



Modulating seed reserve mobilization and antioxidant systems contributes to resilience to water stress in *Mauritia flexuosa* (Arecaceae) seedlings

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Key message

The resilience of *Mauritia flexuosa* seedlings to water stress involves adjustment in dynamics of seed reserve mobilization and efficient antioxidant system.

Abstract

Mauritia flexuosa is an Amazonian palm that has expanded its domains to the seasonal environment of the Cerrado biome. The species produces recalcitrant seeds, but the seedlings are tolerant to water deficit. Our objective was to evaluate the effect of water stress on post-germinative development in the species, focusing on dynamics of seed reserve mobilization and oxidative stress control. Seedlings were grown irrigated with water ($\Psi_w = 0$ MPa) and under moderate ($\Psi_w = -0.9$ MPa) and severe ($\Psi_w = -2.1$ MPa) stress, for 30 days. Biometric, anatomical, histochemical and physiological evaluations (quantification of H_2O_2 and MDA, histolocalization of O_2^- , evaluation of endo- β -mannanase and antioxidant system enzymes activities) were performed. The haustorium (cotyledonary blade) plays a central role in the resilience of seedlings to dehydration, as it adjusts its water potential to maintain water and nutrient absorption. In conditions of water deficit there is an accumulation of partially degraded (and unmobilized) reserves in the digestion zone, which favors water retention in the seed. Endo- β -mannanase activity increases in the endosperm adjacent to the embryo under moderate water stress. Also under moderate stress, root development is prioritized over the aerial part, which contributes to maintain a favorable water status. Seedlings present enzymatic and non-enzymatic antioxidant systems responsive to water stress (in the aerial part and haustorium), which help promote oxidative homeostasis. The resilience of *M. flexuosa* seedlings to water stress involves sophisticated adjustment in reserve mobilization patterns and the action of an efficient antioxidant system, which contribute to their reproductive success and adaptation to seasonality.

Keywords Abiotic stress · Seedling establishment · Palms · Recalcitrant seeds

Introduction

The establishment of seedlings in the environment comprises the most vulnerable phase of the plant life cycle (Tarnawa et al. 2023; Chaib et al. 2023). Biotic (herbivory and pathogen infection) or abiotic (drought, salinity, temperature and metal toxicity) factors can cause stresses capable of inducing structural and metabolic cellular changes, especially during post-germination development. Water deficit is especially harmful, since, after germination, there is usually a reduction in tolerance to desiccation and the translocation pathways of seminal reserves occur in an aqueous medium

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(Dias et al. 2020). In seasonal tropical environments such as the Cerrado biome, germination is, in most cases, allocated to the rainy season (Escobar et al. 2018). However, occasional droughts may occur during these periods, which is increasingly common due to global climate change (Lewandowski et al. 2021; Chaib et al. 2023). Additionally, fires, common in the Cerrado, can further amplify stress (Oliveira et al. 2005). This environmental scenario inevitably restricts seedling development and poses challenges to the perpetuation of native species.

In response to water deficit, seeds and seedlings may present metabolic disorders such as oxidative stress, characterized by the imbalance between the production and removal of reactive oxygen species (ROS), which are highly damaging to cell membrane systems (Mittler 2002; Bailly 2004; Bailly et al. 2008). Enzymes that make up the antioxidant system, mainly catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX), contribute to neutralizing the harmful effects of the main ROS, such as hydrogen peroxide (H_2O_2) and superoxide ion (O_2^-) (Mittler 2002; Das and Roychoudhury 2014). Phenolic compounds are also powerful antioxidants and their production can be stimulated during early plant development in response to dehydration (Chen et al. 2016; Xu et al. 2018). On the other hand, when well controlled, ROS act as chemical messengers and promote the activity of enzymes related to the mobilization of seed reserves (Das and Roychoudhury 2014; Wojtyla et al. 2016; Dias et al. 2018). The understanding of the physiological responses to oxidative stress, promoted by dehydration, in species from tropical environments, is still limited, especially in the seedling establishment stages.

Arecaceae seeds are considered models for the study of reserve mobilization, due to the variety and abundance of compounds stored in the symplast and apoplast (Dias et al. 2018, 2020). During the post-germinative development of palms, antagonistic development pathways are established: the growth of the haustorium (cotyledonary blade specialized in the reserve mobilization) and the degradation and translocation of endosperm compounds to the vegetative axis (Mazzottini-dos-Santos et al. 2017). The endosperm of Arecaceae is rich in mannans, in the cell walls, whose mobilization is mediated by endo- β -mannanase, the main hydrolytic enzyme in the apoplast (Buckeridge et al. 2000; Buckeridge 2010; Dias et al. 2020). The haustorium is the centralizing organ of the digestive process, responsible for the activation of mannanases (previously stored in the endosperm symplast), a process mediated by ROS (Dias et al. 2018). In addition, this structure acts in catabolism, absorption, transport and temporary storage of reserves (DeMason et al. 1985; Alang et al. 1988; Ribeiro et al. 2015). Although the mechanism of reserve mobilization in palm seeds is known, the studies considered optimal water conditions,

therefore, it is not known what the influence of drought is on the dynamics of seed reserves, especially on how mannanase operates under water stress conditions.

Mauritia flexuosa, buriti, is considered the most abundant palm in Brazil; of Amazonian origin, the species also occurs in the Cerrado biome (with a markedly seasonal climate), in marshy environments (veredas) (Silva et al. 2014; Porto et al. 2018). Buriti seeds are recalcitrant (i.e. sensitive to desiccation) and the species' adaptation to constantly humid environments has led to a unique reserve metabolism, with dependence on high levels of hydration, especially for the digestion of thick cell walls (Dias et al. 2020). Recent studies demonstrate resilience to dehydration in young *M. flexuosa* plants (Almeida et al. 2024), however, post-germinative development has not yet been investigated, especially from the perspective of the dynamics of seed reserve mobilization. The expansion of *M. flexuosa* into the Cerrado is of great ecological importance, as the species is considered key to flooded ecosystems known as veredas, which are crucial for the balance of the hydrological cycle and the maintenance of fauna. In this context, studying the transition between recalcitrant seeds and water-deficit-tolerant seedlings can provide valuable information for understanding the species' adaptive process and predicting its behavior in future scenarios.

Our objective was to evaluate the effect of water stress during post-germinative development in recalcitrant seeds of *M. flexuosa*. The following questions were addressed: (i) What is the dynamics of mobilization of seminal reserves under water deficit conditions? (ii) How do antioxidant enzymes (SOD, CAT, and APX) respond to the tissue accumulation of O_2^- and H_2O_2 in seedlings subjected to water stress?

Materials and methods

Collection and preliminary procedures

Newly dispersed fruits of *M. flexuosa* were collected in September 2023, in a vereda located in the Rio Pandeiros Environmental Protection Area, municipality of Bonito de Minas, Minas Gerais, Brazil (15°19'49.70"S; 44°57'20.20"O). The yellowish abscission scar was considered indicative of recent dispersal (Silva et al. 2014). The fruits were stored in water for 15 days to facilitate pulping and, using a scalpel, they were manually pulped and the seeds were disinfected with a 6% sodium hypochlorite solution for 15 min, followed by three washes in running water. Seeds showing signs of deterioration by microorganisms and/or predation by insects were discarded, the remaining seeds were stored in plastic bags at a temperature of 20 °C for up to 30 days, until the

experiments were implemented (Veloso et al. 2016). The water content determined, in five replicates of five seeds, by the oven method, comparing their fresh and dry masses after dehydration at 105 °C, for 24 h (Brasil 2009). The viability of the batch was evaluated by the germination test, in five replicates of 10 seeds, which had the operculum removed, under aseptic conditions, to overcome dormancy (Silva et al. 2014) and cultivated in plastic containers (17 cm x 12 cm x 6 cm) with lid, containing autoclaved sand and moistened to 80% of field capacity (Fig. 1b, c). The material was kept in a germination chamber (EL 202/4, Eletrolab, São Paulo, Brazil), at 30 °C, in the dark (Silva et al. 2014; Dias et al. 2020). The 2 mm protrusion of the cotyledonary petiole was

considered as a morphological indication of germination (Ribeiro et al. 2011).

Determination of water potential of seedling structures

The veredas are unique phytophysionomies that emerge in the Cerrado (neotropical savannah), with the presence of the outcrop of the water table. In these environments, there are variations in soil water availability that lead to the formation of transitional zones between savanna and swamp that, together with seasonality, modulate the dynamics of seed and seedling banks (Porto et al. 2018; Salvador et al.

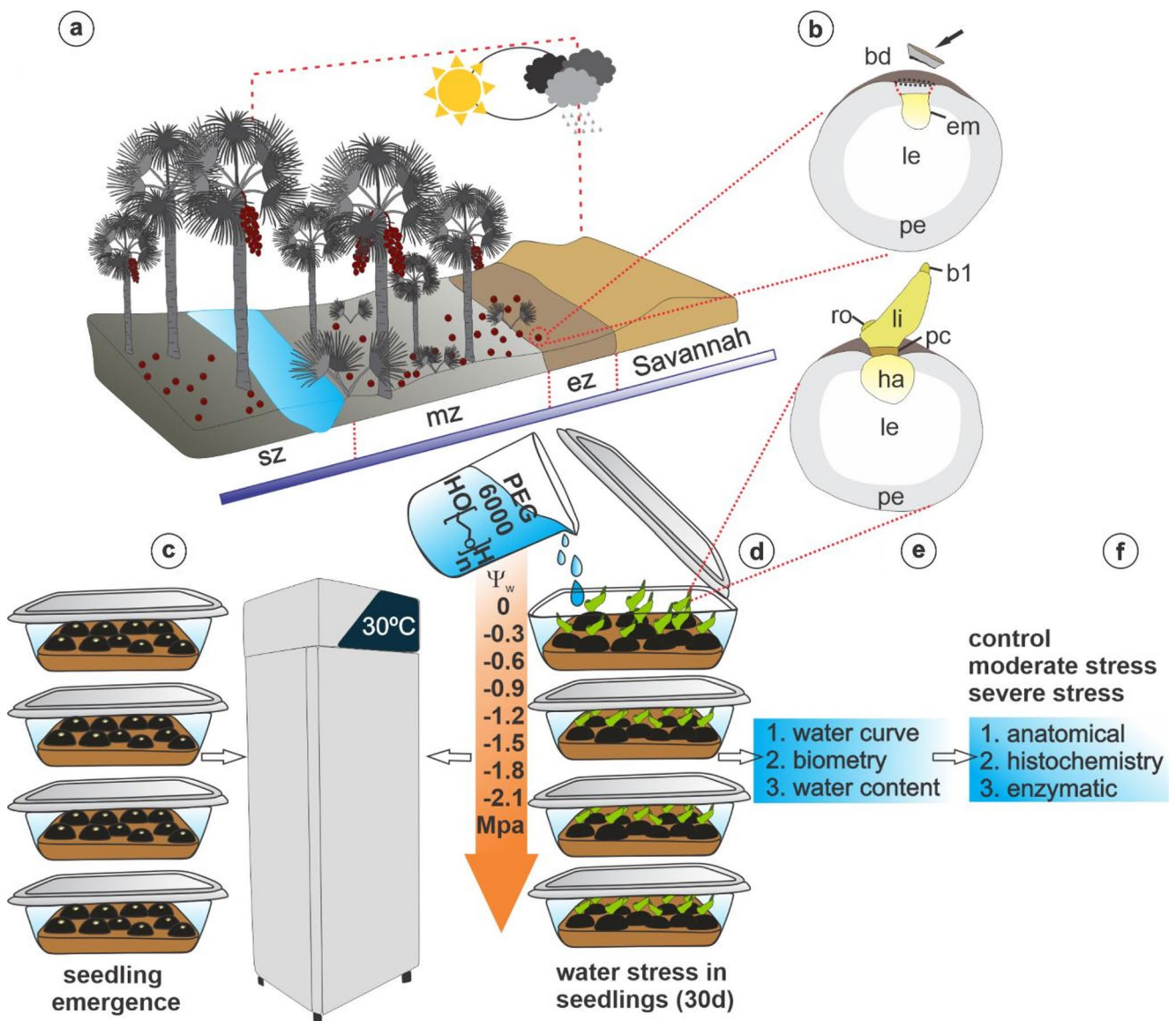


Fig. 1 Scheme representing vereda environments, highlighting the transitional zones (a). Longitudinal section model of the *M. flexuosa* seed, the black arrow points to the removal of the operculum, as a technique used to break dormancy (b). Synthetic scheme of the experi-

mental design (c-f). Longitudinal section of the *M. flexuosa* seedling. b1 first leaf sheath, bd breaking dormancy, em embryo, ez edge zone, ha haustorium, le lateral endosperm, li ligule, mz middle zone, pc cotyledonary petiole, pe peripheral endosperm, ro root, sz marshy zone

2022) (Fig. 1a). Considering these variations, we evaluated the development of *M. flexuosa* seedlings subjected to a water gradient, experimentally simulated, from the perspective of the dynamics of seminal reserves and oxidative stress control.

The *M. flexuosa* seeds were again disinfested and cultivated as previously described, until the emission of the first leaf sheath (Fig. 1b-d). The isolated seedlings were sectioned, separating the aerial part and haustorium and the water content was determined by the oven method in four replicates of five structures (Brazil 2009). To determine the water potential (Ψ_w), the seedling parts were immersed in osmotic solutions, prepared with polyethylene glycol (PEG 6000), an inert, non-toxic osmotic agent that does not penetrate cell membranes, in concentrations adjusted to induce a water deficit corresponding to $\Psi_{ws} = -0.3; -0.6; -0.9; -1.2; -1.5; -1.8$ and -2.1 MPa (Michel and Kaufmann 1973). The solutions were prepared with ultrapure water produced by the Milli-Q Direct 8/16 System. The material was kept in the dark at 25 °C, as a reference temperature. The fresh masses of the seedling structures were measured at the initial condition and after 6, 12 and 24 h of immersion in the osmotic solutions, using four replicates of 5 seedlings for each PEG concentration, in order to determine the moisture content and construct water absorption curves. The variations in water contents of the haustorium and aerial part were calculated and expressed on a dry basis. The Ψ_w was estimated in each evaluated time interval, considering the equilibrium condition of the water flow, by adapting the quadratic equation and $\Psi_{w\text{-seedling}} = \Psi_{w\text{-solution}}$, indicating the stoppage of the water flow (Gonçalves et al. 2020).

Biometric and seedling survival assessments

Seedlings, obtained as previously described, were transferred to plastic containers with lids and sealed with plastic film, containing autoclaved sand moistened with ultrapure water (control) and osmotic solutions formulated with PEG (Fig. 1d). Assessments of length and diameter, in the median portion, were carried out in the aerial part, haustorium and main root (Silva et al. 2014; Fig. 1b, e), after 15 and 30 days of cultivation. The water content of these structures and the endosperm + seed (Fig. 1e) coat was measured using the oven method (Brasil 2009). The evaluations were carried out in four replicates of five seedlings for each osmotic treatment. The morphology of the seedlings, in longitudinal section, was documented with a digital camera (sx520 hs, Canon, Tokyo, Japan), after 15 days of cultivation. Additionally, after 30 days of cultivation in osmotic solutions, four replicates of five seedlings were transferred to cultivation in sand moistened with water, at 80% of field capacity, for another 30 days, to evaluate the survival and recovery

potential of the seedlings through the development of the aerial part and root, by visual parameters.

Definition of Ψ_{ws} for structural and physiological evaluations

For the structural and physiological evaluations described below, Ψ_{ws} capable of inducing moderate and severe stress, in addition to the control condition ($\Psi_w = 0$ MPa), in hydrated seedlings were selected. According to Walters (2015), it is important to consider the imposed osmotic stress as a way of quantifying the cellular response to dehydration. The stresses were defined based on the seedling's water absorption or loss curve, in the haustorium and aerial part (Fig. 2), as follows: moderate stress: restricts water absorption by the seedling, but does not block it completely ($\Psi_w = -0.9$ MPa); severe stress: blocks water absorption and retention capacity by the cells, so that there is water flow from the cell to the PEG solution ($\Psi_w = -2.1$ MPa). The solutions were prepared with ultrapure water produced by the Milli-Q Direct 8/16 System.

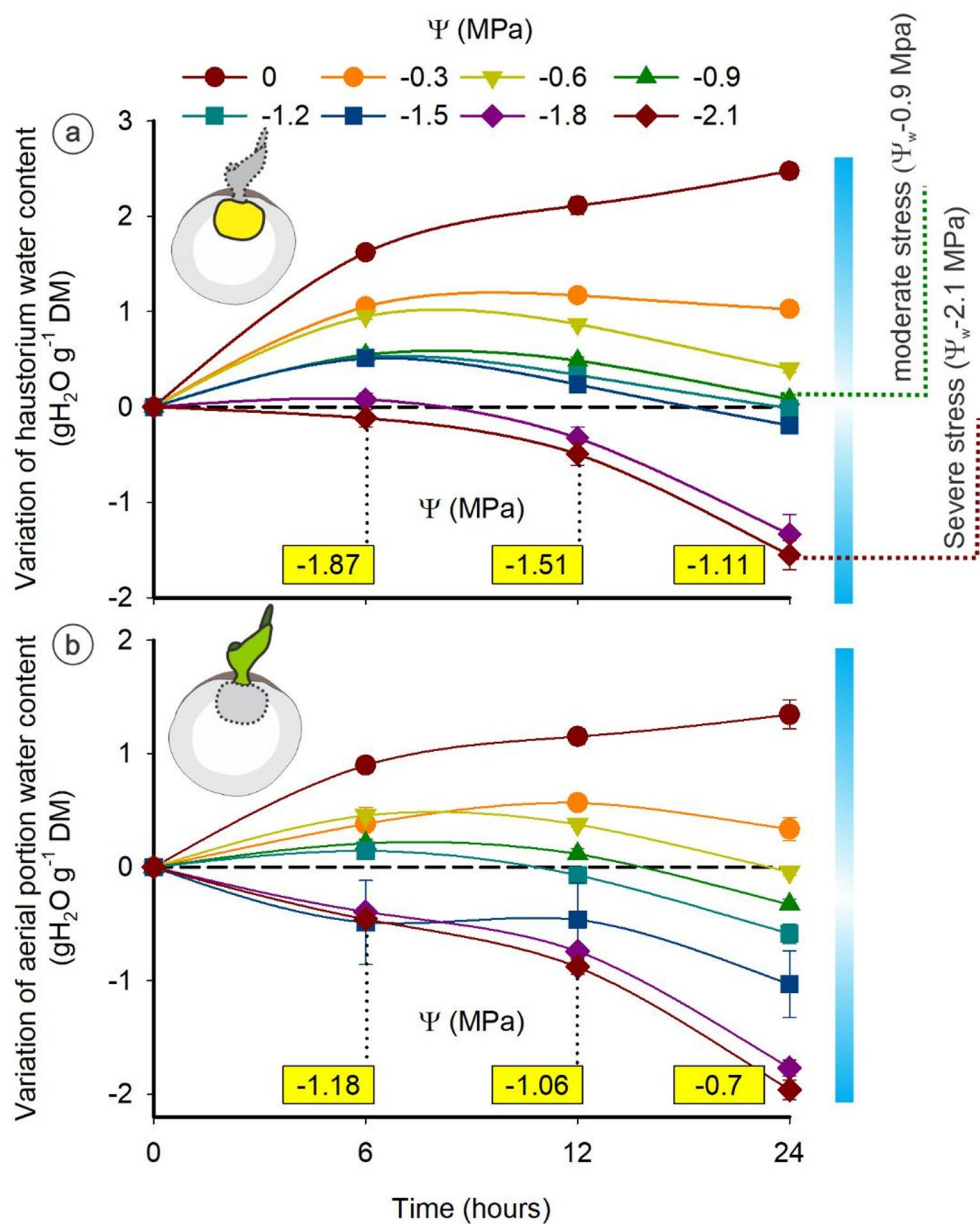
Anatomical and histochemical evaluations

For each of the selected treatments ($\Psi_w = 0, -0.9$ and -2.1 MPa), cross fragments of the median portion of the haustorium attached to the surrounding endosperm were sectioned with the aid of a guillotine and stylet after 3, 7 and 15 days of culture and were fixed in Karnovsky's solution (Karnovsky 1965), dehydrated in an ethyl series and embedded in (2-hydroxyethyl) methacrylate (Leica Microsystems, Heidelberg, Germany), according to Paiva et al. (2011). Cross Sect. (5 μm thick) were obtained using a rotary microtome, stained with 0.05% toluidine blue, pH 4.7 (modified from O'Brien et al. 1964) and the slides were mounted in acrylic resin (Itacril, Itaquaquecetuba, Brazil).

The storage compounds of *M. flexuosa* seeds are mostly heteromannans in the cell walls of the haustorium and endosperm cells, and mucilages of mixed composition (proteins and polysaccharides) in vacuoles (Silva et al. 2014; Dias et al. 2020). Phenolic compounds are also found in the haustorium, especially in response to water stress in embryos (Dias et al. 2024). Thus, histochemical tests were performed with Xylidine-Ponceau (Vidal 1970) for identification of proteins and periodic acid-Schiff reagent (PAS) (Feder and O'Brien 1968) for polysaccharides. Toluidine blue was used to locate phenolic compounds (Ribeiro and Leitão 2020). The sections were evaluated under a light microscope and documented under a photomicroscope (Scope A1/Axiocam 105 Color, Zeiss, Jena, Germany).

To evaluate the effect of treatments on endosperm reserve mobilization, micromorphometric evaluations were

Fig. 2 Variation in water content in $\text{g H}_2\text{O g DM}^{-1}$ and Ψ_{ws} (MPa) in the haustorium (initially $4.94 \text{ g H}_2\text{O g DM}^{-1}$) (a) and in the aerial part (initially $6.49 \text{ g H}_2\text{O g DM}^{-1}$) (b) of *M. flexuosa* seedlings, 10 days after germination, evaluated over 24 h of immersion in different osmotic solutions. The Ψ_{ws} values of the osmotic solutions are represented above. The values in the rectangles represent the Ψ_{ws} in each evaluated time. The Ψ_{ws} capable of promoting moderate and severe stresses are highlighted at the right of the water curve



performed on the processed material, as described for the anatomical and histochemical evaluations. In cross-sections, the extent of the endosperm digestion zone was measured in two distinct regions: the collapsed cell zone and the hyaline cell zone. The evaluations were performed in three replicates using image analysis software (Zen, Zeiss, Jena, Germany).

Physiological evaluations

Evaluation of endo- β -mannanase activity

Endo- β -mannanase is the main enzyme involved in the mobilization of reserves in palm seeds (Mazzottini-dos-Santos

et al. 2017; Dias et al. 2020). We sought to understand the influence of water stress on the activity of this enzyme and its possible contribution to the control of osmotic regulation. From seedlings subjected to different experimental conditions, after 15 and 30 days of cultivation, 5 mg samples were obtained from four biological replicates, each composed of five fragments of endosperm adjacent to the haustorium. The evaluations were carried out according to the protocol established by Pinho et al. (2014). The samples were macerated with a crucible and homogenized in 1 mol L^{-1} sodium acetate buffer. The material was centrifuged for 45 min at $16,000 \text{ g}$ at 4°C . The supernatant was removed, added to a solution of Locust Bean Gum Galactomannan 0.25% (Sigma, USA) prepared in 0.1 mol L^{-1} of sodium acetate

buffer, pH 4.7, and kept in a thermostatic bath with magnetic stirring (SI-153, Solab, Piracicaba, Brazil) at 40 °C for 3 h. The sugars formed from hydrolysis were subjected to a 0.5% (w/v) solution of hydroxybenzoic acid - acid hydrazide (Sigma, USA), 5% (w/v) prepared in an acidic medium HCl 0.5 mol L⁻¹ and diluted in an alkaline medium NaOH 0.5 mol L⁻¹ and the mixture kept for 5 min in a thermostatic bath at 95 °C. In the solution used to eliminate interferents from the extract that react with acid hydrazide (blank), Locust Bean Gum Galactomannan (Sigma, USA) was not added. The analyses were performed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan), at a wavelength of 398 nm. The results obtained from the calibration curve were converted to μM equivalents of reducing sugars per min/gram of fresh mass.

Quantification of hydrogen peroxide (H₂O₂)

Reactive oxygen species, such as H₂O₂ and O₂⁻, can cause potentially fatal oxidative stress to plant cells under water stress. The effect of treatments on the presence of these ROS was evaluated in the tissues of *M. flexuosa* seedlings. After 3, 7 and 15 days of cultivation, four replicates of 30 mg of the haustorium and aerial part from each treatment were macerated in liquid nitrogen. In 2 mL Eppendorf tubes, 400 μL of 0.1% (m/v) trichloroacetic acid (TCA) were added. The plant extract was homogenized by vortexing for 1 min and centrifuged at 10,000 $\times$ g for 15 min at 4 °C. A 250 μL aliquot of the supernatant was extracted and added to 250 μL of 100 mM potassium phosphate buffer (pH 7.5) and 1000 μL of 1 M potassium iodide. The tubes with the solution were placed on ice in the absence of light for 1 h. The samples remained in the dark, at room temperature for 20 min, to stabilize the reaction. The samples were analyzed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 390 nm and the amount of H₂O₂ was expressed in mM g⁻¹ of fresh mass, based on a pre-established H₂O₂ standard curve (Alexieva et al. 2001).

In situ histochemical localization of the superoxide anion radical (O₂⁻)

Four seedlings were separated from the endosperm using a guillotine and a scalpel, after 3 and 7 days of cultivation, for each treatment. The seedlings were immersed in 10 mM sodium phosphate (Na) buffer (pH 7.8) containing 0.1% NBT (v/v). They were then illuminated for 30 min to form dark blue spots (blue formazan precipitates). The material was stored in a glycerol–ethanol solution (1:4, v/v) until photographed (Shi et al. 2010).

Evaluation of the activity of antioxidant system enzymes

After 3, 7 and 15 days of cultivation, 20 mg samples of haustorium and aerial part, from each treatment, obtained in four biological replicates, were macerated in liquid nitrogen. The plant extract was homogenized in 10% m/v PVPP, 500 μL of 50 mM sodium phosphate buffer, pH 6.8, 10 μL of 100 μM EDTA and 490 μL of deionized water, by vortexing for 1 min. The extracts were centrifuged at 10,000 g, at 4 °C, for 15 min. Catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities were determined in 100 μL of the supernatant.

SOD activity was determined by adding the extract to a solution containing 13 mM methionine, 75 μM NBT, 100 μM of 2 μM riboflavin EDTA and 50 mM potassium phosphate buffer pH 7.8. The tubes were illuminated in chambers composed of 15 W fluorescent tubes at 25 °C. After 10 min of incubation, the end of activity was determined by the interruption of light. Control reactions were kept in the dark for 10 min. The blue formazan compound was analyzed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 575 nm. One unit of SOD was defined as the amount of enzyme required to inhibit 50% of the photoreduction of NBT. The results were demonstrated in U min/mg protein (Giannopolitis and Ries 1977).

To determine CAT activity, the extract was added to 50 mM potassium phosphate buffer, pH 7.0, with freshly prepared H₂O₂ (12.5 mM) added at the time of analysis. The analysis was performed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 240 nm for 1 min. CAT activity was estimated using the molar extinction coefficient (ϵ) equal to 39.4 mM⁻¹ cm⁻¹ (Cakmak and Horst 1991) and the results were expressed in μM H₂O₂ min/mg protein.

APX activity was determined by adding the extract to potassium phosphate buffer (50 mM, pH 6.8) and at the time of analysis, 0.25 mM ascorbate and 1.0 mM H₂O₂. The oxidation rate of ascorbic acid was monitored for 1 min, recording absorbance values at 290 nm every 10 s in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). APX activity was determined using ϵ equal to 2.8 mM⁻¹ cm⁻¹ and the results were expressed in μM ascorbate min/mg protein (Nakano and Asada 1981).

Quantification of the lipid peroxidation indicator malondialdehyde (MDA)

After 3, 7 and 15 days of cultivation, four replicates of 30 mg of the haustorium and aerial part, from each treatment, were macerated in liquid nitrogen and added to 1.5 mL of 0.1% (m/v) trichloroacetic acid (TCA). After vortexing for 1 min, the samples were centrifuged at 12,000 rpm for 15 min. A 1

mL aliquot of the supernatant was added to a 3 mL aliquot of 0.5% (m/v) thiobarbituric acid solution prepared in 20% TCA. The solution remained for 60 min at 95 °C and was then cooled on ice for 10 min. The samples were analyzed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at wavelengths of 532 and 660 nm. Interferents were eliminated by subtracting the values 532–660 and the amount of MDA was expressed in nM/g fresh mass (Health and Packer 1968, adapted).

Statistical analysis

The experimental design was completely randomized and evaluated in a factorial scheme, considering factor A as the cultivation time and factor B as the applied water potentials. The means were subjected to analysis of variance and, when significant differences were found, they were grouped by similarity using the Scott-knott test for biometric parameters. For biochemical evaluations (oxidative stress and endo- β -mannanase activity), the means were compared by the Duncan test ($P \leq 0.05$). For micromorphometric evaluations, means were compared using the Tukey test ($P \leq 0.05$). The R software version 4.2.2 Easyanova package, version 8.0. was used.

Results

The newly dispersed seeds of *M. flexuosa* had a water content of 48.7% (0.95 g H₂O g⁻¹ DM). The preliminary test showed that 100% of the seeds germinated within eight days after mechanical removal of the operculum (treatment to overcome dormancy).

Water relations and definition of osmotic stress levels in seedlings

In the first leaf sheath emission phase, 10 days after germination, the haustorium of the seedlings presented a water content of 4.94 gH₂O gDM⁻¹ and $\Psi_w = -1.5$ MPa, after 24 h of evaluation (Fig. 2a). When subjected to solutions with Ψ_w equal to or greater (less negative) than -1.8 MPa, in the period from 0 to 6 h, the haustorium was able to absorb water. Between 6 and 12 h, water absorption by the haustorium was observed in solutions with Ψ_{ws} equal to or greater than -1.5 MPa, so that the capacity for water absorption was gradually reduced over time and, at the end of 24 h, solutions with Ψ_{ws} equal to or less than -1.2 MPa were able to remove water from the structure.

The aerial part presented a water content of 6.49 gH₂O gDM⁻¹ and, $\Psi_w = -1$ MPa, after 24 h. In the period between 0 and 6 h, the aerial part absorbed water in solutions with Ψ_{ws}

equal to or greater than -1.2 MPa, with a gradual reduction in water capacity, so that, between 12 and 24 h, Ψ_{ws} equal to or less than -0.9 MPa were capable of removing water (Fig. 2b). For the evaluations described below, for both structures, based on the water absorption/loss curve (Fig. 2a-b), in addition to the control treatment ($\Psi_w = 0$ MPa), moderate ($\Psi_{ws} = -0.9$ MPa) and severe ($\Psi_{ws} = -2.1$ MPa) stresses were defined, which, respectively, reduces the water absorption capacity, and completely blocks the capacity to absorb and retain water.

Growth and survival rate of seedlings under different levels of water stress

The seedlings in the initial condition, 10 days after germination, presented a bulbous structure, corresponding to the expanded ligule (Moura et al. 2019), which involves the first leaf sheath (Fig. 3a). Post-germative development is of the adjacent ligular type, characterized by restricted elongation of the cotyledonary petiole. The haustorium grows towards the interior of the endosperm and, at the interface, the digestion zone is established. The seed coat is dark brown and covers the endosperm, which has a grayish peripheral portion and a whitish lateral portion, both extremely rigid.

M. flexuosa seedlings grown under different water conditions showed differences in the morphological pattern of development over time (Fig. 3a-f). After 15 days of cultivation, seedlings not subjected to osmotic stress showed pronounced growth of the first leaf sheath, haustorium and main root (Fig. 3b). With the application of decreasing water potentials, there was a gradual stagnation of seedling development (Fig. 3c-f). Severe stress ($\Psi_w = 2.1$ MPa) promoted the formation of a conspicuous digestion zone (Fig. 3f).

The means of the biometric attributes were grouped by similarity, using the Scott Knott test, with the control as a reference. The absolute values and the reduction percentage in relation to the control, are presented in Figs. 4a-f and 5a-g.

All applied Ψ_{ws} reduced the shoot length growth capacity, compared to the control (Fig. 4a). There was a statistical difference between 15 and 30 days, evidencing the shoot development capacity up to the Ψ_{ws} of -0.6 MPa, over time. After 30 days of cultivation, group 'd', which included moderate stress, had reduced 73% of its growth capacity compared to the control, while for group 'e', which underwent severe stress, this reduction was 82.2%. The diameter was smaller at 30 days, compared to 15 days, in all Ψ_{ws} applied, except in the control condition (Fig. 4d). The dry mass of the aerial part increased in seedlings subjected to the control treatment and when they were subjected to $\Psi_w = -0.3$ MPa, between 15 and 30 days (Fig. 5a). At 30 days, seedlings subjected to Ψ_{ws} lower than -0.6 MPa presented dry mass of

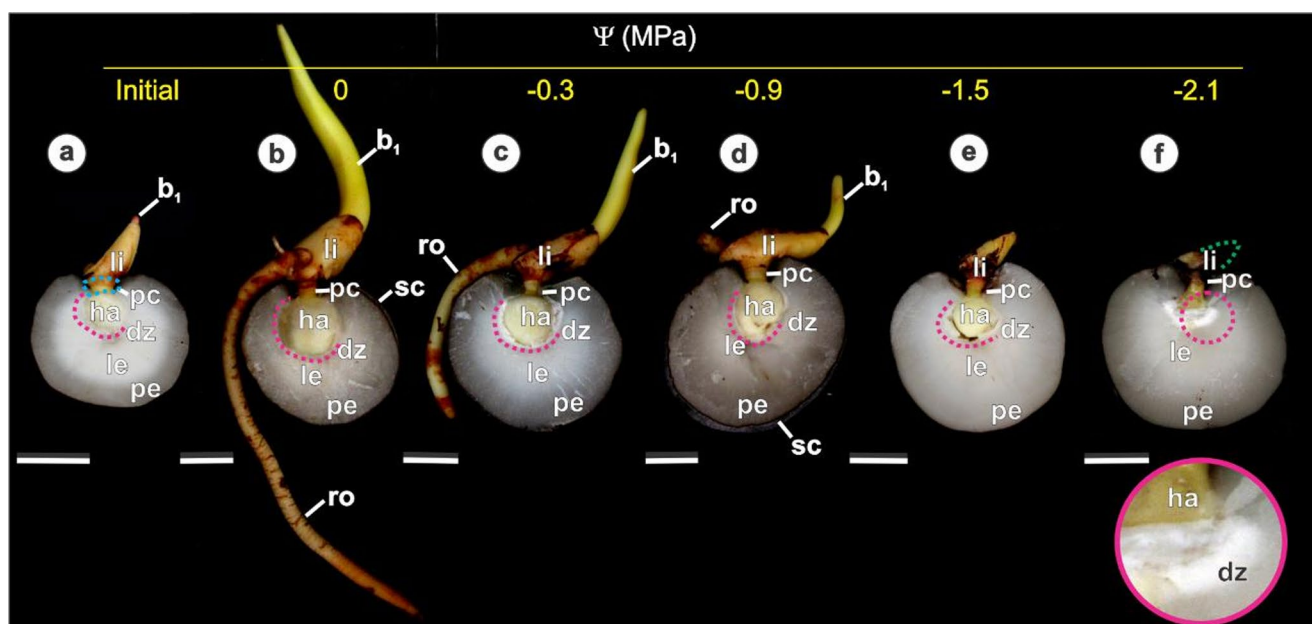


Fig. 3 Seeds (sectioned longitudinally) and seedlings of *M. flexuosa*, 10 days after germination (initial condition) (a) and after cultivation for 15 days under different Ψ_{ws} (b–f). The scale bars correspond to 1 cm. The blue dashed line indicates the cotyledonary petiole, the pink

dashed line highlights the digestion zone. b1 first leaf sheath, dz digestion zone, ha haustorium, le lateral endosperm, li ligule, pc cotyledonary petiole, pe peripheral endosperm, ro root, sc seed coat

the aerial part similar to that observed at 15 days, with a reduction of 85%, compared to the control. The water content of the aerial part did not vary between 15 and 30 days ($M=3.83 \text{ gH}_2\text{O gDM}^{-1}$ $P=0.28$), however, it reduced by 55% with the application of Ψ_{ws} that promoted moderate and severe stress (group 'd'), in a similar way to each other (Fig. 5d).

The haustorium showed continuous development in length, regardless of the Ψ_w used, over time (Fig. 4a). All Ψ_{ws} applied promoted a reduction in the length of the haustorium, compared to the control, so that, after 30 days of cultivation, the haustorium of seedlings subjected to moderate and severe stresses had reduced 23% and 36% of their development capacity, respectively, in relation to the control. The growth in haustorium diameter was limited, between 15 and 30 days, with the application of Ψ_{ws} lower than -0.9 MPa (Fig. 4e). At 30 days, the haustorium diameter had reduced by 51%, similarly, for seedlings subjected to moderate and severe stress. The dry mass of the haustorium increased between 15 and 30 days in seedlings of the control treatment and when $\Psi_{ws} = -0.3, -0.6, -0.9$ and -2.1 MPa were applied (Fig. 5b). After 30 days of cultivation, haustoria of seedlings subjected to moderate and severe stress had reduced their dry mass by 58 and 69%, respectively, in relation to the control (Fig. 5b). Haustorium water content increased by 6% between 15 and 30 days; there was no significant interaction between time and Ψ_w ($P=0.0972$) (Fig. 5e). At 30 days, there was a difference between the Ψ_{ws} , with a 9% increase in the water content of haustoria

of the control treatment in relation to the initial condition and a gradual reduction resulting from the application of all negative Ψ_{ws} , except in the case of severe stress, condition in which there was recovery of the water content to levels similar to those found in the haustorium of seedlings under moderate stress (Fig. 5f).

The roots showed continuous growth in length between 15 and 30 days, with the imposition of Ψ_{ws} equal to or greater than -0.9 MPa (Fig. 4c). At 30 days, moderate stress had caused a 78% reduction in root growth potential, compared to the control condition, and due to severe stress, this reduction was 92% (group 'e'). Only $\Psi_w = -2.1$ MPa promoted a reduction in root diameter (Fig. 4f). There was no statistical difference between the mean root diameters over the evaluated times ($M=2.79 \text{ mm}$; $P=0.8299$). The dry mass of the roots increased in a manner similar to the growth in length, between 15 and 30 days, which indicates that, even under Ψ_{ws} up to -0.9 MPa, there was an increase in mass over time (Figs. 4c and 5c). At 30 days, root dry mass had reduced by 72% and 93%, due to moderate and severe stress, respectively (Fig. 5c). The reduction in root water content was gradual, in relation to the applied Ψ_{ws} (Fig. 5g). There was a 41% reduction in root water content for seedlings under moderate stress and 78% under severe stress. The endosperm did not show any variation in water content with the application of Ψ_{ws} ($M=0.8674 \text{ g H}_2\text{O g DM}^{-1}$; $P=0.4108$) and time ($M=0.8674 \text{ g H}_2\text{O g DM}^{-1}$; $P=0.3361$).

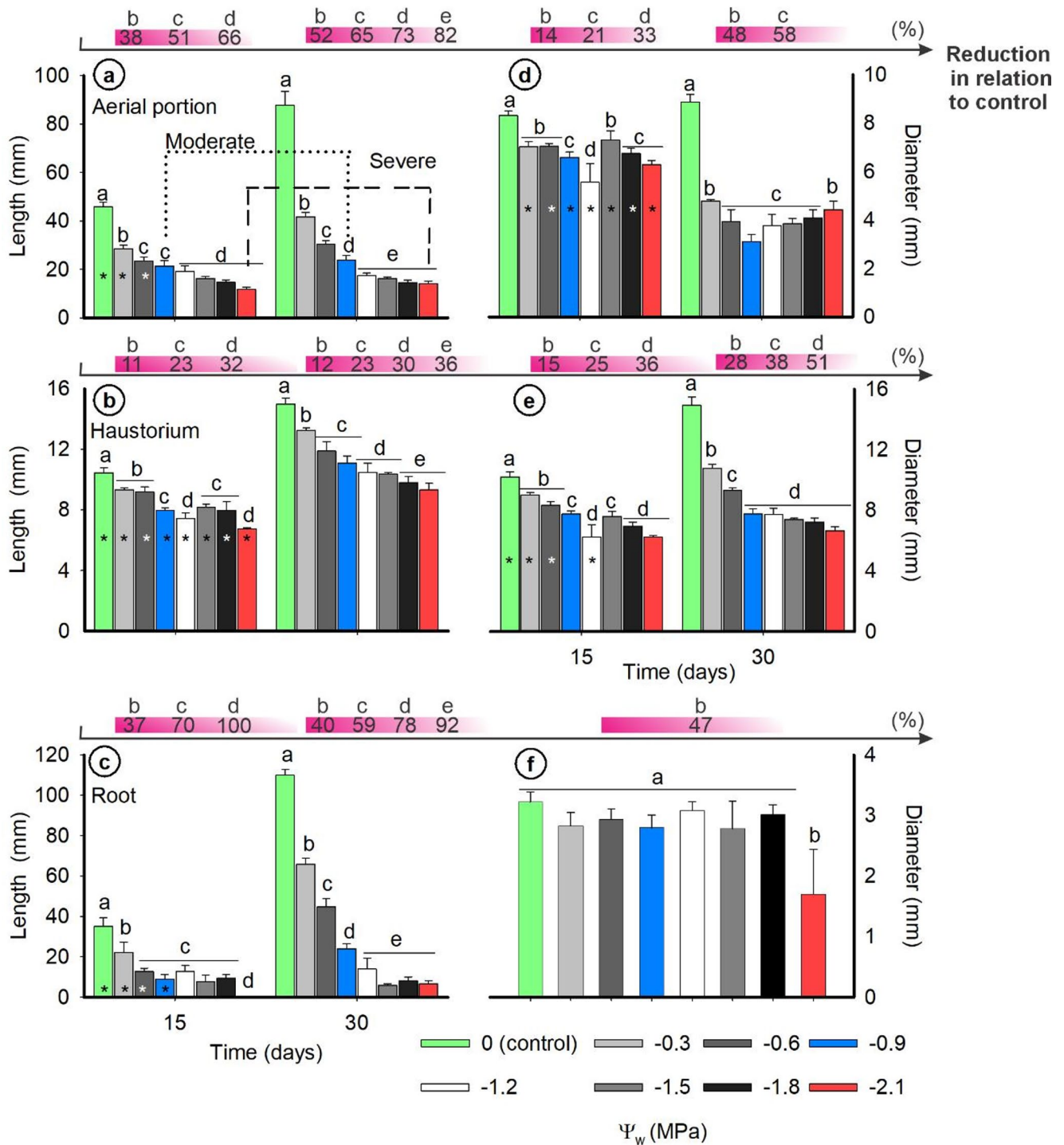


Fig. 4 Biometric characteristics of *M. flexuosa* seedlings after 15 and 30 days of cultivation in osmotic solutions with different Ψ_{ws} . Length of aerial part (a), haustorium (b) and main root (c); diameter of shoot (d), haustorium (e) and main root (f). Different letters, within each time evaluated, indicate that the treatments differed statistically from

each other. The asterisk (*) indicates a difference between 15 and 30 days, by the Scott Knott test ($P \leq 0.05$). The pink bars (above the graphs) indicate the percentages of reduction, in relation to the control. The vertical bars represent the standard error of the mean

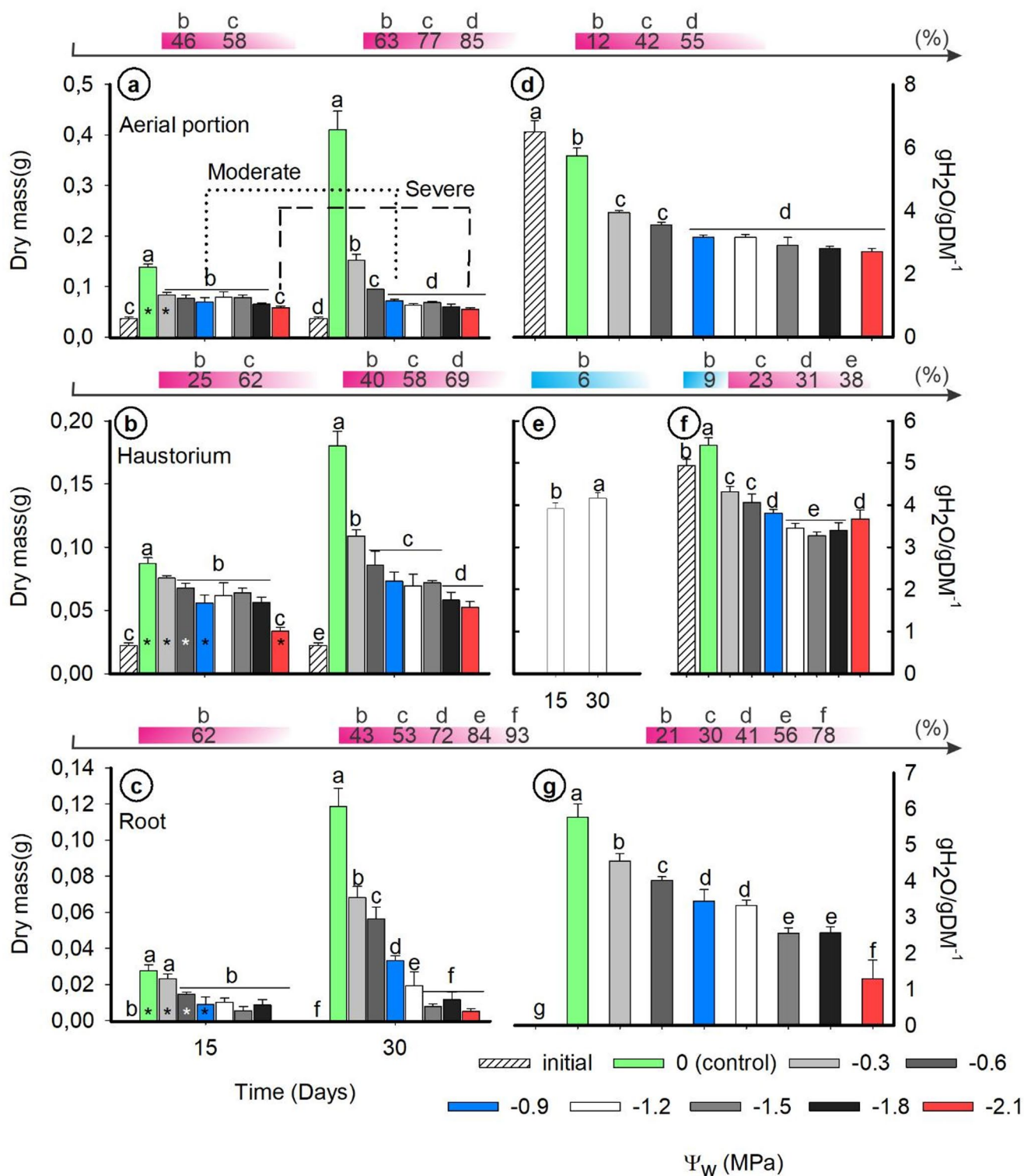


Fig. 5 Biometric characteristics of *M. flexuosa* seedlings after 15 and 30 days of cultivation in osmotic solutions with different Ψ_w . Dry mass of aerial part (a), haustorium (b) and main root (c); water content of aerial part (d), haustorium (e-f) and main root (g). Different letters, within each time evaluated, indicate that the treatments differed statis-

tically from each other. The asterisk (*) indicates a difference between 15 and 30 days, by the Scott Knott test ($P \leq 0.05$). The pink and the blue bars (above the graphs) indicate, respectively, the percentages of reduction and increase, in relation to the control. The vertical bars represent the standard error of the mean

Seedlings rehydrated for 30 days, after undergoing moderate and severe stress, presented a survival percentage of 85 and 34%, respectively, and all showed recovery of the aerial part development. However, of the total number of surviving seedlings, 80% and 25% showed recovery of root development, respectively, due to moderate and severe stress.

Anatomical responses to water stress

The haustorium of *M. flexuosa*, after three days of seed cultivation, showed radially elongated epidermal cells, juxtaposed, and with evident nuclei (Fig. 6a). The parenchyma cells of the haustorium were globose with a gradual increase

in size in a centripetal direction. Epidermal and parenchymal cells adjacent to the epidermis showed indications of cell divisions; vascular bundles occurred along the periphery of the haustorium. The lateral endosperm showed cells with remarkably thick cell walls and a voluminous vacuole, containing intensely purple-stained mucilage. The cytoplasm was restricted to a thin layer at the periphery of the protoplast, containing numerous primary pit fields, connecting the cells. The cytoplasm was restricted to a thin layer at the periphery of the protoplast and numerous primary pit fields connected adjacent cells. The endosperm adjacent to the haustorium presented the digestion zone, composed of collapsed cells at the interface with the haustorium and layers of surrounding hyaline cells, with content already

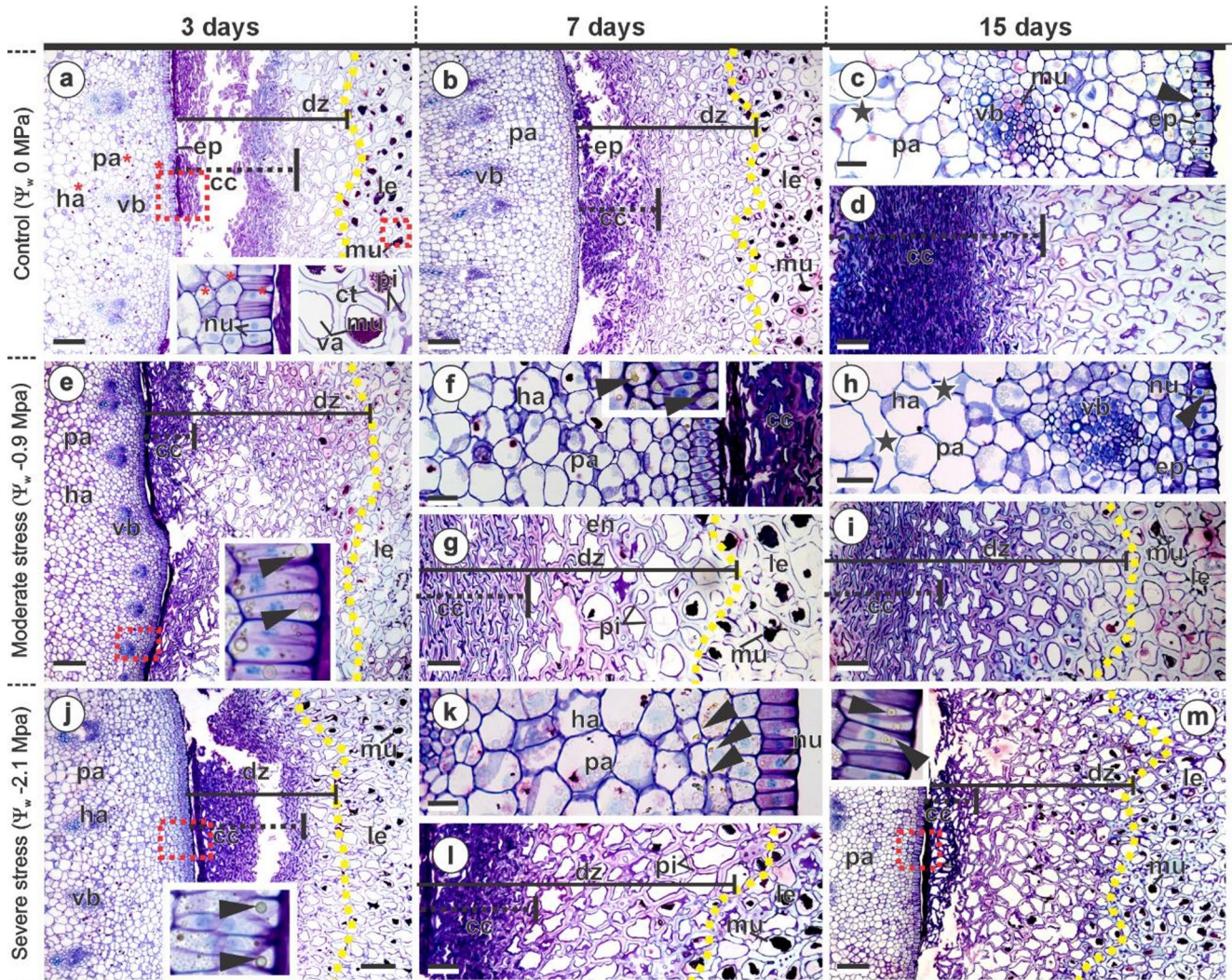


Fig. 6 Cross sections of the median portion of the haustorium and adjacent endosperm of *M. flexuosa* seedlings after 3, 7, and 15 days of culture under control conditions (a-d) or subjected to osmotic solutions promoting moderate (e-i) and severe (j-m) stress. The scale bars correspond to 200 μm . The yellow dashed line delimits the digestion zone; the solid black bar demarcates the radial extension of the digestion zone; the dashed black bar delimits the portion of collapsed

cells; the dashed red rectangles indicate enlargement of points shown in the insets; black arrowheads indicate phenolic compounds; black stars indicate intercellular spaces; red asterisks indicate cell divisions. dz digestion zone, cc collapsed cells, en endosperm, ep epidermis, ha haustorium, le lateral endosperm, mu mucilage, nu nucleus, pa parenchyma, pi primary pit field, vb vascular bundle

mobilized. After seven days of culture, under control conditions, no visible changes were observed in the haustorium (Fig. 6b). The adjacent endosperm digestion zone showed a small increase in the number of hyaline cell layers, compared to three days. After 15 days, in seedlings maintained under control conditions, the haustorium presented phenolic compounds in the epidermis, mucilages were marked in the vascular bundles and intercellular spaces were evident in the parenchyma (Fig. 6c). In the endosperm, the number of collapsed cell layers increased significantly, compared to previous periods (Fig. 6d).

Seedlings subjected to moderate stress ($\Psi_w = -0.9$ MPa), after three days of cultivation, showed accumulation of phenolic compounds in the haustorium epidermis and numerous cell divisions, in the epidermis and parenchyma (Fig. 6e). The endosperm digestion zone was larger, compared to that observed in seedlings subjected to the control treatment. After seven days of culture under moderate stress, no changes in haustorium anatomy were observed compared to three days (Fig. 6e-f). However, the endosperm digestion zone was visibly smaller compared to the previous evaluation period (Fig. 6e, g). At 15 days of culture under moderate stress, the haustorium presented conspicuous intercellular spaces in the parenchyma, and phenolic compounds were marked in the epidermis (Fig. 6h). The digestion zone had a similar extension to that observed at three days, however, compared to the control, the collapsed endosperm cells indicated a lower degree of digestion (Fig. 6d, i).

Seedlings subjected to severe stress ($\Psi_w = -2.1$ MPa), after three days of cultivation, presented haustorium anatomy similar to that observed in seedlings subjected to the control condition (Fig. 6a, j). The digestion zone presented only two to three layers of hyaline cells. After seven days of culture, there was accumulation of phenolics in the haustorium parenchyma (Fig. 6k), and the number of hyaline cell layers increased in the endosperm, compared to three days of culture (Fig. 6j, l). After 15 days of cultivation, in seedlings subjected to severe stress, the haustorium epidermis showed numerous anticlinal divisions and slightly expanded cells, compared to that observed for the control treatment (Fig. 6c, m). In the endosperm, only a thin layer of collapsed cells was observed surrounding the haustorium; the other layers of hyaline cells in the digestion zone had thinner cell walls, compared to what was observed at 15 days in seedlings not subjected to stress and seedlings subjected to moderate stress (Fig. 6d, i, m). These results indicate that the low growth potential of the haustorium limited the absorption and crushing of adjacent endosperm cells under severe stress conditions.

Reserve mobilization under water stress

At all evaluation times, depending on the applied water potentials, proteins were identified in the periphery of the haustorium (epidermal cells, vascular bundles and subepidermal parenchyma), and in the endosperm (Fig. 7a-o). After three days of cultivation, in seedlings subjected to control conditions, proteins were evidenced composing the mucilage, in the vacuoles of the lateral endosperm cells and, in the thin layer of the cytoplasm, restricted to the periphery of the protoplast (Fig. 7a, c). The transition region between lateral endosperm and adjacent endosperm was clearly evidenced by the mobilization of the vacuole contents and the presence of proteins that are internalized into the cell walls from the cytoplasm (Fig. 7c). The continuity of this process established the digestion zone (Fig. 7a, d-e, g-h, j, l, m-n, o-p).

After three days, in seedlings grown under control conditions, neutral polysaccharides, labeled with PAS, were present in the haustorium cells composing the cell walls, and in the protoplast of the epidermal cells (Fig. 8a). Starch grains were also evident in the cells of the epidermis and haustorium parenchyma. In the lateral endosperm, carbohydrates were labeled in the vacuole, composing the mucilage, in the thin layer of cytoplasm and in the inner portion of the cell wall. In the adjacent endosperm cells (digestion zone), the entire length of the cell wall was stained intensely. This carbohydrate detection pattern was observed for all culture conditions, over time (Fig. 8a-n). Variations in the abundance of starch grains were observed, highlighting the significant increase at seven and 15 days, in seedling haustoria, under severe stress (Fig. 8l, n), compared to the same evaluation periods in seedlings subjected to the control condition (Fig. 8b-c) and moderate stress (Fig. 8f, h). Micromorphometric evaluations after 15 days of culture corroborated the structural evaluations and showed a gradual reduction in the extent of the collapsed cell region of the digestion zone under moderate and severe stress, respectively (Fig. 9a). The hyaline cell region showed greater extent when severe stress was applied, compared to the control and moderate stress (Fig. 9b).

Endo- β -mannanase activity

At 15 days of cultivation, no difference was observed between the means of endo- β -mannanase activity in the adjacent endosperm of seedlings subjected to different Ψ_{ws} ($M = 84.74 \mu\text{M}$ equivalents of reducing sugars $\text{min}^{-1} \text{g}^{-1}$ MF; $P = 0.1081$). However, at 30 days of cultivation, there was an increase in enzymatic activity in seedlings subjected to moderate stress (Fig. 10).

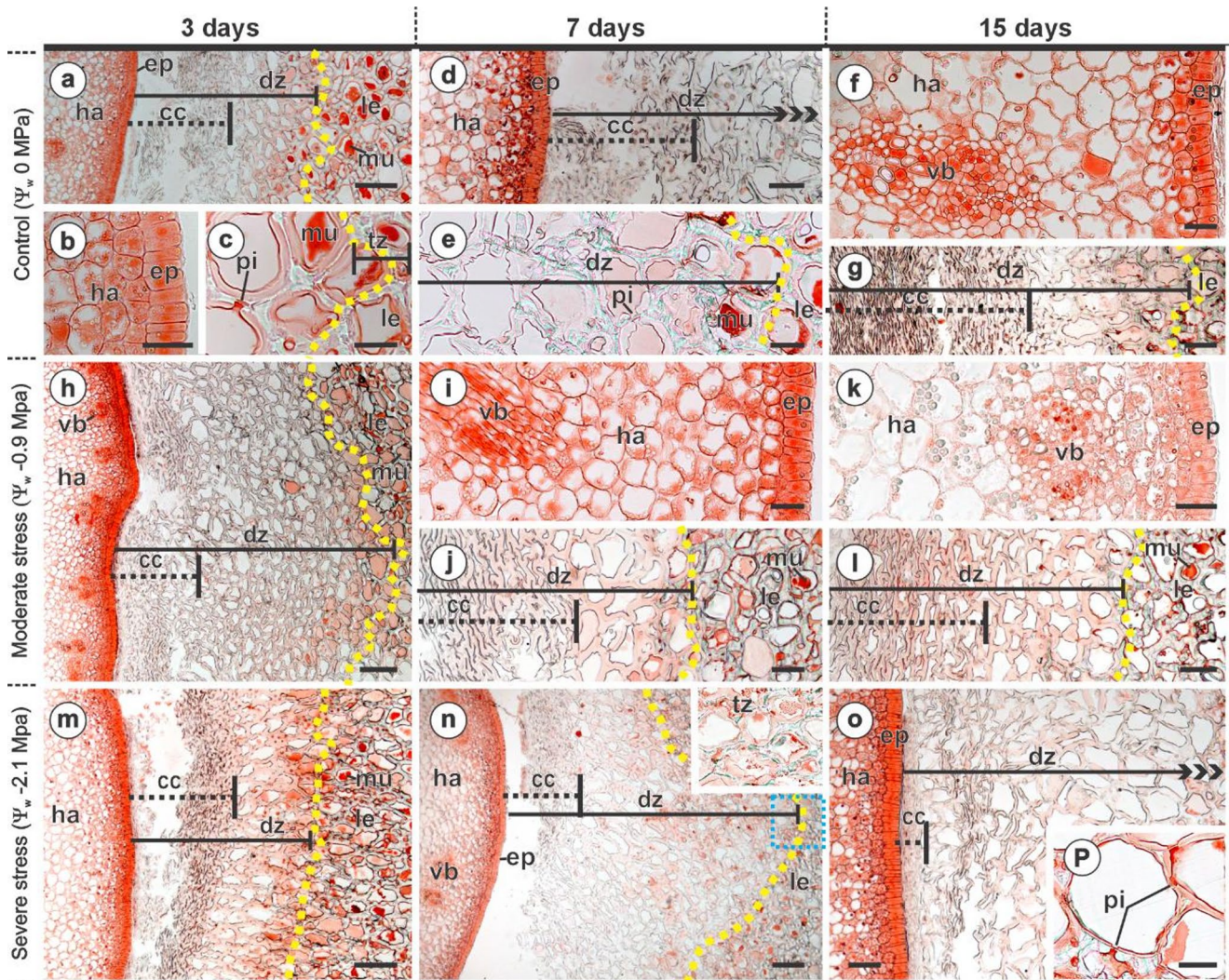


Fig. 7 Cross sections, stained with XP for protein identification, of the median portion of the haustorium and adjacent endosperm of *M. flexuosa* seedlings after 3, 7, and 15 days of culture under control conditions (a–g) or subjected to osmotic solutions promoting moderate (h–l) and severe (m–o) stress. The scale bars correspond to 200 μm . The yellow dashed line delimits the digestion zone; the solid black

bar demarcates the radial extension of the digestion zone; the dashed black bar delimits the portion of collapsed cells. dz digestion zone, cc collapsed cells, en endosperm, ep epidermis, ha haustorium, le lateral endosperm, mu mucilage, nu nucleus, pi primary pit field, tz transition zone, vb vascular bundle

In situ histochemical localization of the superoxide anion radical (O_2^-)

Superoxide anion was found only in the haustorium of control seedlings and seedlings subjected to moderate and severe stress, at 3 and 7 days of cultivation (Fig. 11).

Oxidative stress evaluation

Hydrogen peroxide levels increased in the aerial part of seedlings subjected to severe stress (Fig. 12a), however, there was no difference between the times evaluated ($M=0.56 \text{ mM g}^{-1} \text{ MF}$; $P=0.0822$). CAT activity increased concomitantly with the increase in H_2O_2 in the aerial part of

seedlings subjected to severe stress (Fig. 12b). There was no statistical difference in CAT activity between the times evaluated ($M=610 \text{ } \mu\text{mol min}^{-1} \text{ mg prot}^{-1}$; $P=0.0626$). The APX activity data for the aerial part did not follow the normal distribution according to the Shapiro Wilk test ($P=0.0014$) and were therefore analyzed using the Kruskal-Wallis test, which indicated a difference only between the times evaluated. There was an increase in enzyme activity at 7 days (Fig. 12c), as indicated by the multiple comparisons test (T-test). The application of water potentials did not influence the enzyme activity in the aerial part ($M=0.2574 \text{ } \mu\text{mol AsA mg prot}^{-1} \text{ min}^{-1}$; $P=0.3141$). SOD activity did not vary in the aerial part, depending on the application of Ψ_{ws} ($M=0.0512 \text{ Umin}^{-1} \text{ g prot}^{-1}$; $P=0.4368$), however, it

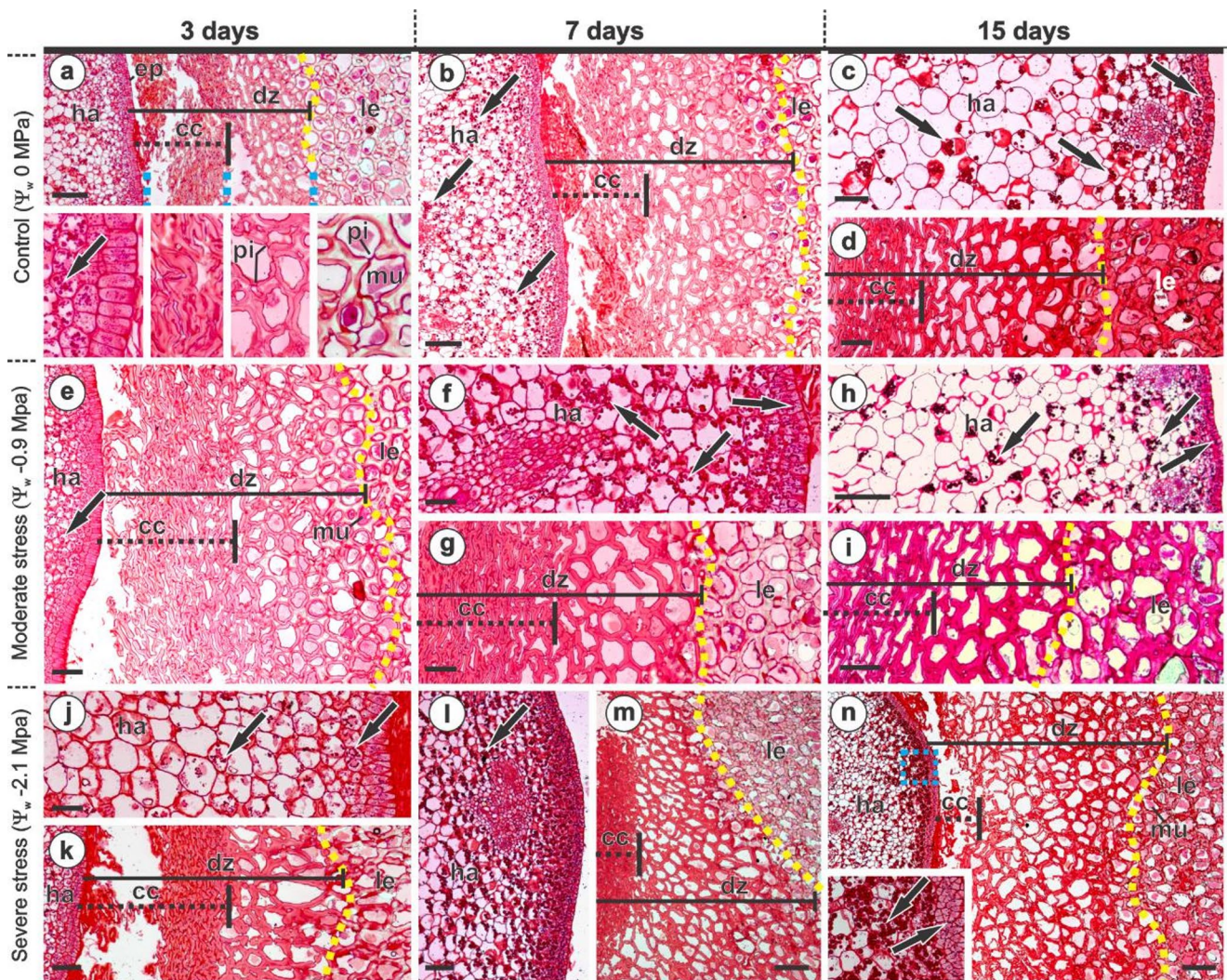


Fig. 8 Cross sections, stained with PAS for carbohydrate identification, of the median portion of the haustorium and adjacent endosperm of *M. flexuosa* seedlings after 3, 7, and 15 days of culture under control conditions (a–d) or subjected to osmotic solutions promoting moderate (e–i) and severe (j–n) stress. The scale bars correspond to 200 μm . The yellow dashed line delimits the digestion zone; the solid black bar

demarcates the radial extension of the digestion zone; the dashed black bar delimits the portion of collapsed cells; the dashed blue rectangles indicate enlargement of points shown in the insets; black arrows point to starch grains in the haustorium. dz digestion zone, cc collapsed cells, en endosperm, ep epidermis, ha haustorium, le lateral endosperm, mu mucilage, nu nucleus, pi primary pit field, vb vascular bundle

was influenced by time, with greater activity at 15 days of cultivation, compared to three and seven days (Fig. 12d). The MDA contents did not vary in the aerial part, as a function of time ($M = 268.69 \text{ nM g}^{-1} \text{ MF}$; $P = 0.2038$) and applied water potentials ($M = 268.69 \text{ nM g}^{-1} \text{ MF}$; $P = 0.2213$).

In the haustorium, the average H_2O_2 levels did not show differences over time ($M = 0.1443 \text{ mM g}^{-1} \text{ MF}$; $P = 0.1078$) and, as a function of the application of Ψ_{ws} ($M = 0.1443 \text{ mM g}^{-1} \text{ DM}$; $P = 0.6664$). Time ($M = 654.27 \text{ } \mu\text{mol/min mg prot}$; $P = 0.741$) and Ψ_{ws} ($M = 654.27 \text{ } \mu\text{mol/min mg. prot}$; $P = 0.776$) also did not influence CAT activity. The same occurred with the APX activity for the time ($M = 0.341 \text{ } \mu\text{mol AsA mg prot}^{-1} \text{ min}^{-1}$; $P = 0.99$) and application of Ψ_{ws} ($M = 0.341 \text{ } \mu\text{mol AsA mg prot}^{-1} \text{ min}^{-1}$; $P = 0.1587$).

SOD activity increased in the haustorium with the application of moderate and severe stress, similarly, compared to the control (Fig. 12f). However, enzyme activity was higher at 3 days compared to other times (Fig. 12g). MDA levels gradually increased in the haustorium with the imposed stress (Fig. 12e), however, there was no difference over time ($M = 16.9 \text{ nM g}^{-1} \text{ MF}$; $P = 0.3593$).

Discussion

This work reports the first in-depth study integrating structural and physiological aspects on the influence of water deficit on the mobilization of reserves in palm seeds and

Fig. 9 Micromorphometry of the endosperm digestion zone (collapsed cells and hyaline cells) after 15 days of cultivation of *M. flexuosa* seedlings, maintained under control conditions or in osmotic solutions with different Ψ_{ws} . Different letters indicate that the treatments differed statistically from each other, according to Tukey's test ($P \leq 0.05$). Vertical bars represent the standard error of the mean

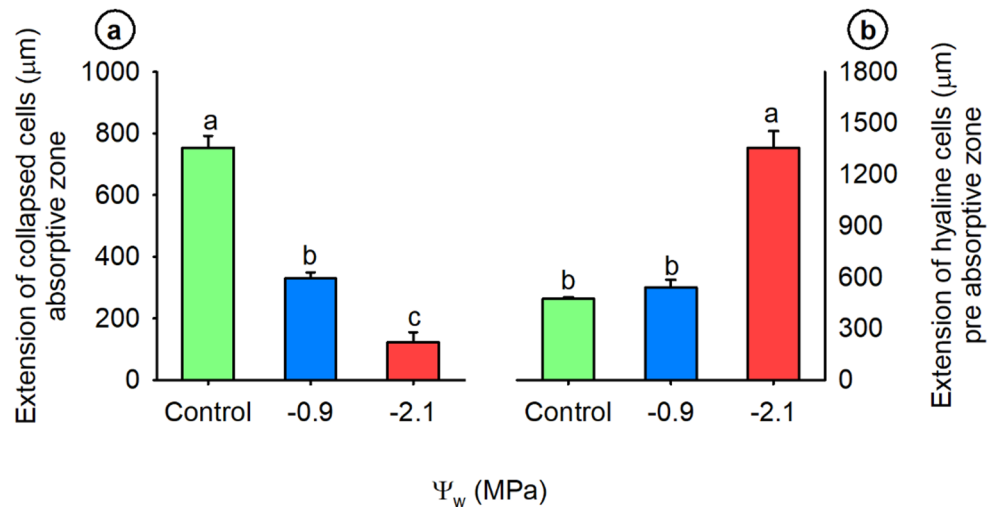


Fig. 10 Endo- β -mannanase activity expressed on a fresh basis, after 30 days of cultivation of *M. flexuosa* seedlings, maintained in control conditions or under osmotic solutions with different Ψ_w . Different letters indicate that the treatments differed statistically from each other, by Duncan's test ($P \leq 0.05$). The vertical bars represent the standard error of the mean

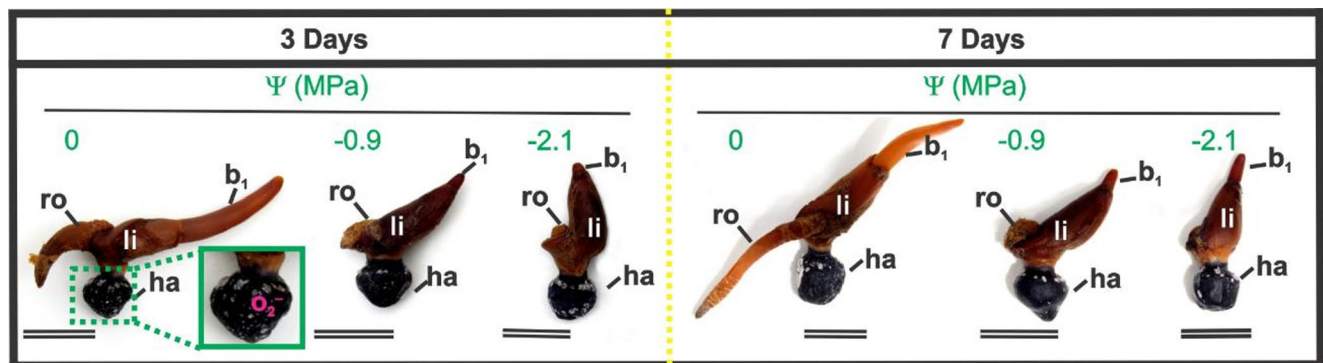
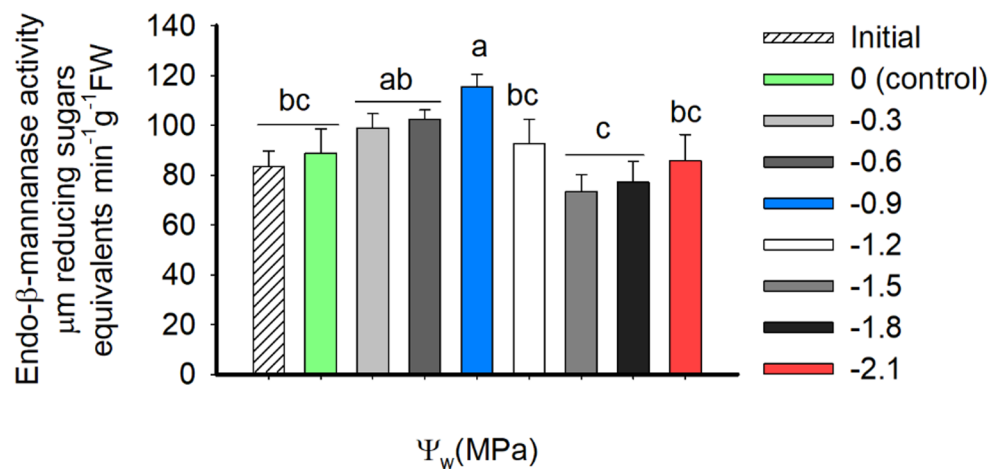


Fig. 11 Histolocalization of superoxide anion after 3 and 7 days of cultivation of *M. flexuosa* seedlings, maintained in control condition or in osmotic solutions with different Ψ_{ws} . The scale bars correspond

to 1 cm. The green dotted line (inset) highlights the intensely stained haustorium region, indicating the presence of the free radical. b1 first leaf sheath, ha haustorium, li ligule, ro root

also in recalcitrant seeds. Dias et al. (2024) reported that *M. flexuosa* embryos, although highly recalcitrant, respond positively to moderate water stress, by increasing metabolism, culminating in eventual germination and post-germinative development under these conditions. In addition, other studies have demonstrated the ability of this species to

form seedlings banks in drier microenvironments of veredas (Fig. 1a) (Porto et al. 2018; Salvador et al. 2022; Almeida et al. 2024). However, the mechanisms involved in seedling resilience to water stress are still poorly understood. The results obtained in this study indicate that adjustments in the mobilization of reserves and the control of oxidative stress

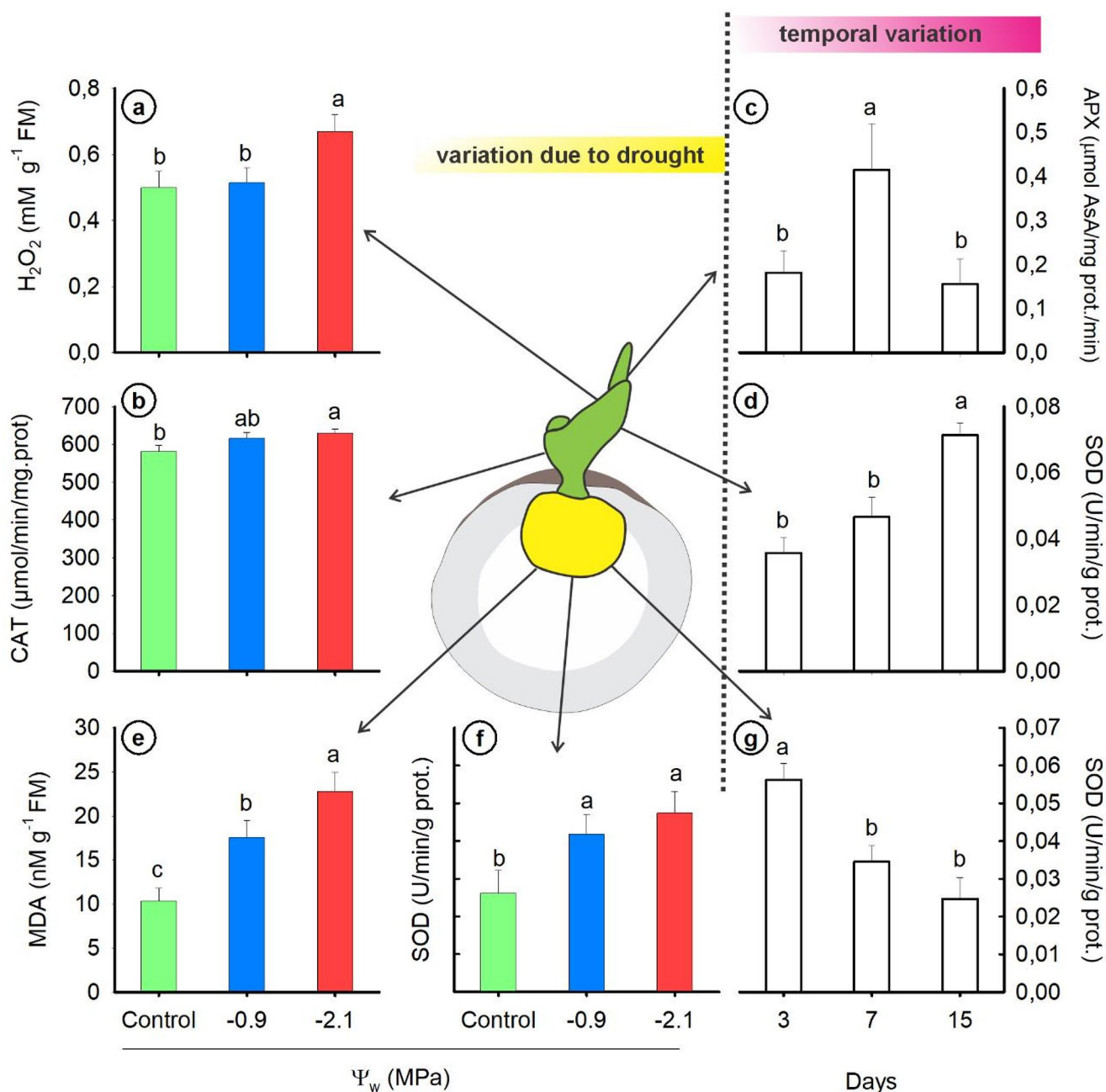


Fig. 12 Oxidative stress indicators in *M. flexuosa* seedlings after 3, 7 and 15 days of cultivation under osmotic solutions with different Ψ_{ws} . H₂O₂ levels (a), CAT (b), APX (c) and SOD (d) activities in the aerial part and MDA levels (e) and SOD activity in the haustorium (f-g).

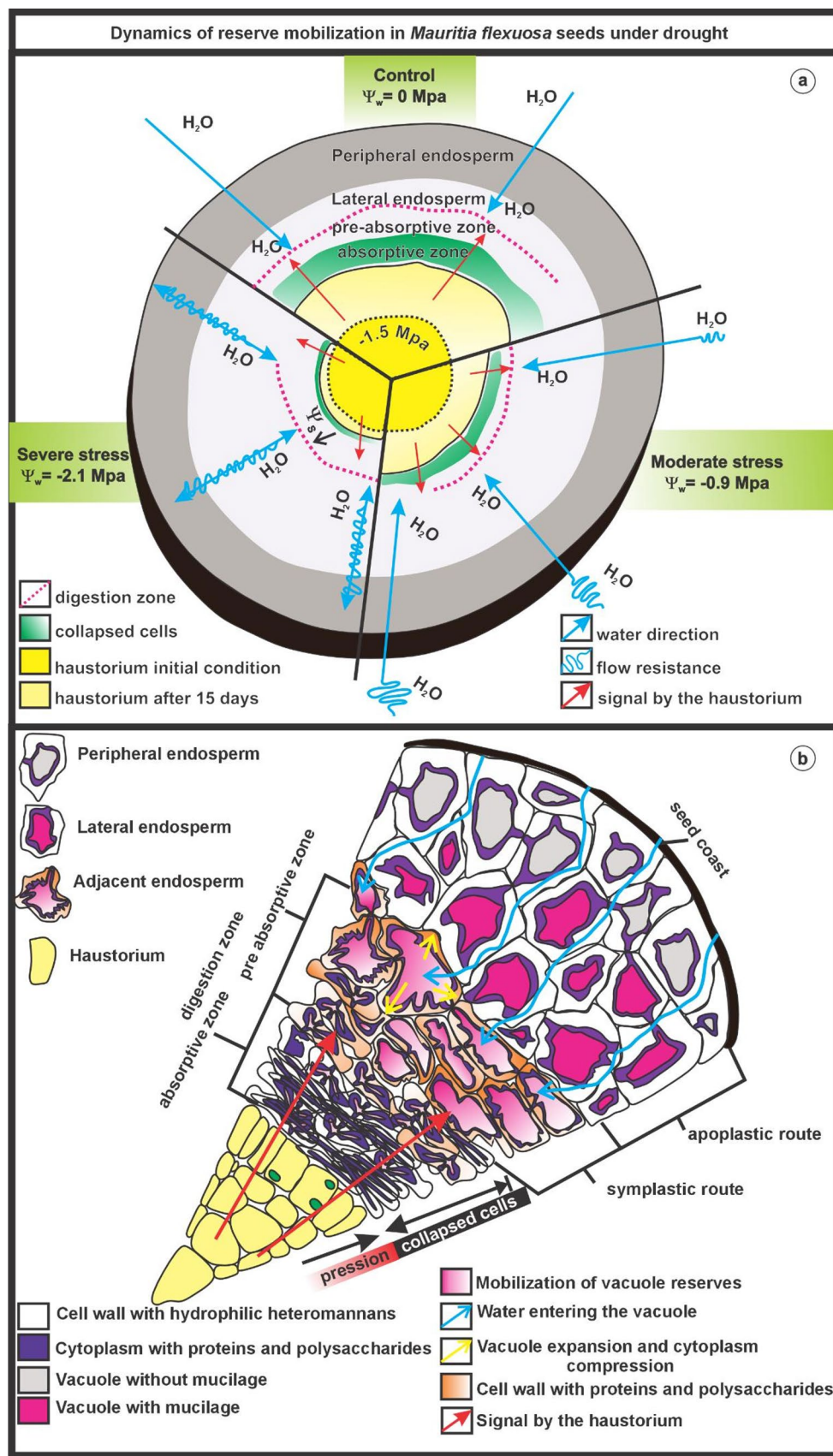
Different letters indicate that the treatments differed statistically from each other, by Duncan's test ($P \leq 0.05$). The vertical bars represent the standard error of the mean

constitute important strategies for tolerance to water stress. A synthetic representation of the main effects of water stress on the dynamics of reserves, as well as the mechanisms of resilience in *M. flexuosa* seeds, can be seen in Fig. 13.

Dynamics of seed reserve mobilization under water deficit conditions

The haustorium plays a central role in the resilience of *M. flexuosa* seedlings to dehydration. Ten days after germination, the haustorium presented $\Psi_w = -1.5$ MPa, which is capable of inducing the absorption of water and nutrients, even under conditions of low water availability (Figs. 2a

Fig. 13 Synthetic diagram of the dynamics of reserve mobilization in *M. flexuosa* seeds under water gradient (hydrated seedlings; under moderate water stress and under severe water stress) (a). Histological diagram of seed reserve mobilization (b)



and 13a). It is interesting to note that the haustorium is the only structure of the seedling that, even when subjected to water scarcity, develops over time (Fig. 4b, e). Several studies had already established the absorption functions of palm haustoria (DeMason et al. 1985; Alang et al. 1988; Mazzottini-dos Santos et al. 2017; Dias et al. 2018, 2020; Fonseca et al. 2024), however, none of them determined the Ψ_w that defines water capacity, i.e., the capacity to absorb or retain water (Gonçalves et al. 2020), which is crucial for the dynamics of seed reserves.

In conditions of water deficit, the partially degraded reserves not absorbed by the haustorium of *M. flexuosa* are concentrated in the digestion zone. This mechanism promotes the reduction of Ψ_w and favors water retention in the seed. Blocking water entry in seedlings under severe stress (Fig. 2a-b) limits haustorium growth (Figs. 3f, 4b and e and 5b), thus, pressure against the adjacent endosperm is attenuated and the zone of collapsed cells (absorptive zone) is not continuously established (Fig. 9a). However, signals for the mobilization of the endosperm adjacent to the haustorium are emitted, which promotes an extensive radius of cells with hydrolyzed content in the pre-absorptive zone, with a reduction in the osmotic potential (Ψ_s) (Figs. 6m, 7o-p, 8n, 9b and 13a). The high water content, characteristic of recalcitrant seeds (Berjak and Pammenter 2007), is then directed to the digestion zone, by the flow of free energy, whose force of attraction between the molecules becomes expressive enough to promote water homeostasis of the seed (Fig. 13a-b). Furthermore, the presence of highly hydrophilic mucilages in the vacuoles of endospermic cells of *M. flexuosa* also contributes to water retention in the symplast (Dias et al. 2020, 2024).

In the case of seedlings under moderate stress, the dynamics are similar, however, the force of attraction generated by the osmolytes in the digestion zone allows external water, even conditioned by resistance ($\Psi_w = -0.9$ MPa), to be allocated to the inside of the seed (Fig. 13a), providing attenuated seedling growth (Figs. 3d and 4a-c). It is known that the mobilization of reserves in palm seeds is initiated by signals coming from the haustorium (DeMason et al. 1985; Mazzottini dos Santos et al. 2017). Dias et al. (2018) would suggest that the O_2 assimilated in the embryos, from stomata in the cotyledonary petiole (Silva et al. 2014), and subsequently directed to the haustorium, participates in the signaling routes, through its modified and reactive forms, the ROs. These molecules can induce the activation of endo- β -mannanases, pre-stored in vacuoles of adjacent endosperm cells (DeMason et al. 1985; Sekhar and DeMason 1990; Zienkiewicz et al. 2014; Mazzottini-dos Santos et al. 2017) and thus initiate a continuous flow of mobilization. Although the mechanism of digestion zone formation is known, especially for orthodox palm seeds, no work to date

has investigated the effects of water scarcity on its dynamics for recalcitrant Arecaceae seeds.

Dias et al. (2020) suggested a model for the mobilization of mannan-rich cell walls in the endosperm of *M. flexuosa* under optimal water conditions, corroborated by our results (Fig. 13b). The process begins with the mobilization of mucilage into the vacuole via the symplast and, with the increase in osmolytes, water is internalized, favoring the turgidity of the vacuole and the pressure against the thin layer of cytoplasm, restricted to the periphery of the protoplast (Figs. 7c and p and 13b). This region houses the endo- β -mannanases, which are, this way, introduced into the cell wall and promote the centrifugal digestion of mannans. In addition to this model, our results allow us to propose that the translocation of water from the peripheral layers of endospermic cells to the cells of the digestion zone is preferentially via the apoplastic route, due to the hydrophilic properties of heteromannans (Buckeridge 2010; Rodríguez-Gacio et al. 2012). These polysaccharides are the main components of the thick cell wall matrices in palm endosperm, capable of retaining large amounts of water, which can be translocated through the pores formed during the deposition of the compounds (Buckeridge et al. 2000; Buckeridge 2010). According to Buckley (2015), apoplastic transport predominates over symplastic transport during transpiration, which reinforces our proposition. Furthermore, in situations of water deficit, the water trapped in the cell walls of the endosperm of *M. flexuosa* can be required more easily, without the controlling action of the plasma membrane. On the other hand, it is likely that the symplastic pathway is predominant in the digestive zone for the translocation of reserves through the numerous primary pit fields after signaling influenced by the haustorium (Fig. 13b).

Under severe stress conditions, the haustorium of *M. flexuosa* acts in the transient storage of significant amounts of starch (Fig. 8l, n), which is proven by the increase in its dry mass between 15 and 30 days (Fig. 5b). On the other hand, under optimal water conditions, Dias et al. (2020) did not observe a significant increase in the dry mass of the haustorium during the mobilization of *M. flexuosa* seed reserves, indicating that starch does not accumulate significantly due to the rapid metabolization of the cell walls and the continuous transport of hydrolysis products to the vegetative axis. We consider that starch storage, under water stress conditions, may constitute a defense to prevent nonspecific glycosylation of cellular elements, which occurs when sugar monomers are free (Buckeridge et al. 2000). In addition, starch may be a strategic energy source, of rapid metabolism, when the optimal water percentages are reached in the buriti seed, after rehydration.

Endo- β -mannanase activity levels increase in the adjacent endosperm of *M. flexuosa* seeds under moderate water

stress, which aids in the recruitment of osmolytes from cell wall digestion (Fig. 10). Increased levels of mannanase activity were also observed by Dias et al. (2024), when embryos were subjected to water stress, which appears to be a response trend of this enzyme in buriti seeds. On the other hand, in fenugreek seeds, mannanase activity was decreased when water stress was imposed (Spyropoulos and Reid 1988). There are several mannanase isoforms in nature, however, only a small percentage has been described for plants, thus, the species-specific regulatory mechanisms lack further studies (Rodríguez-Gacio et al. 2012).

The roots of *M. flexuosa* appear to be the most vulnerable organs to severe dehydration, since seedlings under these conditions, after 30 days of cultivation, with subsequent rehydration for another 30 days, showed a small percentage of regeneration and, of these, only 25% resumed root development. To overcome this fragility, in situations of moderate stress, at the seedling's water absorption threshold (Fig. 2a-b), root development is prioritized over the aerial part, possibly as a strategy to reestablish hydration (Figs. 4a and c and 5. a, c).

Physiological responses to oxidative stress due to water stress

M. flexuosa seedlings present antioxidant systems responsive to water stress, both in the aerial part and in the haustorium, which help promote homeostasis. Inefficient enzymatic antioxidant systems are one of the main problems affecting longevity in recalcitrant seeds (Berjak and Pammenter 2007; Obroucheva et al. 2016; Leprince and Buitink, 2015). Salvador et al. (2022) and Dias et al. (2024) reported that *M. flexuosa* presents embryos with efficient antioxidant systems, regulated by enzymatic and, especially, non-enzymatic (phenolic) pathways, which are responsive to abiotic stresses and allow the maintenance of seeds in the soil for long periods. Our results indicate that seedlings preserve these mechanisms, since H_2O_2 levels and CAT activity were related in the aerial part when severe water stress was imposed (Fig. 11a-b). The efficient activity of the enzyme constitutes a crucial pathway for controlling lipid peroxidation, as demonstrated by the levels of MDA, which did not change in the tissues. APX and SOD activities were not related to water stress, but varied over time, integrating the response to oxidative stress. According to Das and Roychoudhury (2014), in cases of abiotic stress, enzymatic antioxidant systems are the main pathways for eliminating ROs in plants.

In the haustorium, on the other hand, there was an accumulation of superoxide (Fig. 11), a free radical that is extremely damaging to membrane systems (Bailly 2004). In response to stress SOD levels increased (Fig. 12f), but were

not sufficient to prevent damage to cell membranes, indicated by the gradual increase in MDA levels (Fig. 12e), a marker of lipid peroxidation (Heath and Packer 1968; Hasanuzzaman et al. 2020). Noteworthy was the presence of the superoxide radical in haustoria of the control condition, this molecule can act in processes involved in the controlled death of parenchymal cells in the haustorium, with the aim of promoting the formation of schizolysigenic intercellular spaces, important for the accumulation of reserve O_2 , in case of hypoxia due to flooding (Dias et al. 2020). It is interesting to note that H_2O_2 levels were kept stable in the haustorium, which possibly enabled the triggering of events that control reserve mobilization dynamics, even under water stress, since controlled levels of ROS act as chemical messengers, efficient in activating enzymes related to reserve mobilization (Das and Roychoudhury 2014; Wojtyla et al. 2016; Dias et al. 2018; Bailly 2019). It is likely that phenolic compounds observed in seedling haustoria (Fig. 6f, h, k, m) contribute to oxidative homeostasis in stress situations, as occurs in buriti embryos subjected to drought (2024).

M. flexuosa seedlings in post-germinative development are tolerant to conditions of progressive water deficit, as are young plants (Almeida et al. 2024). The dynamics of reserve mobilization, together with responsive antioxidant systems, favors the formation of the species' seedling bank in dry vereda microenvironments, as a complementary strategy to the formation of the persistent seed bank in humid environments (Porto et al. 2018; Salvador et al. 2022). The results presented here may help in understanding the factors that enabled the colonization of the Brazilian Cerrado, typically savanic, by a palm tree originally from the Amazon (Melo et al. 2018). It is important, however, to emphasize that other factors such as rising global temperatures and changes in the hydrological cycle resulting from local anthropogenic influences may affect the survival of *M. flexuosa* in this environment. In this challenging context, expanded reproductive studies could contribute to supporting the conservation and sustainable use of this iconic South American palm.

Conclusions

The haustorium plays a central role in promoting the resilience of *M. flexuosa* seedlings to dehydration, due to its $\Psi_w = -1.5$ MPa, capable of inducing the absorption of water and nutrients, even in conditions of low water availability. Reduced growth of the haustorium, in dry conditions, favors the accumulation of partially degraded and unabsorbed reserves, which are concentrated in the digestion zone; this mechanism contributes to water retention in the seed. Endo- β -mannanase activity increases in the adjacent endosperm of *M. flexuosa* seeds under moderate water stress,

which aids in the recruitment of osmolytes from the digestion of the cell wall, which is rich in mannan. In situations of moderate stress, root development is prioritized over the aerial part, between 15 and 30 days, as a strategy for reestablishing hydration. *M. flexuosa* seedlings, in post-germinative development, present enzymatic and non-enzymatic antioxidant systems responsive to water stress, both in the aerial part and in the haustorium, a condition that contributes to the maintenance of oxidative homeostasis. Together, these characteristics favor the establishment of seedlings in microenvironments subject to water stress and contribute to the reproductive success and adaptation of the species in seasonal environments.

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Author contributions GPD: conceptualization, experimental design, investigation, writing of original draft, and manuscript review and editing. HCMS and YRFN: investigation. LMR and MGCF: investigation, supervision, project administration, funding acquisition, final manuscript review and editing.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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