Environmental and Experimental Botany 226 (2024) 105930

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research paper

Water stress resilience in *Mauritia flexuosa* (Arecaceae) embryos: New insights into the persistence of recalcitrant seed banks

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ARTICLE INFO

Keywords:
Germination and dormancy
Oxidative stress
Palm seeds
Seed ecophysiology
Sensitivity to desiccation

ABSTRACT

The neotropical palm *Mauritia flexuosa* produces seeds that show the association between recalcitrance and dormancy. Despite the intolerance to desiccation, the seeds can maintain persistent banks in flooded environment soils (veredas) in the Cerrado biome. As the mechanisms involved in the persistence of recalcitrant seed banks are still poorly understood, the objective of this work was to evaluate the response of *M. flexuosa* embryos to water deficit and saturation stresses. Embryos of *M. flexuosa* with water content typical of dispersion or subjected to hydration were exposed to moderate and severe water potentials (Ψ_w= −1.5 MPa and Ψ_w= −2.1 MPa), in addition to water saturation (Ψ_w= 0 MPa). Anatomical, histochemical and ultrastructural evaluations were performed on the embryos after 24 h. Membrane integrity estimation, endo-β-mannanase activity and oxidative stress indicators (H_2O_2 and MDA contents, CAT, SOD and APX activity) were also evaluated. The endosperm structure contributes to the maintenance of embryo hydration, while abundant mucilage reserves favor resilience to desiccation. Post-dispersal hydration makes embryos less vulnerable to oxidative stress, which is due to the non-enzymatic antioxidant system. Both moderate water stress and post-dispersal water absorption induce an increase in metabolism and the mobilization of reserves, which indicate that hydration/dehydration cycles can favor overcoming dormancy. *M. flexuosa* embryos show resilience to water deficit, and that is crucial for the persistence of seeds in the soil in seasonal environments, however, successful germination is dependent on high hydration, which prevents structural and physiological damage.

1. Introduction

Recalcitrant seeds dispersed with high water contents, do not tolerate dehydration below a certain limit (0.33 g H_2O g^{-1} DM), which compromises their ability to maintain seed banks in the soil, their use in propagation and the conservation of genetic resources (Hoekstra et al., 2001; Obroucheva et al., 2016; Marques et al., 2018). The loss of viability in these seeds, when dehydrated, is usually related to changes in the plasticity of cell walls (Woodenberg et al., 2015; 2018) and lipid peroxidation of membranes, due to oxidative stress (imbalance between production and removal of reactive oxygen species ROS) (Bailly, 2004; Berjak and Pammenter, 2008). There is great variability in terms of desiccation sensitivity and longevity among recalcitrant seeds, and this is due to structural and cytological particularities, the degree of maturation during dispersion and the efficiency of the antioxidant system (Varghese et al., 2011; Subbiah et al., 2019). Despite advances in research in recent decades, knowledge about the cytological and physiological responses of recalcitrant seed embryos to water stress is still limited, especially for neotropical tree species (Marques et al., 2018).

In regions with seasonal tropical climates, soils commonly go through cycles, with alternation between high precipitation and drought that usually cause water stress (WS), either due to lack or excess of water for the seeds incorporated into the soil seed bank (Baskin and Baskin, 2014; Gonçalves et al., 2020). Oxidative stress due to WS is often fatal to recalcitrant seeds. However, if controlled by enzymatic pathways, especially by catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity, and non-enzymatic pathways, oxidative stress can generate ROS levels capable of inducing metabolic responses related to mobilization of reserves (Bailly, 2004; Wojtyla et al.,2016; Dias et al.,2018), dormancy control (Bewley et al., 2013) and tolerance

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https://doi.org/10.1016/j.envexpbot.2024.105930
Received 8 April 2024; Received in revised form 31 July 2024; Accepted 2 August 2024
Available online 6 August 2024

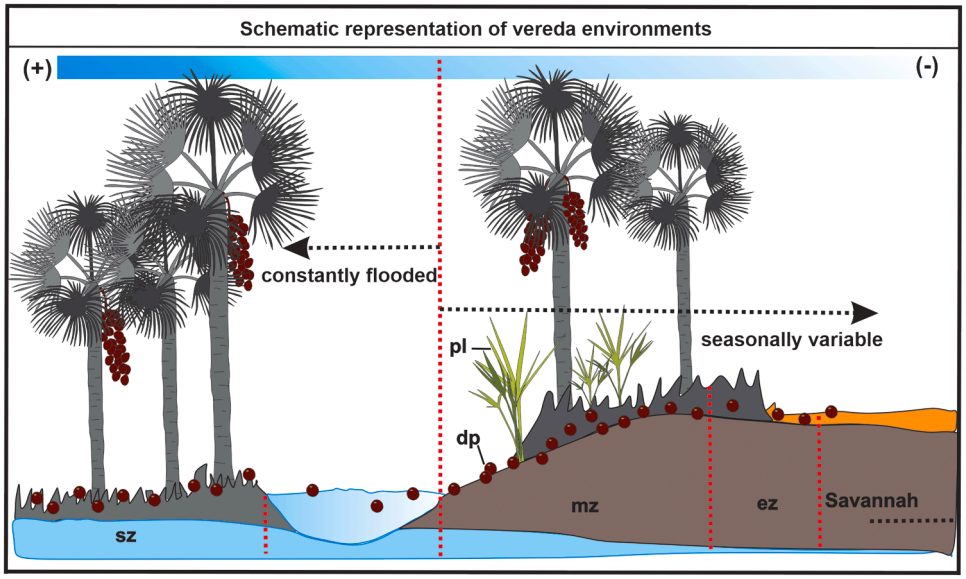

Fig. 1. Scheme representing vereda microenvironments in the Cerrado biome. The blue bar represents the variation in water availability in microenvironments. ez edge zone, dp, diaspore, mz, middle zone, pl, seedling, sz, swampy zone.

to abiotic stresses (Huang et al., 2008: Ozfidan et al., 2012). This way, the control of oxidative stress in recalcitrant seeds is crucial, both for their persistence in the soil and for germination control, which directly affects the dynamics of seed banks.

Mauritia flexuosa L. f. (buriti or aguaje) is widely distributed in tropical South America and is the most abundant palm in Brazil, occurring in the Amazon biome and in the Cerrado, in wetlands known as veredas (Silva et al., 2014; Melo et al., 2018). The species has ecological and socioeconomic importance, as well as agro-industrial potential (Lorenzi et al., 2010; Horn et al., 2012; Endress et al., 2013). The seeds of *M. flexuosa* show the combination of recalcitrance and dormancy, which is a rare physiological aspect among angiosperms (Tweddle et al., 2003; Silva et al., 2014; Veloso et al., 2016), but which has been identified in several species of Arecaceae (Jaganathan, 2021). Trait associated with resistance to desiccation were observed in the embryo, such as slow dehydration and abundant symplastic reserves, especially protein and mucilage (Veloso et al., 2016; Dias et al., 2020). Recent studies have shown the ability of *M. flexuosa* to form a persistent seed bank (Porto et al., 2018; Salvador et al., 2022), in which seeds can remain viable for more than a year (a concept from Thompson et al., 1998 and Csontos and Tamás, 2003). This characteristic indicates a peculiarity between species with recalcitrant seeds (Tweddle et al., 2003; Marques et al., 2018), and raises questions about long-term resilience strategies.

In the seasonal climate of the Cerrado, the veredas show fluctuations in the soil water content, which favors the formation of xeric to hydromorphic microenvironments (Porto et al., 2018) (Fig. 1). As *M. flexuosa* seeds are sensitive to desiccation, this variation in water availability can impose stressful or even lethal conditions (Gonçalves et al., 2020; Salvador et al., 2022). Contrastingly, water variation in the soil can act as a signal for metabolism activation related to overcoming seed dormancy (El-Maarouf-Bouteau and Bailly, 2008; Porto et al., 2018). Understanding the response of *M. flexuosa* embryos to WS may contribute knowledge expansion about the biology of recalcitrant seeds, in addition to providing insights for the conservation of flooded environments, which are threatened by local anthropization and global climate change.

The objective of this research was to evaluate the response of *M. flexuosa* embryos to stresses of water deficit and excess, simulated experimentally. The following questions were addressed: 1) What are the water dynamics like in embryos under water stress? 2) What are the structural, cytological and physiological responses of embryos to stress? 3) What are the effects of post-dispersal hydration on embryos regarding metabolism and stress responses?

2. Materials & methods

2.1. Collection and preliminary procedures

Freshly dispersed fruits of *M. flexuosa* were collected in May 2020, in a natural population, located in the Environmental Protection Area of Rio Pandeiros, municipality of Bonito de Minas, Minas Gerais, Brazil (15°19'49.70"S; 44°57'20.20"O). The fruits were collected from the ground, after natural abscission, from more than 100 individuals, arranged along a route of approximately 2000 m. The yellowish abscission scar was considered as indicative of recent dispersion.

The fruits were stored in water for three days and were pulped, with the aid of a high-pressure washer (Powerwash Eco EWS30, Electrolux, Sweden). Seeds obtained that showed with signs of deterioration by microorganisms and/or insect predation were discarded. The selected seeds were disinfected with a 6 % sodium hypochlorite solution for 15 min, washed three times in running water and treated with a 2 % fungicide solution (Derosal plus, Bayer). The water content was determined in 5 replications of 10 seeds, using the oven method, comparing their fresh and dry weights after dehydration at 105°C for 24 h (Brasil et al., 2009); the results were expressed on a dry basis. The viability of the lot was evaluated by the germination test in 5 replications of 10 seeds, which had the operculum removed to overcome dormancy (Silva et al., 2014), under aseptic conditions, and grown in plastic containers (17 cm×12 cm x 6 cm) with lid, containing autoclaved sand moistened at 80 % of field capacity. The material was kept in a germination chamber (EL 202/4, Eletrolab, São Paulo, Brazil), at 30ºC, in the dark (Silva et al., 2014; Dias et al., 2020). The protrusion of the cotyledonary petiole was considered as a morphological indicator of germination (Ribeiro et al., 2011).

After preliminary tests, the seeds were stored in impermeable polyethylene bags (400μm thick), in a cold room at 20ºC (conditions that allow the maintenance of the physiological status of the seeds, Veloso et al., 2016) for up to 30 days, until the beginning of of the experiments.

2.1.1. Embryo water relations

Mauritia flexuosa is a basal palm belonging to the subtribe

Mauritiinae, subfamily Calamoidae (Dransfield et al., 2008; Reis et al., 2017). The species has a papyraceous and thin endocarp. After fruit abscission, the oleaginous mesocarp and the endocarp are quickly consumed by dispersers and the isolated seed persists in the environment, consisting of the secondary dispersal unit. Dormant seeds incorporated into the seed bank, are subject to water cycles in the soil, inducing water saturation or scarcity (WS) in seeds (Fig. 1). Considering this dynamic, we evaluated the response to WS in isolated embryos. Embryos from newly dispersed seeds were also considered 'newly dispersed', while newly dispersed embryos submitted to immersion in ultrapure water for 24 h were considered 'hydrated', and this terminology will be used from here on. The water content of newly dispersed or hydrated embryos was determined, as previously described, by the oven method (Brasil et al., 2009), in 5 replicates of 10 embryos.

For the determination of water potential (Ψ_w) and simulation of WS conditions, newly dispersed or hydrated embryos were immersed in different osmotic solutions prepared with polyethylene glycol 6000 (PEG 6000), an inert, non-toxic osmotic agent that does not penetrate in cell membranes (Michel and Kaufmann, 1973). The PEG 6000 concentrations were adjusted to provide Ψ_w= 0, −0.3, −0.6, −0.9, −1.2, −1.5, −1.8 and −2.1 MPa - potentials that occur in soils ranging from waterlogged to extremely dry, according to Kirkham (2005). Embryos were kept in the dark at 25ºC, as a reference temperature (Michel and Kaufmann, 1973). The fresh weights of the embryos were measured in the initial condition and after 6, 12 and 24 h of immersion in the osmotic solutions, using 5 replicates of 10 embryos for each concentration of PEG 6000. Water absorption curves were prepared. The Ψ_w was estimated at each evaluated time interval, by adapting the quadratic equation, considering that the absence of water flow indicates Ψ_w embryo = Ψ_w solution (Gonçalves et al., 2020).

2.1.2. Viability evaluation

After 24 h of immersion of the embryos in the osmotic solutions, the tetrazolium test was used to assess viability. The embryos were immersed in a 1 % 2,3,5-triphenyl tetrazolium chloride solution for 4 h, in the absence of light at a temperature of 35 ºC, and viability was defined by staining patterns (Spera et al., 2001; Ribeiro et al., 2010). Additionally, the embryonic respiratory activity was estimated after the period of immersion in tetrazolium. Five mL of 95 % alcohol were added to each tube containing the embryos and the material remained incubated for 15 days, at 10°C, for triphenylformazan extraction. A 3 mL aliquot was taken from the solution and analyzed in a spectrophotometer (Uv-1800, Shimadzu, Kyoto, Japan), at 490 nm (Sershen et al., 2016). The absorbance values obtained were adjusted, considering a blank prepared using boiled dead embryos. The dry mass of the embryos was obtained after dehydration in a forced air circulation oven and the results expressed in absorbance g^{-1} DM.

Intact seeds were immersed in solutions with the same water potential applied to embryos and germination was evaluated. For analyzes corresponding to newly dispersed embryos, intact seeds were submerged in osmotic solutions for 10 days. After this period, to overcome dormancy, the operculum was removed and the seeds were cultivated, as described in the preliminary evaluations of the seed lot. Moreover, the seeds had the operculum previously removed and were cultivated immersed in ultrapure water for 3 days to induce extra water absorption and change in the embryos water status. After this period, the seeds were submerged in the same osmotic solutions for 10 days and transferred to conventional cultivation conditions. In both conditions, germination was evaluated after seven days (Silva et al., 2014) and embryos from non-germinated seeds were submitted to the tetrazolium test.

2.1.3. Definition of Ψ_w for cytological and physiological evaluations

For the cytological and physiological evaluations described hereinafter, the following were selected: the Ψ_w=0, applied to hydrated embryos and the Ψ_w capable of providing moderate and severe WS (Gonçalves et al., 2020, adapted). We considered the hydration curves of the embryos after application of osmotic treatments (Fig. 3A-B) and the results of the viability test (Fig. 3C). Moderate WS was considered to be that which induced reduction in water capacity, i.e., ability of newly dispersed embryos to absorb water and hydrated embryos to retain water (Fig. 3A-B), combined with a reduction in viability to average levels close to 60 % (Fig. 3C). Severe WS was considered to be that which caused a reduction in viability to approximately 30 %. Therefore, both for newly dispersed and hydrated embryos, the Ψ_w that provided moderate and severe WS were, respectively, −1.5 and −2.1 MPa.

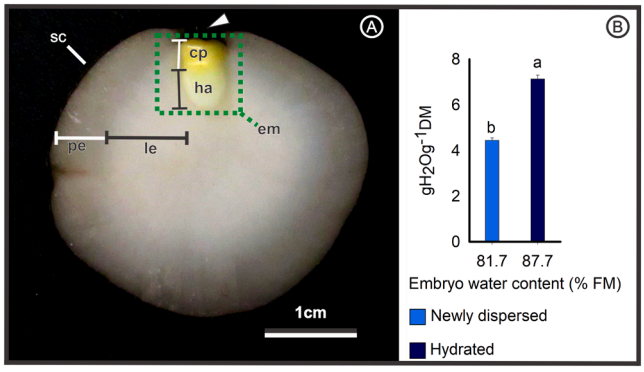

Fig. 2. Longitudinal section of a newly dispersed *Mauritia flexuosa* seed; white arrowhead indicates the operculum (A). Water status of embryos used in the experiments expressed on a dry basis (B). Different letters indicate statistical difference between treatments using the Duncan test ($P \leq 0,05$). Bars represent standard error of the mean. cp, cotyledonary petiole; em, embryo; ha, haustorium; le, lateral endosperm; pe, peripheral endosperm; sc, seed coat.

2.2. Cytological evaluations

For each of the described treatments, cubic fragments (3 mm edges) of the median portion of the haustorium (Fig. 2A) were fixed in Karnovsky's solution (Karnovsky, 1965), dehydrated in an ethylic series and included in (2-hydroxyethyl) methacrylate (Leica Microsystems, Heidelberg, Germany), according to Paiva et al. (2011). Cross-Section (5 μm thick) were obtained using a rotating microtome and stained with 0.05 % toluidine blue, pH 4.7 (O'Brien et al., 1964), modified); the slides were mounted in acrylic resin (Itacril, Itaquaquecetuba, Brazil). (Fig. 3)

Histochemical tests were performed with periodic acid and Schiff's reagent (PAS) (Feder and O'Brien, 1968), to identify polysaccharides; choriphosphine, under UV light (Weis et al., 1988), for pectins; Xilidine-Ponceau (Vidal, 1970), for proteins; Fehling's reagent (Sass, 1951), for reducing sugars; neutral red, under UV light (Kirk, 1970), for lipids and ferric chloride (Johansen, 1940) for identification of phenolic compounds. The sections were evaluated under a photomicroscope (Scope A1/Axiocam 105 Color, Zeiss, Jena, Germany).

For transmission electron microscopy analyses, cubic fragments (2 mm edges) from the median region of the haustorium, including the protoderm and adjacent ground meristem, were fixed in Karnovsky's solution (Karnovsky, 1965) for 24 h, transferred to phosphate buffer of sodium, 0.1 M, pH 7.3 and kept at 8 ºC. The material was post-fixed in 1 % osmium tetroxide, in 0.1 M phosphate buffer, pH 7.2 (Roland, 1978). Ultrathin cross sections were stained with uranyl acetate and lead citrate and examined in a transmission electron microscope (TEM) (Tecnai G2–12-Spirit TEM, Philips/FEI CoMPany, Netherlands), at 80 kV.

Micromorphometric evaluations were carried out on haustorium fragments from 10 embryos from each treatment, processed as described for histochemical evaluations. In cross-sections, the area of 10 randomly selected cells was evaluated in each of 4 regions: protoderm; outer layer of ground meristem; lateral ground meristem and central ground meristem (Fig. 9A). The evaluations were performed using image analysis

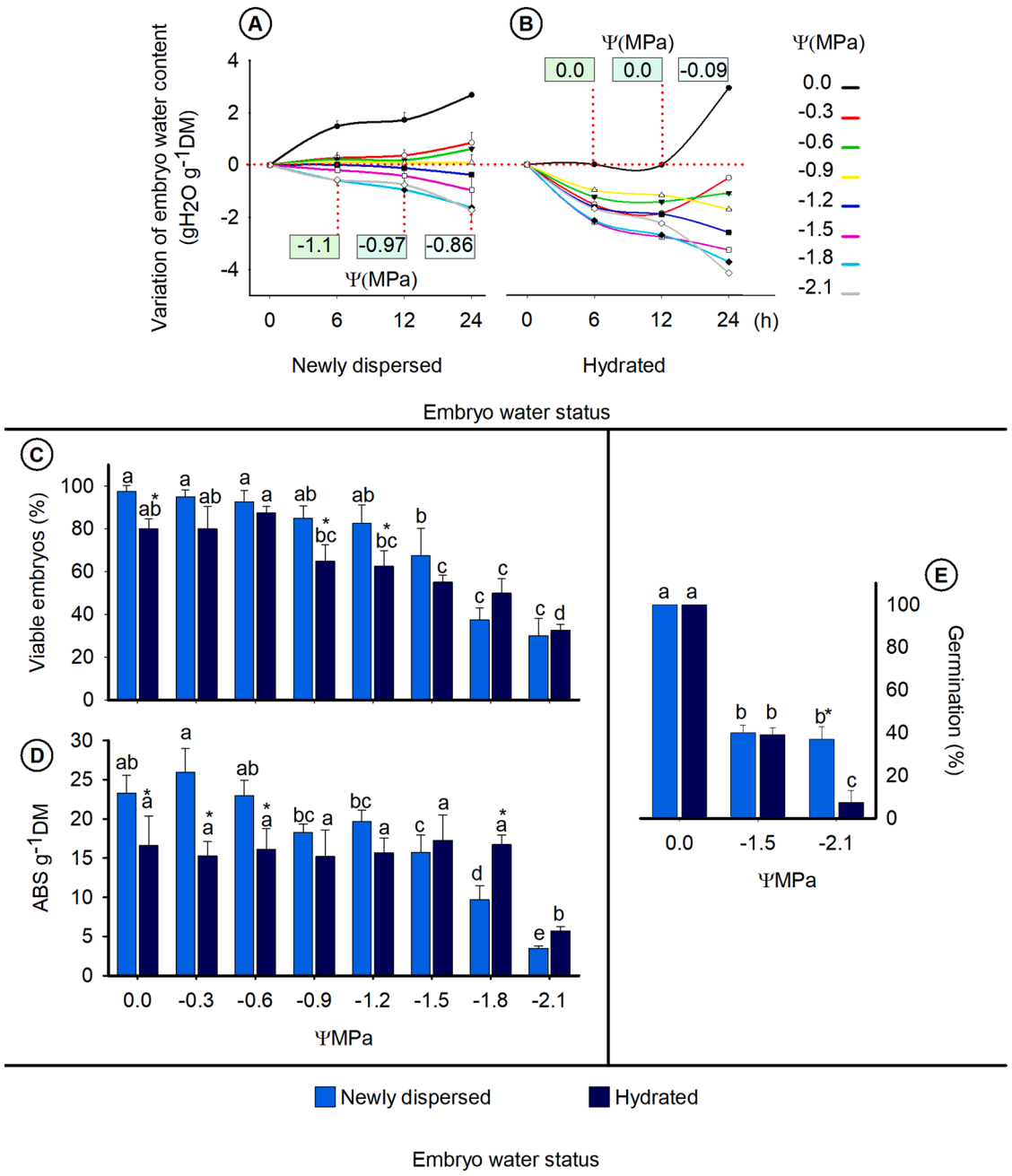

Fig. 3. Variations in water content and Ψ_w of *Mauritia flexuosa* embryos, freshly dispersed (A) or hydrated after dispersion (B), evaluated over 24 h after immersion in different osmotic solutions. The Ψ_w values of the osmotic solutions are represented at the end of the curves. The values in the rectangles represent the Ψ_w of the embryo in each time evaluated; Viability percentages (C) and triphenylformazan absorbance rates (D); Germination percentages after immersion of freshly dispersed or hydrated *Mauritia flexuosa* seeds in osmotic solutions with different Ψ_w (E). Different letters within each freshly dispersed or hydrated condition indicate that the osmotic treatments differed statistically from each other; the asterisk (*) indicates the difference between freshly dispersed and hydrated treatments, according to the Duncan test, ($P \leq 0{,}05$). Bars represent standard error of the mean.

software (Zen, Zeiss, Jena, Germany).

2.3. Physiological evaluations

2.3.1. Membrane integrity estimation

Four replicates of 10 embryos from each treatment were immersed in ultrapure water for 4 h and kept in a germination chamber at 30°C. Electrolyte release was evaluated by means of quantification of electrical conductivity, using a conductivity meter (AKSO, AK83, São Leopoldo, Brazil), according to the methodology adapted from Gomes-Copeland et al. (2012). Results were expressed on the basis of dry mass.

2.3.2. Evaluation of endo-β-mannanase activity

Endo-β-mannanase is the main enzyme involved in the mobilization of mannans, the most abundant carbohydrates in the cell walls of palm seeds (Buckeridge et al., 2000; Buckeridge, 2010). The enzime activity was evaluated, considering the possible role in cell wall carbohydrate dynamics in the response to WS and/or the activation of developmental pathways related to germination. For each treatment, 5 mg samples were obtained from four replicates of five haustoria each. Evaluations were performed 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 g at 4 °C. The supernatant was removed, added to the Locust Bean Gum Galactomannan 0.25 % solution (Sigma, USA) prepared in 0.1 mol L^{-1} of sodium acetate buffer pH 4.7 and maintained in a thermostatic bath with magnetic stirring (Sl-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 hydroxydobenzoic acid - acid hydrazide (Sigma, USA), 5 % (w/v) prepared in an acid medium HCl 0.5 mol L^{-1} and diluted in NaOH 0.5 mol L^{-1}; the mixture was 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 (white), Locust Bean Gum Galactomannan (Sigma, USA) was not added. Readings were performed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 398 nm. The results obtained from the calibration curve were converted into μM equivalent of reducing sugars min^{-1} g^{-1} FM.

2.4. Oxidative stress evaluation

2.4.1. Quantification of hydrogen peroxide (H_2O_2)

Four replicates of 30 mg of embryos from each treatment were macerated in liquid nitrogen. In 2 mL Eppendorf tubes, 400 μL of trichloroacetic acid (TCA) 0.1 % (w/v) were added. The plant extract was homogenized in a vortex for 1 min and centrifuged at 10,000 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 dark for 1 h. Samples remained in the dark at room temperature for 20 min for reaction stabilization, and were analyzed in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 390 nm. The amount of H_2O_2 was expressed in mM g^{-1} FM, based on a pre-established standard curve (Alexieva et al., 2001).

2.4.2. Evaluation of the antioxidant system enzymes activity

Four replicates of 20 mg of embryos from each treatment were obtained after maceration in liquid nitrogen. The plant extract was homogenized in polyvinylpyrrolidone (PVPP) 10 % m/v, 500 μL of 50 mM sodium phosphate buffer, pH 6.8, 10 μL of 100 μM edetic acid (EDTA) and 490 μL of deionized water, in vortex, 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) activity were evaluated in 100 μL of the supernatant.

SOD activity was determined by adding the extract to a solution containing 13 mM methionine, 75μM nitroblue tetrazolium (NBT), 100μM EDTA, 2μM riboflavin 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, activity termination was determined by light interruption. 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 SOD unit was defined as the amount of enzyme required to inhibit 50 % of NBT photoreduction. Results were demonstrated in U min mg^{-1} protein (Giannopolitis and Ries, 1977).

To determine the CAT activity, the extract was added to a 50 mM potassium phosphate buffer, pH 7.0, with freshly prepared H_2O_2 added (12.5 mM) at the time of analysis. The analysis was performed in a spectrophotometer 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 H_2O_2 min^{-1} mg^{-1} 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 H_2O_2. The ascorbic acid oxidation rate was monitored for 1 min, recording the absorbance values at 290 nm every 10 s in a spectrophotometer. APX activity was determined using ε equal to 2.8 mM^{-1} cm^{-1}, and the results were expressed in μM ascorbate min^{-1} mg^{-1} protein (Nakano and Asada, 1981).

2.4.3. Malondialdehyde (MDA) quantification

Four replicates of 30 mg of embryos from each treatment were macerated in liquid nitrogen and added to 1.5 mL of trichloroacetic acid (TCA) 0.1 % (w/v). 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 a 0.5 % (w/v) thiobarbituric acid solution prepared in 20 % TCA. The solution remained for 60 min at 95 °C and then cooled on ice for 10 min. The samples were analyzed in a spectrophotometer at wavelengths of 532 and 660 nm. Interfering elements were eliminated by subtracting the values obtained at 532–660 nm and the amount of MDA was expressed in nM g^{-1} FM (Heath and Packer, 1968, adapted).

2.5. Statistical analysis

Data were evaluated in a factorial scheme, considering factor A, as the Ψ_w employed, and factor B as the hydric status of the newly dispersed or hydrated embryo. Means were submitted to analysis of variance and, when significant differences were found, they were compared using the Duncan test ($P \leq 0.05$). Biochemical data for newly dispersed or hydrated embryos were subjected to Spearman correlation analysis ($P \leq 0.05$). The R software version 4.2.2 (Easyanova package, version 8.0) was used.

3. Results

The *M. flexuosa* seed lot showed 100 % germination, in eight days, after mechanical removal of the operculum (treatment to overcome dormancy). The seeds were dispersed with a water content of 48.7 % (0.95 g H_2O g^{-1} DM).

3.1. Embryo water relations

The newly dispersed seeds of *M. flexuosa* are globose and have a dark brown and thin seminal envelope, which covers the abundant and whitish endosperm with distinct shades between the peripheral and lateral portions (Fig. 2A). The endosperm completely surrounds the embryo, which is located in the micropylar region and adhered to the operculum. The embryo is divided into two regions: the yellowish proximal one, corresponding to the cotyledonary petiole, and the whitish distal one, corresponding to the haustorium (cotyledonary blade). The dry mass of the whole seed is 6.41 g and the dry mass of the isolated embryo is 0.012 g. Thus, the embryo represents 0.2 % of the dry mass of the seed.

Freshly dispersed embryos had 4.44 g H_2O g^{-1} DM and, when subjected to Ψ_w= 0 MPa for 24 h, increased water content to 7.12 g H_2O g^{-1} DM (Fig. 2B). With the gradual reduction of the Ψ_w of the osmotic solutions, there was a reduction in the water absorption capacity of the newly dispersed embryos, however, even in the embryos subjected to Ψ_w= −0.9 MPa, it was possible to observe increases in the water content (Fig. 3A). Reductions in the water content of newly dispersed embryos occurred after treatment with Ψ_w= −1.2 MPa, and the osmotic solution capable of providing severe stress (Ψ_w= −2.1 MPa) induced a reduction in water content in the embryo to 2.72 g H_2O g^{-1} DM after 24 h.

Embryos hydrated and submitted to Ψ_w= 0 MPa, for another 24 h, markedly increased the water content (gain of 2.93 g H_2O g^{-1} DM) which provided hyperhydration between 12 and 24 h of immersion (Fig. 3B). All applied Ψ_w caused a reduction in water content in hydrated embryos, however, in the cases of application of Ψ_w= −0.3 and −0.6 MPa, there was water absorption and partial recovery of water status, from 12 h of immersion. When Ψ_w= −2.1 MPa was applied, the intensity of reduction in water content of the hydrated embryo was lower between 6 and 12 h, when compared to the effects of Ψ_w = −1.5

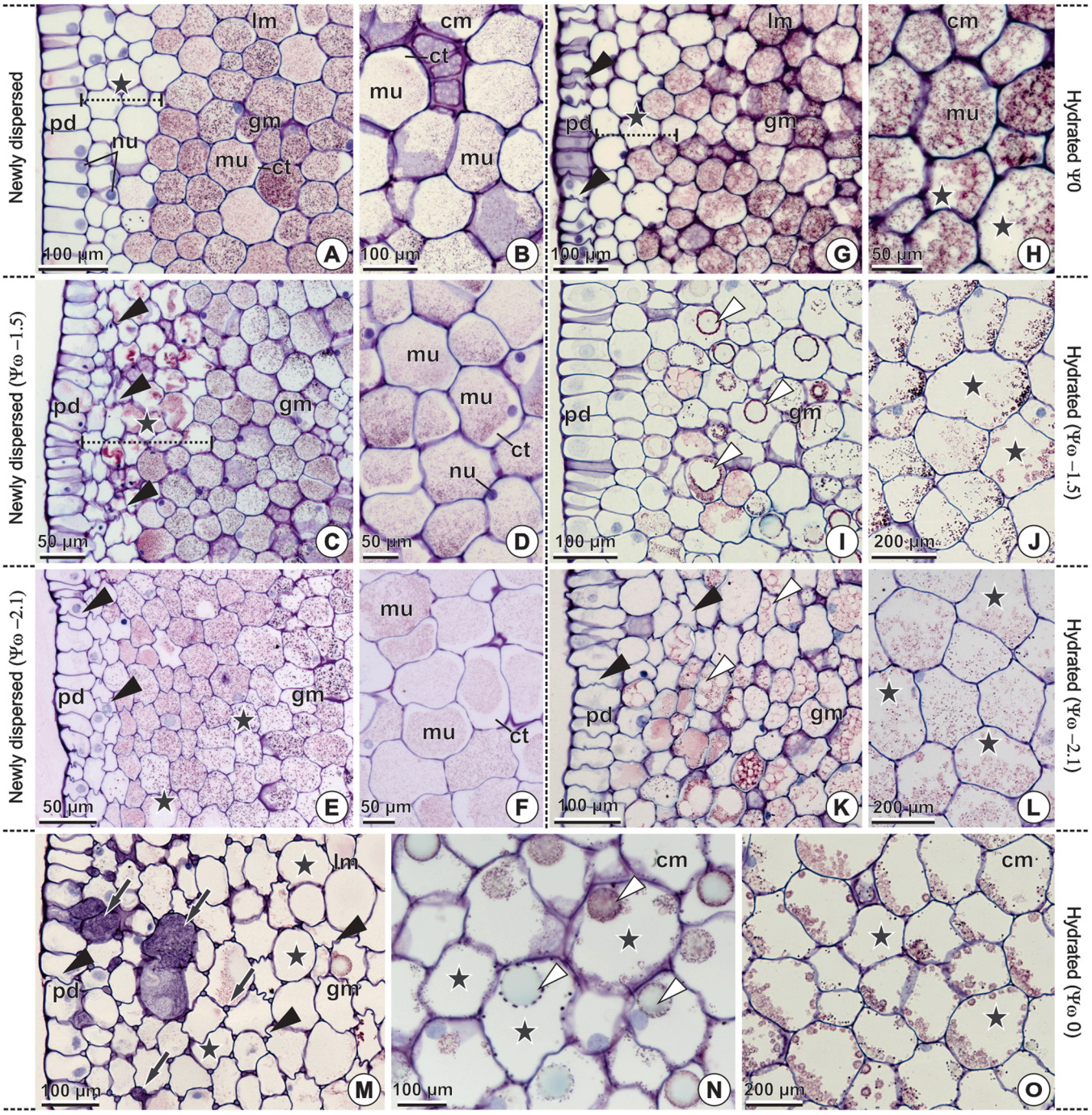

Fig. 4. Cross sections of the haustorium of freshly dispersed or hydrated *Mauritia flexuosa* embryos, after immersion in osmotic solutions with different Ψ_w. Region of the protodermis and lateral ground meristem (A, C, E, G, I, K, M). Ground meristem in the central region of the haustorium (B, D, F, H, J, L, N, O). Layers with hyaline cells adjacente to protodermis (dashed lines); mobilization of simplastic content (stars); association of phenolic compounds and reserve content (white arrowheads); cell wall sinuosities (black arrowheads); disruption of cell walls and accumulation of substances in the intercellular space (black arrows). cm, central ground meristema; gm, ground meristema; lm, lateral ground meristema; mu mucilage; pd, protoderm.

and −1.8 MPa. However, after this period, the reduction in water content was intensified in embryos submitted to $\Psi_w = -2.1$ MPa.

The Ψ_w of the newly dispersed embryo increased by 22 % between 6 and 24 h, with an average between the evaluated times, equal to −1 MPa (Fig. 3A). Hydration promoted an increase of about 90 % in the Ψ_w of the embryos, which reached −0.09 MPa, after 24 h (Fig. 3B).

3.2. Viability and germination

Both in newly dispersed and hydrated embryos, viability began to reduce after the application of $\Psi_w = -1.5$ MPa, with an approximate average of 60 %, with no difference between conditions (Fig. 3C). Viability was reduced to about 30 %, in embryos with both water status, with the application of $\Psi_w = -2.1$ MPa. Freshly dispersed embryos showed greater viability, when compared to hydrated ones, when submitted to $\Psi_w = 0$, −0.9 and −1.2 MPa.

Embryonic respiratory activity gradually decreased in newly dispersed embryos, from the application of $\Psi_w = -1.5$ MPa (Fig. 3D). In the case of hydrated embryos, embryonic respiration was reduced only when they were subjected to severe stress. Freshly dispersed

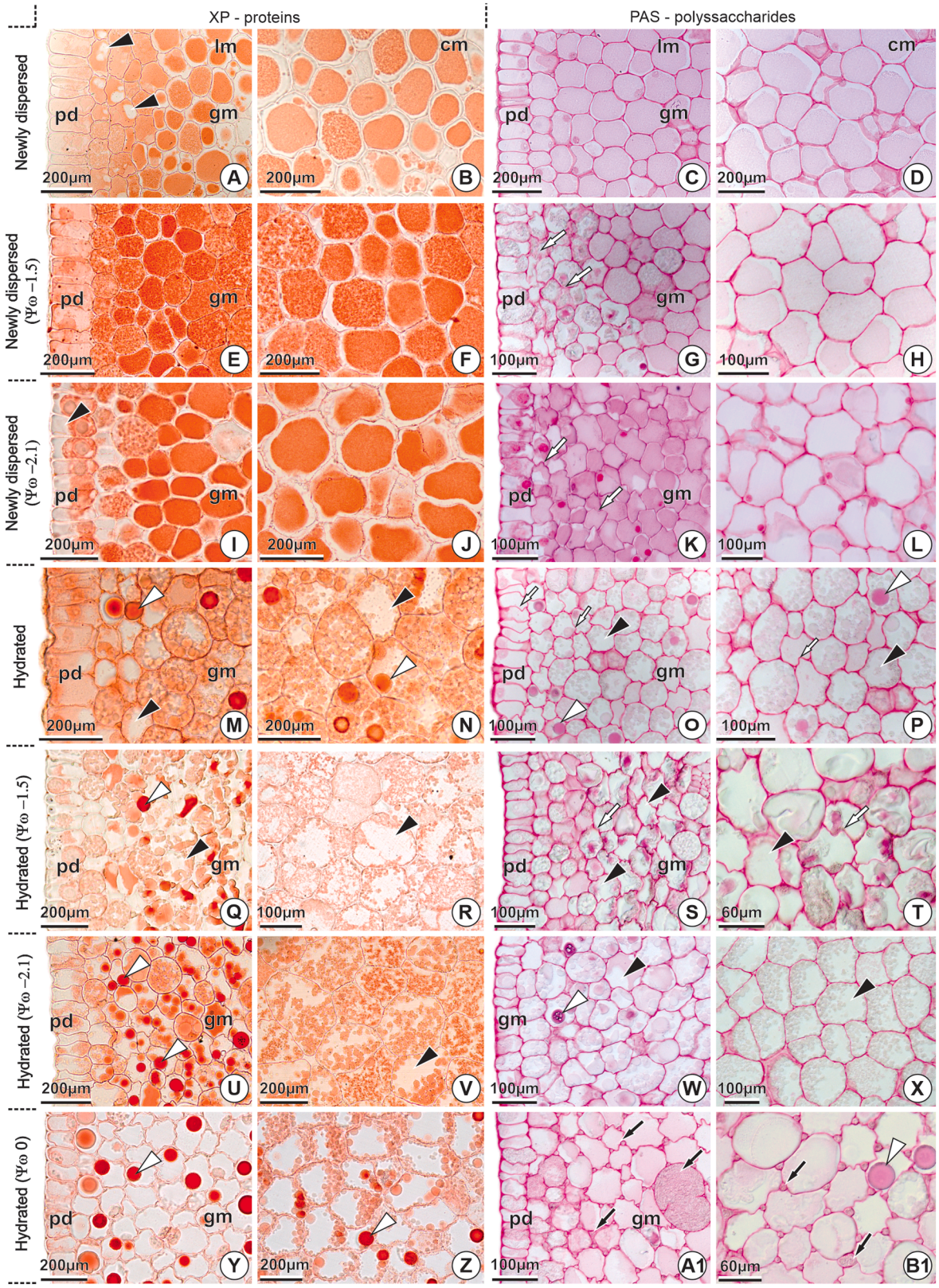

Fig. 5. Cross sections of the median portion of the haustorium of freshly dispersed or hydrated *Mauritia flexuosa* embryos, after immersion in osmotic solutions with different Ψ_w. Proteins associated with the protodermis and lateral ground meristem, revealed by the reddish color in reaction to the Xylidine-Ponceau (XP) reagent (A, E, I, M, Q, U, Y). Proteins associated with the central ground meristem of the haustorium (B, F, J, N, R, W, Z). Carbohydrates associated with the cell walls and protoplasts of the protodermis and lateral fundamental meristem, revealed by magenta staining with periodic acid and Schiff reagent (PAS) (C, G, K, O, S, V, A1). Carbohydrates stored in the central ground meristem cells of the haustorium (D, H, L, P, T, X, B1). Vacuolation indicating mobilization of reserves (black arrowhead); association of proteins or carbohydrates with phenolic compounds (white arrowhead); disruption of the cell wall and accumulation of carbohydrates in the intercellular space (black arrow); cell wall sinuosities (white arrow). cm, central ground meristem; lm, lateral ground meristem; pd, protoderm; gm, ground meristem.

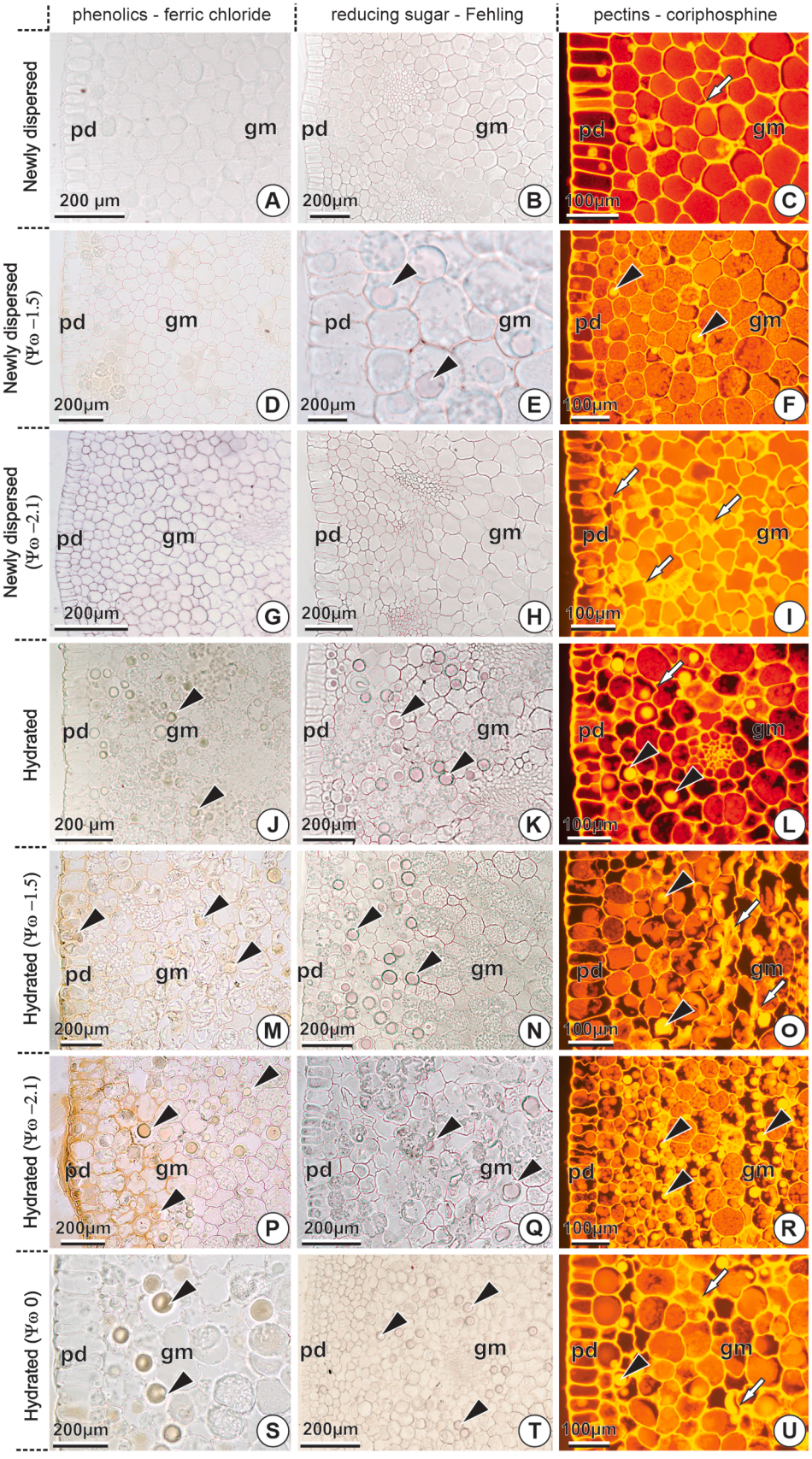

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Fig. 6. Cross sections of the protodermis and ground lateral meristem, in the median portion of the haustorium of freshly dispersed or hydrated *Mauritia flexuosa* embryos, after immersion in osmotic solutions with different Ψ_w. Phenolic compounds marked brown in reaction to ferric chloride (black arrowhead) (A, D, G, J, M, P, S). Reducing sugars revealed by red color, in reaction to Fehling's reagent (black arrowhead) (B, E, H, K, N, Q, T). Acidic polysaccharides revealed by the orange secondary fluorescence reaction, after reaction with corifosphina reagent, under UV light (C, F, I, L, O, R, U). Cell wall (white arrow); association of acidic polysaccharides and phenolic compounds (black arrowhead). pd, protoderm; gm, ground meristem.

embryos, compared to hydrated ones, maintained higher respiratory rates after treatments with Ψ_w= 0, −0.3, −0.6 MPa. The opposite occurred in embryos subjected to Ψ_w= −1.8 MPa, a situation in which the respiratory rate was higher for hydrated embryos.

Freshly dispersed seeds showed 100 % germination when cultivated with pure water, and there was a reduction in the germination rate to 38 %, when they were cultivated in osmotic solutions with Ψ_w= −1.5 and −2.1 MPa (Fig. 3E). In the case of previously hydrated seeds, the percentage of germination reduced when subjected to Ψ_w= −1.5 MPa, remaining similar to newly dispersed seeds. However, when osmotic solution that induced severe stress was used, the germination percentage dropped dramatically, with an average of 7.5 %. Thirty and 20 % of the newly dispersed seeds, respectively, did not germinate when subjected to Ψ_w= −1.5 and −2.1 Mpa. However, 80 and 50 % of the embryos from these seeds showed viability (respectively to Ψ_w= −1.5 and −2.1), when evaluated by the tetrazolium test. The other seeds, freshly dispersed or hydrated, that did not germinate showed a deteriorated embryo, a condition that prevented the evaluation of viability by the tetrazolium test, therefore, they were considered dead after going through the water deficit.

3.3. Anatomical evaluation

The newly dispersed embryos presented radially elongated protodermal cells, juxtaposed, and with evident nuclei (Fig. 4A). Two to three layers of subprotodermal cells in the ground meristem were hyaline; the other cell layers showed a voluminous vacuole containing mucilage with a floccular appearance, peripheral extravacuolar cytoplasm and the intercellular spaces were inconspicuous (Fig. 4 A-B). In embryos subjected to moderate stress (Ψ_w = −1.5 Mpa), there was vacuolation in the cells of the lateral ground meristem, indicating mobilization of reserves and the cell walls showed sinuosity (Fig. 4C). In the central region of the ground meristem, there were no evident cellular alterations (Fig. 4D). When the embryos were subjected to severe stress (Ψ_w= −2.1 Mpa) the cells of the protoderm and underlying ground meristem showed sinuosity in the cell wall, and only a few cells of the ground meristem showed vacuolation (Fig. 4E) and the stored content remained preserved (Fig. 4F).

Hydrated embryos showed sinuosity in the cell walls of the protoderm cells and partial mobilization of reserves (Fig. 4G-H). In response to moderate and severe stress in hydrated embryos, there was mobilization of reserves stored in ground meristem cells (Fig. 4I-L) and accumulation of droplets with mixed content (phenolic compounds and mucilages) in the vacuoles (Fig. 4I, K). Severe stress induced sinuosity in the cell walls of the protoderm and lateral ground meristem (Fig. 4K). Embryos that underwent hyperhydration showed disruption of the cell wall with extravasation of contents into the intercellular space in the ground meristem (Fig. 4M). Reserve material in the vacuoles was scarce and was usually associated with mixed phenolic compounds (Fig. 4M-O).

3.4. Histochemical evaluation

The newly dispersed *M. flexuosa* embryos showed mixed reserve content composed of proteins and carbohydrates in the protoplast of the protoderm cells and in the vacuoles of the ground meristem cells (Fig. 5A-D). Some cells of the lateral ground meristem, close to the protoderm, showed vacuolation indicating mobilization of proteins (Fig. 5A) and cell walls composed of carbohydrates (Fig. 5C-D). In the haustorium of newly dispersed embryos subjected to moderate stress (Ψ_w= −1.5 MPa) no obvious changes were observed in relation to protein reserves (Fig. 5E-F). Carbohydrates were mobilized in some layers of the lateral ground meristem, underlying the protoderm; cell walls presented sinuosities (Fig. 5G), and no alterations were observed in the central region of the ground meristem (Fig. 5H). In embryos submitted to severe stress (Ψ_w= −2.1 MPa) the configuration of the reserves remained similar in comparison with the newly dispersed embryos (Fig. 5I-L), however, the protoplasts underwent retraction (Fig. 5I) and cell walls were sinuous (Fig. 5K).

Hydration of the embryos promoted partial mobilization of reserves, accumulation of mixed protein and carbohydrate compounds in association with phenolic compounds (Fig. 5M-P) and cell wall sinuosities (Fig. 5O-P). All treatments to which the hydrated embryos were submitted induced mobilization of reserves and accumulation of phenolics associated with proteins and carbohydrates (Fig. 5Q-B1). The exception was observed in embryos subjected to moderate stress, in which mixed carbohydrates with phenolics did not occur and the cell walls showed sinuosity (Fig. 5S-T). In the hydrated embryos that remained in the water, there was disruption of the cell wall in the fundamental meristem and leakage of the contents into the intercellular space (Fig. 5 A1-B1).

The newly dispersed embryos did not show accumulation of phenolic compounds (Fig. 6A) and reducing sugars (Fig. 6B). Pectins were labeled as structural components in protodermal cell walls and ground meristem (Fig. 6C). With the application of moderate stress, there was no labeling of phenolics (Fig. 6D), but reducing sugars and pectins were present in ground meristem cells (Fig. 6E-F). In embryos subjected to severe stress there was no identification of phenolic compounds and reducing sugars (Fig. 6G-H), but pectic cell walls showed sinuosity (Fig. 6I). All treatments applied to hydrated embryos promoted the accumulation of phenolics conjugated with reducing sugars and pectins (Fig. 6J-U). Lipids were not detected by the neutral red test in any of the evaluated conditions.

3.5. Ultrastructural evaluation

In newly dispersed embryos, the protoderm showed cells with an outer periclinal wall thicker than the others, and plasmodesmata connecting adjacent cells (Fig. 7A-B). The protoplast was poor in organelles, containing mitochondria with poorly developed cristae, reduced vacuoles and dispersed lipid droplets. The cytoplasm was filled with floccular mucilages and small vesicles were present on the periphery of the cell. Ground meristem cells presented a voluminous vacuole containing mucilage (Fig. 7C). The extravacuolar cytoplasm was not very dense and restricted to a narrow band containing some plastids and lipid droplets (Fig. 7C, D). In newly dispersed embryos submitted to moderate stress (Ψ_w= −1.5 Mpa), the external periclinal cell wall of the protoderm presented layers with different electron densities, and there was an accumulation of vesicles in the periplasmic space and in the periphery of the cytoplasm (Fig. 7E). The cytoplasm was dense, rich in organelles, especially mitochondria with developed cristae and plastids that predominantly occurred around the nucleus (Fig. 7F-G). Small vacuoles containing reserve remnants were commonly flanked by lipid droplets (Fig. 7G). In the ground meristem it was possible to observe cell wall sinuosities (Fig. 7H), the formation of vacuoles and the presence of small mitochondria and proplastids in the periphery of the cytoplasm and around the nucleus (Fig. 7I-J). Severe stress (Ψ_w= −2.1 MPa) caused major changes in the embryo cells (Fig. 7K-S), especially the strong retraction of the plasma membrane, with accumulation of substances in

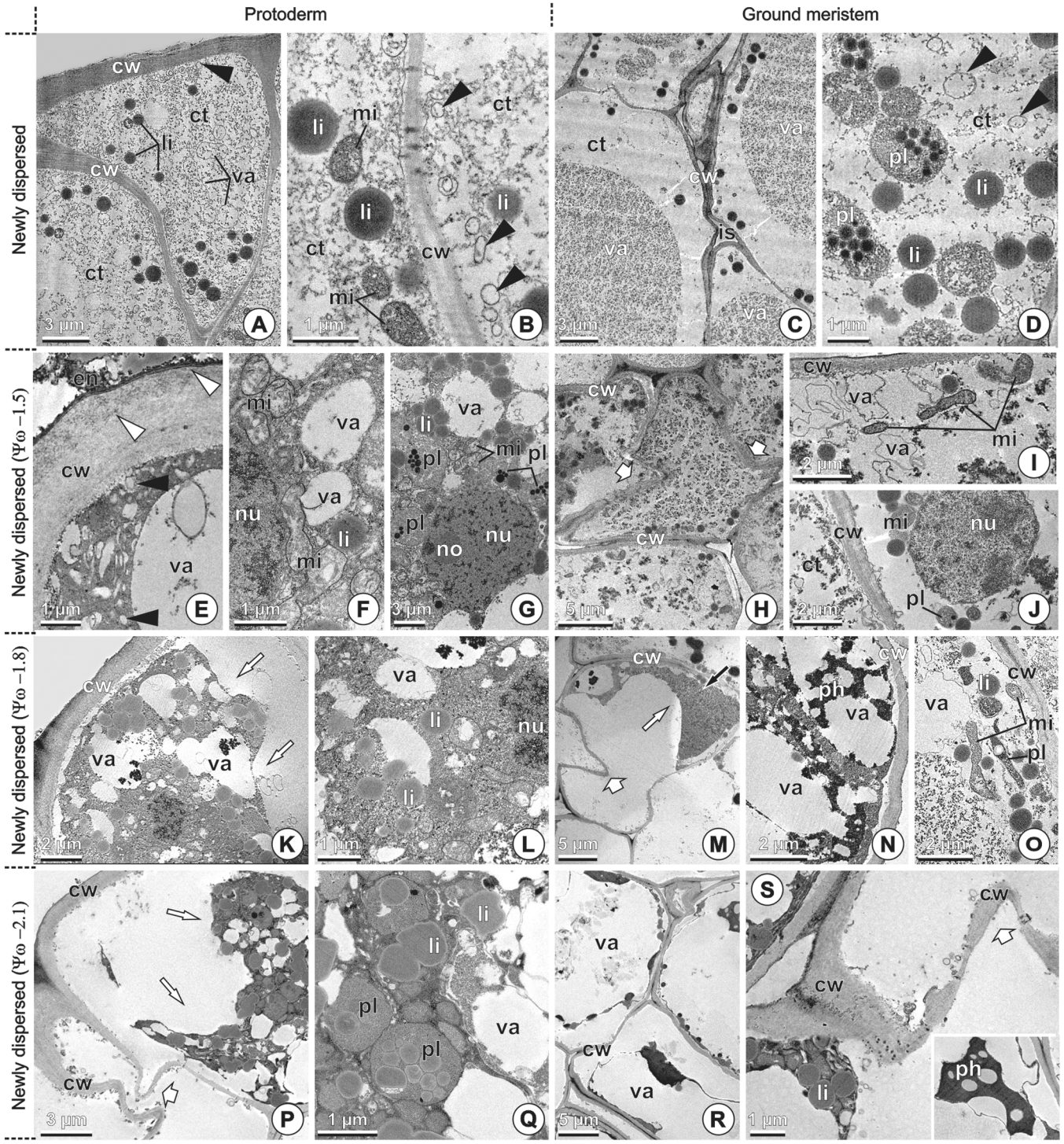

Fig. 7. Ultrastructure of the protodermis and ground meristem of the haustorium, after immersion of freshly dispersed *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Cells with a thick outer periclinal wall, mucilage in the cytoplasm, lipids and poorly developed mitochondria (A-B). Cells with thin walls, large central vacuole containing mucilage (C). Periphery of the cell with small vesicles (black arrowhead), plastids and lipid droplets (D). Proliferation of mitochondria with developed cristae; plastids and lipid droplets around the nucleus; vacuolization; more electron-dense regions in the wall (indication of substance transport) (white arrowhead); sinuosities in the cell wall indicating cell collapse (wide arrow); cell periphery and periplasmic space with small vesicles (black arrowhead) (E-J). Retraction of the plasma membrane (white arrow) and condensation of the protoplast with accumulation of lipid droplets and plastids; vacuolation and accumulation of phenolic compounds in the vacuole; protoplast contraction (black arrow); sinuosities in the cell wall indicating cell collapse (wide arrow) (K-S). ct, cytoplasm; cw, cell wall; en, endosperm; is, intercellular space; li, lipids; mi, mitochondria; no, nucleolus; nu, nucleus; ph, phenolic compounds; pl, plastids; st, starch; va, vacuole.

the periplasmic space (Fig. 7K, P), conspicuous sinuosity in the cell wall (Fig. 7M, P, S), dense, peripheral cytoplasm and accumulation of phenolic compounds in the vacuoles (Fig. 7 K, L, N, S). The nucleus presented a destructured envelope (Fig. 7K-L).

In hydrated embryos, there was a reduction in the reserve content, with evidence of mobilization, associated with plasma membrane retraction, and accumulation of substances in the periplasmic space (Fig. 8A-B). Lipid droplets and plastids with lipid accumulation occurred

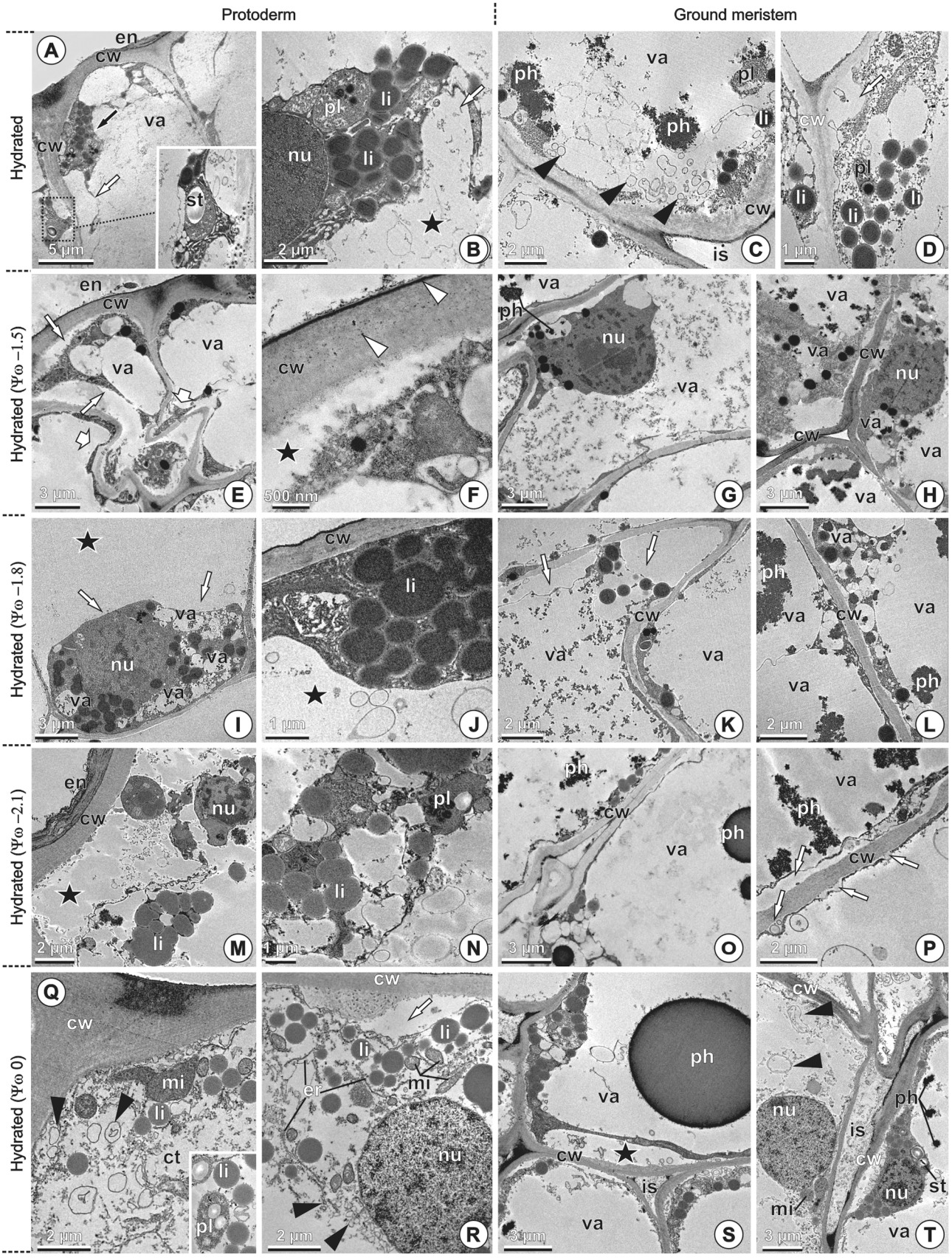

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Fig. 8. Ultrastructure of the protodermis and ground meristem after immersion of hydrated *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Vacuolation showing mobilization of reserves; the inset indicates starch grains; contraction of the dense protoplast (black arrow); plasma membrane retraction (white arrow); periplasmic space with accumulation of substances (black star); lipid droplets and plastids with stored material, close to the nucleus (A-B). Accumulation of vesicles at the periphery of the cytoplasm (black arrowhead); phenolic compounds in the vacuole; plasma membrane retraction (white arrow); lipid droplets and plastids were present in the cytoplasm (C-D). Damaging effect of treatment with strong cell wall sinuosity (wide arrow); plasma membrane retraction (white arrow); indication of substance transport (white arrowhead); periplasmic space with accumulation of substances (black star); collapse of the plasma membrane and nucleus (E-F). Maintenance of cellular structure, with reserves of mucilage and phenolics in the vacuole, peripheral cytoplasm with an evident nucleus (G-H). Strong retraction of the plasma membrane (white arrow); dense and peripheral cytoplasm; substances in the periplasmic space (black star); nuclear envelope collapse; coalescence of lipid droplets, phenolics in vacuoles (I-P). Membrane retraction and accumulation of substances (white arrow). Cytoplasm rich in organelles; notable proliferation of the endoplasmic reticulum, usually associated with ribosomes, adjacent to the plasma membrane and around the nucleus; numerous vesicles dispersed in the cytoplasm and around the nucleus (Q-R). Strong cell wall sinuosity, accumulation of substances in the periplasmic space, phenolic compounds in the vacuole (S-T). ct, cytoplasm; cw, cell wall; em, endosperm; is, intercellular space; li, lipids; mi, mitochondria; no, nucleolus; nu, nucleus; ph, phenolics; pl, plastids; st, starch; va, vacuole.

around the nucleus, and amyloplasts were dispersed in the cytosol. In the cells of the ground meristem there was a slight retraction of the membrane; plastids, lipid droplets, and numerous vesicles occurred at the cell periphery, and phenolic compounds were present in the vacuole (Fig. 8C-D). Embryos hydrated and subjected to moderate and severe stress, in general, contained less stored reserve content compared to cells from freshly dispersed embryos (Fig. 8 E-P), however, vacuolization and accumulation of phenolic compounds were observed (Fig. 8H, L, N, O). The greatest structural deleterious effects were related to cell wall sinuosity (Fig. 8E) and damage to the plasma membrane with protoplast retraction (Fig. 8F, I, K). Severe stress caused disruption of the nuclear envelope of protodermal cells (Fig. 8I, M).(Fig. 9)

In hyperhydrated embryos, protoderm cells showed enhanced metabolic activity (Fig. 8Q-R). Many vesicles, mitochondria, amyloplasts and endoplasmic reticulum were observed close to the prominent nucleus. In the ground meristem cells there were phenolic compounds stored in the hyaline vacuole and some vesicles were also observed (Fig. 8S-T). Starch grains and lipid droplets occurred in the peripheral cytoplasm.

3.6. Micromorphometry

The cross-sectional areas of the haustorium protoderm cells did not show variations in the embryos evaluated in the two water statuses, freshly dispersed or hydrated, after being submitted to different values of Ψ_w (M= 533.33 μm^2; *P*= 0, 5766) (Fig. 9A). The same occurred in the cells of the lateral ground meristema (*M*= 1244.65 μm^2; *P*= 0.9801). In the outer region of the ground meristem, the cells showed greater area in hydrated embryos, compared to newly dispersed embryos (Fig. 9B), however, the applications of osmotic treatments did not influence this characteristic. In the central ground meristem, the cells showed an increase in area with hydration (Fig. 9C), however, with the application of solutions that induced moderate and severe stress, no variations were observed, compared to the initial condition. In hydrated embryos, the cellular area remained constant when they were submitted to different osmotic solutions. Hydrated embryos showed greater cell area in the central region of the fundamental meristem, in the control condition and after being submitted to severe water stress.

3.7. Estimation of the integrity of cell membranes

A significant increase in electrical conductivity was observed in the testing solutions only when the newly dispersed embryos were subjected to Ψ_w= 0 (hydration), and also when they were subjected to severe stress with an osmotic solution of −2.1 MPa (Fig. 10). Moderate stress did not alter the functionality of the cell membranes of the embryos, compared to the freshly dispersed condition. In the case of hydrated embryos, immersion in ultrapure water (hyperhydration) and in the evaluated osmotic solutions, promoted increases in solute leaching. Hydrated embryos submitted to different Ψ_w had higher levels of solute leaching, compared to freshly dispersed embryos.

3.8. Activity of endo-β-mananase

In newly dispersed embryos, submitted to different Ψ_w, the activity of endo-β-mannanase was not verified (Fig. 11). However, for hydrated embryos, severe stress (Ψ_w= −2.1 MPa) induced enzyme activity.

3.9. Oxidative stress

Hydration of newly dispersed embryos promoted reductions in H_2O_2 contents, whereas with the application of Ψ_w= −1.5 and −2.1 MPa the contents increased (Fig. 12A). For hydrated embryos, hyperhydration did not affect the levels of H_2O_2, however, in embryos under moderate and severe stress, there was a significant increase in the levels of this molecule. The production of H_2O_2 was significantly higher in newly dispersed embryos, in relation to those hydrated, in the control treatment (initial condition), and when they were submitted to osmotic solutions that caused moderate and severe stress.

APX activity was higher in the freshly dispersed embryos than in the hydrated embryos, respectively, 5.025 and 2.33 μmol of ascorbate min^{-1} mg^{-1} protein (*P*=0.0457); there was no effect from the application of osmotic treatments (*P*= 0.906). CAT activity did not show significant variations within each condition, except for a reduction in its activity in hydrated embryos subjected to moderate stress Ψ_w=-1.5 MPa (data not shown). There was no difference in CAT activity between freshly dispersed and hydrated embryos, M= 5360.8 μMol H_2O_2 min^{-1} mg^{-1} protein (*P*= 0.75). SOD activity did not vary among newly dispersed embryos subjected to the stressed conditions (Fig. 12B). However, for hydrated embryos, SOD activity increased significantly, similarly under conditions of moderate and severe stress.

The concentration of the lipid peroxidation indicator MDA increased significantly when newly dispersed embryos were subjected to moderate and severe stress conditions (Fig. 12C). For hydrated embryos, no osmotic condition altered MDA levels. MDA levels were higher in newly dispersed embryos, submitted to moderate and severe stress compared to hydrated embryos.

A significant correlation was found between SOD activity and the amount of MDA (S= 0.754, P= 0.005) in newly dispersed embryos. In the case of hydrated embryos, the correlations between SOD activity and amount of MDA (S= 0.628. P=0.029); SOD activity and amount of H_2O_2 (S= 0.685, P=0.017) and amounts of MDA and H_2O_2 (S= 0.856, P= <0.001) were significant.

4. Discussion

This is the first work addressing the influence of soil water cycles, experimentally simulated, on recalcitrant and dormant seeds, which form persistent seed banks. Through structural and physiological evaluations in embryos, characteristics that influence the longevity of *M. flexuosa* seeds were described. Although embryos show resilience to water stress, their persistence and successful germination are dependent on highly hydrated conditions, which highlight their vulnerability to environmental changes. A summary of the main responses to water

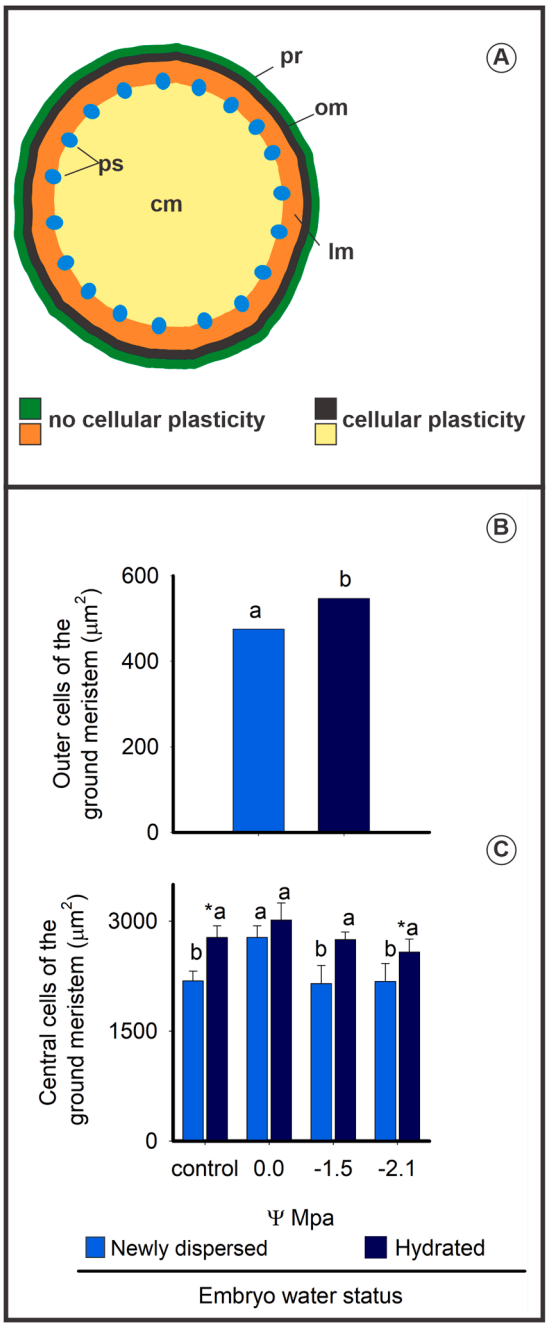

Fig. 9. Cellular micromorphometry of the median portion in cross section of the haustorium after immersion of freshly dispersed or hydrated *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Cross-sectional diagram of the median region of the haustorium showing the four regions evaluated (A). Cross-sectional area of cells in the outer layer of the ground meristem (B). Cross-sectional area of the central cells of the ground meristem (C). Different letters within each freshly dispersed or hydrated condition indicate that the treatments differed statistically from each other; asterisk (*) indicates difference between freshly dispersed and hydrated treatments by Duncan's test, ($P \leq 0,05$). Bars represent standard error of the mean. cm, central ground meristem; lm, lateral ground meristem; om, outer layer of the ground meristem; pr, protoderm; ps, procambial strands.

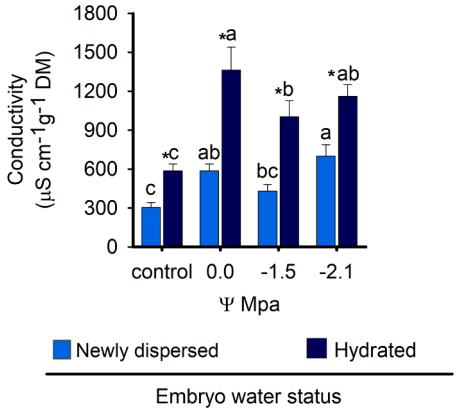

Fig. 10. Electrical conductivity of the test solution, after immersion of freshly dispersed or hydrated *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Different letters within each freshly dispersed or hydrated condition indicate that the treatments differed statistically from each other; asterisk (*) indicates difference between freshly dispersed and hydrated treatments by Duncan's test, ($P \leq 0,05$). Bars represent standard error of the mean.

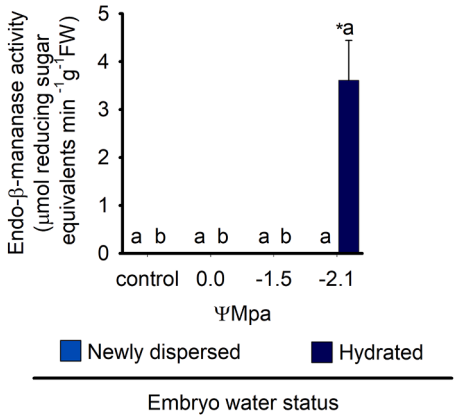

Fig. 11. Endo-β-mannanase activity after immersion of freshly dispersed or hydrated *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Different letters within each freshly dispersed or hydrated condition indicate that the treatments differed statistically from each other; asterisk (*) indicates difference between freshly dispersed and hydrated treatments by Duncan's test, ($P \leq 0,05$). Bars represent standard error of the mean.

stress identified in *M. flexuosa* embryos is presented in Fig. 13.

4.1. Water dynamics in embryos under water stress

Mauritia flexuosa embryos are dispersed highly hydrated (4.44 g H_2O g^{-1} DM) and begin to lose viability with a 22 % decline in water content, when subjected to moderate stress ($\Psi_w = -1.5$ MPa) (Fig. 3A), which are considered indications of high recalcitrance (Berjak and Pammenter, 2007; Obroucheva et al., 2016). However, embryos show remarkable resilience to water stress. Even when subjected to severe stress, some embryos maintain viability (Fig. 3C, D) and seeds germinate at rates close to 40 % (Fig. 3E). It is interesting to note that $\Psi_w = -2.1$, used here to simulate severe stress, is not common in soils and induces permanent wilting in several cultivated species (Roberts and Ellis, 1989; Kirkham, 2005).

The resilience to water deficit in the recalcitrant embryo of *M. flexuosa* is directly related to its ability to absorb and retain water. Most work on recalcitrant seeds has focused on water amounts bordering on desiccation tolerance (Marques et al., 2018; Obroucheva, 2016), however, little is known about the cells' primary responses to dehydration (Walters, 2015). Our results confirm that homeostasis in *M. flexuosa* embryonic cells is dependent on maintaining abundant reserves of highly hydrophilic mixed mucilaginous reserves, composed of carbohydrates and proteins (Silva et al., 2014; Veloso et al., 2016; Dias et al., 2020). In addition, the structure of the endosperm also contributes

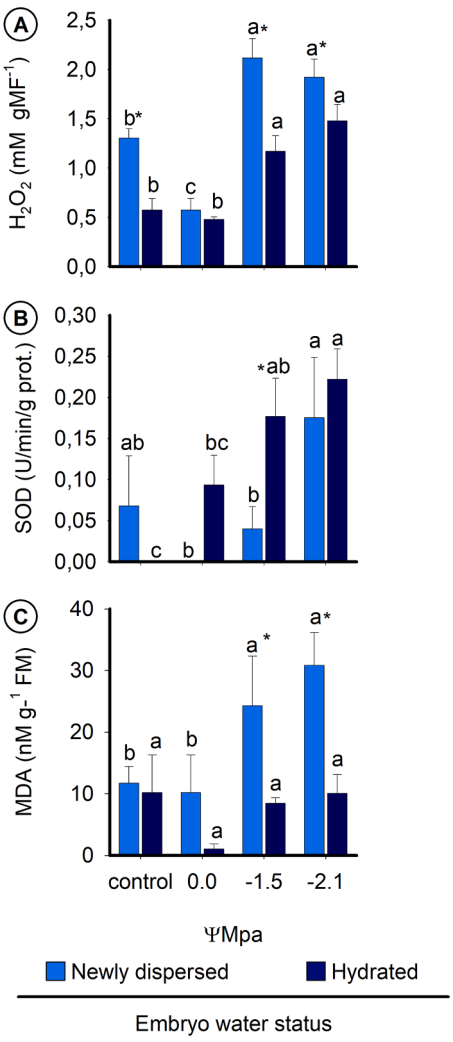

Fig. 12. Hydrogen peroxide (H_2O_2) contents (A), SOD activity (B) and MDA contents (C) after immersion of freshly dispersed or hydrated *Mauritia flexuosa* embryos in osmotic solutions with different Ψ_w. Different letters within each freshly dispersed or hydrated condition indicate that the treatments differed statistically from each other; asterisk (*) indicates difference between freshly dispersed and hydrated treatments by Duncan's test, ($P \leq 0,05$). Bars represent standard error of the mean.

to the preservation of the embryos water status. A recent study demonstrated that the peripheral endosperm preferentially stores water in the vacuoles, while the lateral portion (Fig. 2A; Fig. 13A) is rich in mucilages (Dias et al., 2020). Furthermore, water moves easily through the endosperm by both apoplastic and symplastic flows. Thus, in a situation of water deficit, the peripheral water is more easily lost, due to the greater free energy, while the water present in the internal hydrophilic portions is preserved. This condition creates a buffer system that can be activated in a condition of water deficit, and helps to explain the slow dehydration tendency that the seeds present (Veloso et al., 2016) (Fig. 13A). The ability to keep the embryo hydrated is crucial for the persistence of recalcitrant seed banks in seasonal environments and is related to the reproductive success and wide distribution of *M. flexuosa,* which have been reported in some studies (Porto et al., 2018; Moura et al., 2019; Salvador et al., 2022).

4.2. Structural, cytological and physiological responses of embryos to water stress

Moderate water stress increases the metabolic performance of *M. flexuosa* embryos, which favors the activation of germination pathways. The ultrastructural evaluation of embryos submitted to moderate water deficit shows cells in a more active metabolic state (Fig. 7E-J), compared to the newly dispersed condition (Fig. 7A-D), and the accumulation of reducing sugars (Fig. 6E). In addition, evidence of mucilage mobilization is observed by histochemical evaluations (Fig. 4C). In studies with varied species, it was demonstrated that, in response to mild dehydration, the germination rate increases in recalcitrant seeds (Pammenter et al., 1998; Drew et al., 2000; Eggers et al., 2007). Our results allow us to propose that, under conditions of moderate water deficit, buriti embryos increase metabolism by accumulating osmolytes, such as reducing sugars, which reduces the embryonic Ψ_w, and promotes the absorption of water from the endosperm (Fig. 13B). The absorption of water and nutrients from the endosperm by the embryos is considered one of the initial stages of germination in the species (Dias et al., 2020). Porto et al. (2018) observed that the germination rate and the formation of seedling banks of *M. flexuosa* is higher in drier microenvironments in the veredas, where the persistence of recalcitrant seeds would be compromised. Therefore, we conclude that external water is not required for the germination of these seeds, as occurs with other recalcitrant seeds that eventually germinate when stored (Berjak and Pammenter, 2000; 2013). Additionally, the ability to perceive and react to water signals constitutes an important adaptive strategy for the species in environments with seasonal climates.

Damage caused to newly dispersed *M. flexuosa* embryos subjected to severe water deficit is related to the lack of plasticity of cell walls, the absence of insoluble reserves and deficient antioxidant system (Fig. 13 B, C). In this condition, the protoderm cells show strong protoplast retraction, leading to cytoplasmic condensation, as reported for other species (Hoekstra, 2001). This is particularly harmful to cellular structures, and can lead to molecular interactions that promote membrane fusion and protein denaturation. Rigid cell walls, which do not follow protoplast retraction, as it occurs in buriti embryos (Fig. 7K, P; 9 C), contribute to the harmful effects of desiccation as evidenced by Woodenberg et al. (2018). Although lipids are common in *M. flexuosa* embryos, they probably do not generate volume buffering capacity, essential to alleviate stress damage to the plasma membrane, such as in orthodox seeds, rich in insoluble reserves (Berjak and Pammenter et al., 2013). The lipids present in buriti seeds belong to the terpenoid class (Silva et al., 2014), secondary compounds commonly associated with the stress response.

The enzymatic antioxidant system involving CAT, APX and SOD is not efficient in controlling oxidative stress in *M. flexuosa* embryos under water deficit (Fig. 13C). Although SOD activity is correlated with the amount of MDA, indicating its activation in response to stress, the enzyme action was not efficient in reducing lipid peroxidation. Inefficient antioxidant systems are commonly reported for recalcitrant seeds under water deficit (Berjak and Pammenter, 2007). Here, it was observed that H_2O_2 (one of the main ROS) and MDA (lipid peroxidation indicator) levels concomitantly increase in embryos under severe water stress, which is related to loss of cell membrane functionality and solute leaching. According to Sershen et al. (2016), the extravasation of electrolytes and the degree of vacuolation, which also occurred in these embryos under water deficit, can be used to quantify the damage induced by desiccation. Our results indicate that although *M. flexuosa* embryos show some resilience, the maintenance of their structure and physiology is limited in severe water deficit conditions.

4.3. Effects of post-dispersal hydration on the response to water deficit and embryo metabolism

Mauritia flexuosa embryos that underwent post-dispersal hydration have a lower level of oxidative stress under water deficit, compared to newly dispersed embryos (Fig. 13C). In these embryos, under moderate and severe stress, H_2O_2 and MDA levels are lower compared to newly dispersed embryos (Fig. 12A, C). This condition is not due to the

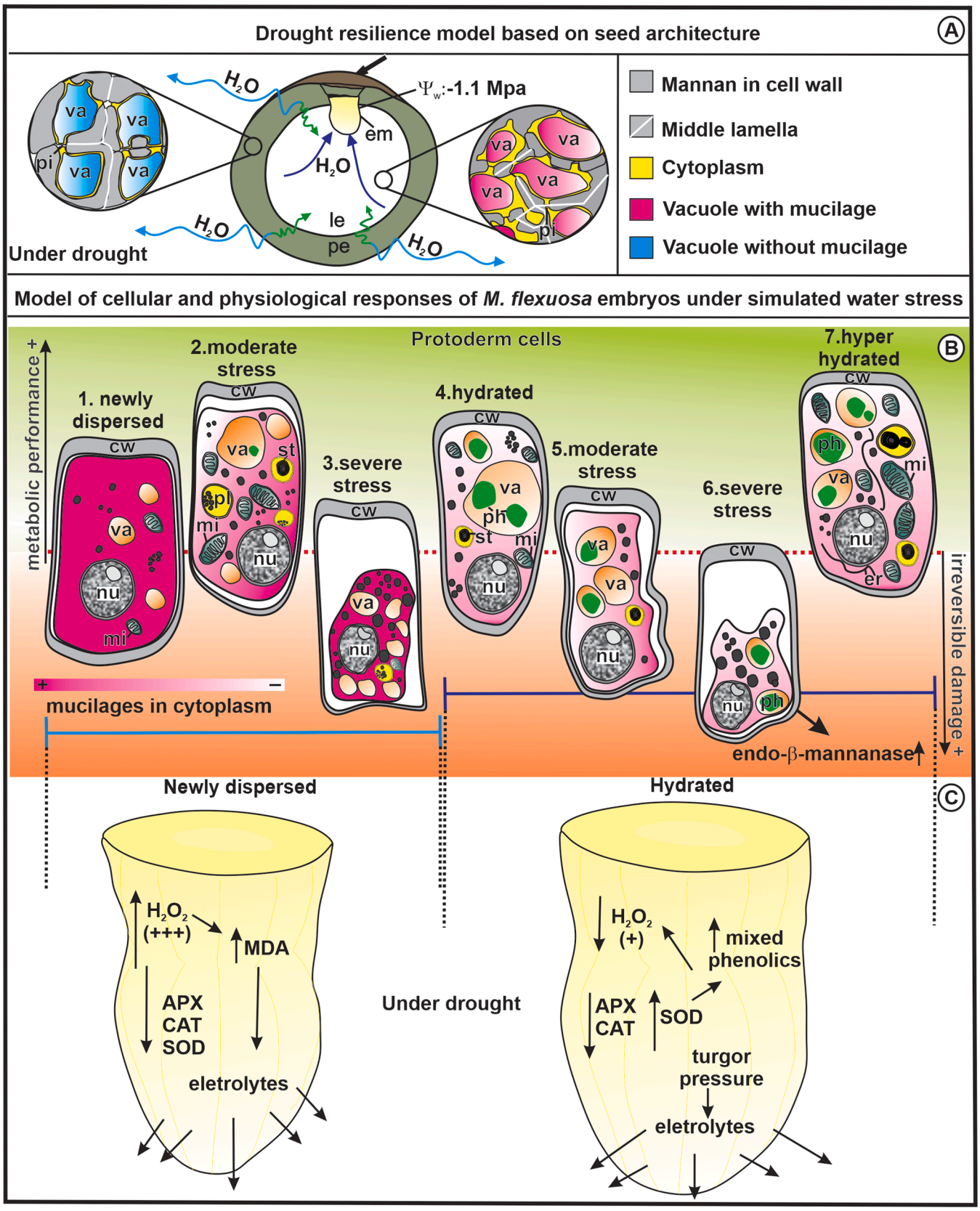

Fig. 13. Scheme representing cytological and physiological responses of *M. flexuosa* embryos to water stress. Dehydration resilience model based on seed architecture (A): water exit from peripheral endosperm in drought conditions (blue arrows); water influx into the lateral endosperm (green arrows); influx of water into the embryo (purple arrows). Protodermal cells of M. flexuosa embryos under different water stress conditions (B): dashed red line, represents the control condition, variations in the position of the cells up or down, represent increased metabolic performance or irreversible damage, respectively. Representation of the physiological status of M. flexuosa embryos under water stress conditions (C). cw, cell wall; em, embryo; er, endoplasmic reticulum; le, lateral endosperm; mi, mitochondria; nu, nucleus; pe, peripheral endosperm; ph, mixed phenolics; pl, plastid; st, starch; va, vacuole.

enzymatic antioxidant system, since, among the main enzymes, only SOD activity was greater in hydrated embryos, and only under moderate stress (Fig. 12B). Furthermore, significant correlations were found between SOD activity and the amounts of H_2O_2 and MDA, which means that although the enzyme is activated, its action is not sufficient to control stress. In hydrated embryos under stress, there is an accumulation of phenolic compounds, especially associated with organic macromolecules (Fig. 6J-U). It is known that phenolic compounds act in the removal of ROS (Xu et al., 2020), especially during seed germination (Gan et al., 2016; Chen et al., 2016; Xu et al.,2018). Some studies have even revealed that the association with carbohydrates, forming conjugates, such as those identified in the present work, increase the antioxidant potential of phenolic compounds (Wang et al., 2016). It is noteworthy that controlled levels of ROS can favor the activation of enzymatic synthesis routes related to the mobilization of reserves and seed germination (Wojtyla et al., 2016).

The viability of post-dispersion hydrated embryos, when subjected to water stress, does not differ from that of freshly dispersed embryos, however, the solute leaching test indicates that the functionality of the membranes is affected (Fig. 10). This is possibly related to sudden variations in intracellular pressure, considering that the isolated embryos were placed directly in the solutions. It is interesting to note that, in an osmotic adjustment attempt, hydrated *M. flexuosa* embryos activate endo-β-mannanase synthesis pathways when subjected to severe water deficit (Fig. 11). Endo-β-mannanase is the main digestive enzyme of the endosperm cell wall in many Arecaceae seeds (Buckeridge, 2010). Under normal water conditions, the activity rates of this enzyme do not change in the haustorium, but rather in the endosperm, during the mobilization of reserves (DeMason et al., 1985; (Mazzottini-dos-Santos et al., 2017); Dias et al., 2020). This is the first record of increased activity levels of this enzyme in palm tree embryos (Fig. 13B). The osmolytes produced as a result of the breakdown of mannans, possibly promote osmotic resistance to the embryos, in the period between 6 and 12 h of immersion in the polyethylene glycol 6000 solution (Fig. 3B).

The hyperhydration of *M. flexuosa* embryos promotes the intensification of metabolism and the mobilization of reserves, traits related to germination. At the end of 48 h of exposure to Ψ_w= 0 MPa, the water content of the embryos increases from 4.4 to 10.1 $gH_2O\ g^{-1}$ DM (Fig. 3A-B), which is related to cell expansion in the central region of the ground meristema (Fig. 9C). Hydraulic capacity (capacity of the cell to store water, according to Dainty, 1976) in embryonic tissues controls cell expansion, a determining factor for the completion of germination in most seeds (Bewley et al., 2013), including buriti (Moura et al., 2019). The dynamics of compounds resulting from the hyperhydration of *M. flexuosa* embryos includes the accumulation of reducing sugars (Fig. 6K, T) and starch grains (Fig. 8A, Q), which constitute a transient reserve during the embryo/seedling transition in palms (Mazzottini--dos-Santos et al., 2017). Moreover, ultrastructural evaluation reveals the intensification of cellular activity, which is considered a marker for changes in the physiological status of embryos (Fig. 13B) (Berjak and Pammenter, 2000).

Under natural conditions, it is likely that the increase in water content of buriti embryos is related to overcoming dormancy. In *M. flexuosa* seeds in which the operculum was mechanically removed, Dias et al. (2020) demonstrated that the water flow and reserves from the endosperm to the embryo is one of the main indicators of the beginning of the germination process. The pronounced morphophysiological dormancy (a concept from Baskin and Baskin, 2014) of buriti seeds, as in other Arecaceae seeds, is determined by the low growth potential of the embryo, which cannot overcome the resistance imposed by the operculum (Ribeiro et al., 2011; 2012; Magalhães et al., 2013; Neves et al., 2013; Oliveira et al., 2013; Silva et al., 2014). Embryo extraction or removal of the operculum eliminates mechanical restriction and exposes the embryos to atmospheric oxygen (Mazzottini-dos-Santos et al., 2018). Oxygen, in its most reactive forms (ROS), participates as a signal in metabolic pathways that increase the ratio between gibberellins (GAs) and abscisic acid (ABA), which promotes germination (Ribeiro et al., 2015; Dias et al., 2018).

In a study of recalcitrant *Aesculum hypocastanum* seeds, dormancy is treated as the inability of the embryo axes to absorb water to the level that triggers germination, even when an ideal supply of water is available (Obroucheva et al., 2004; Obroucheva et al., 2017). Therefore, even in recalcitrant seeds that are dispersed hydrated, water entry into embryos is crucial for the germination completion. According to Salvador et al. (2022), *M. flexuosa* seeds that went through the seed bank showed embryo elongation and an increase in water content, in addition to greater germination capacity, compared to those recently dispersed - indicating a reduction in the dormancy level. The results obtained in the present work corroborate these findings and indicate that the water cycles experienced during the soil seed banks stay, which promote the mobilization of reserves and the modulation of oxidative stress, have a relevant role in attenuating dormancy in the species.

5. Conclusions

The *M. flexuosa* embryo is dispersed, highly hydrated and is sensitive to dehydration, which defines the recalcitrant condition of the seeds. However, seeds show remarkable resilience to dehydration, which is associated with the buffering effect promoted by the endosperm and the accumulation of hydrophilic mucilages in embryonic cells. Both post-dispersion water absorption and moderate water deficit induce the intensification of cellular activity and the mobilization of reserves. Embryos hydrated after dispersion, under water stress, are less vulnerable to oxidative stress due to the non-enzymatic antioxidant system. Although embryos present resilience traits, severe water stress causes structural (loss of cell wall and membrane functionality) and physiological (oxidative stress and blockage of reserve mobilization) damage that leads to loss of germination capacity and, in extreme cases, of viability. The water cycles experienced in the seed bank in the soil probably play a relevant role in overcoming buriti seed dormancy.

CRediT authorship contribution statement

Guilherme Pereira Dias: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Leonardo Monteiro Ribeiro:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Hellen Cássia Mazzottini dos Santos:** Writing – review & editing, Investigation, Formal analysis. **Yule Roberta Ferreira Nunes:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Franca Marcel Giovanni Costa:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the Centro de Microscopia da Universidade Federal de Minas Gerais - CM/UFMG for making its infrastructure available for the electron microscopy analyses. This research was supported by the Long-Term Ecological Research Network (Programa de Pesquisa Ecológica de Longa Duração – PELD-VERE) of the National Council for Scientific and Technological Development – CNPq (Edital 093/2021); the Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (APQ-03371-21); and the Ministry of Science, Technology and Innovations – MCTI, Brazil. The authors are grateful to CAPES for the doctoral scholarship granted to GPD, and to CNPq for the research

productivity scholarships granted to LMR, MGCF and YRFN.

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