



Dormancy overcoming in seeds of cajá-manga (*Spondias dulcis*)

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Abstract

The propagation of 'cajá-manga' (*Spondias dulcis*) is usually performed by seeds. The presence of dormancy is an obstacle for seedling production and the commercial use of its main product, the fruit. This research aimed to evaluate the use of phytohormones associated with the scarification of the distal region of the embryo in dormancy overcoming and in the standardization of germination of 'cajá-manga' seedlings. Endocarps extracted from fruits of six matrices were subjected to the following treatments: control (T1), mechanic scarification in the distal region of the embryonic axis (T2); all following treatments involved scarification and imbibition in a solution of: water for 6h (T3); GA₃ (750 mg L⁻¹) for 6h (T4); GA₃ (350 mg L⁻¹) for 12h (T5); Cytokinin (750 mg L⁻¹) for 6h (T6); Cytokinin (350 mg L⁻¹) for 12h (T7); GA₃ and Cytokinin (750 mg L⁻¹) for 6h (T8); and GA₃ and Cytokinin sowing 25 endocarps in expanded polystyrene trays using a washed sand substrate. The following variables were evaluated: emergence, first emergence count, emergence speed index, mean emergence time, shoot and root length of seedlings, and relative emergence frequency. The experimental design was in randomized blocks, with 9 treatments and four replications consisting of 25 endocarps each. The data obtained were subjected to the F Test at a 1% level of probability, as well as to the Scott-Knott method. The use of mechanic scarification in the opposite region of the embryonic axis, followed by the imbibition in a solution of gibberellin + cytokinin at the concentration of 350 mg L⁻¹ for 12 hours is promising for dormancy overcoming in seeds of *Spondias dulcis*.

Keywords: *Spondias dulcis*, Cytokinin, Gibberellin, Seedlings

Introduction

Within the genus *Spondias*, four species are economically important in tropical America: *S. dulcis*, *S. mombin*, *S. purpurea*, and *S. tuberosa*, which are cultivated and, therefore, are not at risk of extinction, although their genetic diversity can be compromised due to habitat destruction (Mitchell & Daly, 2015). However, the implantation of production fields through seeds is usually hindered by dormancy (Moura et al., 2013; Martins et al., 2019).

Dormancy provides slow and uneven germination, as already noted by other authors for other species of the same genus, such as *Spondias mombin* (Martins et al., 2019) and as reported for native trees (Matos & Ataíde, 2015). Consequently, especially in this genus, combinational dormancy occurs. The first type of dormancy is a physical barrier imposed by the endocarp that acts as a limiting factor, promoting mechanical

resistance to embryo expansion, as well as gas exchange. The second, of a physiological nature, requires the use of phytohormones to overcome this dormancy, normally gibberellic acid (GA₃) (Carvalho & Nakagawa, 2012; Ferreira et al., 2016; Martins et al., 2019).

Mechanical scarification is highlighted among the methods for overcoming physical dormancy, usually performed with a cut in the distal region of the hilum but not reaching the embryonic axis, causing disruption or weakening of the integument and allowing germination (Carvalho & Nakagawa, 2012; Oliveira et al., 2018). This method was successfully employed in *S. mombin* (Martins et al., 2019). Physiological dormancy can be attributed to hormones that inhibit germination (Carvalho & Nakagawa, 2012; Miransari & Smith, 2014). There are other hormones, among them cytokinin, whose efficiency in germination is still little known; however, it is already understood that cytokinin promotes cell

division and, associated with gibberellin, they constitute essential substances that promote germination and the enzymatic process when these are blocked by inhibitors of physiological dormancy (Miransari & Smith, 2014; Piccoloto et al., 2007; Taiz et al., 2017).

It is emphasized that, in nature, dormancy is one of the phenomena that guarantee production over time. For seedling producers, however, it constitutes an obstacle since the seeds are subjected to adverse conditions due to slow germination, also resulting in uneven seedlings (Carvalho & Nakagawa, 2012; Costa et al., 2010).

For dormancy overcoming in 'cajá-manga' seeds, studies are still incipient. Germination in this species is slow and uneven, and the use of dormancy overcoming methods, such as the application of phytohormones, can assist in obtaining quality seedlings and helping in the domestication process of this species (Carvalho & Nakagawa, 2012; Ferreira et al., 2016; Matos & Ataíde, 2015; Rocha et al., 2019).

Thus, the present work aimed to evaluate the use of phytohormones (gibberellins and cytokinins) associated with the scarification of the distal region of the embryo in dormancy overcoming and standardization of seeds of 'cajá-manga'.

Material and Methods

The 'cajá-manga' fruits were harvested in the natural vegetation of the municipality of Jataí, state of Goiás, Brazil, in February 2019, from six matrices with a minimum distance of 3.5 km from each other (Table 1).

Table 1. Georeferencing of and dimensions of height (H) and Diameter at Breast Height (DBH) of matrices of *Spondias dulcis* in the Jataí-GO region.

Matrices	Geographic Coordinates	DBH (cm)	H (m)
M1	17° 52' 47" S 51° 44' 06" O	62.2	14.5
M2	17° 54' 55" S 51° 44' 05" O	65.0	18.5
M3	17° 53' 51" S 51° 43' 24" O	60.4	16.0
M4	17° 51' 39" S 51° 43' 45" O	73.0	22.0
M5	17° 53' 06" S 51° 43' 32" O	64.0	15.0
M6	17° 53' 57" S 51° 44' 05" O	60.2	18.0

Afterward, the fruits were stored in plastic boxes (55 x 36 x 31 cm) and transported to the Agronomic Research Center of the Federal University of Jataí (UFJ).

For the extraction of the endocarp, the fruits were immersed and sprayed with water and sanitized with a 2% sodium hypochlorite solution for 15 minutes. The fruits were subjected to endocarp extraction under running water with the aid of a knife, as recommended for the genus *Spondias* (Brasil, 2013). The endocarps that contained real seeds were denominated seeds, as described in the normative instructions for forest seeds analysis (Brasil, 2013).

The extracted endocarps were subjected to the treatments according to Table 2.

Table 2. Treatments used for dormancy overcoming in seeds of 'cajá-manga' removed from fruits of different matrices of Jataí-GO.

Treatments
T1. Control: without dormancy overcoming treatment
T2. Scarification in the distal region
T3. T2 + Imbibition in water for 6 h
T4. T2 + Imbibition in GA ₃ (750 mg L ⁻¹) for 6 h
T5. T2 + Imbibition in GA ₃ (350 mg L ⁻¹) for 12 h
T6. T2 + Imbibition in Cytokinin (750 mg L ⁻¹) for 6 h
T7. T2 + Imbibition in Cytokinin (350 mg L ⁻¹) for 12 h
T8. T2 + Imbibition in GA ₃ and Cytokinin (750 mg L ⁻¹) for 6 h
T9. T2 + Imbibition in GA ₃ and Cytokinin (350 mg L ⁻¹) for 12 h

For the treatments with scarification in the distal region, a 6-inch bench grinder was used (152 mm of diameter). After the treatments, four blocks with 25 endocarps were individually sown in expanded polystyrene trays (35 x 25 x 5 cm) containing a washed sand substrate with 60% retention capacity, which was previously sieved in a 0.8 mm mesh sieve and sterilized in a forced-air circulation oven at 200°C for two hours (Brasil, 2013).

The experiment was maintained in a plant nursery with an automatic sprinkler irrigation system programmed to be triggered at the beginning and end of the day for an irrigation shift of 40 minutes, evaluating the following characteristics:

Seedling emergence – the most vigorous normal seedlings emerged were evaluated at 60 days, considering that the emergence of more than one seedling per endocarp may occur.

First emergence count – accounting of the most vigorous normal seedlings when reaching 50% + 1 of final emergence.

Emergence speed index (ESI) – performed by counting the most vigorous normal seedlings every five days during 60 days; the data were then subjected to the formula proposed by Maguire (1962).

Mean emergence time (MET) – registered from five to 60 days; the data were subjected to the formula described by Santana & Ranal (2004), with the results expressed in days after sowing.

Shoot and root length of seedlings – assessed by measuring the shoot part, from the base to the insert of the cotyledon, and the main root, from the hood to the base of the most vigorous normal seedling.

Relative emergence frequency (Ref) – the counts from five to 60 days were subjected to the formula described by Santana & Ranal (2004).

A completely randomized block design was

used in the experiment, with treatments distributed into four blocks, with 25 endocarps in each portion. The data obtained were subjected to the normality of data and error homogeneity tests; the F-test at 1% of probability was also employed, and the Scott-Knott method was used for the comparison of means, using the Rbio software, version 119:06/06/2019.

Results and Discussion

There was an effect of the treatments on the variables of emergence, first emergence count, and emergence speed index (Table 3), with no difference

being observed for the mean emergence time and remaining measurements in the obtained seedlings.

The scarification at the distal region, followed by imbibition in GA₃ and Cytokinin (350 mgL⁻¹) for 12 hours (treatment 9), was efficient for overcoming dormancy in 'cajá-manga' seeds (Table 4). This treatment obtained 63% of seedling emergence at 60 days after sowing (d.a.s.) and an emergence speed index of 3.41, values higher than those of other treatments. It is emphasized that, in comparison with the control, this difference was three times higher, demonstrating its efficiency.

Table 3. Summary of the analysis of variance of the data of emergence (E), first emergence count (FEC), emergence speed index (ESI), mean emergence time (MET), shoot length (SL), and root length (RL) of 'cajá-manga' seedlings produced from seeds with different dormancy overcoming treatments.

Source of variation	Mean squares					
	E	FEC	ESI	MET	SL	RL
Treatments	1001.00**	44.45**	2.98**	2.81n.s	21.12n.s	5.93n.s
Blocks	167.7	28.89	1.04	5.40	62.65	11.61
Residues	134.70	15.56	0.35	4.11	31.99	4.63
CV%	38.69	64.54	38.02	4.73	16.01	21.40

**Significant at a 1% level of probability; n.s: not significant at a 1% level of probability; CV: coefficient of variation.

Table 4. Emergence (E), first emergence count (FEC), and emergence speed index (ESI) of 'cajá-manga' seedlings produced from seeds with different dormancy overcoming treatments.

Treatments	E	FEC	ESI
	%	(days)	
T1. Control: without treatment for dormancy	21 d	5 b	1.06 c
T2. Scarification in the distal region	31 c	4 b	1.59 b
T3. T2 + Imbibition in water for 6 h	20 d	4 b	0.95 c
T4. T2 + Imbibition in GA ₃ (750 mg. L ⁻¹) for 6 h	10 d	4 b	0.57 c
T5. T2 + Imbibition in GA ₃ (350 mg. L ⁻¹) for 12 h	33 c	5 b	1.97 b
T6. T2 + Imbibition in Cytokinin (750 mg. L ⁻¹) for 6 h	17 d	3 b	0.85 c
T7. T2 + Imbibition in Cytokinin (350 mg. L ⁻¹) for 12 h	43 b	10 a	2.09 b
T8. T2 + Imbibition in GA ₃ and Cytokinin (750 mg. L ⁻¹) for 6 h	32 c	7 b	1.65 b
T9. T2 + Imbibition in GA ₃ and Cytokinin (350 mg. L ⁻¹) for 12 h	63 a	13 a	3.41 a

Means followed by the same letter, in the same column, did not differ significantly from each other by the Scott-Knott method at a 1% level of probability.

When comparing the values of emergence (63%) and mean emergence time around 43 days, even though there is no statistical difference (Tables 3 and 4), the results in the literature ensure how much the treatment applied in this work was significant. Martins et al. (2019), evaluating *S. mombin*, verified a 68% emergence and a mean emergence time of 273 days after sowing for medium-sized endocarps with beige color and with subjected to mechanic scarification.

At 18 days after sowing, the first germination count resulted in 13% when performing the scarification of the distal region followed by imbibition in GA₃ and Cytokinin (350 mg L⁻¹) for 12 hours (treatment 9), not differing for this variable from the treatment subjected to scarification followed by imbibition in Cytokinin (350 mg L⁻¹) for 12 hours (treatment 7) since each obtained 10 seedlings. Martins et al. (2019), studying the germination of cajá (*S. mombin*) seeds, obtained first germination

count values of 28%; however, this count occurred at 180 days after sowing.

The application of phytohormones on dormancy overcoming or standardization of germination was performed. Majidi et al. (2016) recommend that the application of the GA₃, indoleacetic acid (IAA), and kinetin phytohormones aid in overcoming dormancy and promote quick germination in *Hordeum spontaneum*. In the same way, but not so equitably, the use of an association of phytohormones, such as in the present research, has also been used. Ferreira et al. (2016) efficiently applied dormancy overcoming for seeds of *Annona purpurea* (chincuya) and *A. macrophyllata* (papausa) through soaking in a GA₄₊₇ + BA (6-benzyladenine) mixture for 96 hours. This combination of phytohormones was also employed by Beraud et al. (2016) for *Ugni molinae* Turcz, a fruit species native to Chile.

There is a scarcity of studies evaluating the effect

of cytokinin on *Spondias* spp. Melo et al. (2012), when evaluating the effect of gibberellin, kinetin, and ethylene, verified no differences in seedling emergence for *S. tuberosa*, either with or without mechanic scarification. It is known that cytokinins modulate cell division and differentiation (Rajesh et al., 2019; Taiz et al., 2017). However, the effect of cytokinin on dormancy overcoming has also been checked in another species, for which Lee et al. (2018) found that kinetin had a significant effect on germination promotion and in dormancy breaking of seeds of *Panax ginseng* (ginseng), although a short period of cold stratification is still necessary. For *Arabidopsis*, it was verified that among the functions of cytokinin, there is the control of seed size, including embryo growth, endosperm and seed coating (Mansfield & Bowman, 1993). Miransari & Smith (2014) affirm that cytokinin and its negative interaction with ABA can positively regulate the seed germination and interrupt the seed dormancy.

The action of gibberellins in seed germination is already a fact in the literature as they act in germination promotion and the activation of vegetative growth in the embryo through the mobilization of reserves (Takata et al., 2014; Taiz et al., 2017). Taiz et al. (2017) still reported that the capacity of controlling metabolic processes in plants, from germination to growth, is promoted by gibberellins, through the increase in the number and length of cells.

For treatment 2, the reduction of the physical barrier with mechanic scarification only was not enough

to overcome dormancy in 'cajá-manga', evidencing the combined dormancy of these seeds, that is, a physical barrier imposed by the endocarp as well as a physiological barrier, probably constituted by inhibitory substances that prevent the germination process. This fact has already been confirmed in other *Spondias* species, such as by Melo et al. (2012) in seeds of *S. tuberosa* and by Martins et al. (2019) in *S. mombim*.

When the imbibition for six hours was employed, even in treatment 8, which was very similar to the best treatment, a little effect of the treatment was verified on dormancy overcoming. The interval of six hours of imbibition was probably not enough to overcome the physical barrier of the endocarp as well as to start the seed imbibition process and activation of phytohormones, even if these were used in higher concentrations (750 mgL⁻¹) in these treatments.

Figure 1 depicts the relative emergence frequency of 'cajá-manga' seedlings, in which it is possible to observe that, over time, seedling emergence reaches a maximum point, declines, and go back up; the frequency polygons present a polymodal frequency, with heterogeneous emergence in more than two peaks (Santana & Ranal, 2004). Based on the graphics, it is possible to affirm that the emergence in this species is not yet perfectly synchronized, even with the efficient treatment, spreading out over the time (Oliveira et al., 2017).

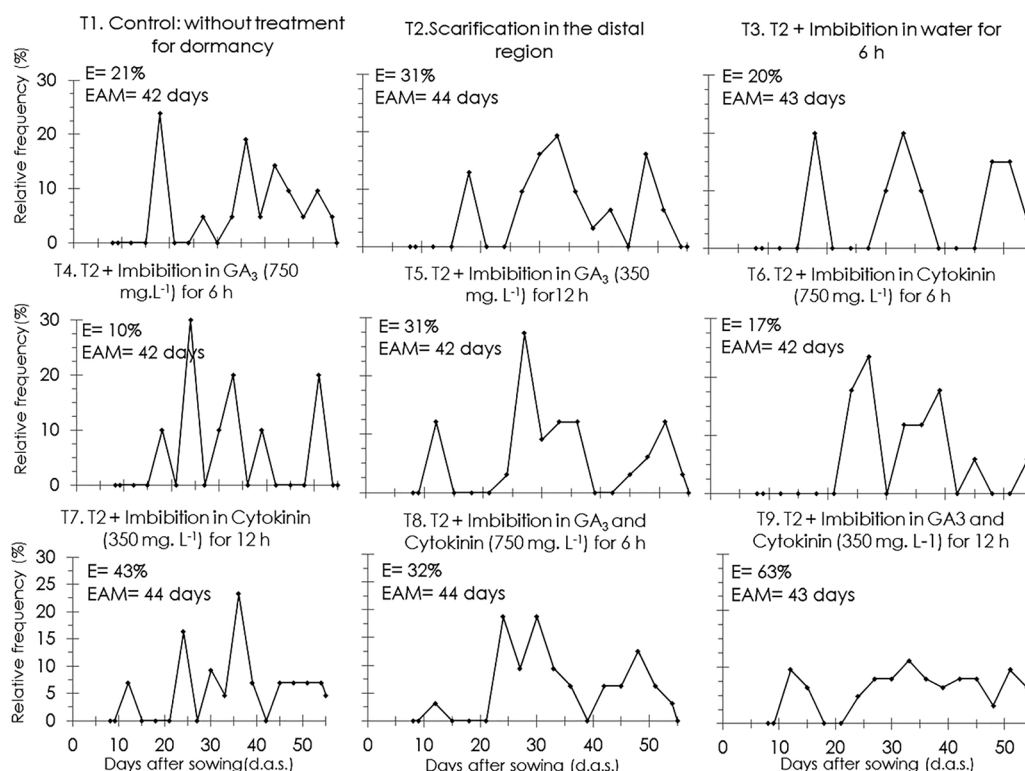


Figure 1. Distribution of the relative emergence frequency of 'cajá-manga' seedlings produced from seeds subjected to different dormancy overcoming treatments (E= seedling emergence percentage; EAM= mean emergence time).

The beginning of seedling emergence occurred around eleven days after sowing, except for the control treatment and for treatments T2, T3, and T6, whose emergence began 20 days after seeding. This period was similar to that reported by Melo et al. (2012) for the seedling emergence of *Spondias tuberosa*, in which the seedling emerged at nine days.

For the mean emergence time, it was verified that there was no difference between the evaluated treatments since the frequency distributions showed that the mean emergence time ranged between 42 and 44 days (Table 3, Figure 1). There was no displacement of the polygon since the mean times were similar, implying that, even uneven, there was no displacement of the polygonal line to the right or to the left of the mean emergence time, that is, there was no delay on the seedling emergence process (Berger, 2014).

It should be noted that, still in Figure 1, treatment 9 (scarification followed by imbibition in GA₃ and Cytokinin (350 mg L⁻¹) for 12 hours) revealed to be promising in the dormancy overcoming of 'cajá-manga', promoting a seedling emergence of 63% and presenting a different behavior from the others, probably for being the only treatment that increased the emergence speed, with an index of 3.41 (Table 3).

Conclusions

The use of mechanic scarification in the opposite region of the embryonic axis, followed by the imbibition in a solution of gibberellin + cytokinin phytohormones in the concentration of 350 mg L⁻¹, for 12 hours, is promising to overcome dormancy in 'cajá-manga' seeds.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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