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ORIGINAL ARTICLE

Physiological behavior trend of *Campomanesia xanthocarpa* (Myrtaceae) seeds under desiccation and their implication for germplasm conservation

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Abstract

Key message **Seed desiccation sensitivity is not determined only by water content threshold but is a consequence of drying rate, which causes rapid viability loss, and this was associated to mechanical/physical damage**

Abstract *Campomanesia xanthocarpa* is a tree from Brazilian Atlantic Forest, one of the world's biodiversity hotspots, that has potential as a model for studies on the physiological behavior of seeds susceptible to desiccation of tropical species. Desiccation sensitivity (DS) seeds are a major concern for ex situ conservation efforts and superficial inferences may generalize their behavior in a wider perspective. Although major physiological responses to stress are shared and relatively well understood, DS seeds responses can be highly variable. In addition, there is a lack of studies concerning DS seeds endogenous polyamines content, antioxidant activity and its relation to desiccation stress and seed viability. Seeds were desiccated and went through germination tests, histological analysis, and estimation of antioxidant enzymatic activity, lipid peroxidation and polyamines content. Due to inherent morphological features, the seeds have a high drying rate and rapidly lose viability within 24 h. During the first 6 h of drying 76% of initial water content is lost, but 96% of the seeds still germinated. Spermidine and H_2O_2-scaveging enzymes activity showed a positive correlation, endorsing polyamines antioxidant role. Although lipid peroxidation increased along seed drying, it was minimal and suggested to be an ongoing process when viability was lost. Due the intensity of tissue water loss it is likely that mechanical/physical damage led to seed viability loss. Assessing the nature of damage and physiological stress response mechanisms contribute to better understand species vulnerability and broad the knowledge of tropical seed behavior.

Keywords Antioxidant defense · Climate change · Desiccation sensitive seed · Ex situ conservation · Lipid peroxidation · Polyamines

Introduction

The global strategies for plant conservation (GSPC) place plants with desiccation sensitive seeds as a major concern to ex situ conservation efforts (Diversity 2012). Seeds have been traditionally classified as their desiccation tolerance (DT) or desiccation sensitivity (DS) based in storage capacity and thresholds of water content (WC) (Roberts 1973). Intermediate seeds are found in between these two contrasting behaviors (Ellis et al. 1990), since they tolerate further drying and storage than DS, but not as DT seeds. These categories are useful for storage purposes, but classifying seeds merely on WC threshold may lead to physiological misinterpretations, as it is not a measure of stress intensity (Walters 2015). Nonetheless, the context of seed physiological behavior is now being considered in a perspective of

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a continuous spectrum of desiccation tolerance/sensitivity (Berjak and Pammenter 2008). Although major physiological responses against desiccation stress are shared among plant organs (Farrant and Moore 2011), unusual DS seed behaviors are emerging and suggest that each species may have its own relation to the environment and respond in its own way against stresses. Therefore, differences in kind or degree may generalize the wider scope and it is necessary looking at seed physiological behaviors as tendencies within the spectrum of tolerance.

In terms of conservation, it is important take into account that DS seeds comprise almost half of the tree species in tropical and subtropical evergreen rainforests and 8% of total world's seed plants (Tweddle et al. 2003; Wyse and Dickie 2017). Due the relative predictive seasons of their environments, DS seeds are thought to have suffered lower selective pressures (Marques et al. 2018), which make them more susceptible to climate changes. Thus, the physiological and ecological strategies of DT seeds are relatively well studied and understood (Dekkers et al. 2015; Leprince et al. 2017), including the climate change context (Walck et al. 2011). On the other hand, only recently researchers started surveying deeper about the ecophysiological aspects of DS seed behaviors. High WC and metabolic activity are one of the main physiological features that comprise DS seeds. It is suggested that they lose viability due three general causes, mechanical/physical damage during water loss and rehydration, metabolism-induced damage along time, and macromolecular denaturation (Umarani et al. 2015). Once shed and exposed to the environment intracellular water is lost and either cellular membranes collapse or the unregulated production of reactive species of oxygen (ROS) compromises cell integrity over time. In this sense, physiological protective mechanisms are determinant to maintain viability, but DS seed constitution and responses to stress are usually highly variable or, in the latter case, even absent (Berjak and Pammenter 2013). In this context, polyamines (PAs) are small polycationic molecules that play important roles in many physiological processes, once they interact to hormones and regulate plant growth and development, and also accumulate as a signal and/or protection against abiotic stresses (Tiburcio et al. 2014). Since PAs stimulate the activity of antioxidant enzymes they may also have an indirect role in scavenging ROS and maintaining cellular homeostasis along the desiccation process. However, there are literally no studies concerning PAs behavior during DS seed desiccation and investigating this process may provide new physiological information and broad the perspective of perception and response of DS seeds to stress.

The seed physiological behavior spectrum must be assessed from broader perspective so that it can be compatible with the in situ and ex situ conservation demands of each species. The Brazilian Atlantic Forest is a biodiversity hotspot that is characterized by a great number of DS seed species (de Souza et al. 2015), especially in later successional stages, nevertheless it is currently highly fragmented and threatened by anthropogenic activities. Myrtaceae is one of the most representative tree families (710 species) in the Atlantic Forest and has a great number of endemic species, which most of them produces DS seeds, but many of these species are disappearing before we even get to know them (Landrum and Kawasaki 1997). In this context, *Campomanesia xanthocarpa* (Mart.) O. Berg, popularly known as guabiroba, is a Myrtaceae tree found in the Southern Brazilian Atlantic Forest. *C. xanthocarpa* is zoochoric, exhibit DS seeds and has economic potential for food, medicinal and ornamental purposes (Lisbôa et al. 2011). Attempts tried to reduce the DS behavior of seeds, but they were unsuccessful (Nunes et al. 2015). Moreover, the ecological and evolutionary context of this species could represent a biological model to understand seed physiological behavior related to desiccation tolerance, looking to improve the knowledge about the spectrum of DS seeds of tropical ecosystems. Seed viability and the success of seedling establishment could be explained by the physiological responses of seeds to the desiccation process. Therefore, the physiological behavior context of the seed desiccation process could supply additional information about viability loss, vulnerability and conservation of related species. In this sense, our work aimed to study the physiological behavior of *C. xanthocarpa* seeds during the dehydration process, to broad the context of tropical DS seeds. We showed the relation between seed dehydration rate and morphophysiological alterations which allow us to assess seed viability, nature of damage and stress degree. In addition, the relation of PAs and antioxidant enzymatic defense with seed viability was here discussed addressing to the metabolic and mechanical/physical damage over seed dehydration. Our study showed seed physiological behavior trend of native species from Brazilian Atlantic Forest, which is crucial to ensure the conservation of many threatened or potential threatened species as well to head in situ and ex situ conservation efforts.

Materials and methods

Plant materials

Mature fruits of *C. xanthocarpa* were harvested from plants population at January 2018 in Santa Catarina state (S 27° 36′ 74″, W 50° 56′ 87″), Brazil. Fruits were transferred to the laboratory and stored under 4–8 °C. Due the nature of their desiccation behavior, seeds had to be immediately submitted through the planned experiments after being extracted from the fruits.

Seed WC and drying

WC of seeds was determined gravimetrically by difference in weight before and after drying at 105 ± 2ºC for 24 h (Brasil 2009) and expressed in dry basis (g H_2O g dw^{-1}). Fresh seeds were submitted through air drying (AD) and silica drying (SD). For AD seeds were incubated at 27 ± 2 °C (55–65% RH), while SD was carried out in sealed plastic containers with dry silica at 27 ± 2 °C. Seed drying rate index (k) was calculated from the amount of water loss in dry basis (g H_2O g dw^{-1}) divided by correspondent elapsed time in hours.

Germination test

After disinfested seeds were placed over Germitest® paper in sterile acrylic boxes (11 × 11 cm), with distilled water and incubated in a germination chamber type BOD (25 ± 2 °C, 60%RH and 12/12 h photoperiod). Seeds were monitored daily and considered germinated with the protrusion of the radicle. Once the test ended germination rate and germination speed index (GSI) (Maguire 1962) were assessed.

Electrolytic leakage

Seeds of each desiccation treatment were submerged in 75 ml of distilled water and leakage of electrolytes was measured over 12 h using a conductivity meter. Final leakage values were expressed as µS cm^{-1} g^{-1}.

Seed osmotic potential

The relation between water potential and WC of *C. xanthocarpa* seeds was estimated based in different concentration of polyethylene glycol (PEG 6000) solutions according to Michel and Kaufman (Michel and Kaufmann 1973). Once whole seeds reached osmotic equilibrium with each PEG solution their WC was estimated gravimetrically.

Light microscopy

Seeds were processed according to the histological methodology described by Steiner et al. (2015). Sections on glass slides were stained with phosphate-buffered toluidine blue. Images were captured under light microscope equipped with digital camera.

Antioxidant enzymes analysis and lipid peroxidation

Seed embryos were used to estimate Superoxide dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX) activities, which were measured using spectrophotometer. SOD activity was estimated by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Giannopolitis and Ries 1977). CAT activity was estimated by the decrease in absorbance of H_2O_2 (extinction coefficient 39.4 M^{-1} cm^{-1}) at 240 nm for 5 min (Peixoto et al. 1999). APX activity was estimated following the decrease in absorbance of A_{290} (extinction coefficient 2.8 mM^{-1} cm^{-1}) at 290 nm for 10 min (Koshiba 1993). The protein contents of the extracts were determined according to Bradford (1976). The level of lipid peroxidation was estimated according to an adaptation of Hodges et al. (1999) from the formation of malondialdehyde–thiobarbituric acid complex (MDA), and measured in spectrophotometer at 532 nm and corrected by subtracting the absorbance at 600 and 440 nm.

PAs analysis

Seed embryos were used to PAs determination according to Steiner et al. (2007). Free PAs were identified by HPLC. PAs content was determined using a fluorescence detector at 340 nm (excitation) and 510 nm (emission). Retention times and peak areas were measured by comparison with standard PAs solution: Putrescine (Put), Spermidine (Spm) and Spermine (Spm). PAs content was expressed as nmol per gram of dry weight.

Statistical analysis

Data were subjected to one-way ANOVA and compared by Tukey ($p < 0.05$). Pearson correlation test ($p < 0.05$) was performed among data. All statistical analyses were performed in R Core Team (2018).

Results

Seed WC and drying

Fresh seeds of *C. xanthocarpa* had an initial WC of 0.684 g H_2O g dw^{-1}. Besides the method, air drying (AD) and silica drying (SD), the desiccation behavior of seeds was similar (Fig. 1a). Half of the initial WC was lost between 2 and 3 h of drying, and after 6 and 12 h seeds had a water loss of 76.2 and 88.5%, respectively (Table 1). In 24 h, AD seeds almost reached a basal stable WC (0.042 g H_2O g dw^{-1}). After 120 h of AD seeds final water loss and WC were 94.6% and 0.037 g H_2O g dw^{-1}, respectively, whilst in SD were 95.9% and 0.027 g H_2O g dw^{-1}, respectively. Significant differences between methods only occurred after 36 h of drying, when seeds of SD reached lower WC. Seeds drying rate (k) during the first

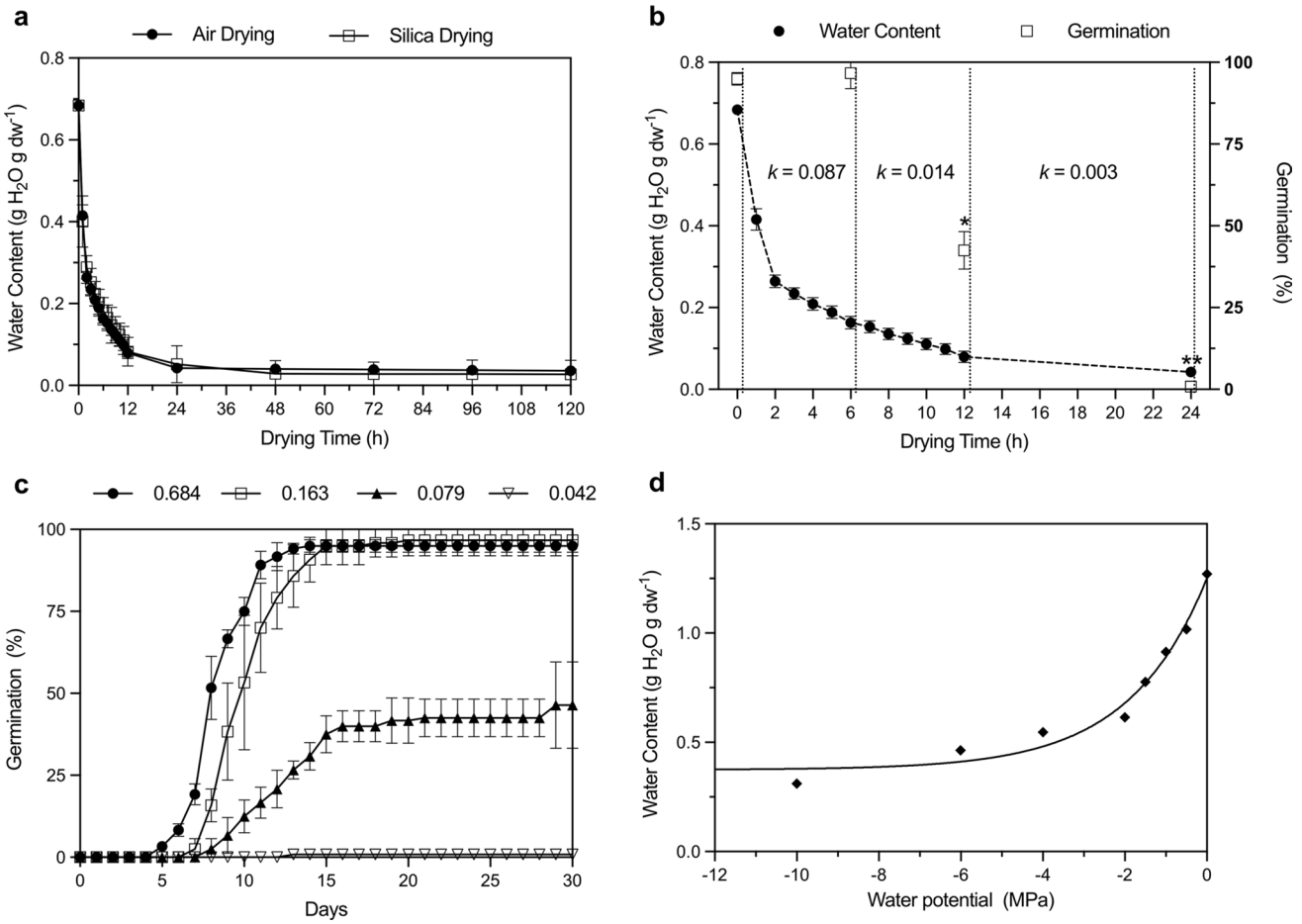

Fig. 1 **a** Drying behavior of *C. xanthocarpa* seeds during air drying and silica drying methods. **b** Air drying behavior of *C. xanthocarpa* seeds during the first 24 h and the relation between water content, time, germination and drying rate (*k*). **c** Germination dynamic of *C. xanthocarpa* seeds submitted to air drying for 0, 6, 12 and 24 h, which corresponds to WC of 0.684, 0.163, 0.079 and 0.042 g H_2O g dw^{-1}, respectively. **d** Water content and water potential relationship of whole seeds of *C. xanthocarpa*. Values are mean of replicates and vertical bars represent ± SD. Asterisks indicate statistical difference according Tukey test ($p < 0.05$)

Table 1 *C. xanthocarpa* seeds behavior trend during air drying, relating its water content, water loss, germination, germination speed index and electrolytic leakage

Drying time (h)	Water content (g H_2O^{-1} g dw^{-1})	Water loss (%)	Germination (%)	Germination speed index (GSI)	Electrolytic leakage (μS cm^{-1} g^{-1})
0	0.684 ± 0.017	–	95 ± 1.92	3.030	32.7 ± 2.27
6	0.163 ± 0.005	76.2	96.67 ± 4.72	2.597	42.27 ± 5.44
12	0.079 ± 0.007	88.5	42.5 ± 5.70*	0.982	73.72 ± 6.16*
24	0.042 ± 0.008	93.9	0.83 ± 1.67*	0.018	91.09 ± 5.07*

Values are mean of replicates and vertical bars represent ± SD

*Indicate statistical difference according Tukey test ($p < 0.05$)

24 h of air drying was 0.027 g H_2O h^{-1}, but it also could be subdivided into three phases. In the initial 6 h, the drying rate was higher (0.087 g H_2O h), from 6 to 12 h decreased (0.014 g H_2O h^{-1}) and almost stabilized from 12 to 24 h (0.003 g H_2O h^{-1}).

Germination test

Considering the high drying rate during the first 24 h, seed desiccation treatments were established based on a time/WC basis, where 0 (fresh), 6, 12 and 24 h of drying corresponded to an average of 0.684, 0.163, 0.079 and 0.042 g H_2O g dw^{-1}

WC, respectively (Table 1). As time progressed and WC decreased, seed germination was reduced (Fig. 1b). Fresh seeds (0.684 g H_2O g dw^{-1}) were able to germinate 95% and drying them for 6 h (0.163 g H_2O g dw^{-1}) did not show any difference in the final germination rate (96.7%). After 12 h, WC reached 0.079 g H_2O g dw^{-1} and germination reduced to nearly half (42.5%), and within 24 h of drying (0.042 g H_2O g dw^{-1}) seeds lost 99% of their viability, as they germinated 0.83%. In addition, during the drying process, seed germination dynamic differed between treatments (Fig. 1c). Fresh seeds germinated faster and reached earlier its maximum value. The protrusion of the radicle in fresh seeds started 5 days after sowing and took 8 days reach 95% of germination. Seeds dried for 6 h showed a lag and started germination at the 7th day and took 12 more days to reach 96.7%. 12 h dried seeds first started to germinate 8 days after sowing and reached final values after 21 days. After 24 h of drying, only one seed germinated. These contrasting dynamics could also be observed by the decrease in GSI (Table 1).

Electrolytic leakage

Leakage of electrolytes remained stable for fresh and 6 h dried seeds (Table 1). After 12 h, leakage raised two fold in comparison to fresh seeds. Seeds that lost viability (24 h of desiccation, 0.042 g H_2O g dw^{-1}) showed the highest values of leakage. Electrolytic leakage showed a strong negative correlation with germination (− 0.9463) (Table S1).

Seed osmotic potential

Seeds embedded with pure distilled water (0 MPa) reached a WC of 1.271 g H_2O g dw^{-1}. The water potential (Ψ_w) of fresh seeds was interpolated from the WC/water potential relation (Fig. 1d) and estimated as − 1.78 MPa at 0.684 g H_2O g dw^{-1}. WC strongly decreases along small changes water potentials prior to − 6 MPa, whilst after this point, substantial changes in water potential are needed to decrease the WC.

Seed anatomy

Seeds of *C. xanthocarpa* are composed by a seed coat that covers the embryo (Fig. 2a), a swollen hypocotyl (Fig. 2b), radicle, small rudimentary cotyledons and lack of endosperm. The seed coat is thin and membranous with an external morphology described as verrucose-glandulose (Fig. 2a), which it is worth mentioning once this external morphology aspect of seeds, has been used as an important taxonomic characteristic to define the genus for Myrtaceae (Landrun 1986). Anatomically is comprised by three distinct tissues (Fig. 2c). The outermost layer of the seed coat, the external tissue (et), is pluricellular, with thin-walled cells

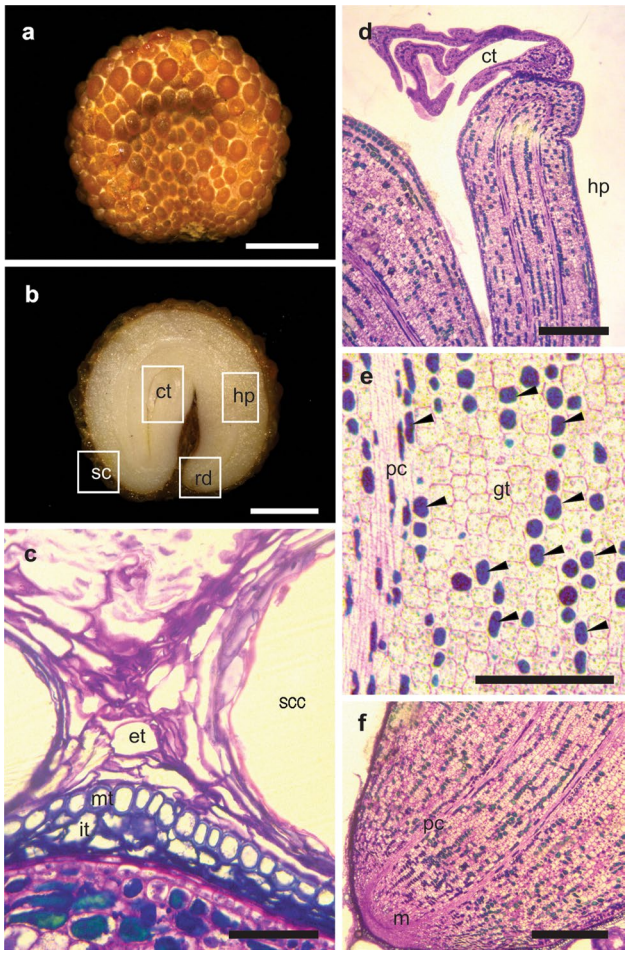

Fig. 2 External morphology of whole (**a**) and longitudinally cut (**b**) *C. xanthocarpa* seed. Longitudinal sections of fresh (**c**, **d**, **e**, **f**) seeds stained with toluidine blue. **a** Overview of the entire seed and its glandular seed coat. **b** Seed longitudinally sectioned showing the thin glandular seed coat and the whole embryo. **c** Seed coat comprised by external, middle and internal tissues. **d** Small folded leafy cotyledons. **e** Hypocotyl ground tissue. Cells exhibit lipidic content that entirely fills intracellular space (arrows). **f** Root apical meristem of the embryo indicate procambium. *ct* cotyledons, *et* external tissue, *gt* ground tissue, *hp* hypocotyl, *it* internal tissue, *mt* middle tissue, *scc* secretory cavity, *pc* procambium, *m* meristem, *rd* radicle, *sc* seed coat. Scale bars: **a**, **b** 2.5 mm; **c** 50 µm; **e** 1000 µm; **d**, **f** 4000 µm

flattened that surround secretory cavities (Gogosz et al. 2010). Afterward, there is a one-layered cell middle tissue (mt) with thickened lignified walls. The inner tissue (it) is irregular of small flattened thin-walled cells. Cotyledons (Fig. 2b, d) are smaller than the other seed components and remain folded in the center of the seed, covered by the swollen spiral hypocotyl (hp). The whole seed is majorly comprised by the hypocotyl filled by ground tissue (gt) (Fig. 2e) with cells that stain green by the metachromatic reaction to TBO indicate polyphenols or lignin (O'Brien et al. 1965; Rogge-Renner et al., 2013). The hypocotyl's ground tissue is comprised by axially elongated cells in the periphery that

tend to be isodiametric or relatively flattened in the middle portion of the organ. Two parallel sets of procambium (pc) cells cross the entire embryo from the cotyledons towards the radicle. Since *C. xanthocarpa* seeds do not have endosperm, its energetic reserves are accumulated among the ground tissue cells. At the radicle apex was possible to verify root apical meristem(m) and procambium (Fig. 2f).

Seed coat tissues were the first to suffer structural changes during desiccation (Fig. 3a, b), where external and internal tissues cell walls exhibited the wavy shape and start to collapse as a result of water removal. On the other hand, the lignified middle tissue did not show any structural changes in all desiccation treatments. Embryo protodermal cells in 6 h dried seeds also showed signs of dehydration when the plasmalemma started to show a detachment from its cell walls (Fig. 3c). Similarly, after 6 h of drying, a few cells of the hypocotyl ground tissue also showed the same characteristics (Fig. 3d). After 12 h of desiccation, plasmalemma detachment were more evident (Fig. 3e), and some cell walls even collapsed due water removal (Fig. 3f). Dehydrating seeds for 24 h led to further detachment of plasmalemma from cell walls, and shrinkage became more frequent in cells of the hypocotyl ground tissue (Fig. 3g).

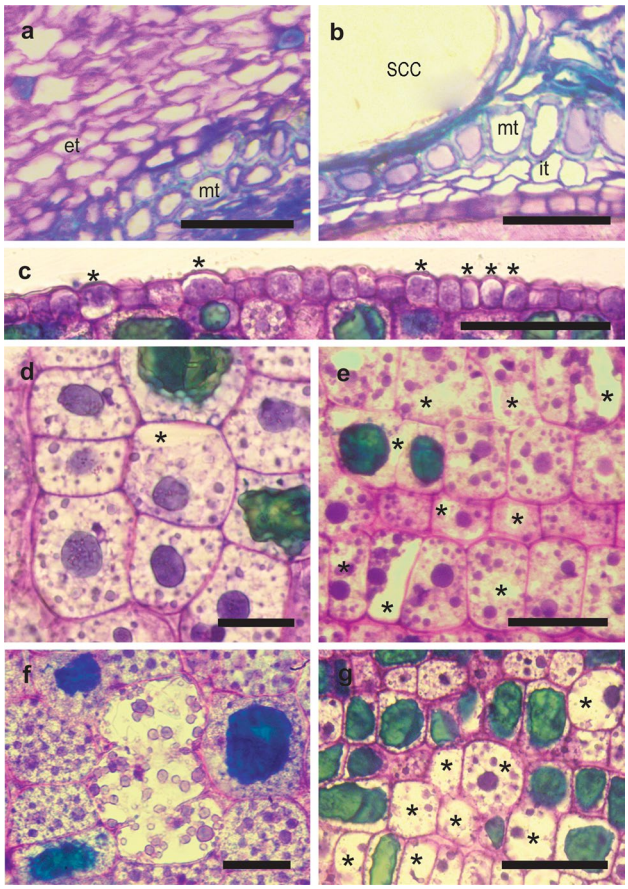

Fig. 3 Longitudinal sections of seed coat (**a**, **b**), protoderm (**c**) and hypocotyl (**d**, **e**, **f**, **g**) of air dried *C. xanthocarpa* seeds, stained with toluidine blue and observed in light microscope. **a** External and middle tissue of the seed coat. Invagination of cell walls of external tissue cells. **b** Middle and internal tissue of the seed coat. Invagination of cell walls of internal tissue cells. **c** Protoderm of a 6 h dried seed. Protodermal cells exhibit separation of the plasmalemma from cellular walls (asterisks). **d** Hypocotyl ground tissue of a 6 h dried seed. Initial evidences of tissue dehydration, detachment of plasmalemma from cellular wall (asterisk). **e** Ground tissue of the hypocotyl of 12 h dried seed. Many cells of the tissue exhibited cytoplasmic shrinkage due water loss, characterized by the separation of the plasmalemma from the cellular walls (asterisks). **f** Cells of the ground tissue of the hypocotyl of seeds that were dried for 12 h. Collapsed cellular walls due the intensity of water loss. **g** Ground tissue of a 24 h dried seed. Along further dehydration cell shrinkage became more evident, leading to a more frequent number of cells with plasmalemma detachment from cell walls (asterisks). *et* external tissue, *it* internal tissue, *mt* middle tissue, *scc* secretory cavity. Scale bars: **a**–**c**, **e**, **g** 50 µm; **d**, **f**, 25 µm

Antioxidant enzymes and lipid peroxidation

SOD activity (Fig. 4a) increased during the first 6 and 12 h of drying, but after 24 h, seeds lost their viability and activity declined. Values of SOD in unviable seeds were almost as half as observed in fresh seeds. In contrast to SOD behavior, APX (Fig. 4b) and CAT (Fig. 4c) shared a common trend. APX activity during the first 6 h of drying declined and after 12 h exhibited a slight increase. When seeds lost their viability APX values were similar as in fresh seeds. A similar behavior was observed for CAT, as its activity significantly reduced during the initial 6 h and then after 12 h started to increase. When seeds lost their viability CAT activity were as observed in fresh seeds. In *C. xanthocarpa* seeds, lipid peroxidation (Fig. 4d) content remained stable after 6 h of drying, had a discrete raise with 12 h and after 24 h significantly increased and statistically differed from other treatments.

PAs

Total free PAs content (Fig. 5a) decreased during the first 6 h of drying, which after 12 h raised and stabilized until 24 h, exhibiting similar values as in fresh seeds. Free Spd was the most abundant PA in *C. xanthocarpa* seeds (Fig. 5b) and exhibited the highest levels through the drying process. As the behavior of total PAs, Spd content decreased after 6 h of drying and started to increase after 12 and 24 h, where it also reached similar values as fresh *C. xanthocarpa* seeds. It was observed a positive correlation during drying between Spd levels and the activity of CAT (0.7429) and APX (0.8205) (Table S1). Free Put and Spm levels were much lower than Spd and showed no significant changes and statistical differences along drying. The Put/(Spd + Spm) ratio (Fig. 5c) tended to decrease during the drying process, mainly driven by the changes in Spd content. The ratio reduction became

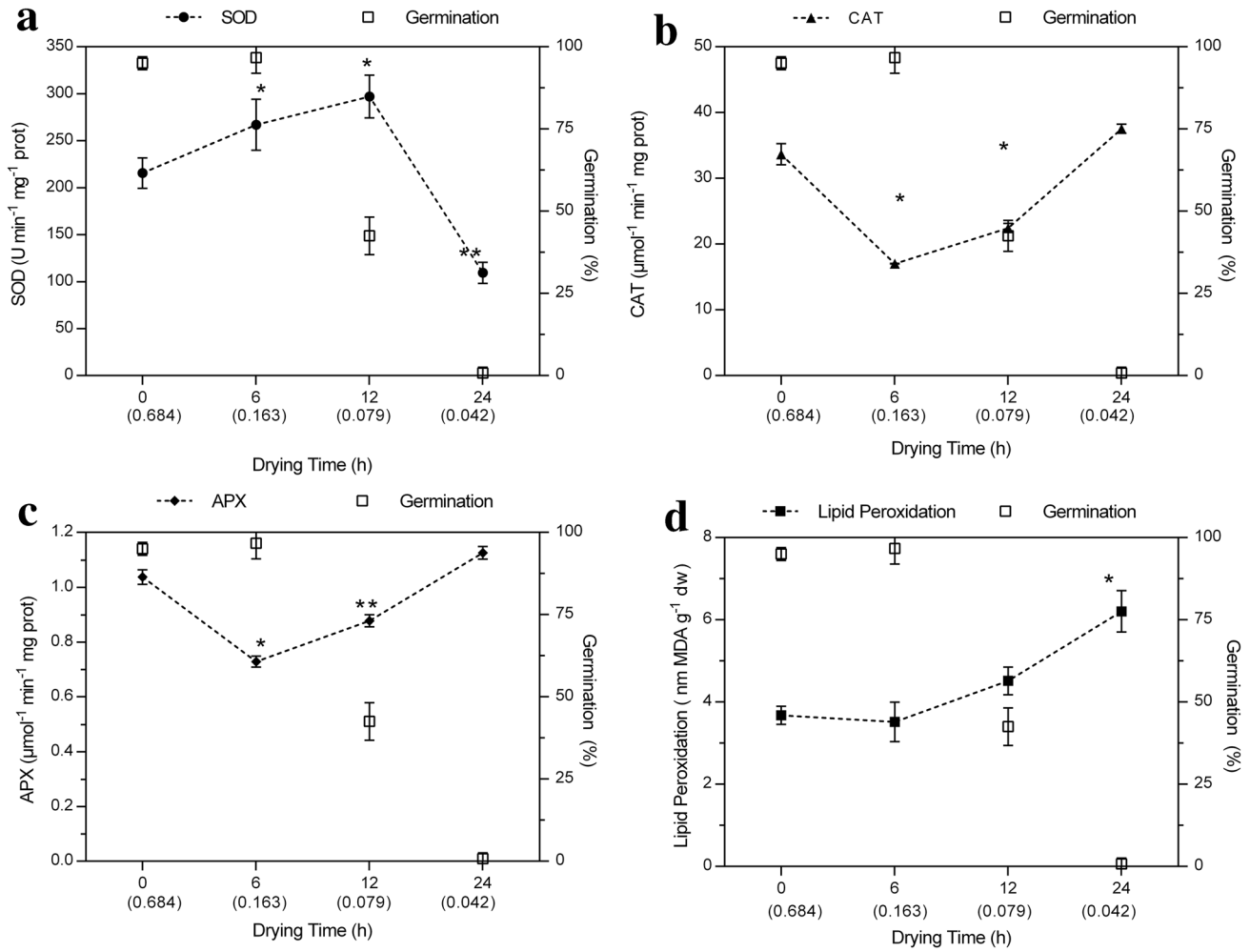

Fig. 4 Enzymatic activity of SOD (**a**), APX (**b**) and CAT (**c**), and lipid peroxidation (**d**) of *C. xanthocarpa* seeds during air drying and in relation to their germination capacity. Values between parentheses under X axis represent seed water content in dry basis (g H_2O g dw^{-1}) at each respective drying time. Values are mean of replicates and vertical bars represent ± SD. Asterisks indicate statistical difference according Tukey test ($p < 0.05$)

more evident after 24 h of drying, a moment that *C. xanthocarpa* seeds lost their viability.

Discussion

After being removed from fruits, *C. xanthocarpa* seeds lost 93.9% of their WC during the first 24 h. In general, most seeds can be either fast or slow dried for distinct purposes. However, water loss occurred rapidly besides the method, thus the drying process was assumed to be naturally fast for *C. xanthocarpa* seeds. For this reason, treatments were defined not only based on seed WC, but also as the time required to reach it, because expressing it along time and drying rate can be more informative when it concerns stress nature, accumulation of damage and seed viability (Varghese et al. 2011; Walters 2015). Nonetheless, it is rare in seed desiccation studies the expression of drying rate along other seed features, and there are no standard methods to calculate it. Hill et al. (2010) tried to propose a single negative exponential model to explain seeds drying behaviors, but almost half of the studied seeds did not fit the model and the physical expectations of water vapor loss. Xia et al. (2012a) expressed the drying rate as the decline in WC during a determinate time period and expressed it as a percentage of water loss. In another study, Xia et al. (2012b) used an index (k) obtained from the slope of the initial drying curve to measure the drying rate. Although there is no consensus in how to calculate or express it, seeds drying rates are extremely useful to give an idea of the physical relation of the environment and intrinsic physiological seeds features. In our work, the concept of drying rate was presented as water loss (%) and as an index obtained from the relation of such water loss along drying time. Seed drying rate depends

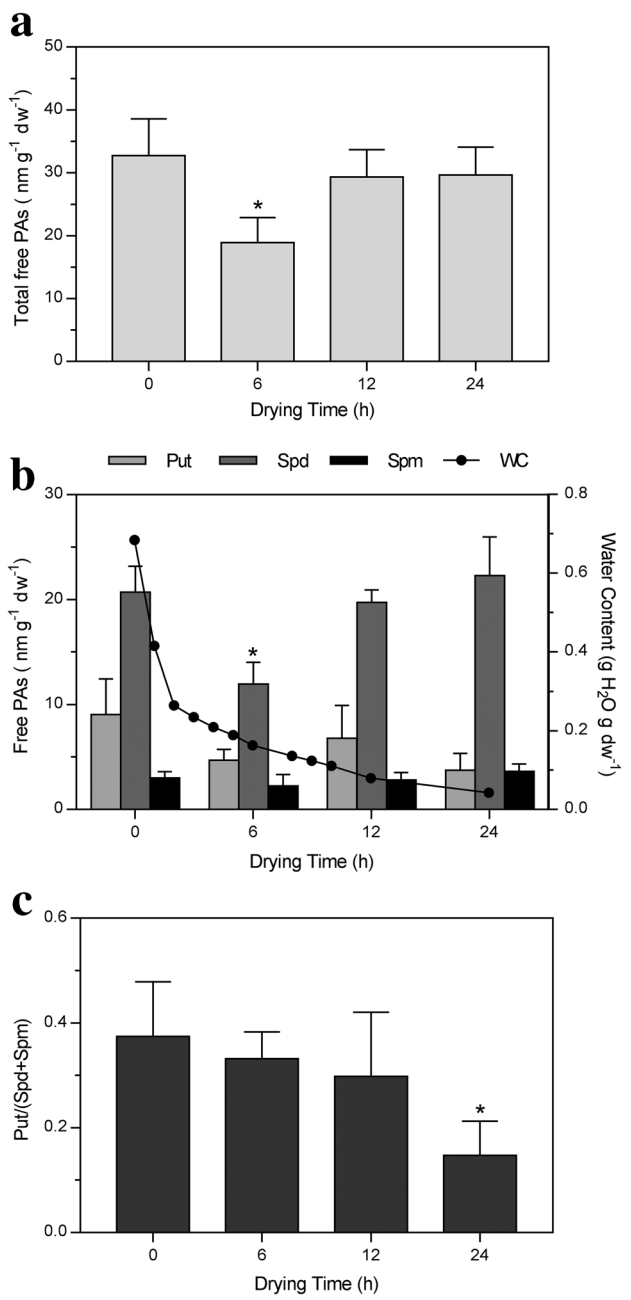

Fig. 5 Total free polyamines (PAs) (**a**) free PAs content (**b**) and ratio between Put/ (Spd + Spm) (**c**) of *C. xanthocarpa* seeds during air drying and different water content. Values are mean of replicates and vertical bars represent ± SD. Asterisks indicate statistical difference according Tukey test ($p < 0.05$)

on their surface area, the hydraulic conductivity of the tissue and the difference in water potential of the external air and seed tissue (Sun 2002). Once the use of silica did not affect the rate of water loss, the drying behavior observed could be due the nature of *C. xanthocarpa* seed coat, which is thin and comprised by unlignified tissues. In fresh *C. xanthocarpa* seeds, the seed coat has a malleable membranous consistency, which upon drying becomes harder and brittle. Conspicuous secretory cavities are all over the surface of *C. xanthocarpa* seed coat, and it has been considered as a distinctive taxonomic trait shared by the entire genus *Campomanesia* (McVaugh 1968). As a matter of fact, the structure that we a priori consider as a glandular seed coat is actually the internal locule-wall of the fruit and is apparently unique in Myrtaceae (Landrum 1982). Interestingly, high drying rates are also observed among seeds of other *Campomanesia*, such as *C. phaea* (Maluf and Pisciottano-Ereio 2005), *C. pubescens* (Dousseau et al. 2011), *C. adamantium* (Dresch et al. 2015), *C. littoralis* (data not published) and *C. reitziana* (data not published). Thus, insufficient dehydration protection by this false seed coat is likely to occur for the whole genus *Campomanesia*, and it could serve as an alert to the vulnerability of these species in terms of seed viability. A typical example is the case of *C. lundiana*, an already extinct *Campomanesia* species from Southeastern Brazil (World Conservation Monitoring Centre 1998).

In our work, after 6 h of drying seeds lost 76% of their WC (0.163 g H_2O g dw^{-1}), but germination (96.7%) was the same as fresh seeds (95%). However, fresh seeds had higher germination speed index (3.030) and the observed lag between imbibition and radicle protrusion of 6 h dried seeds (2.597) may be due the action of mechanisms repairing damages that occurred during the drying process. After 12 h, 88% of the WC was lost (0.079 g H_2O g dw^{-1}), germination was slower (0.982) and reduced below half (42.5%). Finally, after 24 h of drying, seeds lost 93.9% of their initial WC (0.042 g H_2O g dw^{-1}) and seed viability was completely lost. In this sense, Marques et al. (2018) gathered information about seed lowest safe WC that 98 DS and 31 intermediate species could tolerate. The authors assessed that DS seeds on average could be dried to 0.33 g H_2O g dw^{-1} before germination started to decline, whilst intermediate seeds tolerated on average 0.13 g H_2O g dw^{-1}. Thus, according to our results *C. xanthocarpa* seeds would be considered as tending to intermediate behavior, since drying around 0.16 g H_2O g dw^{-1} exhibited no reduction in germination (96.7%). However, we take into account that water loss is so intense during the first hours of drying that WC values could represent just a transient moment along a time that the seed itself did not remain. In fact, it was due this high drying rate that *C. xanthocarpa* seeds were able to tolerate further dehydration and behave out of the classical DS thresholds. This behavior trend even reflects the goals of fast drying methods and helps us to analyze the physiological implications that enable DS seeds survival in lower WC. Therefore, it can be difficult to determine viability loss and to rank DS only considering critical WC. Seeds must be contextualized along their intrinsic morphological, physiological and environmental features (Hill et al. 2012), not only to give an idea of a tendency inside the drying behavior spectrum, but also to bridge the

gap between seed physiologists and community ecologists (Jiménez-Alfaro et al. 2016).

In this context, Neotropical Myrtaceae exhibits a morphological diversity of embryos that has been traditionally used for grouping family subtribes in taxonomy studies (Lucas et al. 2007). Although this variety of embryos does not imply in any success of one structure over another (Landrum and Stevenson 1986), distinct DS behaviors trend can be observed among genera, where some require days to reach a certain WC while others take just a few hours. As an example, *Eugenia*, the most diverse tree genus in the Atlantic Forest (Zappi et al. 2015), due their swollen fused cotyledons exhibit a drying behavior that require several days for seeds to lose viability. Delgado and Barbedo (2007) reported for six *Eugenia* species that seed loss of viability occurred with WC around 0.25 g H_2O g dw^{-1}, which took them on average 456 h of drying. On the other hand, *Campomanesia* small rudimentary cotyledons and seed coat features confers seeds a much faster drying dynamic and a shorter lifespan. Although drying methods may differ slightly between studies, the morphological diversity and responses must be taken into account to assess the real desiccation behavior and seed physiological viability. Surprisingly, from the classical perspective of desiccation thresholds *Eugenia* seeds can be considered as highly DS, once viability is lost at higher WC than most DS seeds, while *C. xanthocarpa* seeds tend to be as intermediate. However, in terms of survival, a 24 h lifespan is much more vulnerable than a hypothetical WC threshold that takes several days to be reached, and distinct sources of damage are likely to be involved. Furthermore, to rank the degree of DS seed and susceptibility of species it is necessary to solve the issues between drying rate and tissue WC, because is difficult to distinguish and to correlate intensity and duration of dehydration stress (Walters et al. 2002). The variation of critical WC along the seed behavior spectrum demonstrates the quantitative nature and capacity of desiccation tolerance (Walters 2015). Thus, the abovementioned issues could lead to a misclassification paradox that neither concerns survival nor nature of damage. Our data underlines the importance of expressing seed WC along a time basis, since distinct desiccation behaviors may imply different interpretations of DS seed survival and physiological stress. In addition, this knowledge amplifies the spectrum of possibilities to study ex situ conservation technologies for tropical seeds.

Furthermore, it is also interesting to point out that such generalization that constrain DS seeds in respect to their desiccation thresholds and physiological behavior may also occur in the ecological context. Seeds that are DS are usually associated with high WC, large size, thin tegument, rapid germination, dispersion in the rainy season and do not form soil seed bank. However, this stereotype does not give us the idea of the idiosyncratic behavior in their natural environments, and as more studies are concerning the diversity of DS seeds the spectrum perspective is getting broader including implications on ex situ and in situ seed conservation. There are examples of DS species with seed features such as dormancy (Gumilevskaya and Azarkovich 2007; Veloso et al. 2016), dispersion in the dry season (Vaz et al. 2016), soil seed bank formation (Ferrandis et al. 2011) and tolerance to flooded anoxic conditions (Calvi et al. 2017; Marques and Joly 2000), which do not fit the classical definitions of DS seeds and may demand different ex situ conservation efforts. In the biodiversity scope, despite the concepts and terminologies limiting the real context of seed behavior, the fact is that conservation of DS seed species is a major concern for ecological and economic issues.

From a physiological perspective, one of the main general causes of viability loss in DS seeds is due mechanical/physical perturbations during water removal and rehydration. When water is removed cells lose turgor, shrink and molecules become spatially closer, which may cause fusion of the membranes and loss of cellular compartmentalization (Walters et al. 2002). However, during fast drying seed water loss may be uneven, and it is likely to occur primarily from apoplast and peripheral cell layers (Berjak and Pammenter 2013). Considering that the seed coat is the main barrier to the external environment, initial water loss of *C. xanthocarpa* seeds seemed to be occurring from peripheral tissues, mainly by its false seed coat. Anatomical evidences from 6 h dried seeds showed signs of dehydration in the false seed coat, since it is relatively hydrated in fresh seeds. Cell walls invaginated in the most internal and external tissues of the seed coat, and the one-layered lignified middle tissue remained unchanged. At this moment, protodermal cells also showed signs of dehydration when its plasmalemma detached from cell walls. The behavior of an initial high rate of water loss followed by a lower rate is associated to changes in covering and internal seed properties (Hill et al. 2010), which also have relation with the distinct types of water in each level of tissue hydration. Although five types of water can be found in tissues, three main types are usually associated with the dehydration process and changes in seed physiology. These types of water are related to tissues hydration level and are usually expressed in terms of water potential (Ψ_w), which is much more informative when concerning water stress (Walters 2015). Type 3 is a free water that form bridges over hydrophobic portions of macromolecules and is detected from − 4 to − 11 MPa, type 2 has a glassy aspect that strong interacts with polar surfaces of macromolecules between − 12 and − 150 MPa, and type 1 occurs at levels below than − 150 MPa and is referred as a molecular theoretical level where water binds as a structural component (Vertucci and Farrant 1995). For *C. xanthocarpa* seeds we were able to establish the water potential of fresh seeds (− 1.8 MPa), but for dried seeds values were estimated

as in the literature (0.163 ~ − 15 MPa; 0.079 ~ − 150 MPa; 0.042 ~ − 200 MPa) (Walters 2015). The total removal of type 3 water is considered to be lethal to DS seeds, because it is related to changes in membrane structure (Vertucci and Farrant 1995). Nonetheless, *C. xanthocarpa* survived to − 15 MPa (0.163 g H_2O g dw^{-1}), a moment where the plasmalemma in most internal tissues were just starting to detach from cell walls. Usually, cells of DS seeds are highly vacuolated and susceptible to volume changes (Walters et al. 2002), thus water in hydrated tissues (type 3) is essential to cell structure maintenance. On the other hand, cells with more dry matter reserves are able to buffer volume changes, and supply water loss tolerance (Walters and Koster 2007). Thus, the great quantity of lipid stored in *C. xanthocarpa* tissues may be contributing to buffer the effects of drying and changes in seed water relations, allowing seeds to maintain viability (96.7%) at lower WC than most of DS species (0.163 g H_2O g dw^{-1}). However, further dehydration and removal of type 2 water can lead to membrane function loss in unprotected systems, which for *C. xanthocarpa* seeds could be observed after 12 and 24 h of drying, where cells plasmalemma detached from walls much more frequently and increased potential mechanical/physical damages to membranes.

Besides mechanical/physical loss of membrane function during dehydration, the other main cause of viability loss in DS seeds is due metabolism aqueous-based degradative processes. The high WC of DS seeds confer the cytoplasm a fluid environment where metabolism is active. Along dehydration metabolism becomes unbalanced and frequently produces excessive quantities of ROS, which can accumulate and compromise cellular membranes, proteins, nucleic acids, cellular integrity and seed survival (Berjak and Pammenter 2008). Usually, DS seeds lose viability when its cytoplasm confers an aqueous environment with relative mobility that can make ROS reach targets far from their production sites (Walters 2015). In this case, the role of antioxidant enzymes is determinant to maintain cellular homeostasis of DS seeds along time, and the first line of defense against ROS is provided by SOD (Gill and Tuteja 2010), which catalyzes superoxide radicals (O_2^-) producing hydrogen peroxide (H_2O_2) and oxygen (O_2). Roach et al. (2008) verified a burst of O_2^- production during the first minutes after excision and drying of DS embryonic axes of *Castanea sativa*. Similarly, *C. xanthocarpa* seeds had an increase of SOD activity when fresh seeds were submitted through 6 and 12 h of drying, which could mean that SOD was responding to the initial intracellular production of O_2^-. On the other hand, when seeds lost viability, after 24 h of drying, SOD activity sharply declined, which may be related to the decrease in O_2^- content also observed by Roach et al. (2008). The main product of SOD activity is H_2O_2, which is a moderately reactive molecule that has relatively long half-life in comparison to others ROS (Gill and Tuteja 2010). Thus, the balance between SOD and H_2O_2-scaveging enzymes (as APX and CAT) is considered to have a central role in maintaining steady cellular contents of O_2^- and H_2O_2 (Quan et al. 2008). Considering that SOD levels increased along desiccation, it could be assumed that its product, H_2O_2, may be also increasing. In this context, it was observed an analogous trend between APX and CAT activities during the drying process of *C. xanthocarpa* seeds. Both enzymes activities decreased in the first 6 h of drying, a moment where there was apparently no substantial damage and change in germination. However, as seed viability began to be lost during the subsequent hours, APX and CAT started to increase and after 24 h of drying reached similar activity as in fresh *C. xanthocarpa* seeds. This behavior of APX and CAT decreasing and returning to fresh seeds values as viability is lost was similar as observed by Chen et al. (2015) for a cultivar of *Camellia sinensis* during drying. The response of enzymatic antioxidant systems of DS seeds can be highly variable among species, and it can significantly differ depending on the drying rate (Luo et al. 2012; Varghese et al. 2011). Fast drying is considered to enhance seed survival, while during slow drying insufficient enzymatic responses increase the likelihood of ROS accumulation. Considering *C. xanthocarpa* seeds, overall antioxidant enzymatic activity somehow tried to respond to dehydration stress and possible ROS production/accumulation, but it remained in lower or equal activities as for fresh seeds when viability began to be lost.

Moreover, PAs are stress-related molecules suggested to play a role in ROS homeostasis and stimulation of ROS scavenging enzymes (Liu et al. 2015). Nonetheless, there is a lack of studies considering endogenous PAs behavior during seed drying, and there are literally no mentions when it comes to DS seeds and its possible relations to seed desiccation stress, antioxidant activity and seed viability. Depending on the source of stress distinct PAs biosynthetic genes are stimulated and in most cases, only one type of the three main PAs is significantly involved (Liu et al. 2015). Spd was the most abundant PA in *C. xanthocarpa* seeds, and it was the main responsible for the overall changes in total free PAs content along the drying process. Since the three main PAs can be interconverted, the observed reduction of Put/(Spd + Spm) ratio shows the induction of Spd metabolism during *C. xanthocarpa* seeds desiccation. Under a broad spectrum of stresses, it has been shown that exogenous Spd increased many stress-responsive genes that improve ROS scavenging capacity in plant tissues and seeds (Parvin et al. 2014; Paul and Roychoudhury 2017; Sudhakar et al. 2015; Yadu et al. 2018). In this context, our study found a significant positive correlation between endogenous free Spd and the activity of CAT (0.7429) and APX (0.8205), indicating its complementary role in the antioxidant defense system. Furthermore, accumulation of PAs is time and stress

dependent, and their biosynthetic genes can be expressed days after stress induction Liu et al. (2011). Since drying *C. xanthocarpa* seeds for 6 h did not affect their germination, it may be that up to that moment the seed itself was not recognizing the changes in WC as stress, thus Spd content decreased. The misperception of stress was probably due the high drying rate of *C. xanthocarpa* seeds, which during the first 6 h of drying most water was lost from seed coat and peripheral cell layers. The increase in free Spd levels after 12 h of drying (0.079 g H_2O g dw^{-1} WC), a moment when germination decreased below half (42.5%), suggests that seeds were perceiving and trying to respond to cells structural changes, which were already becoming generalized in seed tissues. However, *C. xanthocarpa* seeds lost viability before Spd levels could significantly increase, once that 24 h of drying, it only reached similar values as in fresh seeds.

One of the most cited causes of oxidative membrane damages is the formation of MDA due to lipid peroxidation, which in *C. xanthocarpa* seeds only increased after 24 h of drying, when viability was already lost. However, even with this increase, MDA values were much lower when compared to others studies (Chandra and Keshavkant 2018; Parkhey et al. 2012; Sahu et al. 2017), suggesting that lipid peroxidation could be an ongoing process for *C xanthocarpa* seeds. Similar as our results, Xin et al. (2010) found no significant signs of lipid peroxidation during fast drying of DS tissues, and it was suggested the occurrence of mechanical/physical damage, and we suggest as the case of *C. xanthocarpa* seeds. Damage is a function of rate and duration of dehydration, which mechanical/physical perturbations are more likely to occur with high drying rates (Liang and Sun 2002). In this sense, seeds of *C. xanthocarpa* showed a significant negative correlation (− 0.9463) between electrolytic leakage and germination, which is likely the occurrence of membrane structural damages and consequent protoplasm leakage. Thus, at the same rate, *C. xanthocarpa* seeds lose water they may gain and, as consequence, improperly fused membranes during drying may leak intracellular solutes due the inability of membranes transition upon water uptake, leading to seed viability loss.

Structural, physiological and temporal *C. xanthocarpa* seeds changes assessed in this study are summarized in

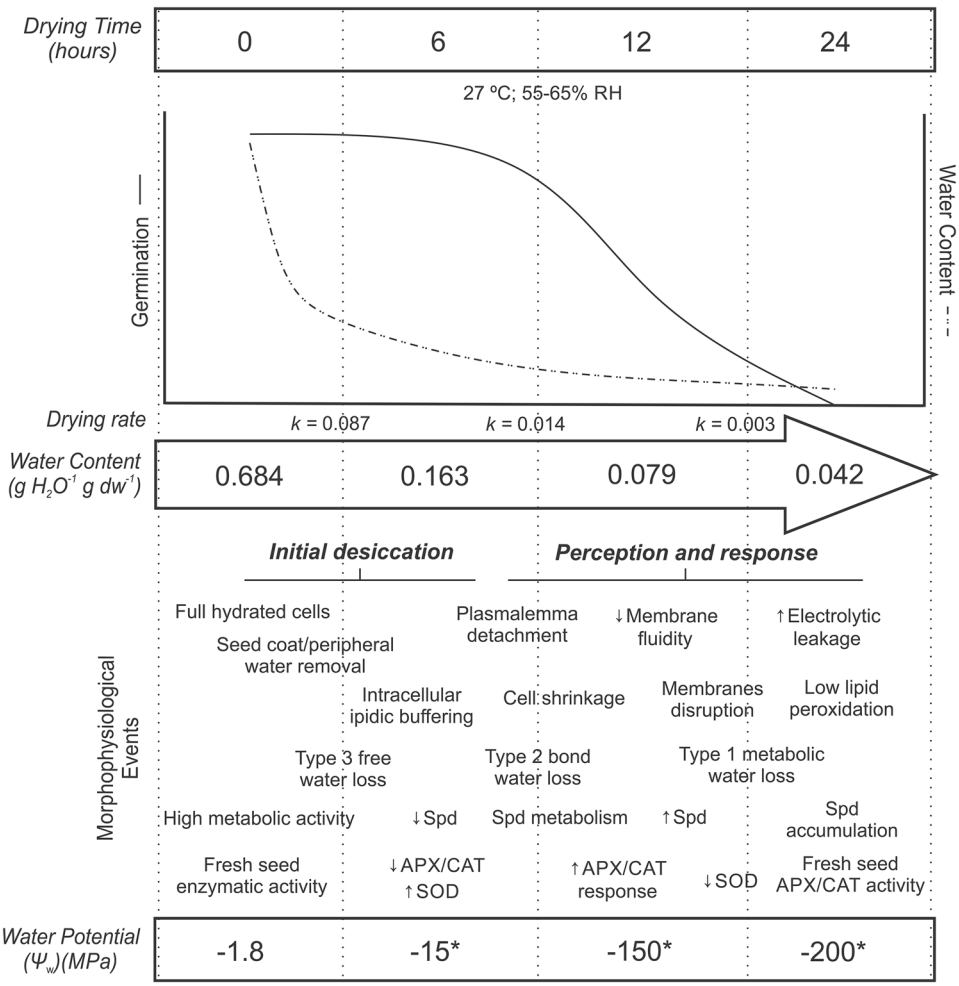

Fig. 6 Overall structural, physiological and temporal features of *C. xanthocarpa* seeds during air drying. Inherent structural and temporal effects lead to a seed behavior trend within the spectrum of desiccation sensitive seeds. *Values estimated according to the literature (Walters 2015)

Fig. 6. This species occurs frequently in one of the two of the world's biodiversity hotspots that are home to many wild plants with real and potential economic and ecological value. Though this schematic figure represents just a glimpse of the real physiological context, it gives an idea of how dynamic, complex and idiosyncratic the processes of perception and response in DS seeds can be. In addition, how structurally and temporally physiological events are associated allow us to identify the tendency of seed behavior within the spectrum of seed tolerance/sensitivity. The proposed idea of a seed physiological behavior trend must take into account intrinsic evolutionary, ecological, morphological and physiological species features, which leads to the expression of a seed tendency before any behavior categorization. Our results give us new insights to develop and study seed conservation technologies which have been a serious barrier for seed of tropical plant species, especially in one of the hotspots holding in Brazil.

Author contribution statement PHMV and NS designed research. PHMV and ROJ performed research and experiments. PHMV, APL, DG and WGV performed biochemical analysis. PHMV and NS analyzed and wrote the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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