

Overcoming *Hancornia speciosa* seed recalcitrance: harvest season and storage time

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Research Article

Keywords: conservation, desiccation sensitivity, enzymatic activity, gene ontology, seed physiology, tropical plants

Posted Date: August 30th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1961756/v1>

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Abstract

Storage of desiccation-tolerant seeds is one of the most effective germplasm conservation strategies; however, several species of tropical and subtropical humid forests have seeds that are sensitive to desiccation, and recalcitrant seeds, making conservation a challenge. Recalcitrant seeds deteriorate during storage due to high respiration rates and metabolic activity, and protocols employing osmoprotective solutions aim to minimize those effects to maintain seed viability for a more extended period. *Hancornia speciosa*, a fruit tree considered a priority for research in Brazil, is a desiccation-sensitive species. Thus, this study aimed to assess the physiological parameters of viability, vigor, and enzymatic activity of *H. speciosa* seeds stored in an osmoprotective solution. Germination percentage, water content, electrical conductivity, shoot, root, seedling length, peroxidase activity, and heat-resistant protein concentration were determined for seeds collected during summer and winter harvests. In addition, gene sequences were explored through gene ontology using Blast analysis to identify the biological and molecular processes associated with enzymatic action during storage. Summer-collected seeds performed better in viability and vigor and are recommended for storage in the osmoprotective solution. After being stored in the solution, seeds collected in the winter improved germination and vigor. *H. speciosa* seeds harvested in the summer or winter and stored in the osmoprotective solution remain viable for up to 90 days. Peroxidase and heat-resistant proteins are active; these enzymes' expression regulation should be investigated in future studies.

Introduction

During seed storage, internal and external factors induce deterioration. Despite being a natural process, seed deterioration is complex, affects the germination rate, and progressively decreases seed vigor (Fotouo-M. et al. 2015; Fatokun et al. 2022).

At storage, seed deterioration rate is determined by molecular and physiological responses (Nagel et al. 2016; Renard et al. 2020; Zhou et al. 2020), influenced by the water content of the seed (Kameswara Rao et al. 2017), and by abiotic factors such as relative humidity, temperature, and the packaging (Wang et al. 2012; Fazeli-Nasab et al. 2022). In addition, biological factors such as viruses, fungi, and insects accelerate seed viability and vigor loss (Parisi et al. 2013; Bueso et al. 2017; Mahesha et al. 2022).

Seed deterioration is a cumulative, irreversible, degenerative process (Zhang et al. 2021). It composes a catabolic event that triggers biochemical reactions that influence the metabolic pathways (Nguyen et al. 2015). These reactions can result in alterations in the cellular constitution, cell and tissue oxidation, loss of performance, development of abnormal seedlings, and/or death.

Among the cellular, metabolic and biochemical changes associated with seed deterioration are chromosomal aberrations, nucleic acid damage, protein and enzyme synthesis, consumption of reserves, and membrane integrity loss (Waterworth et al. 2015; Boniecka et al. 2019; Fleming et al. 2019). The loss of membrane integrity is one of the main causes of the decrease in germination rate and vigor, resulting in the exudation of intracellular electrolytes and affecting longevity (Fotouo-M. et al. 2015).

For enzymatic changes, for example, there is a reduction in the activity of lipases, ribonucleases, proteases, catalases, peroxidases, and dehydrogenases (Colville et al. 2012; Min et al. 2017; Sahu et al. 2017). Hydrogen peroxides and reactive oxygen species (ROS) are produced in several metabolic processes, reaching deleterious levels in seeds. These deteriorative reactions occur faster in seeds dispersed and stored with high-water content, due to high respiration rates, metabolic activity, and consumption of cotyledons reserves (Walters et al. 2010). And, to maintain the viability of these seeds, several protocols aim to reduce respiration and metabolism rates or minimize the deleterious effects of deterioration under storage (Chandra et al. 2019; Pereira et al. 2020).

Despite its challenges, seed storage is one of the most effective strategies for long-term germplasm conservation in desiccation-tolerant seeds. Classified as orthodox, these seeds are dispersed with low water contents and can be desiccated to 6–8% water content and stored at low temperatures (-20°C) (Walters 2015). Under these conditions, the chemical and physical reactions that lead to viability loss are reduced in orthodox seeds.

However, several species from tropical and subtropical humid forests – an estimated 18.5% of known plant species – have recalcitrant seeds (Wyse and Dickie 2017). These humid forest environments are more suitable for immediate germination, and consequently, recalcitrant seeds are dispersed with higher water content and are sensitive to desiccation (Berjak and Pammenter 2013). Due to this characteristic, recalcitrant seeds remain with high respiration rates and metabolism during storage, causing physiological, biochemical, and molecular damages (Berjak and Pammenter 2008; De Vitis et al. 2020).

The methods developed and improved for storage based on the behavior of orthodox seeds created a notion that recalcitrant seeds cannot be stored for longer periods, because of their sensibility to desiccation and low temperatures (Walters 2015). However, the storage of recalcitrant seeds is highly relevant for the agriculture industry and the conservation of native genetic resources. Therefore, alternative strategies have been implemented such as cryopreservation and hydrated storage.

Cryopreservation strategies, such as storing the embryonic axes of seeds under low temperatures (Ballesteros et al. 2014), allow for the conservation of propagules for future in vitro development. Regardless of the advances in cryoprotective techniques and solutions, this strategy still challenges conservation of some species (Santana et al. 2018).

As an alternative, studies have developed hydrated storage strategies for recalcitrant seeds. By performing osmotic conditioning of the seeds, this technique aims to minimize intracellular damage – such as membrane and cell wall degradation, nucleic acid damage, and reactive oxygen species formation (Paparella et al. 2015). Among the most used solutions for osmotic conditioning is polyethylene glycol (PEG) solution, a substance capable of conditioning without penetrating the plant cell, and is an inert and non-toxic compound (Pelegrini et al. 2013; Azerêdo et al. 2016).

Hydrated storage in PEG solution has been employed to extend the viability of recalcitrant seeds of some species (Lahay et al. 2018; Pereira et al. 2020). Among those species is *Hancornia speciosa* Gomes, a native species to Brazil (Silva et al. 2021). The species has high commercial interest due to its fruits (Nunes et al. 2022b) used for the production of pulp and ice cream, produced during two annual harvest seasons. *H. speciosa* is a species threatened by habitat loss (Fajardo et al. 2018; Álvares-Carvalho et al. 2022; Silva et al. 2022) and is considered a priority for research in Brazil (Coradin et al. 2018). Moreover, its recalcitrant seeds have shown an increase in longevity under storage in an osmoprotective solution (Nunes et al. 2022a).

In addition to being recalcitrant, *H. speciosa* seeds have a higher content of lipids in their composition (Santos et al. 2015). This characteristic provides a greater predisposition to the deterioration process during storage, which may lead to the destruction of lipids and the formation of toxic products due to enzymatic hydrolysis and peroxidation (Berjak and Pammenter 2008; Marcos-Filho 2015).

Thus, it is important to investigate the enzymatic processes and pathways occurring in *H. speciosa* recalcitrant seeds, of both harvest seasons during hydrated storage to understand its metabolism better and to support *ex situ* conservation strategies for the species. The objective of this study was to evaluate the physiological parameters of viability, vigor and enzyme activity of *H. speciosa* seeds under storage in an osmoprotective solution.

Material And Methods

Hancornia speciosa var. *speciosa* seeds were obtained from summer (October to December) and winter harvests (April to May) (Coradin et al. 2018) in the area of Restinga vegetation of coastal tablelands within the Atlantic Forest biome in Sergipe, Brazil. The area presents a dry season during summer, composed of temperatures between 25°C and 32°C and 60 mm of monthly average precipitation, and a rainy season during winter, composed of temperatures between 22°C and 28°C and 860 mm of monthly average precipitation (Santos 2011).

Ripe fruits of *H. speciosa*, characterized by green to yellow exocarp with red spots of pigmentation (Pereira et al. 2016; Coradin et al. 2018), were manually processed to obtain seeds within each lot (Nunes et al. 2021), collected at natural populations explored by the traditional community 'Catadoras de Mangaba' in the state of Sergipe (Sergipe 2010).

The seeds were homogenized and sampled for hydrated storage. The storage occurred in a conservative osmoprotective solution for recalcitrant seeds based on the process described in the patent deposit 10 2021 009165 7 (Silva-Mann et al. 2021). The seeds were stored for 0, 30, 60, 90, 120, 150, and 180 days within the solution at $10 \pm 2^\circ\text{C}$ in the absence of light. Afterward, the seeds were analyzed for physiological and enzymatic responses by assessing of the initial quality, germination percentage, water content, electrical conductivity, shoot, root and seedling length, the concentration of peroxidase, and heat-resistant proteins.

Germination percentage (G%): the seeds were germinated on Gernitest® paper moistened with 2.5 times its dry weight with distilled water. The germination occurred at 25°C with a 12h photoperiod (Brasil 2009). The tests were composed of 4 replicates of 25 seeds each and lasted until the 35th day after sowing.

Water content(WG): using the oven method, 10 g of seeds were dried at $105 \pm 3^\circ\text{C}$ for 24h, in duplicate (Brasil 2009).

Electrical conductivity (EC): 25 seeds were weighed and immersed in 75 mL of distilled water, at 25°C for 24h, in the absence of light, using 4 replications (Vieira and Krzyzanowski 1999).

Shoot (SHL), root (RL), and seedling length (SL): at the last day of the germination tests, normal seedlings were photographed using GroundEye® and the measurements obtained (cm).

The concentration of peroxidase: for each storage time, 20 mg of cotyledons was obtained, homogenized for 5 min, and filtered through gauze. The filtrate was centrifuged at 6,800 xg, 4°C, for 15 min, and the removed supernatant was used in the assay. To determine the peroxidase content in the samples, 20 µg of protein + 25mM citrate-phosphate buffer (pH = 5.4) + 1mM guaiacol were used for each 1 mL of the reaction

mixture. The reaction was initialized with the addition of 10 µL of 30% hydrogen peroxidase and the concentration was determined by absorbance at 475 nm at 25°C for 1 min with 10-sec intervals.

Concentration of heat-resistant proteins: 150 mg of extracted embryonic axis were macerated using 1 mL of extraction buffer (50 mM Tris-HCl pH 7.5; 500 mM NaCl; 5 mM MgCl₂; 1mM PMSF). The samples were shaken for 1 min. and centrifuged at 16,000 xg, 4°C, for 30 min. The supernatant was incubated in a water bath at 70°C for 15 min. The protein content was quantified using the Bradford method (Bradford 1976) adding 5 µL of the sample to 195 µL of Bradford's reagent. The samples reacted for 5 min at 25°C. The concentration was determined by absorbance at 595 nm using bovine serum albumin (BSA) as reference. Both enzyme concentrations were determined using a microplate spectrophotometer (Epoch™).

The experiment had a completely randomized design, with four replications. The experiment was composed of a factorial scheme of two harvest seasons (summer and winter) and six storage periods (0, 30, 60, 90, 120, 150, and 180 days). The data were tested for homoscedasticity and normality using Breusch-Pagan and Shapiro-Wilk tests and submitted to an analysis of variance using the F test. A regression analysis was used to analyze the variables throughout the storage time using R (R Core Team 2022).

The data was further analyzed using a correlation matrix. Pearson's linear correlation coefficients (r) were calculated for all physiological and enzymatic data combinations. The t-test ($p \leq 0.05$) was used to determine the significance of the r values. The data was processed and statistically analyzed using R (R Core Team 2022).

Additionally, an analysis of gene sequences sought to further elucidate the possible pathways of seed deterioration of *H. speciosa* during storage. Functional analysis of proteins was conducted through Gene Ontology using the Blast2GO tool (Conesa and Götz 2008; Moothoo-Padayachie et al. 2018). Gene sequences of *H. speciosa* in FASTA format were obtained from the Kew Gardens database for the Tree of Life project (Royal Botanic Gardens), available at treeoflife.org, for input to Blast2GO. Initially, Blast2GO was used to run a Blastx search and consequently employed to map Gene Ontology (GO) and Interpro terms and to annotate the sequences. Blast2GO's automatic annotation was manually revised to ensure accurate assignment. The Blast2GO tool was also used to conduct metabolic pathway analyses of identified proteins according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database (<http://www.genome.jp/kegg/pathway.html>). The Bioconductor R package *rrvgo* (Sayols 2020) was used to visualize the Gene Ontology terms.

Results And Discussion

The analyses revealed a significant difference between harvest seasons for *H. speciosa* seeds in all the parameters assessed. There was a wide difference in germination between the two lots. The summer lot had an 80% germination rate of freshly harvested seeds, similar to other studies (Barros et al. 2011; Oliveira et al. 2018; Nunes et al. 2021). The summer harvest is the main production period of the species when trees produce more and larger fruits (Silva-Júnior and Léo; Coradin et al. 2018). Whereas the winter lot had no germinated seeds before storage. Previous research has reported a low germination rate of freshly harvested seeds of *H. speciosa*, such as 38 (Masetto and Scalón 2014) and 45% (Oliveira and Valio 1992), which is attributed to its recalcitrance (Lorenzi 1992).

Recalcitrant seeds have different characteristics that vary significantly between and within species, as well as seasonally (Berjak and Pammenter 2013), and these results may indicate immaturity during the dispersion of seeds from the winter harvest. Lower maturity at seed dispersal can be attributed to an environment adaptation, presenting seeds at various degrees of recalcitrance (Barbedo 2018), a characteristic that needs further investigation for this species. High relative air humidity and rainfall, according to (MOTA et al. 2005), influence the physical characteristics of *H. speciosa* fruits during the winter harvest and may affect the physiological aspects of its seeds.

Germination increased until 90 days of storage for the winter lot. The storage solution might have allowed seed maturation, and the viability of the winter harvest's immature material increased to levels comparable to the summer harvest at 90 days of storage. In contrast, the germination rate of the summer lot decreased gradually. Despite maintaining seed water content. Ledo et al. 2015 observed a decrease in germination only after four days of storage of *H. speciosa* seeds. Furthermore, seeds of the recalcitrant species *Garcinia gardneriana* (Planch. & Triana) Zappi showed a decrease in germination until 90 days of storage (Viana et al. 2020).

The seeds did not germinate for both lots after 150 days of storage in the solution. After this period, there is also the influence of storage fungi such as *Aspergillus* and *Penicillium* that negatively affect seed germination, as observed during this study and previously reported by Anjos et al. 2009 and Nunes et al. 2022a during storage of *H. speciosa* seeds under different solutions. The presence of internal fungal inoculum is a common condition of tropical and subtropical seeds, which affects hydrated storage due to the high proliferation of these seed-associated fungi (Sutherland et al. 2002).

In a similar behavior to germination, shoot, root, and seedling length (Fig. 2) decreased gradually over storage time for the summer lot, indirectly indicating a decrease in seed vigor, while there was an increase in length for the winter lot until 90 days of storage. The range of seedling length

values until 90 days for the summer lot was similar to that previously reported for *H. speciosa* (7 to 14 cm) after drying seeds up to 144 hours (Santos et al. 2010). Moreover, those values were only found in the winter lot at 90 days of storage.

Masetto and Scalon 2014, observed a decrease in seedling length after osmotic conditioning of *H. speciosa* seeds using a PEG solution. Immature seeds, however, are more responsive to the physiological benefits of osmotic priming for both orthodox and recalcitrant behaviors, resulting in improved seed vigor (Finch-Savage and Bassel 2016). We observed a positive effect of the osmoprotective solution on the vigor of these seeds considering the results of the physiological parameters for the winter lot in germination and seed vigor estimated by seedling length.

Seed water content remained stable throughout the storage period for both harvest seasons. There was no statistical difference between storage time, and the summer harvest had an average of 53% seed water content, while the winter harvest had an average of 49%.

Water content is directly related to seed viability in desiccation-sensitive species, including *H. speciosa* (Oliveira and Valio 1992), which is dispersed with 48–56% of water content, and a reduction to 38% or below causes a gradual loss in viability (Santos et al. 2010). A reduction of *H. speciosa* seed water content to 15% led to a decrease of 30% in radicle protrusion in a previous study (Dresch et al. 2016). Consequently, due to the species' desiccation sensitivity, maintaining high-water content in an osmoprotective solution during storage may be beneficial for its prolonged viability.

However, water content is intrinsically related to embryonic metabolic activity (Vertucci 1989), which may result in oxidative processes and seed deterioration. An indicative of this process is the leachate exudation of cellular components due to the disruption in cell membrane stability, affecting seed viability. For both harvest seasons, leachate exudation increased with storage time, as indicated by electrical conductivity values (Fig. 3), implying a gradual process of age-induced cellular deterioration.

Previous research on the electrical conductivity of the species found similar initial values of 12.4 $\mu\text{S}/\text{cm}/\text{g}$ (Nunes, 2021). However, in other studies, this parameter ranges up to 54.32 $\mu\text{S}/\text{cm}/\text{g}$ (Barros et al. 2011). In addition, another study found a gradual increase in electrical conductivity of *H. speciosa* seeds during storage until 200 days, regardless of storage method (Nunes et al. 2022a).

The difference in enzymatic response of *H. speciosa* lots may reveal mechanisms of action related to cellular damage caused by oxidative stress in desiccation-sensitive plants. Oxidative stress is caused by excess reactive oxygen species (ROS) production, leading to oxidative damage and seed viability loss in recalcitrant seeds (Chandra et al. 2020). Excessive ROS production causes irreversible damage to proteins, nucleic acids, and lipids. Nunes et al. 2022 hypothesize that the high lipid content of *H. speciosa* seeds causes chemical instability during oxidative processes, resulting in the deteriorative activity of lipid peroxidation (Prudente et al. 2017) and reactions resulting in superoxide.

Besides, recalcitrant seeds may lose viability regardless of water loss; therefore, an antioxidant system capable of regulating intracellular ROS levels may be able to maintain viability (Pammenter et al. 1994). Enzymes such as peroxidase remove potentially toxic ROS (Pandey et al. 2017). Peroxidase and heat-resistant protein activity did not differ significantly across storage times, but winter lot seeds had higher concentrations for both enzymes. The summer lot had an average peroxidase concentration of 0.01 mol/L, while the winter lot had 0.02 mol/L. The concentration of heat-resistant proteins in summer lot seeds was 0.32 mg/mL, while winter harvest seeds had a fivefold higher concentration (1.63 mg/mL).

Heat-resistant proteins decrease during seed germination (Soares et al. 2015) and increase during seed maturation and stress (Han et al. 1997). Despite their chaperone-like properties and macromolecule stabilizer mechanisms (Hara 2010), heat-resistant proteins are insufficient to prevent damage in desiccation-sensitive seeds. Some heat-resistant proteins, such as dehydrins, accumulate in the recalcitrant seeds of *Camellia*, *Castanea*, *Euterpe*, and *Quercus*, extending their viability during storage (Kleinwächter et al. 2014; Radwan et al. 2014). Nonetheless, the role of these proteins in recalcitrant seeds remains not fully elucidated.

The protein activity found corroborates the higher electrical conductivity for the winter lot and further indicates damage in plasma membranes caused by oxidative stress-induced lipid peroxidation (Kumar et al. 2015; Mittler 2017; Chandra and Keshavkant 2018).

Considering the parameters for both lots, there is a strong positive correlation (> 0.81) between seed viability and vigor, as measured by germination, shoot, root, and seedling length (Fig. 4). Furthermore, there is a strong inverse relationship (< 0.84) between viability and vigor parameters, determined by germination, shoot, root and seedling length, and electrical conductivity as a seed deterioration. Because there was no statistical difference between storage periods, peroxidase, heat-resistant protein activity, and water content, which did not show a strong correlation to other parameters.

The Gene Ontology analysis of sequences for this species has identified proteins categorized in several metabolic processes related to structural cellular components and the metabolic pathways of organic, nitrogen, and aromatic compounds. The main categories were binding and catalytic activity, followed by organic cyclic compound binding and heterocyclic compound binding metabolism (Fig. 5).

During hydrated storage, proteins involved in processes such as protein binding, organic cyclic compound binding, and heterocyclic compound binding were up-regulated in the seeds of the desiccation-sensitive *Thrichilia dregeana*. Peroxidase and oxidoreductase activity proteins were also up- and down-regulated. Enzymes that act as ROS scavengers, such as L-ascorbate peroxidase and peroxiredoxin were up-regulated during storage for the species (Moothoo-Padayachie et al. 2018), which are immature at dispersal (Pammenter and Berjak 2014).

Furthermore, the authors proposed a selective antioxidant response system including peroxidase activity during hydrated storage that differs from natural desiccation stress (Moothoo-Padayachie et al. 2018). From the annotated proteins for *H. speciosa*, the following (Table 1) are related to the metabolic processes involved in germination and seed oxidative stress and can be further investigated.

Table 1

Gene Ontology (GO) Terms for *Hancornia speciosa* sequence data related to germination metabolism and oxidative stress in seeds.

Sequence Database Code	GO Terms	Description	Similarity%	GO Names
6270	F:GO:0004322; C:GO:0005739; P:GO:0006783; P:GO:0006811; P:GO:0006879; F:GO:0008198; F:GO:0008199; P:GO:0009060; C:GO:0009507; P:GO:0009793; P:GO:0018283; F:GO:0034986; P:GO:0042542; F:GO:0051537; P:GO:1903329	Frataxin, mitochondrial-like	94.15	F: ferroxidase activity; C: mitochondrion; P: heme biosynthetic process; P: ion transport; P: cellular iron ion homeostasis; F: ferrous iron binding; F: ferric iron binding; P: aerobic respiration; C: chloroplast; P: embryo development ending in seed dormancy; P: iron incorporation into metal sulfur cluster; F: iron chaperone activity; P: response to hydrogen peroxide; F:2 iron, 2 sulfur cluster binding; P: regulation of iron-sulfur cluster assembly
5703	C:GO:0005654; C:GO:0005730; P:GO:0006364; P:GO:0009793; C:GO:0016021; C:GO:0030692; C:GO:0032040	Nucleolar complex protein 4 homolog isoform X1	96.31	C: nucleoplasm; C: nucleolus; P: rRNA processing; P: embryo development ending in seed dormancy; C: integral component of membrane; C: Noc4p-Nop14p complex; C: small-subunit processome
5922	F:GO:0003729; F:GO:0005525; C:GO:0009507; P:GO:0009793; P:GO:0042254; P:GO:0042793	GTPase der-like isoform X1 1461	92.01	F: mRNA binding; F: GTP binding; C: chloroplast; P: embryo development ending in seed dormancy; P: ribosome biogenesis; P: plastid transcription
6717	F:GO:0000287; F:GO:0003729; F:GO:0003924; F:GO:0005525; P:GO:0006364; P:GO:0009416; C:GO:0009570; P:GO:0009658; C:GO:0009706; P:GO:0009793; P:GO:0010027	GTP-binding protein OBGC, chloroplastic 1344	90.79	F: magnesium ion binding; F: mRNA binding; F: GTPase activity; F: GTP binding; P: rRNA processing; P: response to light stimulus; C: chloroplast stroma; P: chloroplast organization; C: chloroplast inner membrane; P: embryo development ending in seed dormancy; P: thylakoid membrane organization
5502	P:GO:0002949; C:GO:0005743; P:GO:0009793; F:GO:0046872; F:GO:0061711	Probable tRNA N6-adenosine threonylcarbamoyltransferase, mitochondrial	84.59	P: tRNA threonyl carbamoyl adenosine modification; C: mitochondrial inner membrane; P: embryo development ending in seed dormancy; F: metal ion binding; F: N (6)-L-threonyl carbamoyl adenine synthase activity
6128	F: GO:0004386	Vicilin-like seed storage protein At2g18540	71.62	F: helicase activity
6265	C:GO:0005829; P:GO:0009793; P:GO:0051301	Tubulin-folding cofactor B	93.15	C: cytosol; P: embryo development ending in seed dormancy; P: cell division
5339	F:GO:0003729; C:GO:0005730; P:GO:0006417; P:GO:0009793	Pumilio homolog	83.78	F: mRNA binding; C: nucleolus; P: regulation of translation; P: embryo development ending in seed dormancy
5347	F:GO:0008320; P:GO:0009793; C:GO:0016021; F:GO:0030943; C:GO:0042721; P:GO:0045039; P:GO:0071806	Mitochondrial import inner membrane translocase subunit TIM22-4-like isoform X1	94.86	F: protein transmembrane transporter activity; P: embryo development ending in seed dormancy; C: integral component of membrane; F: mitochondrion targeting sequence binding; C: TIM22 mitochondrial import inner membrane insertion complex; P: protein insertion into mitochondrial inner membrane; P: protein transmembrane transport

Sequence Database Code	GO Terms	Description	Similarity%	GO Names
5406	P:GO:0000717; F:GO:0003678; F:GO:0003684; F:GO:0005524; C:GO:0005634; P:GO:0006366; P:GO:0006979; P:GO:0009408; P:GO:0009411; F:GO:0016887; P:GO:0033683; P:GO:0045951	DNA repair helicase XPD	87.75	P:nucleotide-excision repair, DNA duplex unwinding; F: DNA helicase activity; F: damaged DNA binding; F: ATP binding; C: nucleus; P: transcription by RNA polymerase II; P: response to oxidative stress; P: response to heat; P: response to UV; F: ATP hydrolysis activity; P:nucleotide-excision repair, DNA incision; P: positive regulation of mitotic recombination
6533	F:GO:0004587; C:GO:0005739; P:GO:0006593; P:GO:0006979; P:GO:0009408; P:GO:0009413; P:GO:0009414; P:GO:0009733; P:GO:0009737; P:GO:0009741; P:GO:0009753; P:GO:0010121; P:GO:0019544; F:GO:0030170; P:GO:0042538; P:GO:0042742; F:GO:0042802; F:GO:0050155; P:GO:0051646; P:GO:0055129	Ornithine aminotransferase, mitochondrial	86.87	F:ornithine-oxo-acid transaminase activity; C: mitochondrion; P: ornithine catabolic process; P: response to oxidative stress; P: response to heat; P: response to flooding; P: response to water deprivation; P: response to auxin; P: response to abscisic acid; P: response to brassinosteroid; P: response to jasmonic acid; P: arginine catabolic process to proline via ornithine; P: arginine catabolic process to glutamate; F: pyridoxal phosphate binding; P: hyperosmotic salinity response; P: defense response to bacterium; F: identical protein binding; F: ornithine(lysine) transaminase activity; P: mitochondrion localization; P: L-proline biosynthetic process
6969	F:GO:0005524; C:GO:0005737; F:GO:0032542; P:GO:0034599; P:GO:0098869	Sulfiredoxin, chloroplastic/mitochondrial	89.11	F: ATP binding; C: cytoplasm; F: sulfiredoxin activity; P: cellular response to oxidative stress; P: cellular oxidant detoxification
5333	F:GO:0000049; P:GO:0002926; C:GO:0005634; C:GO:0005829; P:GO:0006979; P:GO:0008284; P:GO:0009787; P:GO:0009965; P:GO:0010928; P:GO:0031538; C:GO:0033588; P:GO:0035265; P:GO:0048530; P:GO:0051301; P:GO:0080178; P:GO:2000024	Elongator complex protein 1 isoform X1	79.08	F:tRNA binding; P:tRNA wobble base 5-methoxycarbonylmethyl-2-thiouridylation; C: nucleus; C: cytosol; P: response to oxidative stress; P: positive regulation of cell population proliferation; P: regulation of abscisic acid-activated signaling pathway; P: leaf morphogenesis; P: regulation of auxin-mediated signaling pathway; P: negative regulation of anthocyanin metabolic process; C: elongator holoenzyme complex; P: organ growth; P: fruit morphogenesis; P: cell division; P:5-carbamoyl methyl uridine residue modification; P: regulation of leaf development
6946	C:GO:0005747; P:GO:0006979; P:GO:0032981	Probable NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12	96.75	C: mitochondrial respiratory chain complex I; P: response to oxidative stress; P: mitochondrial respiratory chain complex I assembly
5513	F:GO:0016691; F:GO:0016787; P:GO:0098869	Soluble epoxide hydrolase	66.42	F: chloride peroxidase activity; F: hydrolase activity; P: cellular oxidant detoxification

Hydrated storage may be an effective way to maintain an active germplasm collection for some species. Utilizing the entire set of gene bank conservation methods is necessary for effective *ex-situ* plant conservation. To have efficient use of storage space and conserve genetic diversity, it is critical to have information on the storage behavior of seeds based on their various characteristics, such as viability and vigor in relation to seasonality. The present results indicate strategies to extend the longevity of *Hancornia speciosa* seeds by addressing their desiccation sensitivity and seasonality; however, further exploitation of processes related to metabolism-driven deterioration during storage, particularly the mechanisms activated during oxidative damage, is required.

Conclusion

Hancornia speciosa Gomes seeds stored in the osmoprotective solution maintain viability until 90 days for both summer and winter harvest lots. The seeds from the summer lot perform better in terms of viability and vigor. Despite showing lower viability and vigor results, the winter lot shows an improvement in germination until 90 days of storage. We recommend the use of the osmoprotective solution to store lots of seeds with high vigor from the summer harvest. The activity of peroxidase and heat-resistant proteins indicates the presence of oxidative stress processes; the regulation of the expression of these enzymes should be investigated further.

Declarations

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

We thank the National Council for Scientific and Technological Development—Brazil (CNPq), the Coordination for the Improvement of Higher Education Personnel—Brazil (CAPES), Brazilian Innovation Agency (FINEP), and the Research Group on Conservation, Breeding and Management of Genetic Resources (GENAPLANT).

Contributions

JLS conducted the research and wrote the text, VVN, BALF and CCC contributed to obtaining the research data, and in the writing. HOS and RSM guided the research, revised the text, and contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

All the authors of this manuscript declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

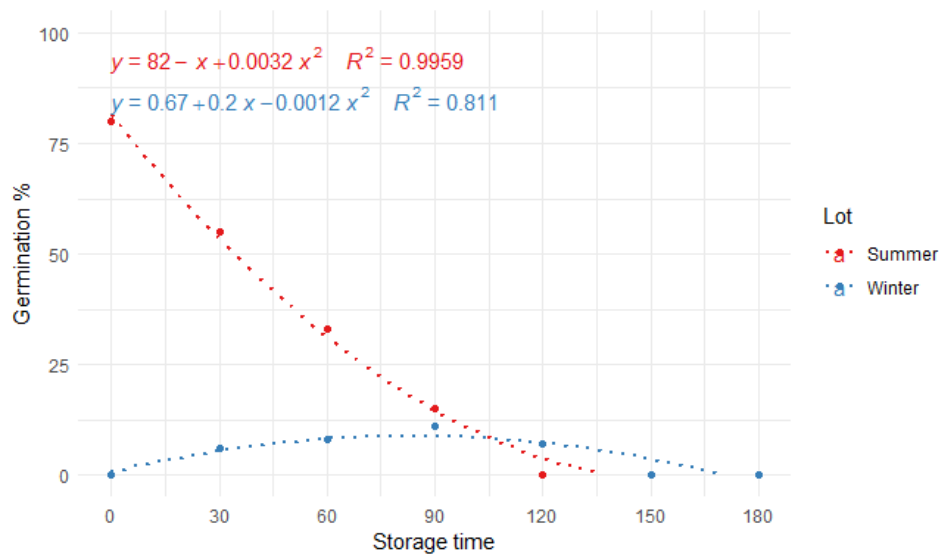


Figure 1

Germination percentage of *Hancornia speciosa* seeds of the summer and winter lots during storage time (days) in conservative solution.

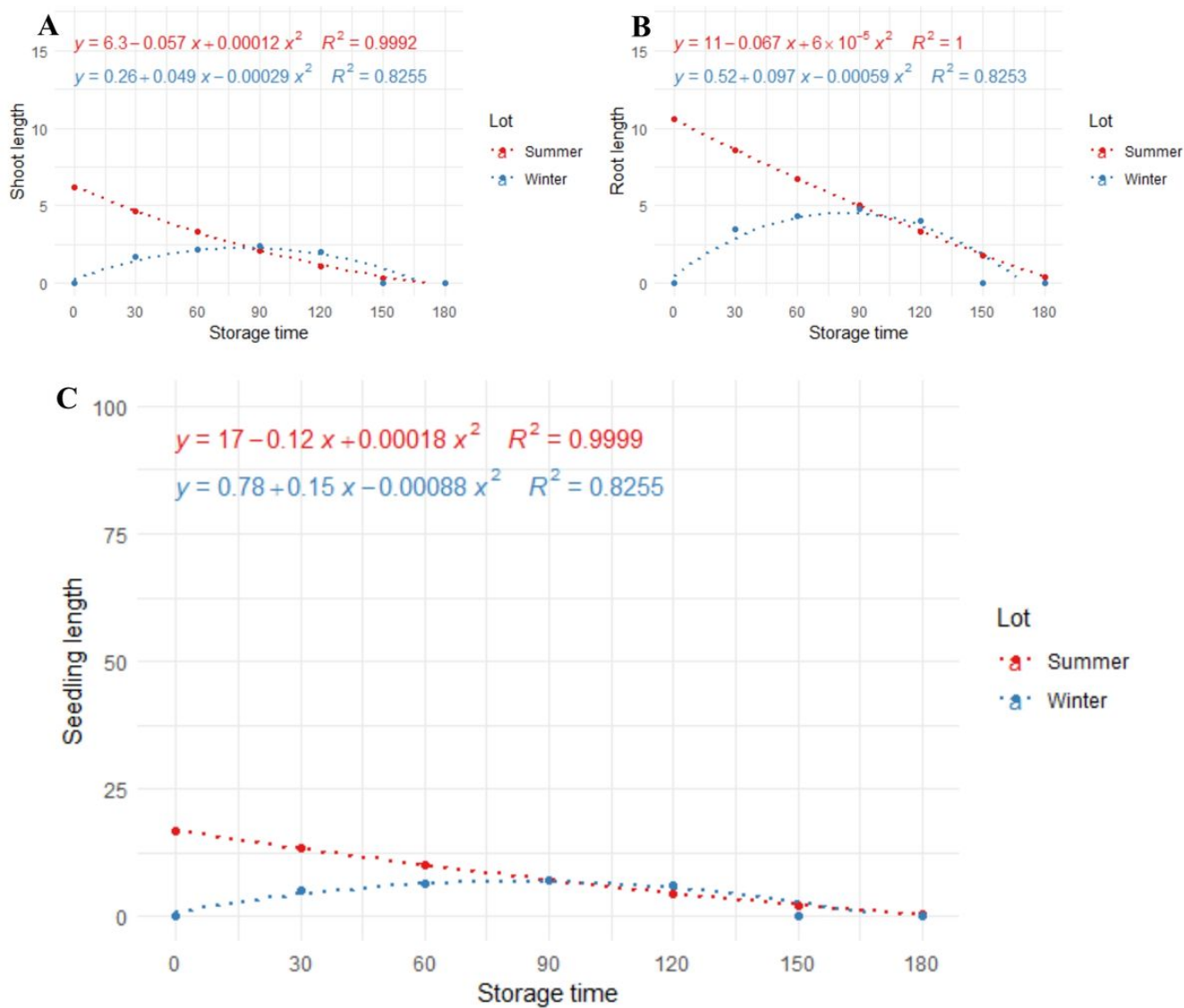


Figure 2

Shoot (A), root (B), and seedling (C) length of *Hancornia speciosa* seeds of the summer and winter lots during storage time (days) in the conservative solution.

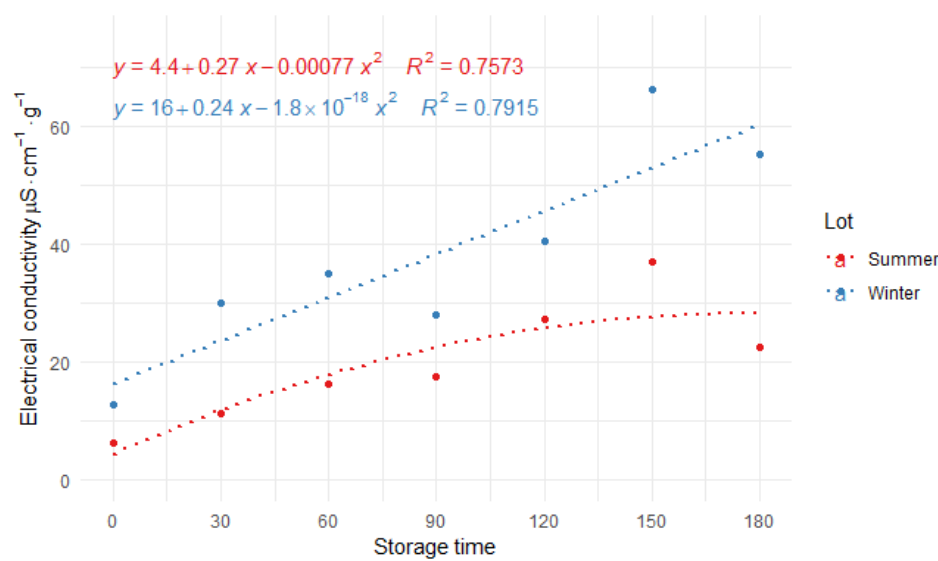


Figure 3
Electrical conductivity of *Hancornia speciosa* seeds of the summer and winter lots during storage time (days) in conservative solution.

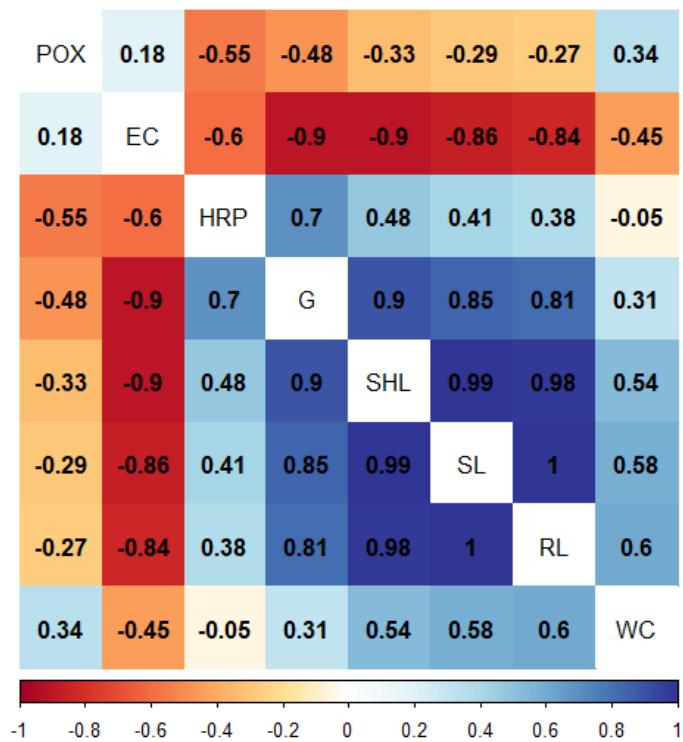


Figure 4
Pearson correlation matrix between the physiological parameters of germination percentage (G), water content (WC), electrical conductivity (EC), shoot (SHL), root (RL) and seedling length (SL), and the enzymatic activity of peroxidase (POX) and heat-resistant proteins (HRP) of *Hancornia speciosa* seeds of the summer and winter lots during storage in conservative solution.

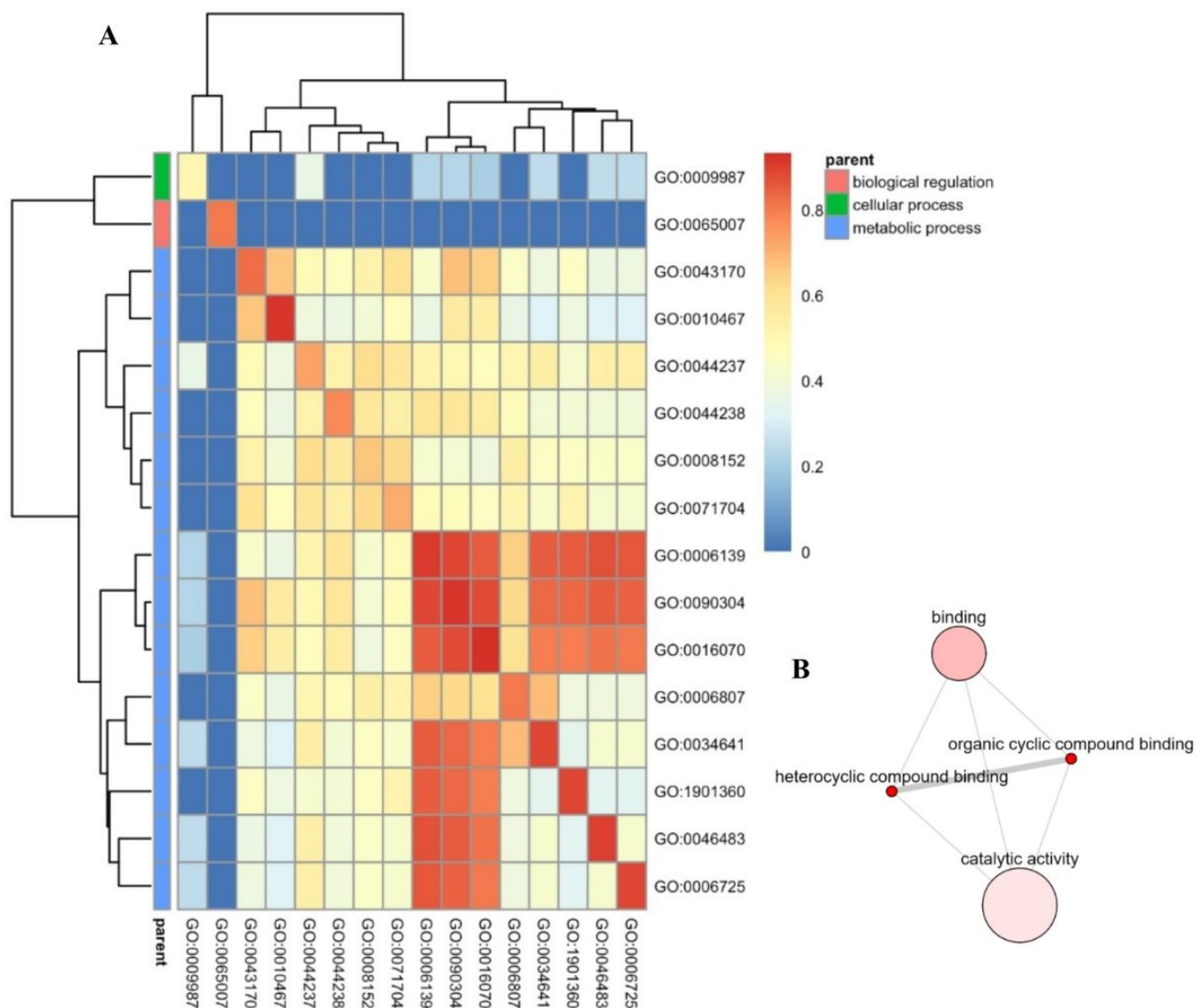


Figure 5

(A) Gene ontology biological processes: Heatmap of *Hancornia speciosa* gene sequences after enrichment analysis. (B) Gene ontology molecular function: Interactive graph of *Hancornia speciosa* gene sequences after enrichment analysis.