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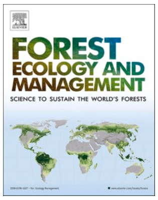

Structural and physiological responses to water availability provide insights into the maintenance of *Mauritia flexuosa* (Arecaceae) seedling banks

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ABSTRACT

Mauritia flexuosa is an ecologically and economically important Amazonian palm. It has an expanded distribution to flooded ecosystems (“veredas”) in the markedly seasonal Cerrado biome. The species’ seeds are sensitive to desiccation, which limits the formation of soil seed banks, although there are indications that *M. flexuosa* can form seedling banks in microenvironments subject to water stress conditions. We evaluated this issue considering both morphological and physiological seedling responses to water availability. *Mauritia flexuosa* seedlings were grown in soils taken from the bottom (organosol) and the edge (gleisol) of a seasonally flooded vereda ecosystem with 0, 40, 60, 80 and 100% available water contents, and their survival and morphologies were evaluated for eight months. At the end of this period, dry mass, photosynthetic parameters, ABA content, leaf ultrastructure, and gene expression associated with aquaporins (MIP family) were evaluated. The seedlings showed phenotypic plasticity, with positive responses to water availability (growth and photosynthetic parameters) as well as the notable ability to survive under water stress in both of the soil types examined. Their responses to water stress were related to ABA accumulation and the maintenance of water homeostasis – with modulation of stomatal control, water use efficiency, and MIP gene expression. *Mauritia flexuosa* can form seedling banks in both flooded and water-stressed environments, which contributes to its reproductive success and wide distribution. The introduction of seedlings produced *ex situ* could be a viable alternative for the recovery of degraded areas.

1. Introduction

Successful seedling establishment is crucial for the conservation and growth of natural populations (Baskin and Baskin, 2014). Post-seminal development constitutes the most critical phase of the plant life cycle, as seedling establishment must occur without the support of structures and physiological mechanisms that will mature only much later (Bewley et al., 2013; Ribeiro et al., 2021; Chaib et al., 2023). Most plant species produce seeds that are tolerant of desiccation and low temperatures (*i.e.*, orthodox; Hong and Ellis, 1996) and can thus form soil seed banks; those seed banks constitute reservoirs of genetic material necessary for the resilience of populations, with seedling emergence (via germination) during windows of climatic opportunity (Long et al., 2014; Souza et al., 2019). Species adapted to humid environments, however, commonly produce seeds that are sensitive to desiccation (*i.e.*, recalcitrant; Hong and Ellis, 1996) and are thus unable to form persistent soil seed banks (Tweddle et al., 2003; Bewley et al., 2013). Nonetheless, in some cases, desiccation-sensitive seeds will germinate quickly and give rise to desiccation-tolerant seedlings – which come to constitute seedling banks, with roles similar to seed banks (Marques et al., 2018). Considering that humid environments are highly threatened by global climate change and local anthropogenic impacts, studies of the mechanisms involved in the formation of seedling banks have become important for biodiversity conservation, as well as constituting an interesting area of plant ecophysiology.

Plants growing in humid environments under seasonal climatic regimes commonly experience both waterlogging and soil water shortage conditions, making water use efficiency and tolerance to water deficits important aspects of seedling bank formation (Martins et al., 2023). Plant-environment water relationships involve a series of anatomical

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structures and complex physiological mechanisms that are still not fully understood, especially in terms of tropical species (Dias et al., 2020; Salvador et al., 2022), with the modulation of photosynthetic activity, the signaling roles of the hormone abscisic acid (ABA), and water flow control mechanisms being particularly important (Tucci et al., 2018; Vogado et al., 2020).

The management of cell water homeostasis is known to involve the accumulation of hygroscopic substances and to rely on the crucial roles of aquaporins (Murata et al., 2000). These membrane intrinsic proteins (MIP) modulate the hydraulic conductivity of biological membranes and function in osmoregulation (Martre et al., 2002; Maurel et al., 2015; Kapilan et al., 2018), with important roles in maintaining water homeostasis under different environmental stress conditions (Wan et al., 2001; Siemens and Zwiazek, 2003; Luu and Maurel, 2005; Galmes et al., 2007; Kapilan et al., 2017). Studies focusing on aquaporins in tropical species, however, are still incipient.

Mauritia flexuosa L. f. (“buriti” or “aguaje”) is an Amazonian palm tree associated with flooded environments and widely distributed in tropical South America (Dransfield et al., 2008; Lorenzi et al., 2010). The species has great ecological importance to regional faunas, as well as economic and social importance to traditional human populations and cosmetic industries (mainly related to the use of its oily fruits) (Endress et al., 2013; Virapongse et al., 2017). *Mauritia flexuosa* populations have expanded to regions of the markedly seasonal Cerrado (neotropical savanna) biome, being predominant in the arboreal component of highly threatened flooded ecosystems (“veredas”) (Fig. 1A) – with important roles in the recycling of organic matter and controlling hydrological cycles (Lima et al., 2014; Melo et al., 2018; Nunes et al., 2022). *Mauritia flexuosa* produces seeds with a rare association of recalcitrance and dormancy (germination blockage) even under favorable conditions (Baskin and Baskin, 2014), which makes their processes of seed bank formation and seedling establishment quite peculiar (Silva et al., 2014). There are indications that *M. flexuosa* tends to form seed banks or seedling banks in permanently humid microenvironments or environments subject to water deficits, respectively, which favor its distribution in seasonal tropical regions (Porto et al., 2018; Salvador et al., 2022). There have been no in-depth examinations, however, of the structural and physiological aspects of the species’ ability to form seedling banks.

We proposed the hypothesis that *M. flexuosa* seedlings have mechanisms that favor their survival over wide ranges of soil water availability and contribute to their establishment in different microenvironments. To test this hypothesis, we cultivated *M. flexuosa* seedlings in soils derived from both hyper-humid microenvironments and those naturally subjected to water deficits, maintaining those substrates at different levels (percentages) of available water. Those seedlings were subsequently examined in terms of their morphologies, physiologies, and ultrastructures; evaluations were also made of gene expression associated with aquaporins.

2. Materials and Methods

2.1. Plant material collection and preliminary procedures

Fruits of *M. flexuosa* were collected from two populations growing in veredas in the Rio Pandeiros Environmental Protection Area, in the municipality of Bonito de Minas in northern Minas Gerais State, Brazil (45°95 ’ - 43°95’W x 15°88’-14°40’S; at 517 m altitude). Fruits that had fallen to the ground after natural abscission and showed yellowish abscission scars (indicative of recent release) were selected (Silva et al., 2014).

The fruit exocarp and mesocarp were removed using a knife; seeds that showed symptoms of attacks by microorganisms or insects were discarded. The healthy seeds were disinfected in a 6% sodium hypochlorite solution for 15 minutes, followed by triple rinsing in running water and subsequent treatment with a fungicide solution (Protreat® 50%). After drying, the seed operculum was removed (using a scalpel) to overcome dormancy, and the seeds were then sown in plastic containers containing sterilized vermiculite hydrated to 80% of its retention capacity with distilled water (Silva et al., 2014). The containers were then kept in a germination chamber at 30ºC for 40 days. After that period, healthy seedlings were selected for experimental treatments (Silva et al., 2014).

2.2. Soil collection and processing

Veredas are flooded environments associated with streams that are generally long and relatively narrow (Fig. 1A), with notable transversal stratifications associated with their slopes (Porto et al., 2018). Organosols that remain waterlogged for most of the year commonly occur in the lowest (bottom) slope sections, associated with bodies of water (Fig. 1B). Gleisols occur on the higher slope sections, at the edge of the vereda (the natural limit of the occurrence of *M. flexuosa* individuals), and are commonly subjected to water deficits in the yearly dry season. Soil collections were made at the lowest (bottom; organosol) slope section and on the edge of the vereda (gleisol) at a depth of 0–30 cm in one of the areas where fruits were collected (45°95’W x 15°88’S).

The soil samples were subjected to texture, fertility, and density analyses (Table 1), and a water retention curve (Fig. 2) was prepared based on the protocols of Teixeira et al. (2017). The mineral fraction of both soils was sandy, with low P, Ca, and Mg levels, and low saturation percentages of exchangeable bases (Table 1). The organosol presented

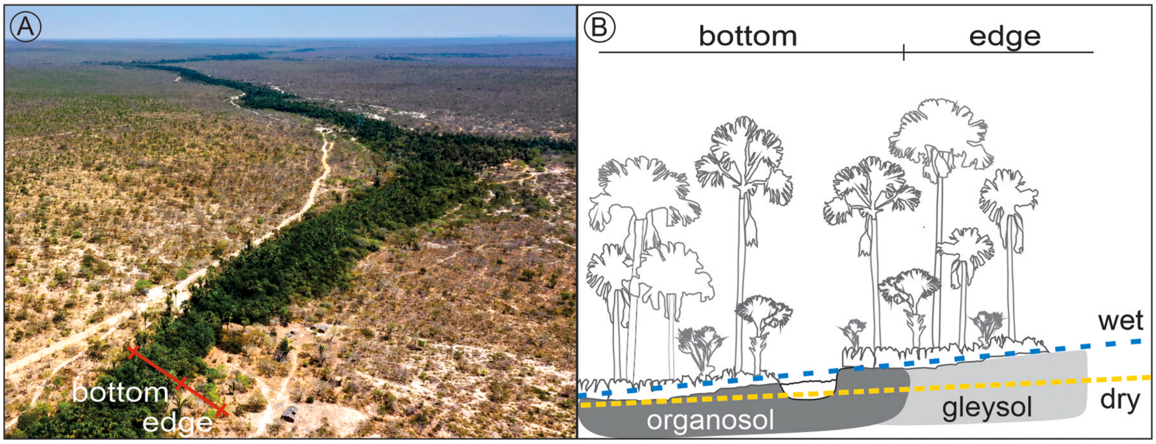

Fig. 1. Characterization of the vereda environment. A) Aerial view of a vereda during the dry season, indicating the microenvironments studied. B) Schematic cross-section of the vereda, indicating the microenvironments studied and the water table heights (dashed lines) in the rainy and dry seasons.

Table 1

Chemical and physical analysis of gleisol and organosol collected in a vereda in the municipality of Bonito de Minas in northern Minas Gerais State, Brazil.

Soil attributes	Organosol	Gleisol
pH	5.50	5.40
P Mehlich (mg dm^{-3})	6.60	0.47
P remaining (mg L^{-1})	27.45	36.21
K (mg dm^{-3})	41.00	5.00
Ca (cmolcdm^{-3})	0.60	1.30
Mg (cmolc dm^{-3})	0,60	0.40
Al (cmolcdm^{-3})	0.00	0.20
H+Al (cmolcdm^{-3})	3.62	3.62
SB (cmolcdm^{-3})	1.30	1.71
t (cmolcdm^{-3})	1.30	1.91
m (%)	0	10.00
T (cmolcdm^{-3})	4.92	5,33
V (%)	26.00	32.00
Organic matter (dag Kg^{-1})	13.60	4.98
Organic carbon (dag Kg^{-1})	7.89	2.89
Coarse sand (dag Kg^{-1})	0.80	30.50
Fine sand (dag Kg^{-1})	81.20	51.50
Silt (dag Kg^{-1})	8.00	6.00
Clay (dag Kg^{-1})	10.00	12.00

SB: Sum of bases (SB= K+Ca+Mg+Na), t: Base exchange capacity at natural pH (t= K+Ca+Mg+Na+Al), m: aluminum saturation (m= Alx100/t), T: Base exchange capacity at pH 7.00 (T= SB+Al+H), V: base saturation (V= SBx100/T).

high levels of organic matter (13.6 dag Kg^{-1}) and organic carbon (7.9 dag Kg^{-1}), resulting in a very high water retention capacity (reaching a moisture saturation of 172%) (Fig. 2). In contrast, the gleisol had a higher proportion of coarse sand (30.5, compared to 0.80 dag Kg^{-1}) but lower levels of organic material (5.0 dag Kg^{-1}) and organic carbon (2.9 dag Kg^{-1}) – which resulted in a water retention capacity at saturation of only 24% (7.2 times lower than the organosol). It is interesting to note that previous studies demonstrated that the development of *M. flexuosa* seedlings is favored by abundant seminal reserves that are consumed over more than six months (Dias et al., 2020; Silva et al., 2014), which minimizes the effect of soil fertility on seedling development.

The soils were dried in the open air and then homogenized. Plastic pots (3.5 L capacity) were weighed, using a mechanical balance, before and after being filled with dry soil. The pots were held in a greenhouse and the soils were irrigated until reaching field capacity.

2.3. Seedling development at different soil water availability levels

Seedlings exhibiting a primary root, a second leaf sheath (Fig. 3), and average root and shoot lengths of 10 and 9.6 cm, respectively, were selected and planted individually in the pots. The potted plants were held under greenhouse conditions (covered with 75% shade cloth) for seven days; soil moisture was subsequently maintained at field capacity for 15 days to promote seedling acclimatization.

Different levels (0, 40, 60, 80, and 100%) of available water with each type of soil [according to the soil water retention curve (Fig. 2) and considering the absorption capacity of the sunflower (*Helianthus annus*) plant model (Teixeira et al., 2017)] were used as treatments. Soil water control was carried out by weighing the pots daily and replacing lost water (lost through evaporation and evapotranspiration). As water replacement was performed daily, differences in the amounts of water incorporated into plant tissues among the different treatments were considered negligible. Two separate experiments were installed, each for a type of soil (organosol and gleisol). In both experiments, a randomized block design was used, with five treatments (percentage of available water), with four replications of five pots with one plant each, total 100 plants per experiment.

2.4. Survival and morphological assessments

Plant survival was assessed daily for eight months. Four plants were removed from each treatment after four months of cultivation to be used for morphological evaluations. The aerial portion, the root system, the haustorium (seedling organ specialized for mobilizing endospermic reserves), and seed remnants (Fig. 3) were dried to a constant weight in a forced ventilation oven at 65° C; dry matter and water content values were subsequently determined. The remaining plants were removed from the pots after eight months of cultivation and the morphometric evaluations were repeated.

2.5. Photosynthesis evaluation

After eight months of cultivation, carbon assimilation, stomatal conductance, and transpiration were evaluated using a portable infrared gas analyzer - IRGA (LCpro-SD, BioScientific, UK) fitted with a coupling chamber for narrow leaves; water-use efficiency was calculated by dividing the values of carbon assimilation by transpiration. These evaluations were carried out using the most developed leaves (four repetitions per treatment), on three successive days, between 10:00 and 12:00 h. Solar radiation during the evaluation days ranged from 350 to 840 mmol m^{-2} s^{-1}. Ultrastructural evaluations of the chlorophyll parenchyma cells of plants grown in organosol for eight months, with 40 and 100% water available, were also carried out. The organosol is the natural environment where *M. flexuosa* seedlings occur most frequently.

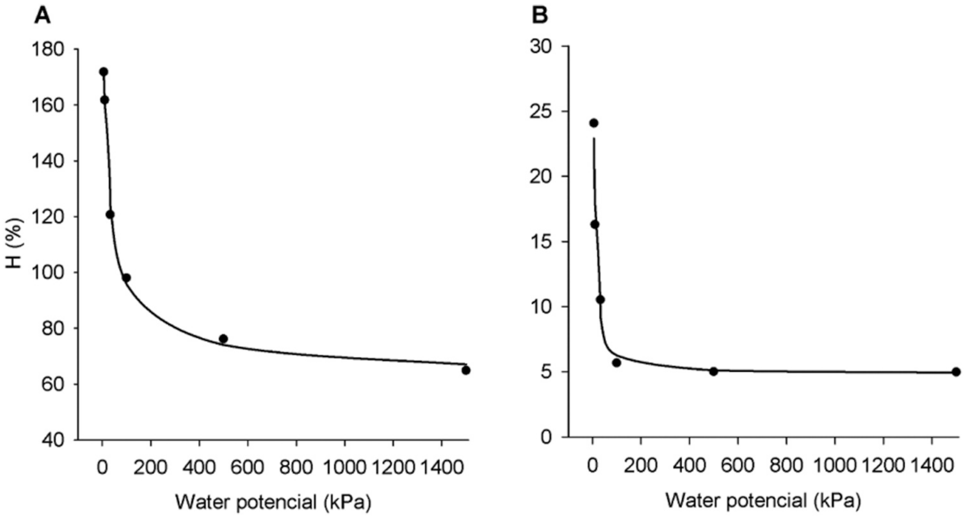

Fig. 2. Soil water retention curves adjusted by the Van Genuchten model (Genuchten, 1980) for organosol (A) and gleisol (B). H, humidity.

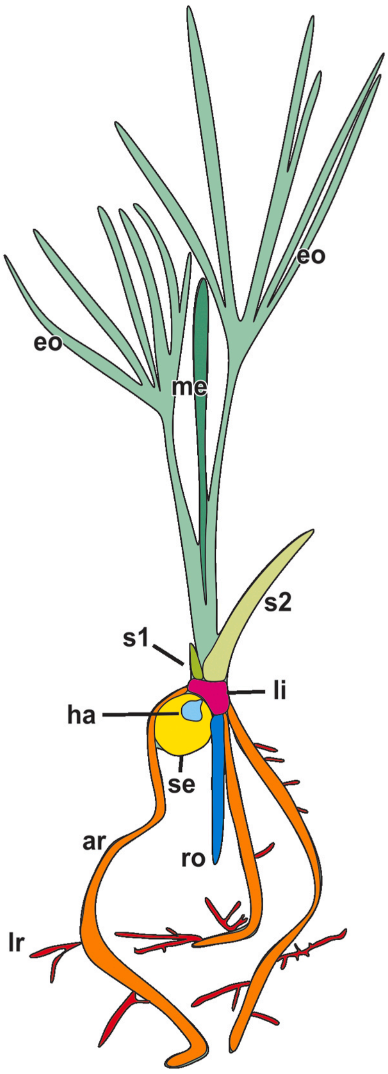

Fig. 3. *Mauritia flexuosa* seedling morphology. ar, adventitious root; eo, eophyll; ha, haustorium; li, ligule; lr, lateral root; me, metaphyll; ro, main root; s1, first leaf sheath; s2, second leaf sheath; se, seed.

Therefore, the characterization of the ultrastructure under these conditions, under different water availability, is more representative. Fragments (3 mm^2) were removed from the median region of the limbus of a leaflet of the most developed metaphyll of three seedlings from each treatment. The material was fixed in Karnovsky's solution, pH 7.2 (Karnovsky, 1965), post-fixed in 1% osmium tetroxide (0.1 M phosphate buffer, pH 7.2), dehydrated in an acetone series, and embedded in Araldite resin (Roland, 1978). Ultrathin sections (50 nm) of that chlorophyll parenchyma were then contrasted with uranyl acetate and lead citrate (Robards, 1978; Roland, 1978) and examined using a transmission electron microscope (Philips CM 100, Philips/FEI Corporation, Eindhoven, Netherlands).

2.6. ABA quantification

ABA evaluations were performed using three replicates of leaflet samples from seedlings cultivated for eight months in all treatments, according to the methodology adapted from Müller and Munné-Bosch (2011). The ABA analytical standard and the sample extracts were analyzed in a UPLC system coupled to a diode arrangement detector (1290 Infinity, Agilent Technologies, Santa Clara, USA). Twenty microliters of each sample were injected into the analytical column (15 cm×4.6 mm, 5 μm) (Kinetex, Phenomenex, Torrance, USA). The elution gradient was run using ultrapure water acidified to 0.05% with acetic acid (solvent A) and acetonitrile with 0.05% acetic acid (solvent B). The column temperature was maintained at 30°C; the flow of the methanol mobile phase was 0.600 mL min^{-1}. Detection used the 250 nm wavelength. The identification of the ABA chromatographic peak in the samples was performed by comparing its retention time with that of the analytical standard. Each evaluation was performed twice for each biological replicate. The results were expressed on a fresh weight basis.

2.7. Assessment of MIP gene expression by qPCR

After eight months of cultivation in each type of soil, the leaves from three plants from each treatment were collected to evaluate the expression of plasma membrane genes associated with aquaporins. Total RNA was isolated from the leaf tissue via the TriReagent® (Sigma-Aldrich, USA), according to the manufacturer's instructions. The purity and concentrations of the isolated RNA samples were checked on 1% (w/v) agarose gels and in a spectrophotometer. Reverse transcription was carried out via the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) and 10 μg of total RNA, according to the manufacturer's instructions. The cDNA concentration was determined spectrophotometrically.

All of the qPCR reactions were performed via Real-Time PCR Systems 7500 Fast (Applied Biosystems, USA), according to the manufacturer's instructions. qPCR primers were designed to avoid the conserved regions of *Arabdopsis thaliana*. Primer sequences are shown in Table 2 (MfPIP1;1, MfPIP1;2, MfPIP1;3, MfPIP1;4, MfPIP2;1, MfPIP2;2, MfPIP2;3 e MfPIP2;4). MfGAPC2 and MfACT2 were used as internal reference genes to normalize expression (Table 2). The reactions were performed in triplicate, containing 10 ng of cDNA, 0.5 μl of each primer (10 pmol), 10 μl PowerUpTM SYBR® Green Master Mix (Applied Biosystems, USA), and sterile Milli-Q water for a final volume of 20 μl. The amplification reactions were performed under the following conditions: (1) activation of Taq DNA polymerase at 50°C for 2 min, (2) initial denaturation at 95°C for 3 min, (3) denaturation at 95°C for 20 s, (4) annealing/extension at 60°C for 30 s. Steps 3 and 4 were repeated for 40 cycles.

The Dissociation Curve 1.0 program (Applied Biosystems, USA) was used to verify that only a single PCR product was generated by the amplification of transcripts. Non-cDNA control reactions were used in all experiments. Gene expression was quantified using the Ct: 2−ΔCt and 2−ΔΔCt comparative methods, with data obtained from three

Table 2
Primers for MIP genes used in the qPCR analysis.

Gene	Primer
MfPIP1;1	F: 5'-CATTCTCATCACAACATCAAACG-3'
	R: 5'-CTGCTAGTCCCTCAAAAACACAA-3'
MfPIP1;2	F: 5'-TCACTCCCGTAGCAAGATCA-3'
	R: 5'-TTTCGCTCGCTCTTCTTCA-3'
MfPIP1;3	F: 5'-CCTCAACTTTCTCGCTACGC-3'
	R: 5'-TGATGAACCTCTCTCTCGCTCT-3'
MfPIP1;4	F: 5'-TCTGCTGTATATGTACAACCCTTCG-3'
	R: 5'- ATAGGAATCGGCCATGAACA –3'
MfPIP2;1	F: 5'- TAGGCGGCAATGCTAAGTTT –3'
	R: 5'- ATGATGAAGAAGGGCGAAGA –3'
MfPIP2;2	F: 5'-GCAAACACAACAGTCGTAGCTCT-3'
	R: 5'-CTTCAACATCCTTCCCCATTT-3'
MfPIP2;3	F: 5'-TGTTGTCATTTTGCTACTCGTTTC-3'
	R: 5'-GGCGTGCCATATTGCTTTTA-3'
MfPIP2;4	F: 5'-TTTCTGTTATTTGTTCGCTTGTGT-3'
	R: 5'-AATGGAAAAATAAAGAGAAGGGTCA-3'
MfACT2	F: 5'- CTCTCCTTGTACGCCAGTGGTC –3'
	R: 5'- TAAGGTCACGTCCAGCAAGGTC –3'
MfGAPC2	F: 5'- TGGTGACAACAGGTCAAGCA –3'
	R: 5'- AAACTTGTCGCTCAATGCAATC –3'

biological replicates and two experimental replicates. The data were subjected to analysis of variance, and the means were compared via Tukey's test at a 5% probability level. The transcripts were validated via the AB 3500 Genetic Analyzer automated sequencer equipped with 50 cm capillaries and POP7 polymer (Applied Biosystems, USA). Sequencing was performed using the BigDye ™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The consensus sequence of 5′ and 3UTR resulting submissions was searched from the Phytozome v12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html) using BLAST and BLASTx.

The sequences were validated using an AB 3500 Genetic Analyzer automated sequencer (Table 3). The data obtained were compared with *Arabidopsis thaliana* genes described in Phytozome v12.1.

2.8. Statistical analysis

The research consisted of two experiments (considering each type of soil) in a randomized block design. A joint analysis of the experiments (soil type and water availability) was performed and, when a significant difference was found using the F test ($p \leq 0.05$), the means were grouped using the Scott Knott test ($P \leq 0.05$). The death of all plants, eight months after starting the experiment, in the 0% water availability treatment (in the gleisol), prevented the joint analysis from being carried out with this treatment. Therefore, in the statistical analysis, at eight months, the 0% water availability treatment was excluded for plant development, physiological characteristics and ABA level. All analyses were performed using R software (R Core Team, 2022).

3. Results

3.1. Seedling survival and development

Buriti seedlings exhibit the typical morphology of palm trees. After germination, the ligule expands and forms a germinative bud, close to the seed, from which the seedling organs are emitted (Fig. 3). The root system is composed of the main root (which is emitted first and is persistent) and numerous adventitious roots, from which lateral roots are emitted. The aerial part is composed of tubular leaf sheaths, from which two eophylls (first leaves with leaflets) are emitted. Metaphylls, leaves with the typical morphology of adult plants, are emitted internally to the eophylls. During seedling development, the haustorium mobilizes and transports abundant seminal reserves to the vegetative axis.

Mauritia flexuosa seedlings showed remarkable tolerance to water deficits. Only seedlings grown in gleisol, with 0% water available (a concept that takes the sunflower plant as a reference, Teixeira et al., 2017), did not survive after eight months (Fig. 4). However, even with this treatment, 38% of the seedlings remained alive for 120 days after the start of the experiment. Seedlings grown in organosol with 0% available water showed 92% survival after eight months. No mortality was observed with the other treatments.

Mauritia flexuosa seedlings showed high adaptation to waterlogged soils, with their development being favored by the increasing availability of water (Fig. 5). Seedlings grown with 60%, or greater, water availability showed negative geotropic root growth, a typical condition for aeration roots or pneumatophores. The seedlings showed greater accumulations of dry mass in the aerial portion as compared to the roots (Fig. 6A-D). After four months of cultivation, seedlings grew more with 100% available water (Fig. 6A). Also, with eight months of cultivation, seedlings showed greater aerial portion development with 100% available water, however the increasing available water (between 40% and 80%) increases the aerial development (Fig. 6B), which does not occur after four months.

There was an interaction between the factors soil type and available water in terms of the dry masses of the roots after four months of cultivation (Fig. 6C). Plants grown in organosol had greater root development when 100, 60, and 40% water was available. In the case of

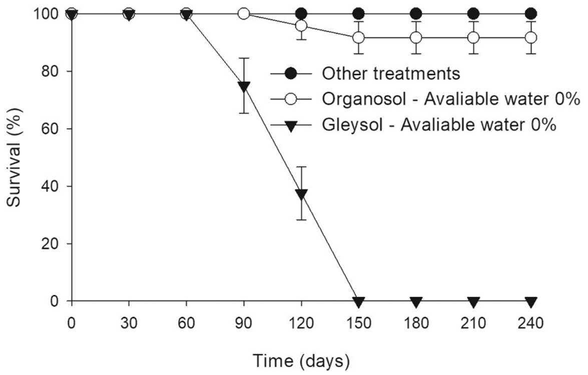

Fig. 4. Survival percentages of *Mauritia flexuosa* seedlings grown for eight months in organosol or gleisol with different percentages of available water.

Table 3
Transcript sequencing of the expression of MIP genes from the Phytozome v12.1 database.

Gene	Product size (bp)	Consensus sequence transcripts 5′-3′UTR
MfPIP1;1	84	CATTCTCATCACAACATCAAACGGCCACAAACAAAAAAGCAAGTGCTTCTTATTGACTGTTTTGTGTTTTTGAGGGACTAGCAG
MfPIP1;2	90	TCACTCCCGTAGCAAGATCAAACAGCTACACGCAAACGACTCAAATCTTTTTTGTCTTTTAGCTAATTAAGTGAAGAAGAGCGAGCGAAA
MfPIP1;3	85	CCTCAACTTTCTCGCTACGCTACATACACAACACAAACACAGCATCTTCTTTGAGAGAATAAGAGAGCGAGAGAGAGGTTCATCA
MfPIP1;4	80	TCTGCTGTATATGTACAACCCTTCGGTGTTTGTGGTTCTTGAATTGTGTATGACTATACATGTTCATGGCCGATTCCTAT
MfPIP2;1	89	TAGGCGGCAATGCTAAGTTTAAATAACACTCAATCATCTCTATAAACTACCCAATAACTACCCTCCGCTTCTTCGCCCTTCTTCATCAT
MfPIP2;2	90	GCAAACACAACAGTCGTAGCTCTTGTTTCTCCCTCTCGTTTAATTAACAAAACAAAAATAAAAGGGAAAAAATGGGGAAGGATGTTGAAG
MfPIP2;3	85	TGTTGTCATTTTGCTACTCGTTTCTACTAGCACTCATGCCTCGAAGATAAATGCTGCTACCATGGTAAAAGCAATATGGCACGCC
MfPIP2;4	85	TTTCTGTTATTTGTTCGCTTGTGTCTATTATGAGATGATGTTTGCTATGATAATTATGACCCTTCTCTTTATTTTTCCATT
MfACT2	162	TCTCGCAACAGCTCTCTTCACCACCGTCGAAGATGTAATCAACACGTTCATCGATCCACCTTCACGTCCTTCCGTTGATCCAAAACATGTCCTCTCTGATAACTTCGCTCCTGTCCTCGACGAGCTTCCTCCAACAGACTGTGAAATCATCCACGGCACTCT
MfGAPC2	134	ATCGACCGGAGAGATTCGAAAGTATATTTACGGAGAGGGGAAATACGGAGGAGAGCCTCTGTTTCTACCTTCCGGTGACGGAGAAGAAGACGGAGGTTACATAATGGTG TTCGTTCACGACGAGGAGAAGGTGA

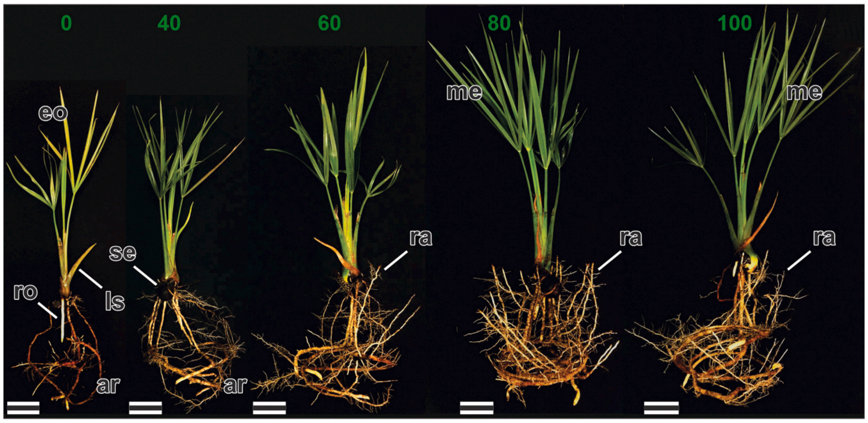

Fig. 5. Morphology of *Mauritia flexuosa* seedlings grown for eight months in organosol with different available water percentages (green numbers). ar, adventitious root; eo, eophyll; ls, leaf sheath; me, metaphyll; ra, aeration root; ro, main root; se, seed.

the gleisol, the best performance occurred in treatments with 100 and 80% of available water. The evaluation carried out at eight months showed that dry mass accumulation in the roots was greater with 100 and 80% water available (Fig. 6D).

The water content of the aerial portion of the seedling in organosol cultivation, four months after the start of the experiment, was higher in seedlings grown with 0% available water in relation to the other treatments, which did not differ from each other (Fig. 6E). In the case of cultivation in gleisol, the opposite was observed, that is, the 0% available water treatment resulted in the lowest seedling aerial portion water content among all of the treatments. After eight months of cultivation, different levels of available soil water did not provide significant differences in terms of this character, with an average value of 73±4,5.

The water contents of the roots of seedlings grown in organosol were not influenced by water availability after four months of cultivation. The water contents of the roots of seedlings cultivated in gleisol with 0% water availability were lower in relation to the other treatments; no differences were observed among the other treatments (Fig. 6G). Eight months after the start of the experiment, the greater influence of water availability was seen in seedlings grown with 100 and 80% available water levels (Fig. 6H).

After four months of cultivation, the dry masses of seeds and haustorium and haustorium water content were not influenced by water availability. For seedlings grown in organosol, the water contents of the seeds were higher when 100, 80, and 40% of water was available, with no differences between these treatments. In the case of the gleisol, no differences were observed due to available water percentages (Fig. 7).

3.2. Photosynthesis evaluation

The capacity of buriti palms to fix carbon (*A*) after eight months of cultivation was not influenced by interactions between the factors soil type and water availability. There were differences between all of the treatments available water (Fig. 8A), with the best photosynthetic performance being associated with greater water availability (80 and 100%). Also, stomatal conductance (gs) was highest when 100 and 80% soil water was available, in relation to other water availability levels (Fig. 8B). Evapotranspiration (E) was not influenced by soil type, and evidenced patterns very similar to those observed for gs when comparing available water percentage levels (Fig. 8C), with the highest values observed in the 100 and 80% treatments. Water-use efficiency (WUE) was not influenced by water availability.

Ultrastructural evaluations of the chlorophyll parenchyma showed that cells from plants grown in organosol with 100% water available had lenticular chloroplasts, were rich in starch grains, and had well-structured internal membrane systems, well-developed thylakoids, and well-defined grana (Fig. 9A-B). Chloroplasts from cells of seedlings subjected to a water deficit of 40% of available water, on the other hand, were globular, more voluminous, had reduced numbers of starch grains, and showed electron-dense stroma and grana with disorganized membranes (Fig. 9C-D).

3.3. ABA quantification

ABA levels in the leaves of *M. flexuosa* plants cultivated for eight months were shown to be influenced by the interaction between soil type and water availability. The ABA concentrations in plants grown in organosol were higher when 40 and 60% water was available, as compared to 80 and 100% (Fig. 10). There were differences between all treatments in the case of cultivation in gleisol with 40–100% water availability, with higher ABA levels under higher water deficit conditions.

3.4. Evaluation of MIP gene expressions

The genes MfPIP1;1, MfPIP1;2, MfPIP1;3, MfPIP1;4, MfPIP2;1, MfPIP2;2, MfPIP2;3, and MfPIP2;4 were expressed in young leaves of *Mauritia flexuosa*, cultivated in organosol and gleisol at different levels of soil water availability (Fig. 11A-B). Among plants grown in organosol, the relative expressions of the MfPIP1;3, MfPIP2;1 and MfPIP2;2 genes increased with a 40% increase in water availability (Fig. 11A); the relative expressions of the MfPIP2;3 and MfPIP2;4 genes increased with a 60% increase in water availability; the relative expressions of the MfPIP1;1 and MfPIP1;4 genes increased with an 80% increase in water availability; the relative expression of the MfPIP1;2 gene increased under conditions of 100% water availability. In terms of the plants grown in gleisol, the relative expressions of the MfPIP2;4 genes increased with a 60% increase in water availability (Fig. 11B); the relative expression of the MfPIP1;1, MfPIP1;2, MfPIP1;4, MfPIP2;1 and MfPIP2;2 genes increased at an 80% level of water availability; and the relative expressions of the MfPIP1;3 and MfPIP2;3 genes increased under 100% water availability conditions.

4. Discussion

The present work addressed the ability of the iconic Amazonian palm tree *M. flexuosa*, considered a key species in vereda environments in the Cerrado biome, to form seedling banks. Veredas are highly threatened flooded ecosystems that are essential for controlling hydrological cycles, maintaining the regional fauna, and providing varied resources for traditional human populations in vast semiarid areas of central Brazil (Fig. 1A). Local anthropogenic impacts (and possibly global climate change) have caused progressive lowerings of vereda water tables and reductions of their soil moisture levels, which have resulted in their

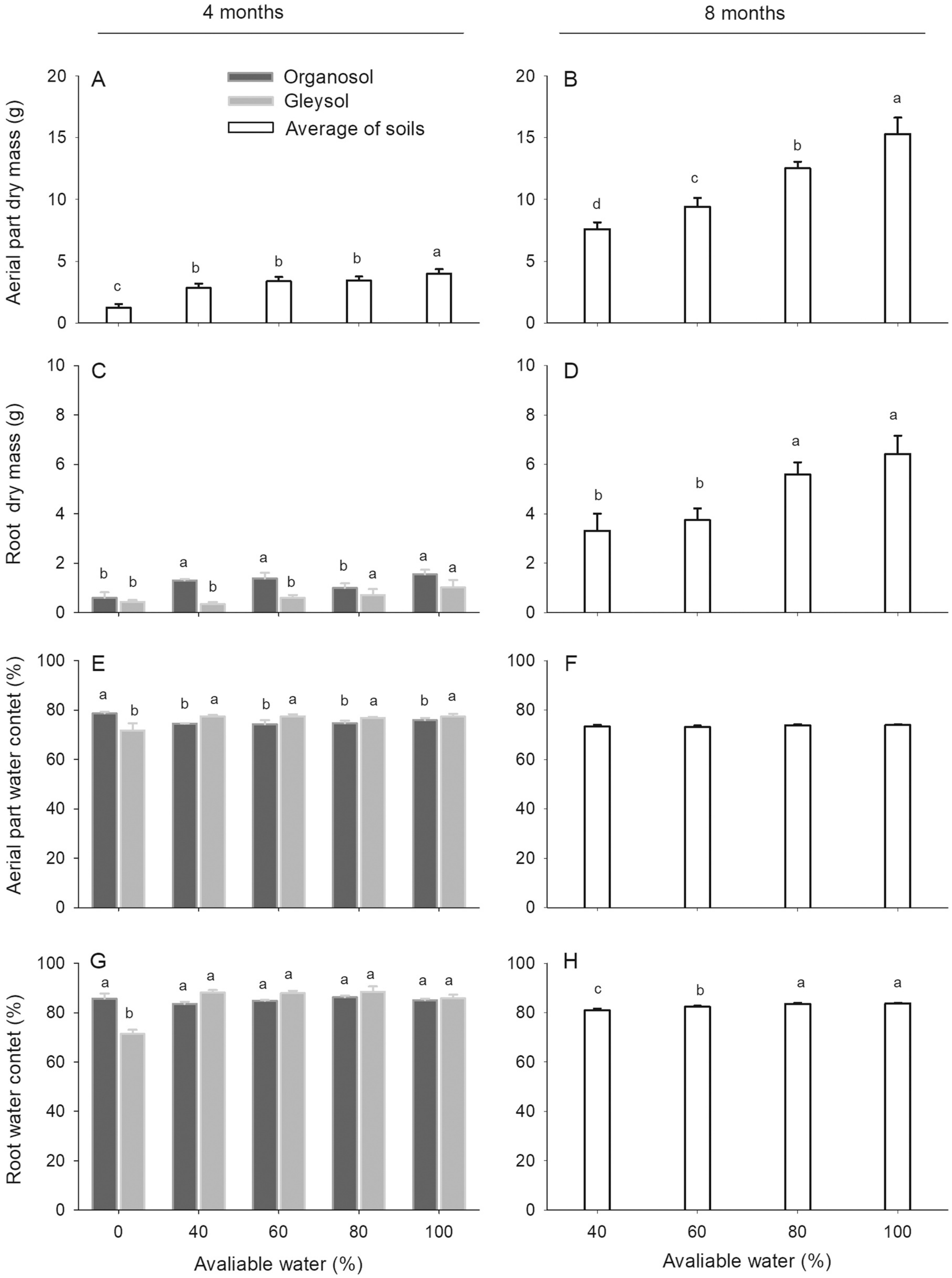

Fig. 6. Dry mass of the aerial portion (A-B) and root portion (C-D), and water content of the aerial portion (E-F) and root portion (G-H) of *Mauritia flexuosa* seedlings cultivated for four and eight months in organosol or gleisol, with different available water percentages. Different letters indicate significant differences between the water percentage classes using the Scott-Knott test ($P<0.05$). Vertical bars indicate the standard error of the mean.

collapse in several locations (Nunes et al., 2022). In this context, the controlled cultivation of *M. flexuosa* seedlings in soils typical of their natural environments, but under different levels of water availability, generated structural and physiological information important for defining the maintenance capacities of its populations in veredas under current and future scenarios.

Seedling survival and structural aspects of their development

Mauritia flexuosa seedlings demonstrated phenotypic plasticity and the capacity for survival and development under a wide range of soil water conditions in two types of soils typical of its natural Cerrado

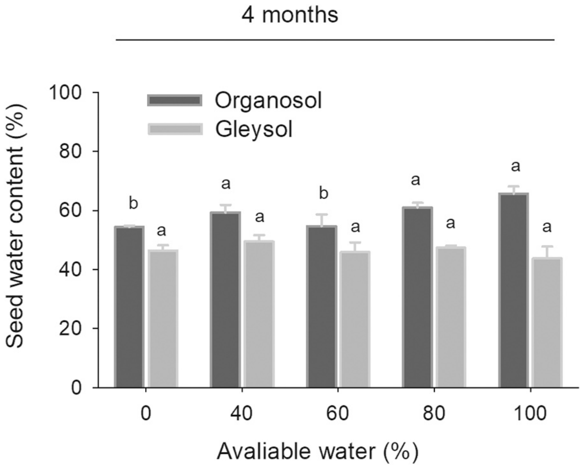

Fig. 7. Water content of the seed of *Mauritia flexuosa* seedlings grown for four months in organosol or gleisol, with different available water percentages. Different letters indicate significant differences between the water percentage classes using the Scott-Knott test (P<0.05). Vertical bars indicate the standard error of the mean.

environment. Its seedlings are adapted to flooded environments and showed greater development under conditions of high water availability. The aerial portion/root portion ratio of the seedlings is greater than 2 (Fig. 6 A-D), which is viewed as an adaptation to vereda environments where water availability is normally high and there is considerable competition between species composing the herbaceous stratum (Silva et al., 2014). It was observed that greater root system growth in response to greater water availability is associated with negatively geotropic root growth (pneumatophores; Pereira et al., 2000) related to aeration (Fig. 5). Shoot and root growth, as well as the water content of seedling tissues, were found to be little affected during the first four months of growth if water availability was reduced from 100% to 40% (Fig. 6C, E, G), probably reflecting abundant seminal reserves still available at that time (Dias et al., 2020) that minimized the impact of water stress on seedling nutrition.

The positive responses of young plants of several palm species endemic to humid environments to high water availability conditions are well known. For *Bactris gasipaes* (“peach palm” or “pupunheira”) and *Euterpe oleraceae* (“açaí”), greater soil water availability favors photosynthesis, vegetative development, and plant vigor (Silvestre et al., 2016; 2017; Tucci et al., 2018). On the other hand, these species are normally intolerant of water stress (as observed with *E. oleracea* seedlings subjected to water regimes of 40 and 70% of field capacity; Silvestre et al., 2016). In the case of *M. flexuosa*, we found that, in addition to good performance under waterlogging conditions, it was notably tolerant to water deficits. It is interesting that this species, together with *Mauritiella armata*, are the only palms of recognized Amazonian origin that have adapted to the Cerrado biome (even if only growing in flooded environments) (Ávila et al., 2023).

Physiological aspects of seedling responses to water availability

Photosynthesis became reduced in *M. flexuosa* seedlings under conditions of low water availability, although their water-use efficiency was not affected (Fig. 8A). The levels of ABA, a hormone directly involved in stomatal control (Weyers and Paterson, 2001), greatly increased in leaves experiencing water restriction conditions (Fig. 10), and were associated with reductions in stomatal conductance (Fig. 8B) and evapotranspiration (Fig. 8C); similar effects were also observed in *B. gasipaes* (Tucci et al., 2018). Stomatal closure represents the primary cause of photosynthetic and transpiration rate reductions under conditions of water scarcity (Tucci et al., 2018; Vogado et al., 2020; Martins et al., 2023), and those reductions led to decreases in the speeds of physiological processes and consequently reduced plant growth (and even death) (Silvestre et al., 2016; 2017; Martins et al., 2023). *Mauritia flexuosa* seedlings, however, were found to be capable of physiological adjustments that maintained the same proportions of carbon fixed per absorbed water under the water availability conditions evaluated. The ability to adjust photosynthetic parameters under stress conditions was likewise reported in young plants of the palm tree *Acrocomia aculeata*, a pioneer species with wide distribution in seasonal tropical environments (Mota and Cano, 2016; Dias et al., 2018; Rosa et al., 2019), and in *Phoenix dactylifera*, a palm species adapted to arid conditions (Arab et al., 2016; Kruse et al., 2019). On the other hand, young plants of *Euterpe edulis*, a species endemic to the Atlantic Rainforest (Gatti et al., 2011) as well as the Amazonian palm *B. gasipaes* (Martins et al., 2023), both evidence low capacities for controlling stomatal conductance and evapotranspiration rates – with low water-use efficiencies which resulted in low tolerances to water stress.

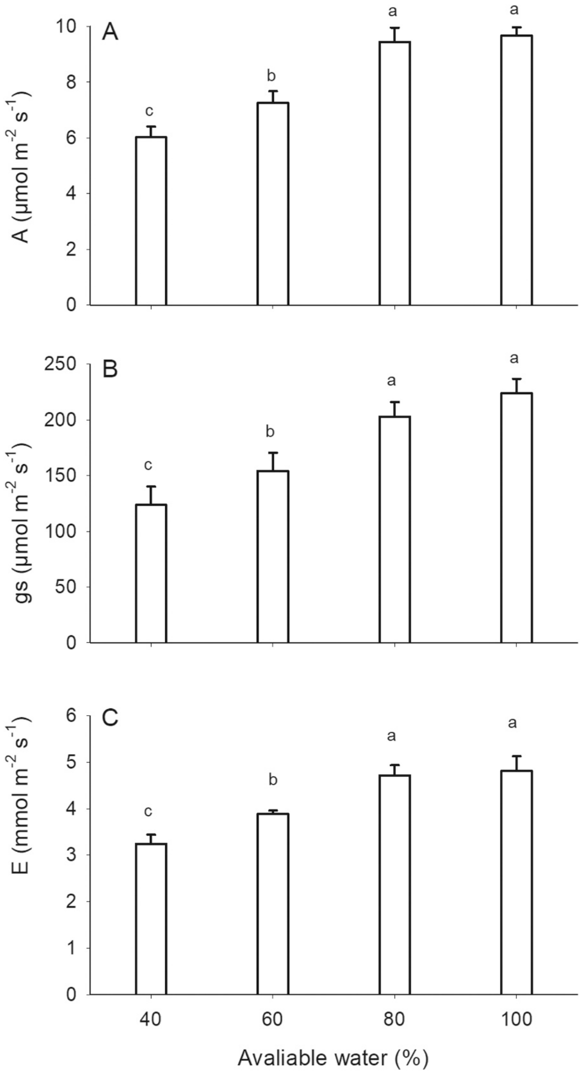

Fig. 8. Photosynthetic parameters of *Mauritia flexuosa* seedlings grown for eight months in organosol or gleisol, with different available water percentages. A) Fixed carbon (*A*). B) Stomatal conductance (gs). C) Evapotranspiration (E). Different letters indicate significant differences between water percentage classes using the Scott-Knott t test (P<0.05). Vertical bars indicate the standard error of the mean.

Changes in the chloroplasts of *M. flexuosa* seedlings were observed under water deficit conditions that included changes in their shapes, increased volumes, reduced starch accumulations, and disorganized membranes (Fig. 9C-D). These changes have been described in several other species under water stress conditions (Grigorova et al., 2012;

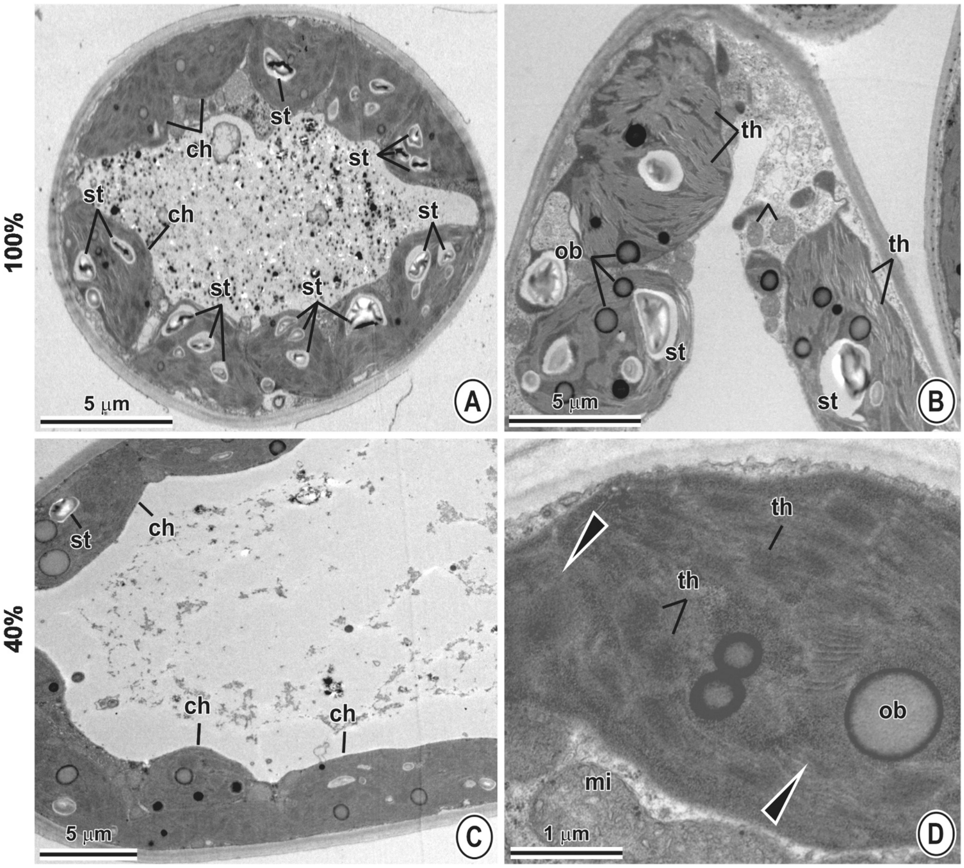

Fig. 9. Ultrastructure of the chlorophyll parenchyma cells in the leaves of *Mauritia flexuosa* seedlings grown for eight months in organosol. The water percentage levels are shown in the left column. A-B) Cell with lenticular chloroplasts, rich in starch grains, with a well-structured internal membrane system, well-developed thylakoids, and well-defined grana. C-D) Cell with voluminous, globular chloroplasts, with a reduced presence of starch grains, electron-dense (arrows) and bulky stroma, and grana with disorganized membranes. ch, chloroplast; cw, cell wall; mi, mitochondria; ob, oil body; st, starch; th, thylakoid; va, vacuole.

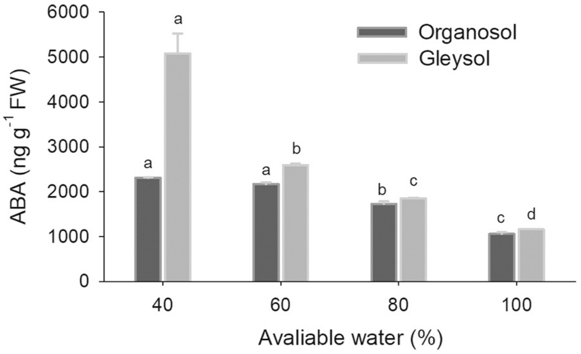

Fig. 10. ABA contents in the leaves of *Mauritia flexuosa* seedlings grown for eight months in organosol or gleisol, with different available water percentages. Different letters indicate significant differences between water percentage classes using the Scott-Knott test (P<0.05). Vertical bars indicate the standard error of the mean.

Zhang et al., 2015; Shao et al., 2016;). Membrane disorganization commonly occurs under water stress conditions, with grana increasing while the stroma increases and becomes electron dense – which affects electron capture and transport as well as photosynthetic efficiency. The decreases in the numbers and volumes of starch grains are related to their degradation and consumption to synthesize osmotically active products that can minimize membrane damage (Grigorova et al. 2012).

The qPCR analyses showed that the MfPIPs evaluated were expressed at different levels in the young leaves of *M. flexuosa* grown in organosol and gleisol under different levels of soil water availability (Fig. 11 A-B). Intrinsic plasma membrane proteins (PIP) constitute the largest subfamily of MIPs in the plant kingdom (Anderberg et al., 2012). Most PIPs have been identified in the plasma membrane and are generally located in organs characterized by high water flow (Kaldenhof and Fisher, 2006; Kapilan et al., 2018). Several studies have put forward physiological and genetic evidence that PIPs are associated with water channels (aquaporins) and have crucial roles in regulating water flow through plant tissues and water homeostasis (Kammerloher et al., 1994; Chaumont and Tyerman, 2014; Maurel et al., 2015; Shinozaki and Yamaguchi-Shinozaki, 2022).

Studies on aquaporins are still incipient in Arecaceae, and the present work represents the first report on the expression of the genes associated with these water channels in a species from the basal subfamily Calamoideae. In general, increased relative expressions of MfPIPs genes in *M. flexuosa* seedlings were evident as a result of increased water availability (Fig. 11A-B), although MfPIP1;3, MfPIP2;1 and MfPIP2;2 gene expressions were identified in plants grown in the organosol even at 40% water availability (Fig. 11A). Although MIP expressions vary greatly among different species and the types and intensities of the stresses that they are exposed to, transcellular water transport mediated by PIPs in plants has an important role in maintaining water homeostasis (Zargar et al., 2017; Kapilan et al., 2018; Pawłowicz and Masajada, 2018). The exposure of plants to water stress conditions commonly results in the inhibition of aquaporin activity as well as water transport at the cell level and in whole organs (Carvajal et al., 1999; Boursiac et al., 2005; Sutka et al., 2011; Hachez et al., 2012; Perrone et al., 2012). The expression of eight MfPIPs genes in *M. flexuosa* seedlings (three of which are expressed even under conditions of low water availability) indicates

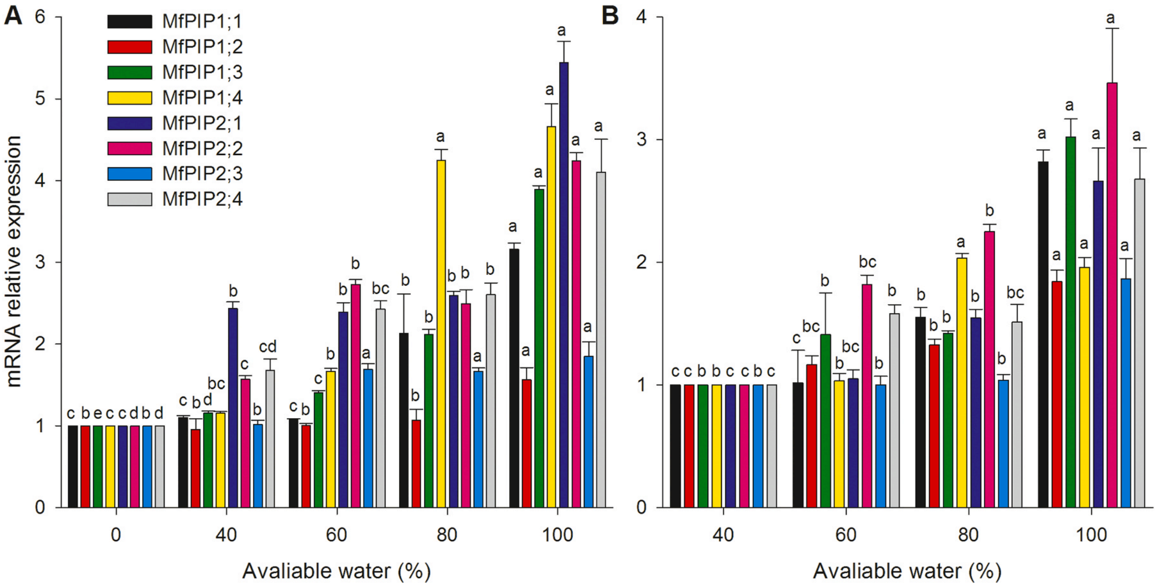

Fig. 11. Relative expression of the genes MfPIP1;1, MfPIP1;2, MfPIP1;3, MfPIP1;4, MfPIP2;1, MfPIP2;2, MfPIP2;3, and MfPIP2;4 measured via qPCR in the leaves of *Mauritia flexuosa* cultivated by eight months in organosol (A) and gleisol (B), under different soil water percentages. MfGAPC2 and MfACT2 were used as endogenous controls. Different letters indicate significant differences between water availability percentages, considering each gene, using the Scott-Knott test ($P<0.05$). Vertical bars indicate the standard error of the mean.

that they play important roles in regulating water transport under stress conditions. Future functional characterization of these MfPIPs will undoubtedly help to elucidate details of water transport in buriti plants and their adaptive mechanisms under stress conditions.

The ability of *M. flexuosa* to form seedling banks in veredas environments

Mauritia flexuosa seedlings demonstrated resilience and the ability to survive and develop under a wide range of water availability conditions in two soil types typical of their seasonal Cerrado environments. Veredas are commonly long and narrow flooded environments associated with streams, and evidence marked stratifications in their transverse planes (Fig. 1A-B; Nunes et al., 2022). This configuration imposes strong edge effects and limits the distribution of *M. flexuosa,* as their seeds are sensitive to dehydration (Salvador et al., 2022; Veloso et al., 2016). Local anthropogenic impacts (and possibly global climate change) have caused a general lowering of Cerrado water tables, further confining hyper-humid vereda areas in semiarid areas of central Brazil (Nunes et al., 2022) and the Amazonian region (Galeano et al., 2015).

Previous studies have demonstrated that *M. flexuosa* seeds have a remarkable ability to perceive environmental signals and that they are capable of differential responses to contrasting vereda microenvironments. The species tends to form seed banks in the bottom areas (hyper-humid sites with organosol) of veredas, with pronounced dormancy of the seeds favoring the species' barochoric and hydrochoric dispersal (Galeano et al., 2015; Porto et al., 2018). The seeds can remain for several years in these hyper-humid environments, as dormancy is only gradually overcome (Salvador et al. 2022). In edge areas (subject to drought and occupied by gleisol), however, dormancy is overcome in just a few months and seedlings soon emerge (Porto et al., 2018). The present work contributes to our understanding of this phenomenon–which develop better in flooded conditions but nonetheless evidence structural and physiological mechanisms related to water stress tolerance. In addition to constituting an example of "bet-hedging" (Porto et al., 2018), the reproductive versatility of *M. flexuosa* constitutes an interesting case of habitat protection, as the maintenance of vereda edge areas contributes to the preservation of humidity in bottom regions where adult populations and seed banks are concentrated.

The lowering of Cerrado water tables (often aggravated by the burning of organic material in the organosol) has been relentless in the semiarid regions of Brazil (and even in the Amazon), and tends to result in seed bank destruction, the death of grown individuals, and increased competition with other species migrating from drained environments (Galeano et al., 2015; Nunes et al., 2022). Our results allowed an integrated characterization of the responses of seedlings to different water availability conditions, and can thus contribute to efforts to conserve and regenerate highly vulnerable vereda ecosystems threatened by local anthropogenic impacts and/or global climate change. Thus, under conditions in which the soil-water balance can be preserved or recovered, the introduction of seedlings produced *ex situ* could aid in the recovery of degraded veredas.

5. Conclusions

Mauritia flexuosa seedlings show great phenotypic plasticity, with positive responses to water availability (in terms of both growth and photosynthetic parameters) – but also a notable ability to survive under water stress conditions in the two soil types typical of vereda sites. The responses of these seedlings to water stress are related to ABA accumulation and the maintenance of water homeostasis through the modulation of stomatal control, water use efficiency, and the expression of MIP genes. *M. flexuosa* can form seedling banks in both flooded and water-stress environments, which contributes to the reproductive success of the species and its wide distribution. In situations where the water balance of the vereda soils can be preserved or recovered, the introduction of seedlings produced *ex situ* could aid in the recovery of degraded areas.

CRediT authorship contribution statement

Paulo Sergio Nascimento Lopes: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Tarcísia da Silva Almeida:** Project administration, Investigation, Resources, Writing – original draft. **Leonardo Monteiro Ribeiro:** Writing – review & editing, Formal analysis, Writing – original draft, Conceptualization. **Cristina de Paula Santos Martins:** Writing – original draft, Methodology, Formal analysis. **Thays Lima Barbosa:** Investigation. **Maria Olívia Mercadante-Simões:** Formal analysis. **Edson de Oliveira Vieira:** Methodology, Formal analysis, Conceptualization. **Yule Roberta Ferreira Nunes:** Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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