dos parâmetros de crescimento analisados, no qual a seca provocou redução do número de folhas, da massa fresca foliar, do caule e da raiz e do diâmetro do coleto. A seca também induziu maior diâmetro do cilindro central nas raízes de plantas originadas de sementes grandes e menor espessura do parênquima cortical nas raízes de plantas originadas de sementes pequenas. O teor de carboidratos solúveis foi reduzido nas raízes de plantas sob seca quando comparado ao controle, verificando-se também bloqueio no transporte da seiva nas plantas sob seca. Os resultados obtidos demonstram que o estresse hídrico causado pela seca reduziu o crescimento e características anatômicas da raiz, no entanto, as plantas oriundas de sementes grandes apresentaram maior massa foliar, massa fresca do caule e seca da raiz e massa seca total e maior razão massa seca da parte aérea/massa seca da raiz. Assim, o tamanho da semente influenciou o desempenho das mudas de *E. uniflora,* tanto sob seca quanto na condição controle.

Palavras-chave: estresse hídrico, mudanças climáticas, respostas morfológicas, anatomia, carboidratos solúveis.

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1. Introduction

Eugenia uniflora L. (Myrtaceae), popularly known as *pitangueira* (Brazilian cherry tree), can be found as a dense, 2- to 4-meter high shrub, or as a 6 to 9-meter high tree, branched, with a round treetop of 3 to 6 meters of diameter, with evergreen or semi-evergreen foliage. This is a species native to Brazil, non-endemic, and can also be found in Paraguay, Argentina, and Uruguay (Bezerra et al., 2018). It is found in the *Cerrado*, Atlantic Forest and Pampa biomes, and in some forests such as Riparian Forest, Perennial Seasonal Forest, and Semideciduous Seasonal Forest (Mazine et al., 2020). *E. uniflora* has high survival capacity in degraded areas, adaptability to the most diverse soil types and climatic/environmental settings, and can be used to recover degraded areas, being cultivated in several regions of the world (Bezerra et al., 2018; Vera Cruz et al., 2024).

Environmental factors such as reduced water availability and extreme temperature events can affect plant establishment. The lack of water availability tends to limit plant growth and development, as it affects several physiological and biochemical processes (Moustakas et al., 2022). Water stress induced by water scarcity impairs cell division, cell elongation and differentiation, and metabolism, causing turgor reduction and altering photosynthetic rates and the entire energy balance (Moustakas et al., 2022). Thus, reduced precipitation and soil moisture, due to climate change, intensely drives physiological changes in the plant (Pezzola et al., 2017).

Over time, significant changes in climate have been observed, due to deforestation and atmospheric pollution, causing an accelerated increase in greenhouse gases, increasing average temperatures and making drought seasons more intense (Pachauri et al., 2014). Thus, an increase in the duration, intensity and frequency of droughts as a consequence of climate change is expected in the coming years, in such a way that it may reach alarming levels (Moustakas et al., 2022). As a result, environmental factors can more intensely affect the morphological and physiological responses of plants, hindering their establishment and growth.

Plants have developed various energy strategies to circumvent/tolerate water scarcity. Increased root growth, increased leaf thickness, decreased leaf area, fewer leaves, reduced stomatal conductance and leaf curling to decrease evapotranspiration are some physiological and morphological adjustments observed in plants (Hesse et al.,2024). The accumulation of osmoregulatory substances such as soluble sugars helps plants to preserve water status, and thus maintain the overall structure and growth of plants (Rosa et al., 2009).

Extreme weather conditions can affect phases related to the life cycle of plants, impacting the early stages of plants life (Klupczyńska and Pawlowski, 2021). Thus, the recruitment of new plants initially depends on the seed germination capacity and establishment of seedlings in the environment. Considering the relationship between climate and recruitment, different species have developed strategies to successfully germinate, which can be altered due to climate change (Cochrane et al., 2015).

Seed germination is sensitive to environmental factor and can occur only under specific conditions, with availability of water, light and temperature (Vásquez-Ramírez and Venn, 2025; Gao et al., 2024). The lack of adequate conditions for germination can cause dormancy, affecting the establishment of new plants.

Seed germination is also related to the characteristics of the seed itself, and seed mass and size may vary depending on the species or even between individuals of the same species (Yi et al., 2019).

Seed size is often indicative of physiological quality (Socolowski et al., 2011). Thus, larger seeds are more vigorous, presenting better germination and vigor in batches containing large- and medium-sized seeds, when compared to smaller ones (Popinigis, 1985; Carvalho and Nakagawa, 2012). Pereira et al. (2013) observed that seedlings from larger seeds may have more developed roots than seedlings from smaller seeds, which may confer a competitive advantage in obtaining soil resources such as water resulting in a greater probability of species and/or seedlings establishment. Moreover, seedlings from larger seeds could have greater tolerance to environmental stress situations, such as drought (Yi et al., 2019). In this context, studying the interaction between seed size and the performance of native plants under water stress conditions can contribute to ecological restoration projects and biodiversity conservation, considering the greater frequency of extreme climate events.

Considering the current context of climate change, with the increasing number of drought periods, this study aimed to analyze the effect of seed size on the emergence capacity and drought response of *Eugenia uniflora* seedlings.

2. Materials and Methods

2.1. Collection of plant material

Mature fruits of *E. uniflora* were collected from trees present in fragments of the Semideciduous Seasonal Forest (23°24'27.9"S 51°55'54.8"W and 23°24'05.7"S 51°56'33.7"W), state of Paraná, Brazil. Plant material was collected in October 2021, and later the fruits were taken to the laboratory, in which seeds were extracted manually.

2.2. Biometrics and seed distribution in classes

The size of the seeds of *E. uniflora* was measured individually with the aid of a digital caliper. In total, 200 seeds were used as a basis for classifying the classes. Malformed seeds with visible signs of predation or pathogen attack were discarded. Seeds were classified into two classes: small seeds (S) and large seeds (L). Class S refers to seeds sized less than the mean size minus the standard deviation (< 9.3 mm), and class L corresponds to seeds greater than the mean size plus the standard deviation (> 9.5 mm). For classification, the standard deviation was used as a parameter, as it is commonly employed in analyses involving measures of mean deviation (Andrade and Ogliari, 2007).

2.3. Emergence analysis according to classes

For the seedling growth test, 100 seeds were used in four replicates of 25 for each size class. Sowing was performed

in trays containing sand and commercial organic substrate (Fertilizare) in a 2:1 ratio, respectively, with each cell of the tray receiving only one seed. Seedling emergence was monitored and recorded daily in a greenhouse, and from the data obtained, percentage of emergence, average emergence time, and seedling emergence speed index were calculated according to Vieira and Carvalho (1994).

2.4. Acclimation and water stress analysis in a greenhouse

To evaluate the responses of plants to water deficit, after emergence, all seedlings were transplanted into 10cm × 20cmseedling bags, containing sand and commercial organic substrate (Fertilizare) in a 2:1 ratio, respectively, and kept in a greenhouse. Each bag received a single seedling, according to size class.

After transplantation, plants were irrigated daily for 50 days for acclimatization. Subsequently, the pots were divided into the following greenhouse treatments: plants originating from small seeds (S) and large seeds (L), and two water regimes (plants irrigated every day and non-irrigated plants). Thus, *E. uniflora* plants were subjected to the treatments: plants originated from S seeds and irrigated daily (CS), originated from L seeds and irrigated daily (CL), originated from S seeds and non-irrigated (HSS) and originated from L seeds and non-irrigated (HSL). Non-irrigated plants were kept without any irrigation for 15 days. After this period, ascent of sap, total soluble carbohydrate content, anatomical characteristics and growth of CS, CL, HSS and HSL plants were analyzed.

2.5. Ascent of sap analysis

For the ascent of sap analysis, a cross-section was performed on the stem, near the root crown, after which the plants were kept in a 1% eosin solution for 4 hours (adapted from Ganthaler and Mayr, 2021). During this period, the rise of the eosin solution from the base of the stem to the leaves was observed. Plants were photographed and then freehand cross-section of the stem was performed. The cuts were deposited on a slide and coverslip, and a drop of 33% glycerin was added. The sections were imaged under Leica EZ4D light microscope with a digital camera attached.

2.6. Quantification of total soluble carbohydrate contents

For the evaluation of total soluble carbohydrates, five root and leaf samples from each treatment were kept in an oven at 60°C for 48 hours, and then the dry weight of 100 mg was determined, from which the soluble carbohydrates and each organ (dried root and leaf) were quantified. The root samples (n=5) were crushed separately to obtain the alcoholic extract, which was evaporated and water was added, obtaining the aqueous extract, from which the contents of the soluble compounds were determined. The same procedure was performed for leaves. Total soluble carbohydrate contents were determined through reactions with anthrone (Clegg, 1956). The reading was performed on a spectrophotometer (Shimadzu UV-1201 spectrophotometer) at 620 nm.

For pigmented extracts, such as the leaf, the previous depigmentation process was necessary, carried out in a separation funnel using chloroform and distilled water. Then, these samples were subjected to an evaporation process and further quantified, as described above.

2.7. Evaluation of anatomical characteristics

For the anatomical analysis, the leaves and roots of *E. uniflora* plants from three individuals of each treatment were used. Root segments located one centimeter from the apical region were collected; for the leaves, segments close to the midrib of the median region of leaves that were between the second and third knots were selected. The material was fixed in FAA, dehydrated in ethyl alcohol series, and included in Leica historesin, according to manufacturer's guidelines, and sectioned in a rotation microtome (Johansen, 1940). Cros-sections were made and stained with toluidine blue (O'Brien et al., 1964).

Histological analyses were performed on Leica EZ4D light microscope with coupled image capture system, and processed using Leica Application Suite software version 1.8.

Based on the images obtained, the following measurements were performed: for root parameters, total root diameter (TD) (µm), central cylinder diameter (CCD) (µm), cortical parenchyma thickness (CPT) (µm) and exoderm thickness (EE) (µm); for leaf parameters, adaxial epidermis thickness (AET) (µm), abaxial epidermis thickness (ABET) (µm), palisade parenchyma thickness (PPT) (µm), spongy parenchyma thickness (SPT) (µm), mesophyll thickness (MT) (µm) and total leaf thickness (TT) (µm). Both images were analyzed using Image-Pro® Plus 4.5 image analysis software (Media Cybernetics, Rockville, MD, USA).

2.8. Growth assessment

Growth evaluation was performed 15 days after the beginning of the water stress period, and 10 plants of each treatment were randomly chosen. The plants were carefully removed from the bags so that they would not be damaged. The bags were ruptured vertically and the substrates removed with running water, to prevent loss of root fragments.

Measures of the following growth variables were considered: shoot height (H); root length (RL); collar diameter (CD);leaf fresh mass (LFM), leaf dry mass (LDM), stem fresh mass (SFM), stem dry mass (SDM), root fresh mass (RFM), root dry mass (RDM), shoot dry mass (SM), total dry mass (TDM) and leaf number count (LN). The ratio between root dry mass/shoot dry mass (RDM/SM) was also calculated.

The height and length of the root were obtained with the aid of a ruler, while for the root crown diameter a digital caliper was used. The number of leaves was obtained by manually counting the fully expanded leaves of each plant. For biomass analysis, the roots, stem and leaves of each plant were separated and weighed on a precision analytical scale to obtain the fresh mass, which were placed in paper packages, and then oven-dried at 60°C for 48 hours. After this period, the dry matter of each organ was obtained on a precision analytical scale.

2.9. Statistical analysis

The results obtained, the growth, anatomical and physiological parameters were subjected to two-way analysis of variance (ANOVA), also verifying the interaction of the factors "seed size" and "drought" on the analyzed parameters. Emergence results were analyzed using one-

way ANOVA. The analyzed characteristics that obtained p ≤ 0.05 were considered significant. Fisher's test was used for a posteriori comparisons. Analyses were performed using the Statistica statistical package version 7.1.

3. Results

Seed size influenced the response of most growth variables, such as plant height and fresh and dry mass. The dry factor alone had a significant effect for most of the parameters analyzed, excepting root dry mass and RDM/SM ratio. However, the interaction of seed size and drought was significant only in the analysis of total soluble carbohydrates and root fresh mass.

3.1. Emergence

The emergence analysis showed that seeds of both classes obtained, on average, a 90%emergence percentage, an emergence speed index of 0.64 seedlings per day and an average emergence time of 35.6 days. Regarding seedling emergence, no significant difference was observed between seed size classes.

3.2. Ascent of sap

By analyzing ascent of sap, it was verified that plants under control conditions, regardless of the seed size, maintained sap transportation intact, with reddish color being observed in the leaves; notably, for plants under water stress, the sap transport was interrupted (Figure 1).

3.3. Quantification of total soluble carbohydrate contents

The analysis of the results obtained for the contents of total soluble carbohydrates showed the significant interaction between treatments and seed size ($p = 0.0018$). The control treatment plants showed higher levels of total soluble carbohydrates in the root, regardless of seed size. However, considering only the root, small seeds under

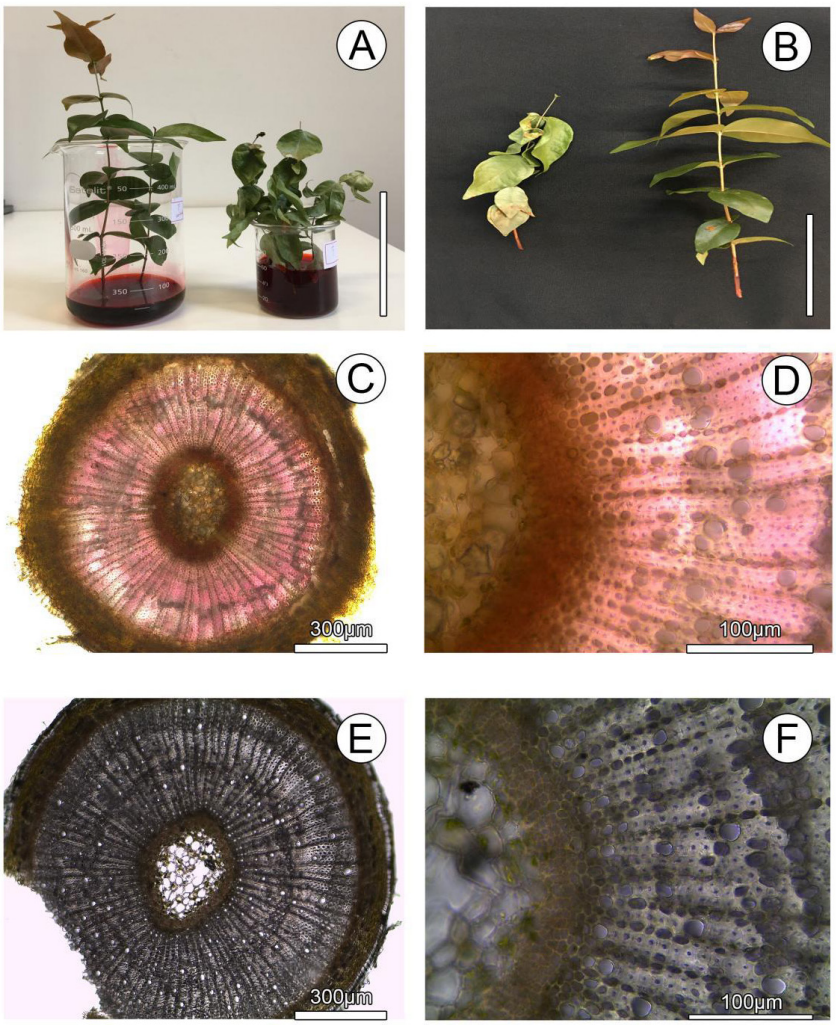

Figure 1. Sap rise of *E. uniflora* 15 days after the start of water stress treatment. (A) CS plants on the left and HSS on the right kept in 1% eosin solution; (B) Plants after 4h in eosin solution, with CS plant on the right and HSS plant on the left; (C and D) cross-section of the stem of CS plants; (E and F) cross-section of the stem of HSS plants. (A and B: scale = 10 cm).

hydric stress (HSS) had higher total soluble carbohydrate content than large seeds under hydric stress (HSL) (Table 1).

Regarding the content of soluble carbohydrates found in the leaves of the control plants, a higher content was observed in the leaves of the CL plants, with no difference in the leaves of plants under stress, regardless of the size of the seed (Table 1).

By analyzing the difference between root and leaf, considering the same treatment and same size, a higher content of soluble carbohydrates was obtained in the root of CS plants, while the opposite was verified in CL plants. For the analyses between different sizes and water conditions, no significant difference was found. (Table 1).

3.4. Anatomy

The radicle of the plants (Figure 2A) from small-seed control groupies composed of uniseriate epidermis and cortex with uniseriate exodermis, spongy cortical parenchyma and endodermis. The central cylinder of this root has uniseriate and parenchymal pericycle and alternating primary xylem and phloem cords; in the central root a spongy parenchyma can be observed, and the root is polyarch (Figure 2A). The root of the plant from the large-seed control group is structurally like the previous plant, except that the endoderm may have cells with dark content, probably phenolic derivate (Figure 2B).

Regarding the anatomical parameters measured of *E. uniflora* roots, no significant differences were observed in total root diameter (TD) between treatments (control and water stress) and between the evaluated sizes (S and L) (Table 2).

There was a smaller central cylinder diameter (CCD) in the HSS plants, when compared to the HSL, with a significant difference considering the same treatment. No significant difference was observed in the CDD for both small and large seeds under control conditions. When evaluating water treatments for the same seed, CL plants presented smaller CCD when compared to the HSL plants; regarding small seeds no significant difference was observed for this parameter.

For cortical parenchyma thickness (CPT), a significant difference was observed only between CS and HSS plants, with roots of CS plants showing thicker CPT than the roots of HSS plants (Tab. 2).

The roots of HSL plants showed thinner exoderm thickness (ET) when compared to the roots of CL plants. There was no significant difference between the plant roots of small-sized seeds (Table 2).

For the roots of HSS plants, it was observed that the epidermis and cortex had compressed and irregular cells; in the cortex the endodermis had a thick internal periclinal wall, and in some samples the epidermis collapsed. In the cortical parenchyma, it was observed that some contiguous cells had increased size and had their cell walls destroyed, generating larger compartments (Figure 2C).

In the roots of HSL, collapsed and partially eliminated epidermis and cortex structures were observed, with already developed periderm; a partially destroyed central parenchyma was also verified (Figure 2D).

The leaf anatomical parameters evaluated showed similar values between treatments and between sizes, and no significant differences were obtained between them

Table 1. Total soluble carbohydrate contents of *Eugenia uniflora,* evaluated 15 days after the start of water suspension treatment.

Treatments	Total soluble carbohydrate contents (mg g^{-1} MS)	
	Root	Leaves
CS	2.39 Aaα ± 0.29	0.78 Bbα ± 0.05
CL	1.79 Abα ± 0,09	2.18 Aaα ± 0.59
HSS	1.48 Aaβ ± 0.16	1.31 Aaα ± 0.22
HSL	0.24Baβ ± 0.02	0.69 Aaβ ± 0.08

*Equal letters do not differ by Fisher's test at 5%. Capital letters considering the same organ among different sizes, lowercase letters comparing organs in the same treatment and seed size. Greek letters comparing water stress and control (considering the same organ and the same seed size) (Means ± SE). CS = plants originated from small seeds and irrigated daily; CL = plants originated from large seeds and irrigated daily; HSS = plants originated from small seeds and non-irrigated; HSL = plants originated from large seeds and non-irrigated.

Table 2. Anatomical parameters of the roots of *Eugenia uniflora* evaluated 15 days after the start of the water stress treatment. Total root diameter (TD), Central cylinder diameter (CCD), Cortical parenchyma thickness (CPT), and Exoderm thickness (EE).

Treatments/Parameters(μm)	TD	CCD	CPT	EE
CS	867.2 Aa ± 66.1	407.3 Aa ± 48	324.0 Aa ± 106	20.1 Aa ± 2.6
CL	733.7 Aa ± 128.3	325.4Ab ± 38.1	207.8 Aa ± 17.5	21.9Aa ± 3.6
HSS	589.9 Aa ± 21.8	249.1Ba ± 37.7	88.7Ab ± 8.6	15.1 Aa ± 2.0
HSL	517.8 Aa ± 21.0	527.7 Aa ± 94.3	125.4 Aa ± 34.9	13.9Ab ± 1.0

*Equal letters do not differ by Fisher's test at 5%. Capital letters considering different seed sizes in the same water condition, lowercase letters considering the same seed size but under different water treatments (Means ± SE). CS = plants originated from small seeds and irrigated daily; CL = plants originated from large seeds and irrigated daily; HSS = plants originated from small seeds and non-irrigated; HSL = plants originated from large seeds and non-irrigated.

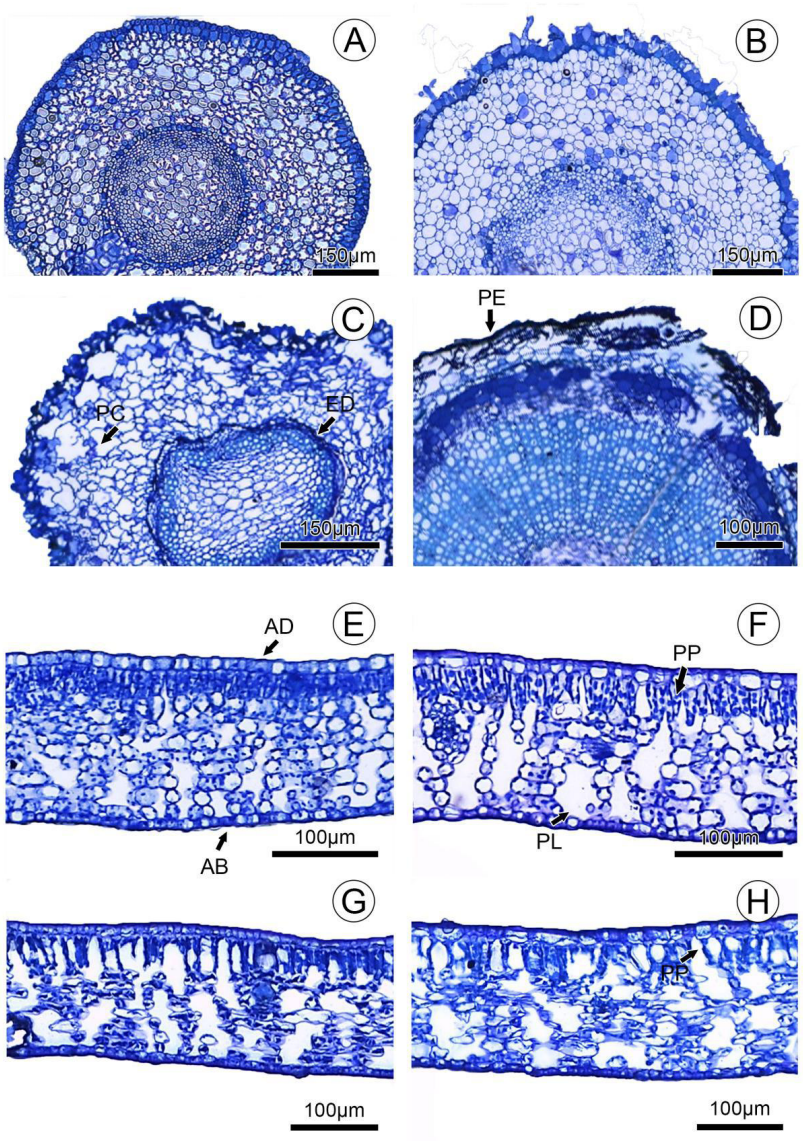

Figure 2. Anatomical structures in cross-sections of root and leaf of Eugenia uniflora 15 days after the start of water stress treatment. (A-D) Root. (A) S control; (B) L control; (C) HSS; (D) HSL; (E-H) Leaf. (E) S control; (F) L control; (G) HSS; (H) HSL. PC- cortical parenchyma; ED- endoderm; PE- periderm; AB- abaxial epidermis; AD- adaxial epidermis; PP- palisade parenchyma; PL- spongy parenchyma.

(Table 3). Notably, it was observed that the epidermis of the adaxial and abaxial faces had more compressed cells in the leaves of plants under water stress (HSS and HSL). In the mesophyll, the palisade parenchyma cells were also compressed and narrower. In the spongy parenchyma, more developed intercellular spaces were observed. The bundle sheath of some of the leaf vascular system cells has also undergone some compression (Figure 2G and 2H).

3.5. Growth

Plants from large seeds, regardless of water condition, presented higher LFM, LDM, RDM, TDM and SM (Table 4).

Considering the control treatment, CL plants still showed higher H, while HSL plants showed higher SDM and RDM/SM than HSS plants (Table 4).

Comparing the effect of water condition on plants from seeds of the same size, it was found that drought negatively affected LN, fresh plant mass and root crown diameter (RCD) in HSS plants (Table 4). HSL plants had lower H, LN, fresh leaf, stem and root mass and lower RCD than CL plants. Overall, water deficit affected the same growth parameters, regardless of small or large seed, compared to the control (Table 4).

4. Discussion

The variation in the size of *E. uniflora* seeds showed that large seeds provided greater LFM, LDM, SDM, RDM, and SM. The higher fresh and dry mass of the plants may

Table 3. Anatomical parameters of *Eugenia uniflora* leaves evaluated 15 days after the start of water stress treatment. adaxial epidermis thickness (AET), abaxial epidermis thickness (ABET), palisade parenchyma thickness (PPT), spongy parenchyma thickness (SPT), mesophyll thickness (MT), and total leaf thickness (TT).

Parameters (μm)/Treatments	CS	CL	HSS	HSL
AET	16.6 Aa ± 1.6	16.6 Aa ± 0.9	13.4 Aa ± 0.9	16.7 Aa ± 1.4
ABET	14.5 Aa ± 1.4	14.7 Aa ± 1.4	12.6 Aa ± 1.2	13.6 Aa ± 0.5
PPT	44.6 Aa ± 4.6	47.7 Aa ± 4.3	39.8 Aa ± 6.0	46.4 Aa ± 1.6
SPT	107.6 Aa ± 21.0	81.9 Aa ± 61.0	87.7 Aa ± 15.6	88.7 Aa ± 13.9
MT	151.7 Aa ± 20.3	129.1 Aa ± 5.8	124.0 Aa ± 20.7	139.5 Aa ± 15.2
TT	178.3 Aa ± 21.8	159.9 Aa ± 7.7	151.3 Aa ± 22.1	165.1 Aa ± 15.6

*Equal letters do not differ by Fisher's test at 5%. Capital letters considering different seed sizes in the same water condition, lowercase letters considering the same seed size, representing the difference between water treatments (Means ± SE). CS = plants originated from small seeds and irrigated daily; CL = plants originated from large seeds and irrigated daily; HSS = plants originated from small seeds and non-irrigated; HSL = plants originated from large seeds and non-irrigated.

Table 4. Growth parameters of Eugenia uniflora plants evaluated 15 days after the start of water stress treatment. Shoot height (H), Root length (RL), leaf number (LN), leaf fresh mass (LFM), leaf dry mass (LDM), stem fresh mass (SFM), stem dry mass (SDM), root fresh mass (RFM), root dry mass (RDM), collar diameter (CD), total dry mass (TDM), shoot dry mass (SM), ratio root dry mass/shoot dry mass (RDM/SM).

Parameters/ Treatments	CS	CL	HSS	HSL
H (cm)	17.11 Ba ± 0.82	20.5 Aa ± 1.08	14.77 Aa ± 0.59	15.53 Ab ± 0.75
RL (cm)	17.60 Aa ± 1.11	18.40 Aa ± 1.47	15.15 Aa ± 1.31	15.14 Aa ± 1.25
LN (g)	17.10 Aa ± 1.53	18.70 Aa ± 2.03	12.80 Ab ± 0.99	12.70 Ab ± 1.14
LFM (g)	1.288 Ba ± 0.130	1.598 Aa ± 0.119	0.361 Bb ± 0.021	0.491 Ab ± 0.038
LDM (g)	0.373 Ba ± 0.037	0.471 Aa ± 0.030	0.291 Ba ± 0.017	0.404 Aa ± 0.031
SFM (g)	0.306 Aa ± 0.031	0.376 Aa ± 0.035	0.098 Bb ± 0.010	0.126 Ab ± 0.009
SD, (g)	0.113 Aa ± 0.011	0.137 Aa ± 0.123	0.076 Ab ± 0.007	0.102 Ab ± 0.009
RFM (g)	0.448 Ba ± 0.050	0.819 Aa ± 0.053	0.107 Ab ± 0.010	0.213 Ab ± 0.025
RDM (g)	0.096 Ba ± 0.012	0.151 Aa ± 0.011	0.078 Ba ± 0.006	0.147 Aa ± 0.013
CD (mm)	1.510 Aa ± 0.07	1.630 Aa ± 0.07	1.000 Ab ± 0.05	1.130 Ab ± 0.05
TDM (g)	0.602 Ba ± 0.064	0.798 Aa ± 0.040	0.472 Ba ± 0.028	0.684 Aa ± 0.045
SM (g)	0.506 Ba ± 0.053	0.647 Aa ± 0.035	0.394 Ba ± 0.024	0.537 Aa ± 0.038
RDM/SM (g)	0.187 Aa ± 0.012	0.235 Aa ± 0.017	0.200 Ba ± 0.013	0.277 Aa ± 0.025

*Equal letters do not differ by Fisher's test at 5%. Capital letters considering different seed sizes in the same water condition, lowercase letters considering the same seed size and different water conditions (Means ± SE). CS = plants originated from small seeds and irrigated daily; CL = plants originated from large seeds and irrigated daily; HSS = plants originated from small seeds and non-irrigated; HSL = plants originated from large seeds and non-irrigated.

indicate a better early development of the seedlings (Antunes et al., 2012). Often, seeds with larger size and/or mass have higher values for the biomass of their seedlings, which can be considered more vigorous. According to Socolowski et al., (2011), the initial growth of seedlings depends on the seeds mass, and these characteristics can also be considered as a reproductive strategy.

Water deficit is one of the most important factors that considerably inhibit growth and metabolic activities, such as photosynthesis (Ashraf and Harris, 2013). During the drought season, to avoid excessive water loss, plants decrease stomatal conductance, thereby reducing carbon uptake for photosynthesis (Lopes et al., 2022), which consequently negatively affects growth. *E. uniflora* plants, originating from small or large seeds and maintained under water deficit, showed a significant reduction in growth parameters, such as H, NL and fresh mass, indicating that the water deficit negatively affected the seedlings growth, regardless of the seed size. Moreover, the results demonstrate that severe drought can compromise the establishment and performance of seedlings of *E. uniflora*.

However, under water stress, plants originating from small seeds had a lower RDM/SM ratio than plants originating from larger seeds, demonstrating that the larger seed size provided greater root growth in relation to the plant shoot. Greater root growth relative to the shoot is a characteristic observed in plants subjected to drought-related water stress, which allows them to obtain water

from deeper soil layers (Lynch et al. 2014). Investment in the root system may be associated with tolerance to water stress, indicating that plants allocate more biomass to roots in soil with lower water availability (Lima et al., 2014; Vera Cruz et al., 2024). Furthermore, other growth parameters were superior in HSL plants compared to HSS ones, such as fresh plant mass and leaf, root and total dry mass, indicating better performance of plants originating from large seeds under water deficit.

The lower RDM/SM ratio verified in the HSS plants indicate the highest investment in the shoot compared to the accumulation of root biomass. Carvalho et al., (2023) reported that species with greater resistance to drought have characteristics such as lower height and larger seeds, which would guarantee the production of more vigorous seedlings due to seed size and mechanisms associated with xylem vulnerability due to the effects of embolism and hydraulic resistance in taller plants.

The analysis of eosin transport in *E. uniflora* plants showed the suspension of transport, due to the discontinuity of the water column in the shoot (Figures 1A and 1B), which may be related to xylem vessel cavitation due to drought. Plants with xylem highly resistant to cavitation due to drought have greater capacity to survive stress (Pivovaroff et al., 2016), however, severe drought imposed on *E. uniflora* plants disrupted the waterflow.

Some woody plants, which show large reductions in transpiration and stomatal conductance when exposed to water stress, increase the synthesis or accumulation of osmotically active solutes in leaf cells (Nepomuceno et al., 2001).The accumulation of these compounds decreases the water potential in the leaf, increasing the absorption capacity of water by the roots, and alleviating the effects of water stress (Albuquerque et al., 2013). Additionally, the accumulation of these sugars during stress provides protection and prevents oxidation of cell membranes and maintains leaf turgidity (Sami et al., 2016, Lopes et al., 2022). Commonly, plants use part of the sugars from photosynthesis or from leaf degradation of starch to stimulate the growth of the underground system, providing greater root growth and greater water absorption capacity (Albuquerque et al., 2013). However, in *E. uniflora* the highest content of soluble carbohydrates in the root was observed in the control treatment plants and in the leaves for large seeds from the control group, indicating that drought reduced photosynthetic activity and carbohydrate synthesis.

In drought conditions, there is a drastic reduction in cell turgor, changes and mesophyll compaction caused by plant dehydration resulting from water stress. Plasmolyzed cells can also be found mainly in the spongy parenchyma, shortening the leaf lamina (Vieira et al., 2022). Deformed cells were found both in the root of HSS plants (Figure 2C) and in HSS and HSL leaf tissues (Figures 2G and 2H). However, there was no significant difference in leaf anatomical parameters in *E. uniflora* under both studied treatments. For measurements observed in anatomical root sections in HSL plants, there was a larger central cylinder diameter than in HSS plants. This can be seen in Figures 2C and 2D, noting the deformed cells in the root cortex of HSS plants, while HSL presented more subtle changes. This reinforces the greater vigor of plants from large seeds, with the maintenance of cell structure, even in severe drought conditions. However, for HSS plants, severe drought induced cell deformation of the cortical parenchyma and central cylinder, which contributed to the reduction of CCD and CPT size.

Changes in the anatomical characteristics of the roots are important for the acquisition of soil water, in addition to providing drought resistance (Zhu et al., 2010). Plants under drought may present root cortical aerenchyma, reducing metabolic costs (Díaz et al., 2018), such as decreased breathing expenses, greater rooting and improved leaf water status (Lynch et al., 2014). Regions with lysis are noted in the root cortex of HSS plants, which may be associated with cell death due to stress. The formation of root cortical aerenchyma would be associated with the limitation of radial conductivity, essential to avoid the water loss from the roots to dry soil (Lynch et al., 2014).

Thus, this study demonstrated that the size of *E. uniflora* seeds significantly influenced the development of seedlings, with larger seeds providing better growth and being more vigorous. On the other hand, regardless of the size of the seed, all plants presented drought survival strategies. Anatomical analysis of the roots revealed structural changes, such as the formation of cortical aerenchyma, which contributes to drought tolerance. These results highlight the importance of seed size and anatomical, physiological and biochemical changes in *E. uniflora* species response to water stress and provide valuable data for understanding the ecology and management of this species in drought-susceptible environments.

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