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Storage potential of *Eugenia uniflora* Lam. seeds incubated in different osmotic solutions and temperatures

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ABSTRACT: The *ex situ* conservation of recalcitrant seeds has been one of the biggest challenges in seed technology. Different strategies have emerged in recent years and among these strategies, osmotic conditioning at controlled temperatures has shown excellent results. Therefore, the objective of this research was to store recalcitrant seeds of *E. uniflora* through osmotic conditioning in order to reduce the metabolism of these seeds and extend their storability. The seeds were stored at temperatures of 10 and 25 °C in PEG solutions at 0.0 (water), -0.5, -1.0, -1.5, and -2.0 MPa, as well as without moistening, for 3, 6, 12, and 18 months. There was a reduction in metabolism in the treatments with PEG, and seed viability was maintained after 18 months at 25 °C and -1.5 MPa.

Index terms: conservation, osmoconditioning, recalcitrant seeds.

RESUMO: A conservação *ex situ* de sementes recalcitrantes tem sido um dos maiores desafios da tecnologia de sementes. Diferentes estratégias surgiram nos últimos anos e dentre essas estratégias, o condicionamento osmótico em temperaturas controladas tem demonstrado excelentes resultados. Portanto, o objetivo desta pesquisa foi armazenar sementes recalcitrantes de *E. uniflora* por meio de condicionamento osmótico, a fim de reduzir o metabolismo dessas sementes e ampliar sua capacidade de armazenamento. As sementes foram armazenadas nas temperaturas de 10 e 25 °C em soluções de PEG a 0,0 (água), -0,5, -1,0, -1,5 e -2,0 MPa e sem umedecimento, durante 3, 6, 12 e 18 meses. Os resultados demonstraram redução do metabolismo nos tratamentos com PEG, e a viabilidade das sementes foi mantida após 18 meses a 25 °C e -1,5 MPa.

Termos para indexação: conservação, osmocondicionamento, sementes recalcitrantes.

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INTRODUCTION

The inclusion of seeds sensitive to desiccation, i.e., recalcitrant seeds, in germplasm banks is one of the main challenges in seed conservation. Such seeds lose viability when dehydrated or kept under refrigerated conditions (Roberts, 1973; Umarani et al., 2015; Wyse et al., 2018). However, sensitivity to desiccation and to low temperatures can vary among species, and up to now, there is no efficient storage method that can include the recalcitrant seeds of all species (Barbedo, 2018; Breman et al., 2021). It is estimated that around 8% of all flora is composed of species that have desiccation-sensitive seeds, and this may rise to 18.5% in tropical and subtropical regions (Wyse and Dickie, 2017). Therefore, developing technologies that allow recalcitrant seeds to be conserved in germplasm banks is an urgent need, especially due to concerns over loss of plant diversity.

Among the attempts to conserve these seeds, water control through osmoconditioning has shown promising potential by regulating the intense metabolism of recalcitrant seeds of some species. Polyethylene glycol 6000 (PEG) has been widely used for that purpose, above all to store seeds of this type (Andréo et al., 2006; Pereira et al., 2020; Pelissari et al., 2022; Cécel and Barbedo, 2023).

Seed metabolism can be indirectly analyzed by respiration rate, allowing more accurate diagnosis of the condition of the seeds under storage conditions (Lamarca and Barbedo, 2012). The amount of oxygen (O_2) consumed by the seeds, as well as the amount of carbon dioxide (CO_2) released, allow the intensity of seed metabolism to be quantified and the degree of efficiency of the seed and environment conditions to be identified for extended storage. The respiratory quotient (RQ), obtained by the quotient between the CO_2 released and the O_2 consumed, then provides information regarding the type of respiration, whether aerobic or anaerobic; and the difference between the O_2 consumed and the CO_2 released indicates whether other oxidation processes are occurring, such as those resulting from the action of reactive O_2 species (Kader and Saltveit, 2002; Bragante et al., 2018).

Eugenia uniflora L. (pitanga), native to the Atlantic Forest, Cerrado, and Pampa biomes and not endemic to Brazil, is of great ecological importance; it produces fruit with pharmacological and food potential and has potential for use as an urban tree (Bourscheid et al., 2011; Moura et al., 2018; Bittencourt et al., 2021; Mazine et al., 2024). The seeds of this species are sensitive to desiccation and prove to have interesting traits for the study of recalcitrant seed conservation in general. The aim of this study was to analyze the changes in respiratory rates of *E. uniflora* seeds resulting from incubation under controlled hydration, as well as the effects of this incubation on the storability of these seeds.

MATERIAL AND METHODS

Obtaining plant material – Ripe fruit from *E. uniflora* was collected from ten mother plants in the state park *Parque Estadual das Fontes do Ipiranga*, São Paulo, Brazil (23°38'30.7" S, 46°37'14.2" W), between 26 Oct. 2021 and 13 Nov. 2021. Seeds were manually extracted from the fruit under running water using trays and sieves. Excess water was removed from the seeds using filter paper and the seeds were stored in transparent polyethylene bags at 8 °C in a BOD incubation chamber until the experiments began, not exceeding seven days.

Physical and physiological determinations – The moisture content (expressed as a percentage, wet basis) and the dry matter content (in $g.seed^{-1}$) were determined using the air-circulation laboratory oven method for 17h at 103 °C (Brasil, 2009). The water potential (expressed in MPa) was measured by a WP4 potentiometer (Decagon Devices, Inc.; Pullman, WA, USA), based on dew point temperature (Delgado and Barbedo, 2012). Both measurements were performed with two replications of three seeds each.

Germination was evaluated by placing the seeds on Germitest® (germination testing) paper previously moistened with tap water, with two sheets as a base and one over the seeds. These papers were rolled up and placed in transparent polyethylene plastic bags with perforations (ten 0.5-mm holes) and placed in a climate-controlled room (25 °C) under continuous light. Weekly evaluations were made up to 60 days, recording the number of germinated seeds (root

emergence of 0.5 cm) and those that produced normal seedlings, i.e., with roots and shoots that developed without defect (Delgado and Barbedo, 2007). Four replications of nine seeds each were used.

Preparation of the osmotic incubation solutions – The osmotic solutions were prepared by dissolving different amounts of solid PEG crystals in distilled water. A magnetic stirrer was used to homogenize the solution in the preparation process. The amounts of PEG were determined from the table proposed by Villela et al. (1991), preparing solutions of -0.5, -1.0, -1.5, and -2.0 MPa.

Analysis of the respiratory rates of seeds incubated in the different osmotic solutions – Seed samples were weighed and distributed into 3 replications of 20 seeds, placed on two sheets of Germitest® paper, and covered by a third sheet. All the seeds were moistened until saturation with PEG solutions at the water potentials of -0.5, -1.0, -1.5, and -2.0 MPa, as well as with distilled water (0.0 MPa). The sheets with the seeds were rolled and placed in 600-mL, transparent, hermetic glass containers (respirometers - Lamarca and Barbedo, 2012; Bonjovani and Barbedo, 2019), with lids sealed using Parafilm® (plastic wrap), and incubated in a BOD chamber at 10 °C and at 25 °C in darkness. Free seeds were also incubated in the respirometers, i.e., seeds without moistening in paper rolls (control).

The beginning of the experiment was considered to be when the containers were sealed (Lamarca and Barbedo, 2012; Bonjovani and Barbedo, 2019). The seed respiration rate was analyzed after 12 hours of incubation, calculated according to consumption of O_2 and release of CO_2 (Kader and Saltveit, 2002). To obtain the O_2 consumption and CO_2 release rates, the gases were collected from inside the containers with a hypodermic needle inserted into a rubber septum in the respirometer lid, as described in Manoel et al. (2024), and measured with the Model 6600 gas analyzer (Illinois Instruments, Inc., Johnsburg, IL, USA). The values obtained as percentage of volume were converted to partial gas pressure and then to µmols by the Clapeyron equation, PV = nRT. The number of mols of each gas was then divided by the seed dry matter of the respirometers and the incubation time. Furthermore, the Respiratory Quotient (RQ) and the oxidation rate were calculated, as previously described. Three replications of fifteen seeds each were used.

Seed storage in the osmotic solutions – Seed samples were distributed at equal distance on two sheets of Germitest® paper (28 × 19 cm, sterilized), for four replications of ten seeds, and covered by a third sheet. All were previously moistened with 30 mL of distilled water (0.0 MPa) or with 30 mL of the previously described PEG solutions. Then the sheets were rolled up and placed in polyethylene bags, which were closed with twist ties and perforated (10 holes) to allow gas exchanges. They were then stored at 2 temperatures (10 and 25 °C) in the dark. The control consisted of seeds placed in perforated plastic bags, without moistening. All the treatments were stored for 3, 6, 12, and 18 months, and at the end of each period, the seeds were evaluated for germination and moisture content, as previously described. At 6 and 18 months of storage, the seeds were also evaluated regarding their water potential, according to the methodology already described.

Analysis of variance (ANOVA) was used on the data, and the means were compared with each other by Tukey's test (5%). When necessary, the data were transformed using √(x+0.5) to provide for normality (Santana and Ranal, 2004).

RESULTS AND DISCUSSION

The recently collected *E. uniflora* seeds had 95% germination, 92% normal seedlings, 52.4% moisture content, 0.17 $g.seed^{-1}$ dry matter, and water potential of -2.56 MPa.

Incubation of the seeds at the different temperatures and osmotic potentials resulted in different respiratory rates (Figure 1). At 25 °C, nearer the natural condition of these seeds, incubation in water or at lower osmotic potentials (-0.5 to -2.0 MPa) resulted in a lower CO_2 release than in the seeds incubated without any moistening (control). That may indicate a higher respiratory rate of the control seeds. Nevertheless, the O_2 consumption data did not show differences among the treatments, indicating that the difference in CO_2 release was probably due to oxidative processes not resulting from respiration. The RQ data and the oxidation rates (Figure 1) support this possibility, since an RQ near 1.0

indicates respiration using carbohydrates as a probable substrate, whereas RQs lower than 0.6 suggest the presence of other oxidative reactions (Lamarca and Barbedo, 2012).

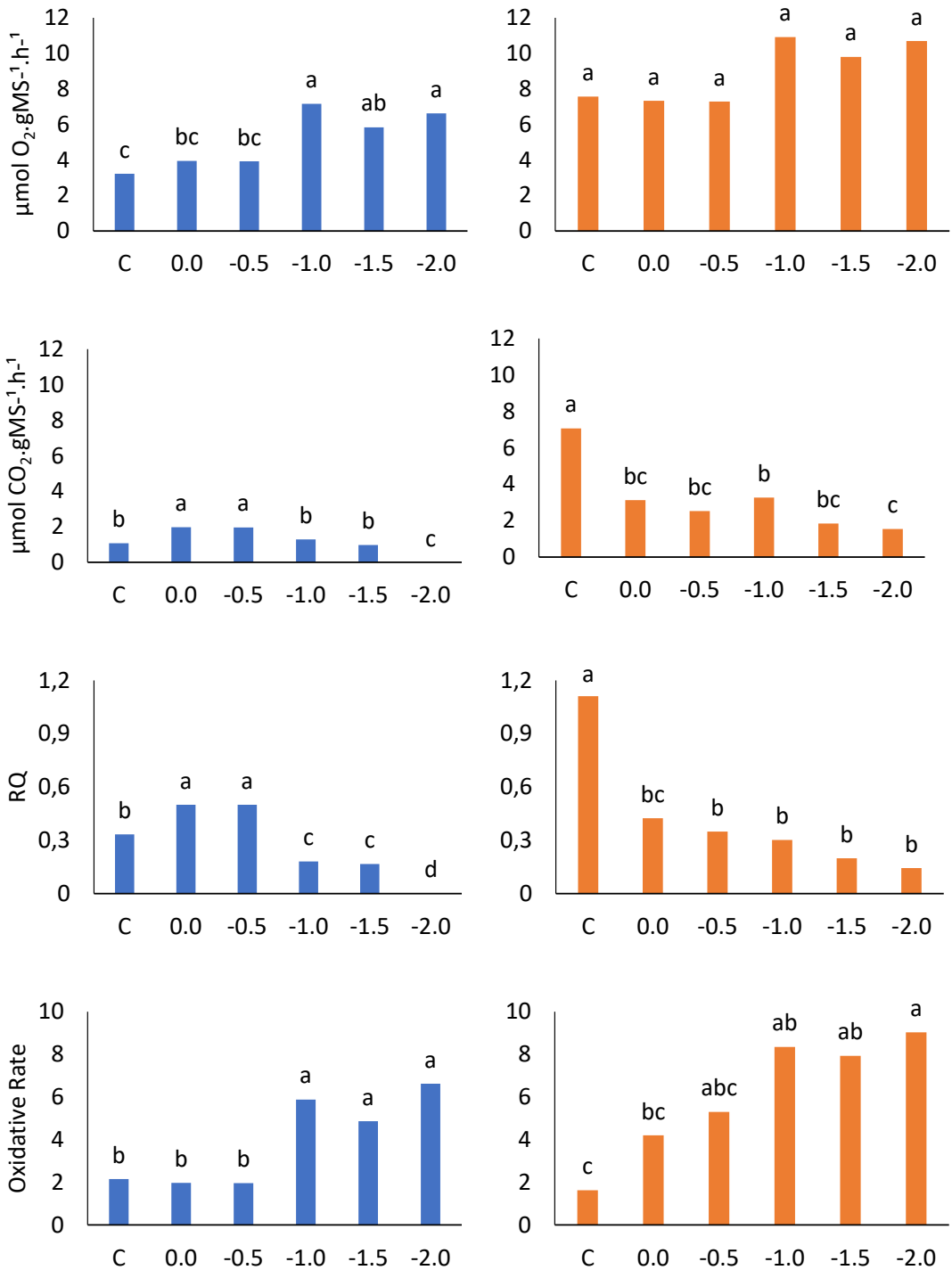

Figure 1. O_2 consumption, CO_2 release, respiratory quotient (RQ), and oxidative rates of *E. uniflora* seeds incubated for 12 h at the temperatures of 10 °C (blue) and 25 °C (orange), in the following treatments: control (C), water (0.0), and PEG at the osmotic potentials of -0.5, -1.0, -1.5, and -2.0 MPa. Columns with the same letter do not differ from each other (Tukey's test, 5%).

The seeds incubated at the lower temperature, 10 °C, had less release of CO_2, especially those of the control (Figure 1), suggesting lower respiratory rates than in the seeds incubated at 25 °C. This lower respiratory intensity could result in greater storage potential. However, the values of O_2 consumption, of RQ, and of oxidative rates show that the reduction in temperature intensified the oxidative processes, and may speed deterioration of the seeds (Chandra et al., 2021). Interestingly, however, at the osmotic potentials of -1.0, -1.5, and -2.0 MPa, the seeds had higher oxidative rates, which may indicate some stress condition from low temperature.

In general, the seeds had lower respiratory rates at 10 °C than at 25 °C, which was also seen for seeds of other species within the family, such as *Eugenia pyriformis* Cambess., *E. brasiliensis* Lam., and *Myrcianthes pungens* (O. Berg) D. Legrand (Lamarca et al., 2020; Guardia et al., 2020; Cécel and Barbedo, 2021; 2023). However, the reduction in temperature brought about an increase in oxidative rates, as also observed for recalcitrant seeds of *Inga vera* subsp. *affinis* (DC.) T.D. Pennington (Bonjovani and Barbedo, 2014). If, on the one hand, reduction in respiratory rates could increase storability, on the other hand, the increase in oxidative rates could have an opposite effect. In fact, there is the recommendation of storing seeds in germplasm banks under low O_2 concentration conditions in the environment (Groot et al., 2015), which could reduce these oxidation rates, as observed in seeds of *Pinus densiflora* (Gerna et al., 2022), of lettuce, and of onion (Schwember and Bradford, 2011).

Storage of seeds under these different conditions showed that, already in the first period (3 months), germination occurred within the container itself (Figure 2). The number of seeds that germinated during storage varied depending on the temperature and the type of incubation. In storage at 25 °C and at the potentials of 0.0, -0.5, and -1.0 MPa, more than 90% of the seeds had already germinated (Figure 3), which would be expected given the high respiratory rates (Figure 1). The reduction in water availability to -1.5 MPa decreased these germination values to around 50%, and an even greater reduction in this availability, to -2.0 MPa, practically impeded germination in the container. Nevertheless, the seeds remained viable. In reducing the storage temperature to 10 °C, there was also generally reduction in seed germination during storage, except for the seeds incubated in water (0.0 MPa) and in PEG solution at -0.5 MPa (Figure 3), which also exhibited higher respiratory rates than the seeds incubated at lower osmotic potentials (Figure 1). The seeds of the control treatment hardly germinated during storage, probably because of reduction in their moisture content (Table 1) to levels below that tolerated by these seeds (Delgado and Barbedo, 2007). It is noteworthy that while incubation without solutions (or water) resulted in rapid reduction in seed moisture content, incubation in solutions with different water potentials resulted in seeds with different levels of hydration, generally lower as the water potentials decreased (Table 1).

All the seeds, whether or not they germinated in the container itself, were placed to germinate in the germination test. The total number that germinated was thus considered as all the seeds that continued the germination that had begun in storage with those that began germination in the germination test. The seeds that germinated during storage but that did not continue their development during the germination test were recorded as not germinated. Likewise, those that had germinated during storage and had already died and that were placed under the germination test were also recorded as not germinated.

Conservation of seed viability during storage was clearly better at the lower temperature, 10 °C, than at 25 °C (Figure 4), as would be expected for recalcitrant seeds (Barbedo, 2018) and also from the lower respiratory rates (Figure 1). Even at the lower temperature, the seeds lost viability already at 6 months when not kept in a moist substrate (control). When kept in a substrate with total water availability, they began to lose vigor after 12 months of storage, as can be seen by their ability to produce normal seedlings (Figure 4). The seeds kept in PEG solutions did not show this same pattern of deterioration (Figure 4); they were likely highly affected by the lower respiratory rates, especially at the potentials below -0.5 MPa (Figure 1).

Seeds stored at the higher temperature deteriorated more rapidly, and not only did the control seeds quickly lose vigor, but also the seeds kept in the water substrate. They did not produce normal seedlings after the first 3 months of storage, whereas seeds kept in a PEG solution at -0.5 MPa deteriorated after 6 months of storage (Figure 4). After 18 months of storage, only the seeds kept in a PEG solution at -1.5 MPa maintained viability and the ability to produce normal seedlings.

Figure 2. Germination and formation of normal and abnormal seedlings from *Eugenia uniflora* seeds. A: germinated seed, with emergence of primary root; B: normal seedling with developed shoots and roots; C: abnormal seedling formed during storage; D: abnormal seedling formed during storage but with resumption of shoot development during the germination test; E and F: seeds removed after 12 months of storage in the control, soaked in water (0.0 MPa), or in PEG solutions with different osmotic potentials (-0.5, -1.0, -1.5, and -2.0 MPa) at 10 °C (E) or 25 °C (F).

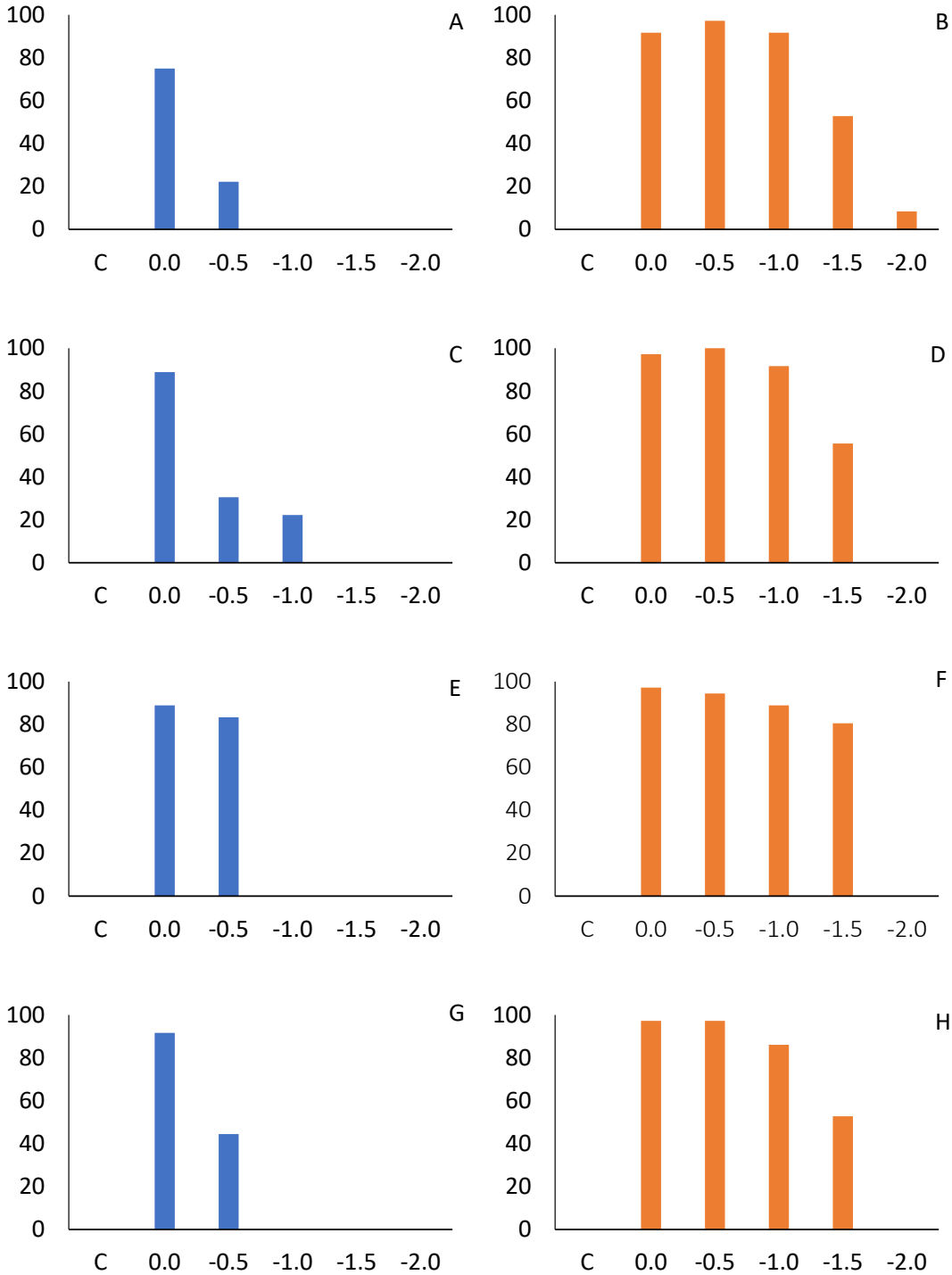

Figure 3. Specific germination (%) of *E. uniflora* seeds after each storage period of 3 (A and B), 6 (C and D), 12 (E and F), and 18 (G and H) months at 10 °C (blue) and 25 °C (orange) in the following treatments: control (C, without moistening), water (0.0), and PEG at the osmotic potentials of -0.5, -1.0, -1.5, and -2.0 MPa.

Table 1. Moisture content and water potential (Ψw) in *E. uniflora* seeds after removal from BOD chambers at the periods of 3, 6, 12, and 18 months in the following treatments: control (without moistening), water (0.0), and PEG at the potentials of -0.5, -1.0, -1.5, and -2.0 MPa, at 10 and 25 °C.

Storage temperature	Type of incubation	Moisture content (%)				Water potential (MPa)	
		Period (months)				Period (months)	
		3	6	12	18	6	18
10 °C	Control	26	13	12	18	-70.5	-40.1
	0.0	57	55	64	69	-1.2	-1.8
	-0.5	56	53	58	45	-2.1	-3.3
	-1.0	49	51	56	56	-3.4	-3.7
	-1.5	56	55	52	50	-4.2	-4.4
	-2.0	52	51	47	48	-4.6	-5.6
25 °C	Control	19	20	15	18	-44.9	-52.0
	0.0	65	71	58	58	-1.1	-2.0
	-0.5	60	65	51	37	-2.6	-20.8
	-1.0	58	63	55	32	-5.1	-22.6
	-1.5	63	56	31	31	-5.3	-23.5
	-2.0	58	47	44	31	-5.9	-26.2

Analyzing the germination and normal seedling production values (Figure 4) together with the respiration and oxidative rates (Figure 1), it is clear that both the reduction in temperature and in water availability result in two important consequences: reduction in respiratory rates, which would be favorable to storage, and increase in oxidative rates, which would negatively affect storage. Perhaps for that reason, some treatments stood out, such as storage at -0.5 MPa when the temperature was low, and storage at -1.5 MPa when the temperature was higher. In both cases, there may be a point at which greater reduction in respiratory rates coincided with a smaller increase in oxidative processes.

Eugenia spp. are well adapted to 25 °C (Gomes et al., 2016). At low temperatures, the mobility of water molecules and metabolic processes are reduced (Nadarajan et al., 2023). That may explain the decrease in germination during storage at 10 °C, as was also observed by Inocente and Barbedo (2019) and Cécel and Barbedo (2021). The high moisture content and accumulation of reserves in seeds at an advanced maturity stage result in germination, and removal of water from the seeds leads to an increase in solutes and, consequently, reduction in metabolism (Bewley et al., 2013, Marcos-Filho, 2015). However, it is noteworthy that when water availability declined using the PEG treatment at -1.5 MPa, even at 25 °C, it was possible to maintain practically 50% of the seeds viable, and many were able to produce normal seedlings, even after 18 months of storage (Figure 4H). This 18-month time is quite significant for recalcitrant seeds stored at a high temperature, since *Eugenia* spp. seeds more quickly become inviable at ambient temperatures (Silva et al., 2019). Moreover, finer adjustment in the PEG concentration, among other factors, will likely allow an even longer storage period. It is important to mention that the seeds germinating during storage did not totally compromise the viability of the treatments with PEG, for the resumption of germination by shoot production resulted in normal seedlings, as already shown in seeds of *E. involucrata* DC. (Inocente and Barbedo, 2021) and in *E. brasiliensis* Lam. (Cécel and Barbedo, 2023). Therefore, the results of the present study indicate the considerable potential of use of osmotic solutions to increase the storability of recalcitrant seeds. The results also show the importance of oxidative processes in the loss of viability of seeds during storage.

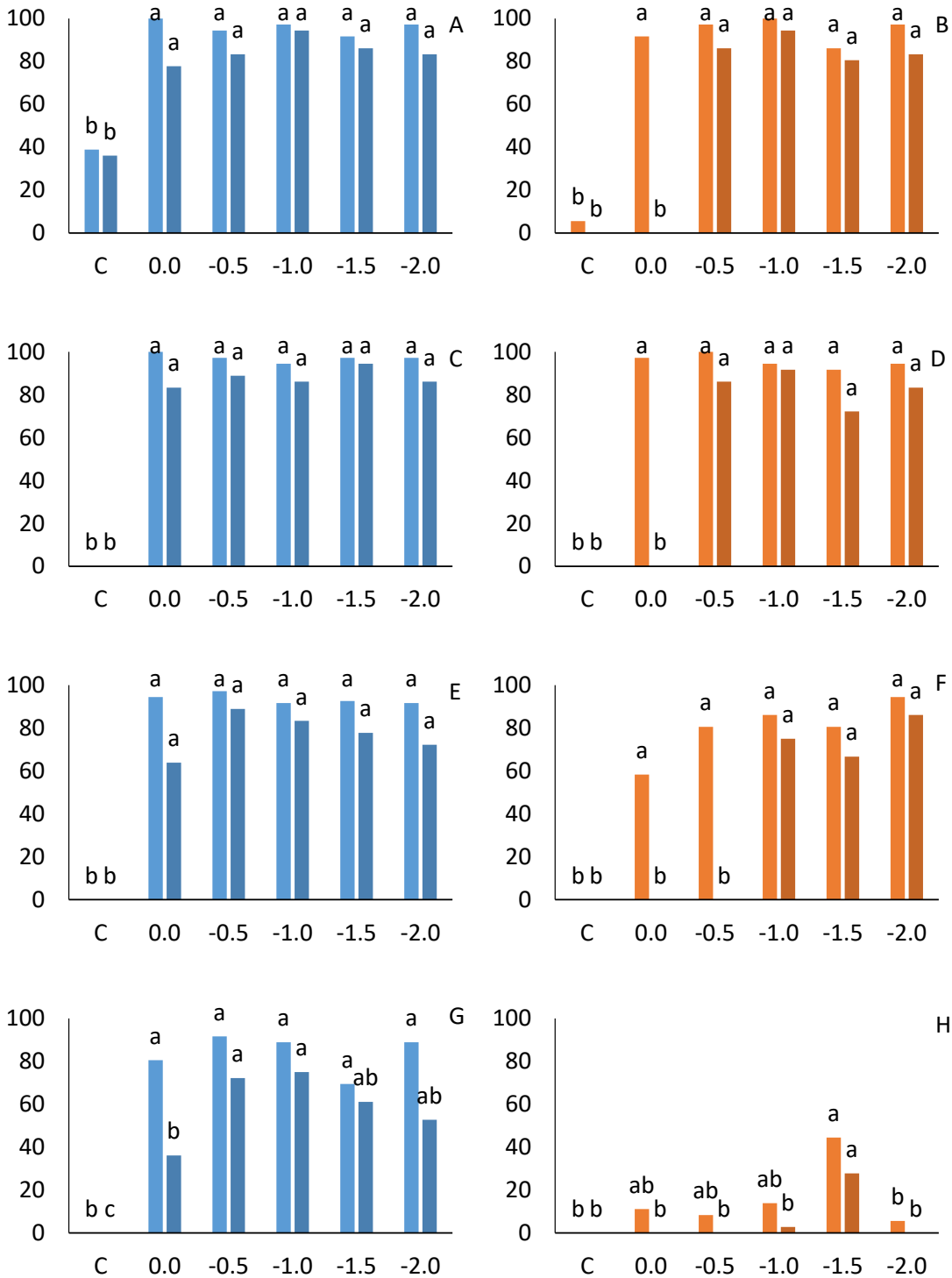

Figure 4. Germination percentages (lighter-colored bars) and development of normal seedlings (darker bars) in the germination test of *E. uniflora* seeds stored for 3 (A and B), 6 (C and D), 12 (E and F), and 18 (G and H) months at 10 °C (blue) and 25 °C (orange) in the following treatments: control (C, without moistening), water (0.0), and PEG at the osmotic potentials of -0.5, -1.0, -1.5, and -2.0 MPa. Columns with the same letter do not differ from each other (Tukey's test, 5%).

CONCLUSIONS

E. uniflora seeds can be stored for up to 18 months at 25 °C when kept at -1.5 MPa.

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REFERENCES

ANDRÉO, Y.; NAKAGAWA, J.; BARBEDO, C.J. Mobilização de água e conservação da viabilidade de embriões de sementes recalcitrantes de ingá (*Inga vera* Willd. subsp. *affinis* (DC.) T. D. Pennington). *Revista Brasileira de Botânica*, v.29, p.309-318, 2006. https://www.scielo.br/j/rbb/a/gsT3rwcTftKX8JYZKHm8Hxd/?format=pdf&lang=pt

BARBEDO, C.J. A new approach towards the so-called recalcitrant seeds. *Journal of Seed Science*, v.40, p.221-236, 2018. https://doi.org/10.1590/2317-1545v40n3207201

BEWLEY, J.D.; BRADFORD, K.J.; HILHORST, H.W.M; NONOGAKI, H. *Germination*. In: BEWLEY, J.D.; BRADFORD, K.J.; HILHORST, H.W.M; NONOGAKI, H. (Eds.) Seeds: Physiology of Development, Germination and Dormancy. New York: Springer, p.133-181, 2013.

BITTENCOURT, A.H.C.; MACHADO, J.S.; SILVA, M.G.; COSENZA, B.A.P. Evaluation preliminary phytochemistry and antimicrobial activity of extracts in *Eugenia uniflora* L. on *Escherichia coli* and *Staphylococcus aureus*. *Research, Society and Development*, v.10, e329101320579, 2021. https://rsdjournal.org/index.php/rsd/article/view/20579/19194

BONJOVANI, M.R.; BARBEDO, C.J. Induction of tolerance to desiccation and to subzero temperatures in embryos of recalcitrant seeds of inga. *Journal of Seed Science*, v.36, p.419-426, 2014. https://www.scielo.br/j/jss/a/ChRn5wMWVvLKjNjnZ6LBKQz/?format=pdf&lang=en

BONJOVANI, M.R.; BARBEDO, C.J. Respiration and deterioration of *Inga vera* ssp. *affinis* embryos stored at different temperatures. *Journal of Seed Science*, v.41, p.44-53, 2019. https://www.scielo.br/j/jss/a/tRbBzPM9tYRNBJZnnyFs9Pv/?format=pdf&lang=en

BOURSCHEID, K.; VIEIRA, N.K.; LISBÔA, G.N.; KINUPP, V.F.; BARROS, I.B.I. *Eugenia uniflora* (pitangueira). In: CORADIN, L.; SIMINSKI, A.; REIS, A. (Eds.) Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro - Região Sul. Brasília: Embrapa, p.170-177, 2011.

BRAGANTE, R.B.; HELL, A.F.; SILVA, J.P.N.; CENTENO, D.C.; FIGUEIREDO-RIBEIRO, R.C.L.; BARBEDO, C.J. Physiological and metabolic responses of immature and mature seeds of *Libidibia ferrea* ((Mart. ex Tul.) L.P. Queiroz) under contrasting storage temperatures. *Brazilian Journal of Botany*, v.41, p.43-55, 2018. https://link.springer.com/article/10.1007/s40415-018-0442-3#citeas

BRASIL. MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. *Regras para Análise de Sementes*. Brasília: Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária, 399p. 2009. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

BREMAN, E.; BALLESTEROS, D.; CASTILLO-LORENZO, E.; COCKEL, C.; DICKIE, J., FARUK, A.; O'DONNELL, K.; OFFORD, C.A.; PIRONON, S., SHARROCK, S.; ULIAN, T. Plant diversity conservation challenges and prospects - The perspective of botanic gardens and the Millennium Seed Bank. *Plants*, v.10, p.1-35, 2021. https://www.mdpi.com/2223-7747/10/11/2371

CÉCEL, A.T.; BARBEDO, C.J. Taxas respiratórias de sementes de *Eugenia brasiliensis* Lam. (Myrtaceae) sob atmosferas modificadas. *Hoehnea*, v.48, p.1-8, 2021. https://www.scielo.br/j/hoehnea/a/Jd8cdVVgMxpz9psky7KcvMh/?format=pdf&lang=pt

CÉCEL, A.T.; BARBEDO, C.J. Storage of recalcitrant seeds of *Eugenia brasiliensis* Lam. under control of water availability. *Journal of Seed Science*, v.45, e202345009, 2023. https://doi.org/10.1590/2317-1545v45264131

CHANDRA, J.; DUBEY, M.; VARGHESE, B.; SERSHEN; KESHAVKANT, S. Towards understanding the basis of desiccation-induced oxidative stress in recalcitrant seeds: the case of *Madhuca letifolia* Roxb. *South African Journal of Botany*, v.142, p.100-105, 2021. https://www.sciencedirect.com/science/article/pii/S0254629921002271

DELGADO, F.L.: BARBEDO, C.J. Tolerância à dessecação de sementes de espécies de *Eugenia*. *Pesquisa Agropecuária Brasileira*, v.42, p.265-272, 2007. https://www.scielo.br/j/pab/a/NsKVTkGhDp5V3ZBsqhBc3Qc/?format=pdf&lang=pt

DELGADO, L.F.; BARBEDO, C.J. Water potential and viability of seeds of *Eugenia* (Myrtaceae), a tropical tree species, based upon different levels of drying. *Brazilian Archives of Biology and Technology*, v.55, p.583-590, 2012. https://www.scielo.br/j/babt/a/XnKQvPP8MwqZ9zdDKM9QC7h/?format=pdf&lang=en

GERNA, D.; BALLESTEROS, D.; ARC, E.; STÖGGL, W.; SEAL, C.E.; MARAMI-ZONOUZ, N.; NA, C.S.; KRANNER, I.; ROACH, T. Does oxygen affect ageing mechanisms of *Pinus densiflora* seeds? A matter of cytoplasmic physical state. *Journal of Experimental Botany*, v.73, p.2631–2649, 2022. https://doi.org/10.1093/jxb/erac024

GUARDIA, M.C.; ASPERTI, L.M.; CANCIAN, G.M.; BARBEDO, C.J. Tolerância à dessecação e armazenamento de sementes de *Myrcianthes pungens* (O. Berg) D. Legrand (Myrtaceae). *Hoehnea*, v.47, p.1-9, 2020. https://www.scielo.br/j/hoehnea/a/h9JFyhQMLPKzsrp39by8FzM/?format=pdf&lang=pt

GOMES, J.P.; OLIVEIRA, L.M; FERREIRA, P.I.; BATISTA, F. Substrato e temperatura para teste de germinação em sementes de Myrtaceae. *Ciência Florestal*, v.26, p.285-293, 2016. https://periodicos.ufsm.br/cienciaflorestal/article/view/21120/pdf_1

GROOT, S.P.C.; GROOT, L.; KODDE, J.; VAN TREUREN, R. Prolonging the longevity of *ex situ* conserved seeds by storage under anoxia. *Plant Genetic Resources: Characterization and Utilization*, v.13, p.18-26, 2015. https://doi.org/10.1017/S1479262114000586

INOCENTE, M.C.; BARBEDO J.C. Germination of *Eugenia brasiliensis, E. involucrata, E. pyriformis and E. uniflora* (Myrtaceae) under water-deficit conditions. *Journal of Seed Science*, v.41, p.076-085, 2019. https://www.scielo.br/j/jss/a/GDzDjGQ7qSxQHHfDzNM3Mpv/?format=pdf&lang=en

INOCENTE, M.C.; BARBEDO J.C. Regeneration of roots and seedlings from *Eugenia involucrata* seeds under water deficit conditions. *Journal of Seed Science*, v.43, p.1-14, 2021. https://www.scielo.br/j/jss/a/vp3dm8tJXYjdr58YwvRMV6p/?format=pdf&lang=en

KADER, A.A.; SALTVEIT, M.E. *Respiration and gas exchange.* In: BARTZ, J.A.; BRECHT, J.K.; WEICHMANN, J. (Eds.) Postharvest physiology and pathology of vegetables. New York: Marcel Dekker, p.7-30, 2002.

LAMARCA, E.V.; BARBEDO, C.J. Short storability of *Caesalpinia echinata* Lam. seeds as a consequence of oxidative processes. *Hoehnea*, v.39, p.577-586. 2012. https://www.scielo.br/j/hoehnea/a/gyYZZ53k8QMwcY3DtSqnyxP/?format=pdf&lang=en

LAMARCA, E.V.; PARISI, J.J.D.; BARBEDO, C.J.Taxas respiratórias de sementes de *Eugenia pyriformis* Cambess. dispersas em diferentes estádios de maturação. *Hoehnea*, v.47, p.1-12, 2020. https://www.scielo.br/j/hoehnea/a/TVKzWsvYGqQdB9ntJvghqQt/?format=pdf&lang=pt

MANOEL, G.F.; TINÉ, M.A.; GUARDIA, M.C.; NIEVOLA, C.C.; BARBEDO, C.J. Atmosphere modification system for respiration analysis of recalcitrant seeds during storage. *Journal of Seed Science*, v.46, e202446004, 2024. https://doi.org/10.1590/2317-1545v46277680

MAZINE, F.F.; BÜNGER, M.; FARIA, J.E.Q.; FERNANDES, T.; GIARETTA, A.; VALDEMARIN, K.S.; SANTANA, K.C.; SOUZA, M.A.D.; SOBRAL, M. *Eugenia*. In: Flora e Funga do Brasil, 2024. Rio de Janeiro: Jardim Botânico do Rio de Janeiro. https://floradobrasil.jbrj.gov.br/FB10560

MARCOS-FILHO, J. *Fisiologia de sementes de plantas cultivadas*. Londrina: ABRATES, 2015. 659p.

MOURA, G.C.; OLIVEIRA, J.I.; BONOME, L.T.S.; FRANZENE, G. *Eugenia uniflora* L.: potential uses as a bioactive plant. *Arquivos do Instituto Biológico*, v.85, p.1-9, 2018. https://www.scielo.br/j/aib/a/XKFppRWGQWvG6RKwXtCWjkq/?format=pdf&lang=en

NADARAJAN, J.; WALTERS, C.; PRITCHARD, H.W.; BALLESTEROS, D.; COLVILLE, L. Seed longevity - the evolution of knowledge and a conceptual framework. *Plants*, v.12, p.1-22, 2023. https://www.mdpi.com/2223-7747/12/3/471

PELISSARI, F.; PEREIRA, W.V.S.; PEREIRA, T.M.; SANTOS, H.O.; FARIA, J.M.R.; JOSÉ, A.C. Effect of PEG and ABA on desiccation tolerance and storage of *Magnolia ovata* (A.St.-Hil.) Spreng. seeds. *Journal of Seed Science*, v.44, p.1-11, 2022. https://www.scielo.br/j/jss/a/Pz5Xy5JG3MhKHtWSKWYLyQL/?format=pdf&lang=en

PEREIRA, L.C.V.; RAFAELLA, C. M.; CAROLINA, R. Z.; ANDERSON C. J.; FARIA J.M.R. Storage of short-lived seeds of *Inga vera* subsp. *affinis* in osmotic medium. *Seed Science Research*, v.30, p.156-160, 2020. https://doi.org/10.1017/S0960258520000185

ROBERTS, E.H. Predicting the storage life of seeds. *Seed Science and Technology*, v.1, p.499-514, 1973.

SANTANA, D.G.; RANAL, M.A. *Análise da germinação: um enfoque estatístico*. Brasília: Universidade de Brasília, 2004, 247p.

SCHWEMBER, A.R.; BRADFORD, K.J. Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. *Seed Science Research*, v.21, p.175-185, 2011. https://doi.org/10.1017/S0960258511000080

SILVA, H.W.; VALE, L.S.R.; SILVA, C.F.; VELASCO, M.F.; MONFORT, L.H.F. Qualidade de sementes de *Eugenia dysenterica* DC. durante o armazenamento. *Revista Engenharia na Agricultura*, v.27, p.12-21. 2019. https://periodicos.ufv.br/reveng/article/view/831/pdf

UMARANI, R.; AADHAVAN, E.K.; FAISAL, M.M. Understanding poor storage potential of recalcitrant seeds. *Current Science*, v.108, p.2023-2034, 2015. https://www.jstor.org/stable/24905571

VILLELA, F.A.; DONI-FILHO, L.; SEQUEIRA, E.L. Tabela de potencial osmótico em função da concentração de polietileno glicol 6000 e da temperatura. *Pesquisa Agropecuária Brasileira*, v.26, p.1957-1968, 1991. https://ainfo.cnptia.embrapa.br/digital/bitstream/AI-SEDE/21308/1/pab18_novdez_91.pdf

WYSE S.V.; DICKIE, J.B. Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology*, v.105, p.1082-1093, 2017. https://besjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/1365-2745.12725

WYSE S.V., DICKIE, J.B.; WILLIS, K.J. Seed banking not an option for many threatened plants. *Nature Plants*, v.4, p.848–850, 2018. https://www.nature.com/articles/s41477-018-0298-3