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# Desiccation Tolerance and germination of *Psidium guajava* seeds from different regions of Brazil

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SILVICULTURE

## ABSTRACT

**Background:** The germination ecology of *P. guajava* seeds collected in two regions of Brazil was investigated aiming to (1) characterize the seed batches; (2) determine the best temperature for germination; and (3) assess variations in seed desiccation tolerance (DT). For each batch, we determined seed water content and initial germination. To evaluate temperature effects, we tested seed germination. After, we generated the imbibition curves for the two provenance. To evaluate the loss of DT, the seeds were left to germinate along different time intervals. We monitored water loss by regularly weighing the seeds until achieving stabilization, keeping them under these conditions for 72 hours. After drying, the seeds were pre-hydrated and submitted to the germination test.

**Results:** We identified variations in physiological characteristics between the seeds from north and southeast batches. At the temperature extremes, the seeds from both provenance failed to germinate. The highest germination percentages were observed at 20 and 25 °C (constant) and between 20-30 °C (alternating) for the two batches and, for the southeast batch specifically, at 30 °C. The imbibition curve displayed a triphasic pattern with a rapid increase in seed fresh weight in the first 48 hours.

**Conclusion:** The southeast provenance seeds lost DT abruptly, until its complete loss after radicle protrusion, which occurred in both batches. The highest values of germination speed index (GSI) of both provenance were observed after a 9-day imbibition period, suggesting seed hydration memory. Drying resulted in necrosis of the root tissue and death of the seeds with visible roots.

**Keywords:** Drying, fruit tree, Myrtaceae, tree seed

## HIGHLIGHTS

Variations in the seed physiological characteristics were observed between the studied provenances.
Extreme temperatures prevented *P. guajava* seeds to germinate.
The x-ray images allowed an assessment of seed internal morphology.
The seeds from north provenance lost desiccation tolerance slower.

# INTRODUCTION

Desiccation tolerance (DT) is commonly described as the ability of organisms and organs, such as seeds, to survive minimum water levels without accumulating lethal damage, while remaining able to recover their normal metabolism after hydration (Leprince and Buitink, 2010; Dekkers et al., 2015). However, throughout their development, the physiological tolerance behavior of orthodox seeds may change as germination advances at different water availability conditions (Bewley et al., 2013; Pereira et al., 2014; Rodrigues-Junior et al., 2015; Maia et al., 2016; Castro et al., 2017). In most species that produce orthodox seeds, DT loss is related to radicle protrusion (Buitink et al., 2003; Bewley et al., 2013).

Seed DT loss entails high susceptibility to variations in environmental conditions because the transition from seed to seedling is a sensitive stage of a plant's life cycle. Seed tolerance to stressful environmental conditions is essential for a species to establish a seed bank and/or germinate when conditions are favorable (Salazar et al., 2011). When seedlings become sensitive to desiccation, they are particularly vulnerable to water deficit effects (especially in the radicle region), which may lead to seedling death (Kalemba et al., 2019). Some protocols have been suggested to attempt at recovering DT in germinated seeds (Vieira et al., 2010; Masetto et al., 2014, 2015; Rodrigues et al., 2015), which could promote the survival of these individuals under natural selection.

Seed sensitivity to desiccation is expected to be more common among tropical and subtropical tree species, predominantly in wet and frost-free habitats (Wyse and Dickie, 2017). Given the variations in relative air humidity, photoperiod, temperature, and precipitation across different biomes, plants can develop adaptations and strategies to survive in low-humidity environments, therefore avoiding, resisting, or tolerating desiccation. Species with a wide geographical distribution, which are subject to this broad environmental variation, may reflect this influence in the sizes, shapes, optimal germination temperatures, maturation periods, and levels of DT among produced seeds (Abe and Matsunaga, 2011; Liu et al., 2017). This highlights the importance of considering environmental conditions to understand seed responses to desiccation (Daws et al., 2006).

Erratic rainfall or frequent droughts, coupled with high temperatures, may hamper seed germination and reduce seedling survival (Ooi, 2012), especially in dry biomes such as the Brazilian Caatinga (dry woodlands) and Cerrado (savannas). Beyond these already stressful conditions, climate change is expected to promote an increase of up to 4.8 °C in the average global temperature (IPCC, 2014). Furthermore, associated decreases in rainfall may result in a 10% expansion in the global dryland extent (Sherwood and Fu, 2014). Different water availability conditions have stimulated, through evolutionary pressure, the development of plant strategies that enable their survival even under unfavorable conditions. These include drought tolerance and drought resistance (Leprince and Buitink, 2010) and can be observed in species occurring across different regions of Brazil.

The natural occurrence of a tree species in a given area depends on its adaptations to local climate and soil conditions and seed dispersal characteristics. Seeds able to germinate under different environmental conditions tend to have a wider geographic distribution (Marques et al., 2014). The species *Psidium guajava* L. belongs to the family Myrtaceae, is native from tropical America, and is composed of more than 100 genera and 3800 species (Haida et al., 2015). It is a widely distributed tree species in Brazil, with an occurrence range spanning the Amazon, Caatinga, Cerrado and Atlantic Forest domains. Its occurrence has been confirmed in the north (Acre and Amazonas), northeast (AL, BA, CE, MA, PE, PI and SE), central-west (MS and MT), southeast (ES, MG, RJ and SP), and south (PR, SC e RS) regions of the country (Flora do Brasil, 2020). Highly esteemed for its edible fruits and medicinal applications. *P. guajava* is often used in restoration projects due to its classification as an early succession species (Brancalion et al., 2010).

Identifying the limiting conditions for tree species survival contributes to a better understanding of vegetation dynamics under climate change and anthropization contexts, while also informing species conservation strategies (Liu et al., 2017). Here, we investigate the germination ecology of P. guajava seeds collected in two regions of Brazil to (1) characterize the seed batches; (2) determine the best temperature for germination; and (3) assess variations in seed desiccation tolerance.

# MATERIAL AND METHODS

## Plant material

Mature *Psidium guajava* L. fruits were collected from at least five trees, in two Brazilian regions: north (city of Manaus, Amazonas state; 3°05′31″S and 59°59′36.5″W) and southeast (district of Macuco de Minas, Itumirim municipality, Minas Gerais state; 21°17′38,9″S and 44°45′29″W). The seeds were sent at the Tree Seed Laboratory of the Federal University of Lavras (UFLA) to conduct the experiments. To separate the seeds, the fruits were manually macerated and washed in running water through a sieve. The seeds were then left to dry at room temperature (20 °C) and later kept in a dry cold chamber at ±10 °C and 40% of relative humidity before the experiments. We determined individual seed weight and thousand-seed weight following Brasil (2009) and using an analytical balance with a precision of $10^{-4}$g.

## Determination of seed water content

Randomly sampled seeds from each batch (four repetitions of five intact seeds) were kept in an oven at 105 ± 3 °C for 24 hours (Brasil, 2009). Seed water content was expressed for each batch in terms of average wet basis moisture content.

## Germination at different temperatures

For the germination test, we first disinfected for 10 minutes the seeds from both batches with sodium

hypochlorite (2.5% v/v). Then, in a gerbox recipient, the seeds were sown on two germination paper sheets moisturized with a water content 2.5 times heavier than the weight of the dry paper. To evaluate temperature effects on germination, the seeds were incubated in a growth chamber at 10, 15, 20, 25, 30, 35, and 40 °C under constant lighting and at alternating temperatures between 20-30 °C with a 12-hour photoperiod.

After the test, we assessed seed germination percentage, germination speed index (GSI) (Maguire, 1962), normal seedling percentage, and percentage of dead and hard seeds. For each treatment, we used four repetitions of 25 seeds. Seeds were considered germinated when the radicle emerged (2 mm long) and seedlings were considered normal when they exhibited well-developed structures (root, stem, and first pair of leaves). Germination was scored daily until test stabilization.

### Imbibition curve

Seeds from both batches were sown in gerbox recipients following the same previously described criteria for germination test. The gerbox recipients were placed inside growth chambers at alternating temperatures between 20-30 ± 2 °C and a 12-hour photoperiod. The seeds were weighed before imbibition and at every other day thereafter. Before weighing, we removed water from the surface of the seeds with the aid of a paper towel sheet and, after weighing, placed them back under germination conditions. For each batch, 40 repetitions of one seed were used.

### Desiccation tolerance

Seeds from the two batches were sown in gerbox recipients following the same criteria as the previously described germination test. We used alternating temperatures between 20-30 ± 2 °C and a 12-hour photoperiod.

Germination was interrupted to initiate drying at different moments, defined based on the imbibition curve, as follows: (1) non-imbibed seeds (control); (2) seeds imbibed for 6 days; (3) seeds imbibed for 9 days; (4) seeds imbibed for 12 days; and (5) seeds right after radicle protrusion. For the north batch seeds, a sixth period was also used: (6) seeds imbibed for 15 days. We included this sixth period because radicles took longer to emerge in the seeds from the north batch. The seeds were weighed, laid out forming a single layer over a mesh screen inside gerbox recipients containing silica gel at the bottom, and placed in growth chambers at 20 ± 2 °C in the dark. The seeds were regularly weighed until they attained the same moisture content as observed before imbibition (target weight). After reaching the target weight, the seeds were kept in that state for 72 hours, as described by Buitink et al. (2003). The silica gel was replaced every 24 hours.

After drying, the seeds were moisturized over a mesh in a closed and water-saturated gerbox for 24 hours (with no direct contact between the water and seeds to avoid imbibition damages) at the same temperature as used in the germination test. The germination test was then retaken following the same above-described criteria.

We assessed seed germination percentage, germination speed index (IVG) (Maguire, 1962), normal seedling percentage, and percentage of dead and hard seeds. The criteria used to define germinated seeds and normal seedlings were the same as previously described. We used normal seedling percentage as a criterion to evaluate DT.

### X-ray test

We obtained radiographic images from four repetitions of 100 seeds fixed on acetate sheets with the aid of a double-faced adhesive tape. We used Faxitron X-Ray equipment with automatic adjustments for radiation intensity (24 kV) and time of exposure to radiation (10 seconds), and at a focal distance of 14.3 cm from the x-ray source. The radiographic images were saved in TIFF format.After obtaining the radiographic images, the seeds were sown following the same germination criteria as described above and arranged in the same position as during the x-ray test. For this test, only the southeast batch seeds were used.

### Experimental design

We used a completely randomized design (CRD) and assessed data normality through a Shapiro-Wilk test. The results were submitted to analysis of variance and the averages were compared using a Tukey test at 5% of probability. All statistical analyses were carried out in the R environment (R Core Team, 2019).

## RESULTS

The seeds from north and southeast batches had, respectively, initial water contents of 8.6% and 10.6% and average germination percentages of 88% and 98%. The average individual seed weight for the north batch was 0.0052 g and for the southeast batch, 0.0097 g. Tab. 1 shows the thousand-seed weight calculated for each batch following Brasil (2009). Germination varied significantly among the different temperatures tested (Fig. 1 and Tab. 2). At the temperature extremes (10, 15, 35 e 40 °C), germination in both batches was either extremely low ($\leq$ 2%) or inexistent. The germination tests conducted at these temperatures resulted in high percentages of dead and hard seeds. At 40 °C, seeds were deteriorate and mortality increased. Classifying the seeds as dead or hard proved difficult due to seed darkening or the presence of fungi, which caused some of them to be wrongly categorized as dead. The seed coats remained rigid even after completion of the germination test.

**Tab. 1** Thousand-seed weight of *Psidium guajava* seeds collected in the north (Manaus, AM) and in the southeast (Itumirim, MG) of Brazil.

|           | Average (g) | Variance  | Standard deviation | Coefficient of variation |
|-----------|-------------|-----------|--------------------|--------------------------|
| North     | 5.618       | 0.000462  | 0.02149            | 3.814157                 |
| Southeast | 9.919       | 0.000578  | 0.024033           | 2.422969                 |

**Tab. 2** Germination characteristics of *Psidium guajava* seeds collected in the north (Manaus, AM) and in the southeast (Itumirim, MG) of Brazil.

| Batch | Temperature (°C) | Onset of radicle protrusion (days) | Germination (%) | Normal seedlings (%) | Abnormal seedlings (%) | Hard seeds (%) | Dead seeds (%) | GSI |
|---|---|---|---|---|---|---|---|---|
| North | 10 | - | 0 Ac | 0 Ac | 0 Aa | 100 Aa | 0 Ab | 0.00 Ad |
| | 15 | 31 | 2 Ac | 2 Ac | 0 Aa | 96 Aa | 2 Ab | 0.02 Ad |
| | 20 | 19 | 89 Aa | 89 Aa | 0 Aa | 10 Ad | 1 Ab | 1.01 Ab |
| | 25 | 14 | 93 Aa | 93 Aa | 0 Aa | 5 Ad | 2 Ab | 1.10 Bb |
| | 30 | 14 | 57 Bb | 57 Bb | 0 Aa | 42 Ac | 1 Ab | 0.68 Bc |
| | 35 | - | 0 Ac | 0 Ac | 0 Aa | 100 Aa | 0 Bb | 0.00 Ad |
| | 40 | - | 0 Ac | 0 Ac | 0 Aa | 78 Ab | 22 Ba | 0.00 Ad |
| | 20-30 | 12 | 96 Aa | 95 Aa | 1 Aa | 3 Ad | 1 Ab | 1.57 Ba |
| Southeast | 10 | - | 0 Ab | 0 Ab | 0 Aa | 100 Aa | 0 Ab | 0.00 Ac |
| | 15 | - | 0 Ab | 0 Ab | 0 Aa | 96 Aa | 4 Ab | 0.00 Ac |
| | 20 | 16 | 96 Aa | 96 Aa | 0 Aa | 3 Ab | 1 Ab | 0.94 Ab |
| | 25 | 11 | 99 Aa | 99 Aa | 0 Aa | 0 Ab | 1 Ab | 1.65 Aa |
| | 30 | 14 | 93 Aa | 93 Aa | 0 Aa | 6 Bb | 1 Ab | 1.10 Ab |
| | 35 | 24 | 1 Ab | 0 Ab | 0 Aa | 89 Ba | 10 Ab | 0.01 Ac |
| | 40 | - | 0 Ab | 0 Ab | 0 Aa | 9 Bb | 91 Aa | 0.00 Ac |
| | 20-30 | 11 | 98 Aa | 98 Aa | 0 Aa | 0 Ab | 2 Ab | 1.78 Aa |

*Averages followed by the same lowercase letter compare different temperatures within the same variable in each of the two batches. Averages followed by the same uppercase letter compare the same temperature within the same variable between the two batches. Tukey test at 5% of probability.
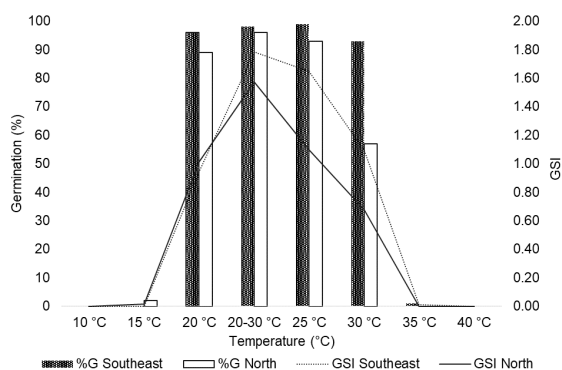


**Fig. 1** Effects of different temperatures on the germination percentage and germination speed index (GSI) of *Psidium guajava* seeds collected in the north (Manaus, AM) and in the southeast (Itumirim, MG) of Brazil.

The onset of radicle protrusion varied with temperature, taking from 11 to 31 days. The cumulative germination curve of the seeds from the two batches revealed an influence of temperature on germination speed (Tab. 2, Fig. 2 and 3). The highest seed germination percentages were observed in the two batches at the constant temperatures of 20 and 25 °C and at alternating temperatures between 20-30 °C, while also at a constant temperature of 30 °C for the southeast batch. The highest GSI values were observed in the two batches at alternating temperatures between 20-30 °C, while also at a constant temperature of 25 °C for the southeast batch.

Because it showed the best germination results for both batches, we used alternating temperatures between 20-30 °C in the other tests. The temperature of 20 °C was considered the minimum necessary to germinate and 30 °C was considered the maximum temperature to promote germination in both batches, although in the north batch, 30 °C resulted in a significant reduction in germination percentage (p>0.05). The imbibition pattern that emerged was triphasic, with a rapid increase in seed fresh weight during the first 48 hours that characterized phase I (Fig. 4).
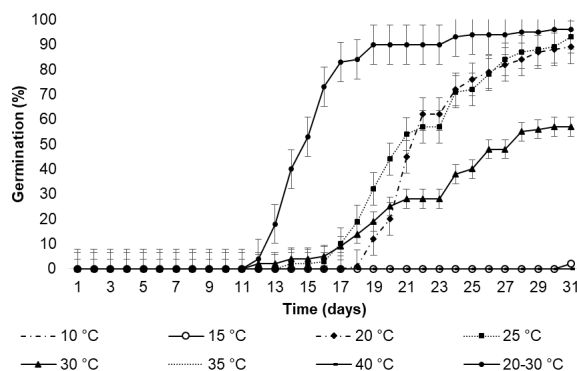


**Fig. 2** Effects of different temperatures on the germination curve of *Psidium guajava* seeds collected in the north of Brazil (Manaus, AM).
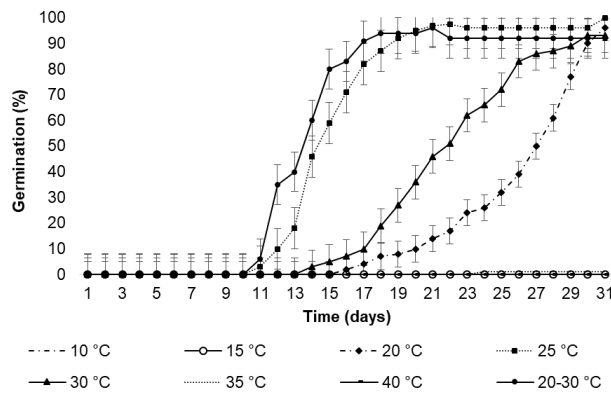
**Fig. 3** Effects of different temperatures on the germination of *Psidium guajava* seeds collected in the southeast of Brazil (Itumirim, MG).

After these 48 hours, we observed a stabilization in seed fresh weight and water absorption (phase II). The radicles of the southeast batch seeds emerged earlier (after 10 days of imbibition) than those of the north batch seeds (after 14 days of imbibition).

After radicle protrusion, seed weight increased (phase III). The southeast and north batches reached 50% of germination after 13 and 18 days, respectively. In both batches, DT was maintained until approximately 9 days of imbibition, with normal seedling percentages above 90% (Tab. 3). After 12 days, DT of the southeast batch seeds severely decreased, whereas the north batch seeds kept normal seedling percentages around 60% even after 15 days of imbibition. After radicle protrusion, we did not observe the formation of normal seedlings with drying and rehydration.

Compared with the southeast batch, the seeds from the north batch had slower DT loss, lower weight gain, and later radicle protrusion (Fig. 5). The southeast batch seeds presented earlier radicle protrusion, higher weight gain, and a faster DT loss, with a steeper decrease around 12 days (Fig. 6). No normal seedlings were produced after radicle protrusion in the two batches. Drying caused the germinated seeds rootlets to become necrotic, which
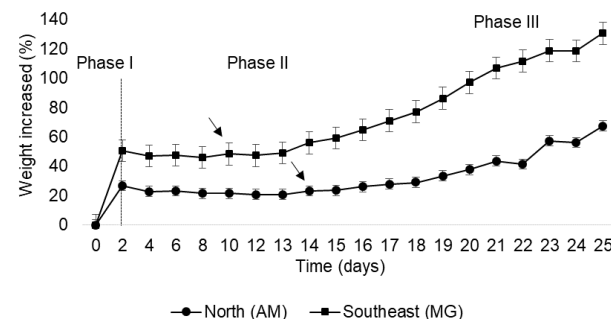


**Fig. 4.** Imbibition curve of *Psidium guajava* seeds collected in the north (Manaus, AM) and in the southeast (Itumirim, MG) of Brazil and germinated at alternating temperatures between 20-30 °C. The arrows indicate the onset of radicle protrusion in each batch.

impeded the growth and development of the seedlings. However, we observed that during imbibition periods that preceded radicle protrusion (9 and 12 days), even with necrosis of the root tissue, seeds from both batches produced roots.

The x-ray images (Fig. 7 and 8) allowed an assessment of seed internal morphology. The seeds with lower internal filling did not produce normal seedlings after the germination test. These dead or hard seeds did not germinate under optimal temperature conditions due to the small amount of endosperm tissue or defects in embryo formation. From the 400 radiographed seeds, 97.25% germinated, of which 97% formed normal seedlings. Only 2.75% of the seeds (which could be identified in the radiographic images) were classified as dead or hard. Seed water content during the test was 10.14%.
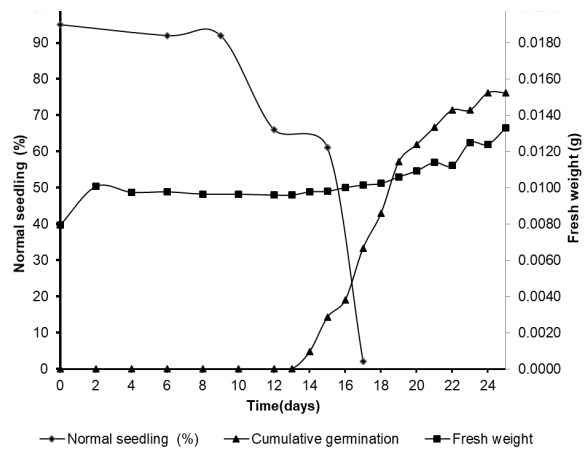


**Fig. 5** Normal seedling percentage, cumulative germination, and fresh weight of *Psidium guajava* seeds collected in the north region of Brazil (Manaus, AM) in response to a desiccation treatment after different periods of imbibition.
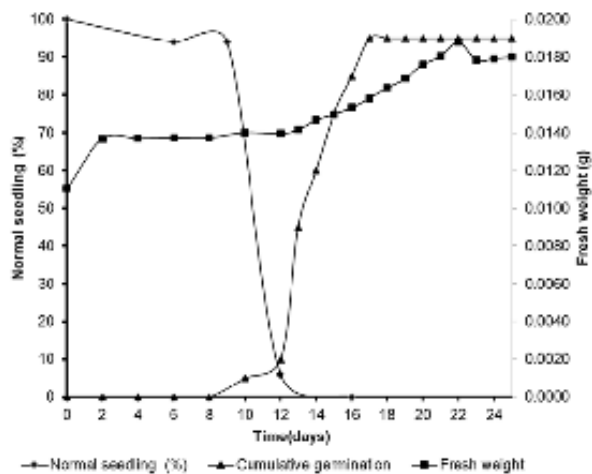


**Fig. 6** Normal seedling percentage, cumulative germination, and fresh weight of *Psidium guajava* seeds collected in the southeast region of Brazil (Itumirim, MG) in response to a desiccation treatment after different periods of imbibition.

**Tab. 3** Normal seedling percentage (%) and germination speed index (GSI) of *Psidium guajava* seeds collected in the north (Manaus, AM) and in the southeast (Itumirim, MG) of Brazil, imbibed during different periods followed by drying and rehydration.

| Imbibition period (days) | Normal seedlings (%) | | GSI | |
|---|---|---|---|---|
| | North | Southeast | North | Southeast |
| 0 | 95 aA | 100 aA | 1.43 bB | 1.73 bA |
| 6 | 92 aA | 94 aA | 1.43 bB | 1.62 bA |
| 9 | 92 aA | 94 aA | 1.72 aB | 2.06 aA |
| 12 | 66 bA | 6 bB | 1.36 bA | 0.28 cB |
| 15 | 61 b | - | 1.58 b | - |
| Radicle protrusion | 2 cA | 0 bA | - | - |

*Averages followed by the same lowercase letter on the columns and uppercase letter on the rows (within each variable) do not differ statistically according to a Tukey test at 5% of probability.
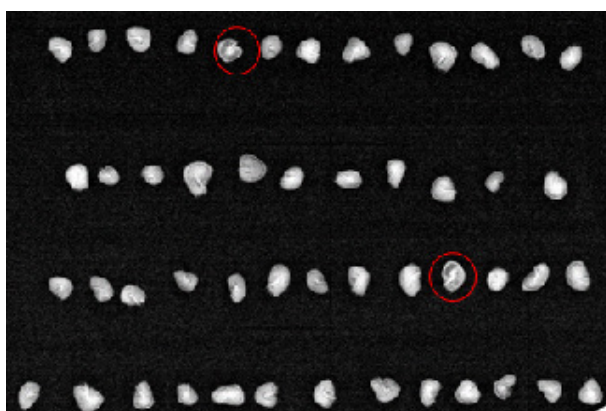


**Fig. 7** X-ray image of seeds from the southeast batch. Highlighted seeds were classified as dead in the germination test.
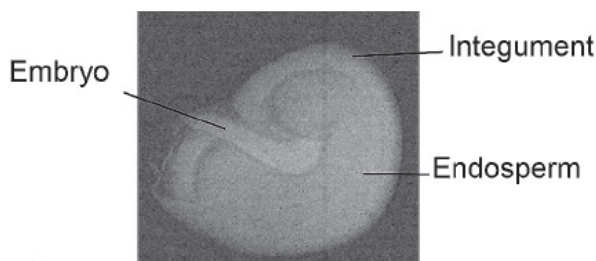


**Fig. 8** Detail of the internal morphology of a Psidium guajava seed obtained through an X-ray test.

## DISCUSSION

The seeds collected in both regions exhibited variations in physiological characteristics. Previous studies have shown that different species may alter patterns of seed mass or morphology as an adaptation to climate (Gorden et al., 2016). Seed size and weight may vary according to environmental conditions, which can also affect seed responses to the environment (Bewley et al., 2013; Oliveira et al., 2019) and can be observed in seed desiccation tolerance and germination responses at different temperatures.

At the extremes temperature (10 and 15 °C and 35 and 40 °C), Psidium guajava seeds did not germinate. Temperature also influenced germination speed in the two batches. Temperature plays an important role in seed germination and plant establishment (Liu et al., 2017) for influencing cell permeability, hampering seed water uptake and gas exchange and, consequently, affecting seed germination intensity and speed (Gualtieri and Fanti, 2015). The influence of high temperatures on seed viability is related to their role in enzyme and protein degradation (Whitmore, 1982). According to our results, the best temperatures for *P. guajava* seeds to germinate are between 20 and 30 °C. This interval is also recommended for the germination of various other tropical and subtropical tree species (Whitmore, 1982). Constant temperatures of 25 °C for Cerrado and Atlantic Forest species and 30 °C for Amazon species are also recommended in previous study (Brancalion et al., 2010). However, in our assessment, the germination percentage of the north batch seeds significantly reduced at 30 °C ($p > 0.05$).

Submitting the seeds to alternating temperatures promoted seed germination in both batches. The use of photoperiod favors pioneer species because light stimulate seed germination (Gualtieri and Fanti, 2015). Moreover, temperature fluctuations (involved in the alternating temperatures) cause the seed coat to expand and shrink, which may lead to crack openings that facilitate gas and water uptake and, consequently, seed germination (Bewley et al., 2013). Both batches displayed the highest GSI values under the alternating temperatures treatment. In the southeast batch, these values did not differ significantly from the results at 25 °C.

Seed germination responses to environmental conditions directly contribute to species distribution patterns (Ribeiro and Kolb, 2016). A single species may present different germination patterns in response to slight differences in environmental conditions (Abe and Matsunaga, 2011; Liu et al., 2017). In *P. guajava*, germination occurred (and varied) in a narrow temperature range. Although the north region of Brazil experiences high temperature and humidity conditions all year round, the north batch seeds did not have better results at higher

temperatures. This can be relate to the region, or even to the genetic material.

Global temperatures are expected to increase considerably until the year 2100. The most optimistic scenarios project a global temperature increase of 0.3 to 1.7 °C, while the most pessimistic project predict an increase of 2.6 to 4.8 °C (IPCC, 2014). Moreover, the global dryland extent is expected to increase by 10%, given predicted decreases in rainfall (Sherwood and Fu, 2014). Focusing on South America, climate change projections are even more pessimistic, with forecasted precipitation decreases of up to 40% in the tropical regions of the continent, including the Amazon and northeast regions of Brazil. A warming of 4 to 6 °C is also expected in the region (Marengo et al., 2012).

Altogether, these forecasts indicate that climate changes may limit the establishment or force *P. guajava* plants to adapt, especially those originating from the north region of Brazil. These changes may affect *P. guajava* ecology, given that variations in temperature and humidity directly influence germination (Walck et al., 2011; Ooi, 2012).

The role of genetic and phenotypic plasticity on seed germination in different regions and under different environmental conditions should also be regarded. Such plasticity may protect the populations against some of the climate change effects (Walck et al., 2011; Liu et al., 2017). Our results revealed higher germination plasticity among the southeast batch seeds compared with the north batch seeds. The imbibition curves presented a triphasic model. The seeds showed rapid imbibition during the first 48 hours, characterizing phase I, which is when the seed reserves are consumed and the energy required for phase II is produced (Marcos-Filho, 2005). Phase II began in the two batches at the same time, but had different durations. The southeast batch seeds regained weight earlier (around the 14th day) than the north batch seeds, indicating an earlier end of phase II. Germination onset also occurred earlier in the southeast batch (10th day).

Early germination favors seedling establishment before unfavorable periods (Rubio de Casas et al., 2017). The transition from seed to seedling is a high-risk period in the life cycle of most plants. Therefore, seed ability to tolerate stressful environmental conditions is essential for a species to establish a seed bank and germinate when conditions become favorable (Salazar et al., 2011).

The north batch seeds remained desiccation-tolerant for up to 15 days, with seed germination percentages above 60%, smaller seed sizes, and slower germination. The southeast batch, on the other hand, showed a drastic reduction in germination after 12 days of drying (15%), larger seeds, higher water content, and faster germination.

As germination advances, orthodox seeds tend to progressively lose DT, which is usually completely lost after radicle protrusion. This was the case for P. guajava seeds in our study, and likewise for Anadenanthera colubrina, Leucaena leucocephala, Peltophorum dubium, and Senna multijuga in previous studies (Rodrigues-Junior et al., 2015; Ribeiro et al., 2016; Castro et al., 2017). The mechanisms involved in desiccation tolerance are gradually deactivated during germination, with a complete loss of tolerance for many species usually coinciding with radicle protrusion (Buitink et al., 2003; Bewley et al., 2013). Sometimes, DT loss happens after radicle protrusion, as has been observed in Sesbania virgata and Handroanthus impetiginosus (Vieira et al., 2010; Martins et al., 2015; Masetto et al., 2015; Costa et al., 2016). In both batches, the highest GSI values were observed after 9 days of imbibition followed by drying. Previous studies that investigated hydration and dehydration cycles suggest that stress tolerance may increase after seeds are exposed to repeated cycles of hydration followed by drying (Hora and Meiado, 2016; Lima and Meiado, 2017; Lima et al., 2018).

DT is attributed, among other mechanisms, to an accumulation, during hydration and dehydration cycles, of LEA proteins that are responsible for tolerance increase. Therefore, imbibition may act as physiological conditioning that increases stress tolerance by creating a form of seed hydration memory (Chen and Arora, 2013).

Hydration memory is the ability of seeds to retain biochemical and physiological alterations stimulated by discontinuous seed hydration (Lima et al., 2018). P. guajava seeds may display this trait, which agrees with the highest GSI values observed after 9 days of imbibition, but this requires further investigation.

Although we observed root meristem necrosis, root systems were formed. When seeds with recently emerged radicles were dried, root tissue necrosis was so advanced that regeneration was no longer possible. Seeds of *Handroanthus impetiginosus* were able to develop adventitious roots even when radicles had become necrotic (Vieira et al., 2010). In *Sesbania virgata*, secondary (not adventitious) roots were formed after desiccation (Costa et al., 2016). In our study, we were not able to assess which case applied to *P. guajava* seeds because they were not dissected.

The portion of the embryonic axis that contains the root tissue in a seed is its most sensitive part to dehydration (Kalemba et al., 2019). By disrupting the seed coat and forming the primary root, this is the first structure to emerge during germination. This entails high sensitivity of this structure (formed by meristematic tissue) to environmental conditions (Costa et al., 2016). Despite dehydration damages, *P. guajava* seeds that were dried before radicle protrusion were able to form a root system, although with an unknown origin.

The radiographic images of the southeast batch seeds revealed their internal morphology. We were able to visualize seed coat thickness, endosperm (seed reserve tissue), and seed embryo. We could also associate seed physiological quality with the x-ray images. The use of optical analysis techniques, such as x-rays, for the assessment of seed physical quality, is highly advantageous due to its non-destructive and practical approach (Brasil, 2009; Grasso et al., 2018). In tree species, this technique can contribute to the management of seedling production by guiding the separation of seed batches with higher physical quality (Lima et al., 2018), through the observation of tissue formation and presence of empty or predated seeds.

Lower density seeds are less likely to germinate and more likely to produce low-quality seedlings because

higher seed density is linked to greater tissue integrity and reserve content, which are essential for germination and seedling formation (Medeiros et al., 2020).

Recent reports have show the usefulness of radiographic images for embryo abnormality detection, identification of seed coat mechanical damages, determination of seed maturation stage, full seed selection, and general assessment of seed quality (Amaral et al., 2019; Gibbert et al., 2019; Medeiros et al., 2019; Medeiros et al., 2020). X-ray tests are recommended by the Rules for Seed Testing (Brasil, 2009) and by the International Seed Testing Association (ISTA, 2003) aiming to detect full, empty, damaged, or insect-predated seeds. In *P. guajava* seeds, radiation intensity, period to radiation and a focal distance used in this reserch were enough to allow the visualization of internal seed morphology, indicating that these conditions can be applied to batch classification.

## CONCLUSIONS

We observed variations in the physiological characteristics between the batches collected in the north and southeast regions of Brazil. The seeds from the north batch lost desiccation tolerance slower than the seeds from the southeast batch, which were more sensitive to desiccation. Desiccation tolerance was completely lost in both batches after radicle protrusion.Extreme temperatures (below 15 °C and above 35 °C) prevented *P. guajava* seeds to germinate.

## AUTHORSHIP CONTRIBUTION

Project Idea: ACF, ACJ, JMF

Database: ACF

Processing: ACF, OAOT

Analysis: ACF, OAOT, ACJ

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