

RESEARCH NOTE

Investigation of dormancy and storage potential of seeds of yellow passion fruit¹

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ABSTRACT – *Passiflora actinia* Hooker is a passion fruit species native to Brazil, typically found in the Atlantic and Araucaria Forests. It has economic relevance (edible fruit) and medicinal value (sedative and anxiolytic properties), in addition to being used for vegetative propagation, as rootstock for other *Passiflora* species. This study aimed at investigating the occurrence of dormancy in *P. actinia* seeds, and at evaluating their storage potential. The germination test considered different combinations of temperature and lighting regimes, whereas seed dormancy was assessed using different germination-inducing treatments (tegument cutting, warm water immersion, and gibberellin application). Seed viability during storage was also appraised. The results showed that seed germination should be conducted on paper at the alternating temperature of 20-30 °C, without lighting. Also, newly-harvested seeds presented physical and physiological dormancies. The immersion of seeds in water at 40 °C or 50 °C (for 5 or 10 minutes) proved to be efficient in breaking the physical dormancy. Physiological dormancy, in turn, was successfully interrupted by applying 100 mg. L⁻¹ of gibberellic acid on the substrate paper. The storage of seeds under refrigeration, inside hermetically sealed polyethylene packaging, preserved their physiological quality for up to nine months.

Index terms: *Passiflora actinia*, fruit-bearing species, germination, gibberellin, propagation.

Investigação de dormência e potencial de armazenamento de sementes de maracujazeiro amarelo nativo

RESUMO – *Passiflora actinia* Hooker é uma espécie nativa de maracujazeiro, típica da Mata Atlântica e da Mata com Araucária, que apresenta valores econômico (fruto comestível), medicinal (atividades sedativa e ansiolítica), além de ser usada em propagação vegetativa (porta-enxerto para outras espécies de *Passiflora*). Este trabalho teve por objetivos investigar a existência de dormência em sementes de *P. actinia* e avaliar o potencial de armazenamento destas. O teste de germinação foi conduzido por meio de combinações de temperatura e regimes de luz, enquanto a presença de dormência foi investigada através de diferentes tratamentos para promoção da germinação (corte no tegumento, imersão em água quente e aplicação de giberelina). Analisou-se, também, a viabilidade das sementes durante o armazenamento. Neste caso, o teste de germinação de sementes foi feito sobre papel, a 20-30 °C, sem o fornecimento de luz. Sementes recém-colhidas apresentaram dormência física e fisiológica. Os tratamentos com imersão das sementes em água a 40 °C ou 50 °C (5 ou 10 minutos) foram efetivos para quebrar a dormência física. A dormência fisiológica pôde ser superada com a aplicação de 100 mg. L⁻¹ de ácido giberélico sobre o substrato de papel. O armazenamento em refrigerador, dentro de embalagens de polietileno hermeticamente fechadas, preservou a qualidade fisiológica por até nove meses.

Termos para indexação: *Passiflora actinia*, espécie frutífera, germinação, giberelina, propagação.

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Introduction

Passiflora actinia Hooker, popularly known as yellow passion fruit, is a heliophile plant, native to Brazilian dense ombrophilous forest and mixed forest (the Atlantic and Araucaria Forests, respectively). It is typically found in the states of *Espírito Santo*, *Rio de Janeiro*, *São Paulo*, *Paraná*, *Santa Catarina*, and *Rio Grande do Sul* (Cervi et al., 2009), inhabiting both the inner part as well as the edges of the forests, where it grows until its branches reach the highest strata, in order to get direct sunlight (Mondin et al., 2011).

The yellow passion fruit has economic value, as it is edible, and can also be employed for ornamental purposes. The yield per plant is expressive, and the fruits are filled with a flavorful pulp. In folk medicine, the leaves serve as the base for sedative and anxiolytic concoctions (Kurtz et al., 2003), and they recently proved to bear neuropharmacological properties (Santos et al., 2016). Additionally, as it is more tolerant to low temperatures, it can be used as rootstock for *Passiflora alata* Curtis and *Passiflora quadrangularis* L., (Lima et al., 2007). Nevertheless, despite its enormous potential, the species has been threatened by the progression of deforestation in native areas. Thus, preserving it in seedbanks is imperative to protect the species and to promote its sexual propagation (Teixeira et al., 2016).

Basic genetic research approaching populational aspects and breeding programs are still scarce for the majority of *Passiflora* species (Cerqueira-Silva et al., 2014). Seeds of *P. actinia* – as well as those of other indigenous representatives of the genus – might exhibit dormancy, once they have not undergone genetic selection to annul such impediment. Therefore, it might be an obstacle for large-scale seedling production, and researches on the type of dormancy and possible treatments for promoting germination are crucial for further commercial use.

As for the mechanisms through which this phenomenon occurs, the following two are most significant: embryo dormancy (endogenous) and dormancy imposed by the outer layers of the seed (exogenous). The first one includes the cases of metabolic inhibition and immature embryos, and it is divided into physiological, morphological, and morphophysiological (Cardoso, 2009).

Plant growth regulators are commonly used to overcome physiological dormancy. The gibberellic acid (GA_3) is one of these compounds, and it is applied to the outside of seeds to stimulate germination (Shu et al., 2016). It acts primarily on the gene expression of hydrolytic enzymes responsible for weakening the tegument (Taiz et al., 2017) and for degrading reserve substances (Rego et al., 2018). These conditions allow the embryo to retake its growth and break the dormancy (Castro et al., 2005).

On that account, treatments with GA_3 have been suggested to tackle the physiological dormancy of several *Passiflora* species, including *P. cincinnata* Mast. (Zucareli et al., 2009; Moura et al., 2018), *P. ligularis* (Cadorin et al., 2017), *P. caerulea* (Hossel et al., 2018), *P. edulis* f. *flavicarpa* (Lima et al., 2009; Cárdenas et al., 2013), *P. suberosa*, *P. morifolia*, and *P. tenuiflora* (Marostega et al., 2017). Different doses of this plant regulator are used, as seed dormancy is linked to the genotype (Santos et al., 2015).

Conversely, the exogenous dormancy is caused by tissues of the seed itself (extraembryonic), such as the tegument or parts of the fruit, and it can be associated to physical, mechanical, and chemical factors. Physical dormancy, specifically, is caused by the impermeable character of the tegument of both seeds and fruit, which totally or partially restricts water diffusion to the embryo. The literature on *Passiflora* seeds contain some promising treatments against it, which include the use of potassium nitrate (KNO_3) at 1%, for *P. eichleriana*, scarification with sandpaper, for *P. micropetala* (Marostega et al., 2017), and scarification with sandpaper and water bath at 50 °C for 5 minutes, for *P. cincinnata* (Oliveira-Júnior et al., 2010).

It is worth mentioning that a single species can present different forms and levels of dormancy. Cases in which more than one type occur are known as complex dormancy, and they have been observed in *P. mollissima* and *P. nov* sp. (Delanoy et al., 2006).

The present work aimed at investigating the occurrence of both physical and physiological dormancies in seeds of *P. actinia*. Their storage potential was withal assessed.

Material and Methods

The plant material was gathered from *P. actinia* individuals cultivated in the experimental area of the Agricultural Sciences Sector of the *Universidade Federal do Paraná*, in *Curitiba*. This species has simple, alternate leaves (Figure 1A), which have entire and ovate blades, entire edges, obtuse apex, and rounded base. The leaf surface is subcoriaceous textured, completely glabrous, and discolor, with the adaxial side exhibiting a green hue (Kurtz et al., 2003). The *P. actinia* flower is lush white and has a corona composed of purple-white striped filaments, arranged in five series. They assume a reddish color near their base, which attracts pollinating agents (Figure 1B).

In order to obtain the seeds, yellow-rind fruits (Figure 1C) with translucent-yellowish pulp (Figure 1D) were harvested – these characteristics qualified them as ripe. They had their content manually removed, and the seeds were separated from the fleshy part by rubbing them against a sieve (Figure 1E). They were then washed under running water and let to drying in ambient

laboratory conditions for seven days. After that, the seeds were once again processed with the aid of the sieve, until the total removal of the aril.

For the germination tests, combinations of four temperatures (20, 25, 20-30 and 30 °C) and two light regimes (with or without continuous supply) were considered. The seeds were sown over blotting-paper sheets placed inside plastic boxes (11.0 x 11.0 x 3.5 cm). The paper had been previously moistened with water at the quantity of 2.5 times the dry substrate weight. The normal seedlings were counted periodically, until steady germination, and the results were expressed in percentage.

The interruption of physical dormancy was evaluated following the procedures described in Rules for Seed Testing (Brasil, 2009). In this case, the following treatments were appraised: control; seeds cross-cut at the region opposite to the embryonic axis (Figure 1F); seeds immersed in water for 24 hours (with and without the cross-cut); seeds immersed in water at 40 °C (for 5 or 10 minutes); and seeds immersed in water at 50 °C (for 5 or 10 minutes).

The breakage of physiological dormancy was assessed using two forms of gibberellic acid administration: imbibition, via immersion of the seeds in a GA₃ solution for 5 hours; or moistening, by laying them over blotting-paper sheets soaked

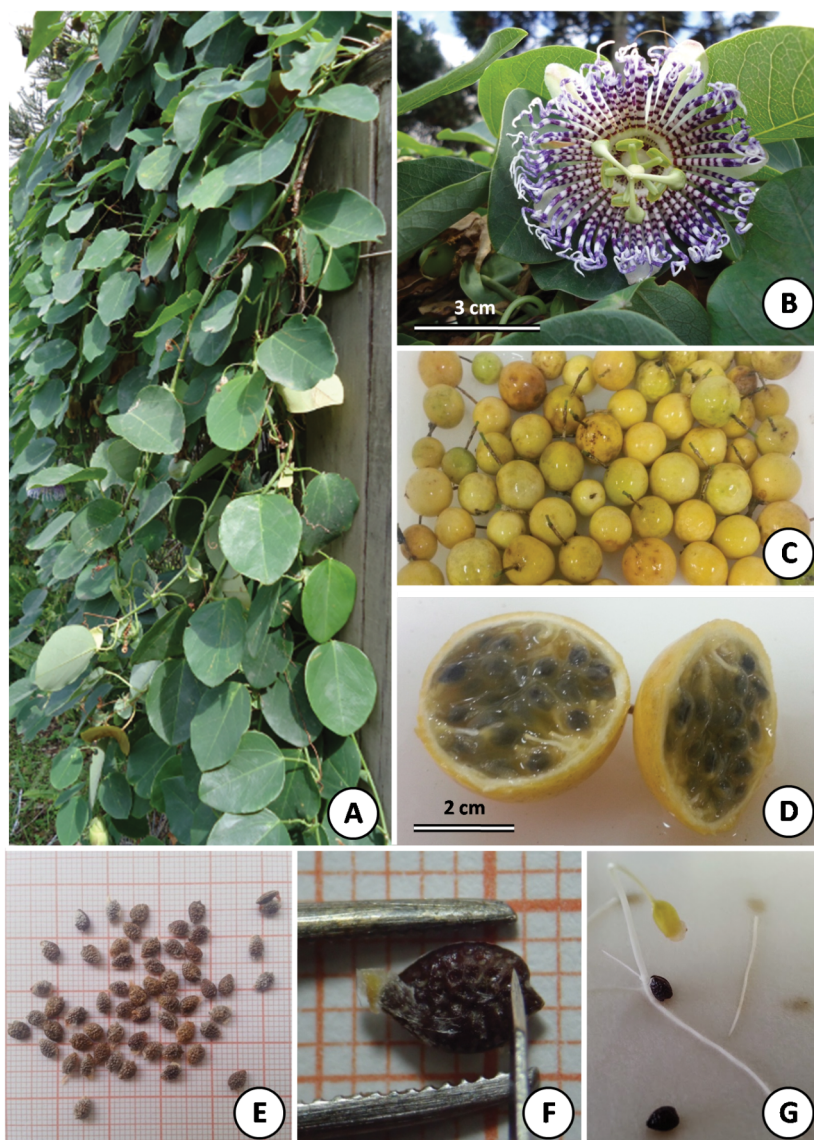


Figure 1. Details of *P. actinia* – Leaves (A); flower (B); ripe fruits at the point of harvest (C and D); processed seeds (E); cross-cut made in some seeds at the region opposite to the embryonic axis, for inducing germination (F); normal seedling, as used in the germination tests (G).

with a GA₃ solution at the proportion of 2.5 times the dry substrate weight. In both methods, the GA₃ concentrations of zero (water), 100, 200, 300, 400 and 500 mg.L⁻¹ were tested.

After the germination-inducing treatments had been applied, the yellow passion fruit seeds were placed over blotting-paper sheets, inside plastic boxes (11.0 x 11.0 x 3.5 cm). Next, they were kept at alternating temperatures of 20-30 °C, without light. Emerged normal seedlings (Figure 1G) were periodically counted, until germination become stable. Together with the germination test, the germination speed index (GSI) was withal calculated, according to the guidelines of Maguire (1962).

The storage potential of the seeds was evaluated by stowing them in hermetically sealed polyethylene packages, which were maintained under two conditions: refrigeration (at 7.3-8.5 °C and 49-52% RH) or laboratory ambient (at 19.6-23.1 °C and 53-62% RH). Seed physiological quality was gauged after zero, three, and nine months of storage, via germination and vigor (germination speed index) tests, carried out over paper, at 20-30 °C, in the absence of light.

The evaluation of both physiological and physical dormancy complied with a completely randomized design, with four replications. The data gathered were subjected to analysis of variance, and the means were compared by the Scott-Knott's test ($p \leq 0.05$). When significant differences were detected, a regression model with the best trend fit to the GA₃ concentration curve (physiological dormancy) was applied. In turn, the germination study followed a completely randomized design with a factorial scheme (temperature x light regime), whereas the storage potential was tackled in subdivided parcels, with four replications, having the means been contrasted by the Tukey's test ($p \leq 0.05$).

Results and Discussion

The germination test conducted without any promoting treatment revealed that the combination of the alternating temperature of 20-30 °C and absence of light accounted for the highest percentage of normal seedling emergence (Table 1), which reached 60%. A similar result was found for the germination speed index (Table 1), confirming that this treatment promoted the best seed performance.

In studies with other *Passiflora* species (namely, *P. alata* Dryander, *P. cincinnata* Mast., *P. edulis*, and *P. ligularis*), the alternating temperature of 20-30 °C resulted in a high germination percentage (Osipi and Nakagawa, 2005; Zucareli et al., 2009; Cadorin et al., 2017; Torres-G, 2018). This condition also led to a high germination speed index, in *P. incarnata* L. (Zucareli et al., 2015), and to an expressive seedling emergence, in various cultivars of *P. edulis* (Souto et

al., 2017). As pointed by Brancalion et al. (2010), a routine of alternating temperatures might help in breaking both physical and physiological seed dormancies.

It could also be verified that the lowest germination percentages were obtained at 30 °C (Table 1). Such outcome is possibly related to the fact that the species is native to subtropical regions, thus it does not endure hot temperatures – the alternating ones used in this research are actually closer to those of its natural habitat.

The assays with *P. actinia* additionally showed that, despite germinating under light, this species scored higher values of seedling emergence and speed index when kept in the dark. The absence of a lighting supply also triggered a better performance of other representatives of the genus, such as *P. incarnata* L. (Zucareli et al., 2015), *P. cincinnata* (Zucareli et al., 2009), and *P. edulis* (Ribeiro et al., 2017).

Table 2 contains the variables tested to assess physical dormancy of yellow passion fruit seeds. The control exhibited a 72% germination rate in the experiment carried out at 20-30 °C, without light supply. However, this number was significantly upgraded when the seeds were soaked in water at 40 °C or 50 °C, for either 5 or 10 minutes. In those conditions, normal seedling emergence achieved 81% and 88%, indicating the existence of dormancy. As for the germination speed index, the hot-water immersions also proved to be efficient, which corroborates previous works with *P. edulis* (Torres-G, 2018) and *P. cincinnata* (Oliveira-Júnior et al., 2010).

Table 1. Germination and germination speed index (GSI) of *P. actinia* seeds, at 20, 25, 30 and 20-30 °C, either with or without light.

Temperature (°C)	Without light	With light
	Germination (%)	
20	39 bA	0 bB
25	32 bA	20 aB
30	5 cA	3 bA
20–30	60 aA	17 aB
CV (%)	16.12	
	GSI	
20	0.091 dA	0.033 bcA
25	0.506 bA	0.186 aB
30	0.210 cA	0.000 cB
20–30	0.665 aA	0.141 abB
CV (%)	18.20	

Means followed by the same letter (lowercase, in the column, and uppercase, in the line) do not differ, according to the Tukey's test ($p \leq 0.05$).

It could be withal noticed that seed preparations that included a cross-cut at the region opposite to the embryonic axis and water immersion for 24 hours (and the combinations of these factors) negatively affected seedling emergence (Table 2). Cutting the seeds reduced both the percentage and the speed of germination in other *Passiflora* species as well, probably due to the enhanced sensitiveness to pathogen attack caused by the injury (Cárdenas et al., 2013). Soaking in water for 24 hours, in turn, might have caused damage by imbibition, which, in combination with the incision, led to the inferior results obtained.

Table 2. Results of the germination test and germination speed index (GSI) at 20-30 °C, either with or without light, after the treatments for breaking physical dormancy of *P. actinia*.

Treatments	Germination (%)	GSI
Control	72 b	0.67 b
Cross-cut at the region opposite to the Embryonic axis	45 d	0.63 b
Immersion in water for 24 h	60 c	0.65 b
Immersion in water for 24 h + cross-cut	40 d	0.56 b
Immersion in water at 40 °C for 5 min	87 a	0.76 a
Immersion in water at 40 °C for 10 min	81 a	0.81 a
Immersion in water at 50 °C for 5 min	81 a	0.70 a
Immersion in water at 50 °C for 10 min	88 a	0.76 a
CV (%)	9.17	13.07

Means followed by the same letter do not differ statistically, according to the Scott-Knott's test at a 5% probability level ($p \leq 0.05$).

When the physiological dormancy was looked into – first by using the plant growth regulator and next performing the germination test at 20-30 °C, in the absence of light (Figure 2) – it became clear that, in terms of seedling emergence (Figure 2A), moistening the substrate paper with a GA_3 solution generated better results than the immersion treatment. This outcome likely has to do with the more prolonged contact between seeds and plant regulator, made possible by the soaking substrate.

At 100 $mg.L^{-1}$ of GA_3 , 89% of normal seedlings emerged, a figure that is statistically higher than those found in the control (77%) and other treatments. It hence proves that yellow passion fruit seeds exhibit physiological dormancy. The results of GSI (Figure 2B) endorse that the concentration of 100 $mg.L^{-1}$ of GA_3 applied to the substrate paper speeded up the germinative process, thus being the most indicated in cases of physiological dormancy. Nevertheless, a germination decline might occur due to excessive doses of gibberellic acid (Taiz et al., 2017).

Dispersing gibberellin through seed immersion proved to be efficient for breaking the physiological dormancy in many other species of *Passiflora*, including *P. suberosa*, *P. morifolia*, *P. tenuifolia* (Marostega et al., 2017), *P. edulis* (Santos et al., 2013), and *P. cincinnata* Mast (Zucareli et al., 2009). On the other hand, the utilization of gibberellin in *P. caerulea* was ineffective, maybe because of the brief contact between seeds and solution – only 30 minutes (Hossel et al., 2018).

Figure 3 depicts the mean percentage of normal seedlings emerged per day, during the germination tests conducted at the alternating temperature of 20-30 °C, without light. The graph in Figure 3A shows that, in the case which no stimulating treatment was used, the germination stabilized 104 days past sowing, when the final count was then performed. Also, the first seedlings came out by the 20th day, and the peak occurred on the 43rd day. On the other hand, in the most promising treatments for breaking dormancy (Figure 3B), the study could be concluded within 59 days, with the number of normal seedling reaching the maximum after 20 and 29 days of planting. Those conditions proved to be capable of accelerating the whole process.

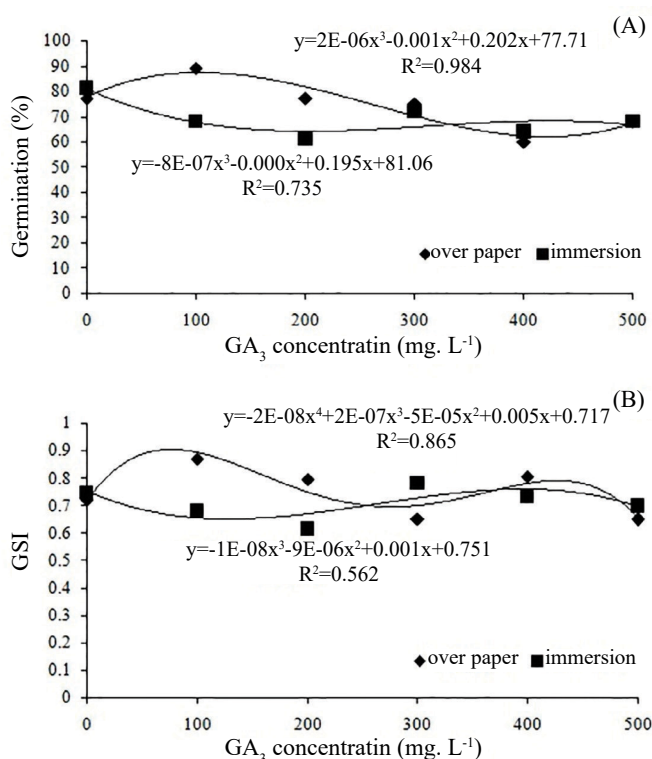


Figure 2. Germination (A) and germination speed index – GSI (B) of *P. actinia* seeds at 20-30 °C, either with or without light supply, after treatment with gibberellic acid (GA_3) for breaking the physiological dormancy. The acid was applied by immersion or via substrate paper.

Notably, the best treatment against physiological dormancy (100 mg.L⁻¹ of GA₃, applied over paper) lasted only 49 days (Figure 3C), with the germination peaking on the 26th day after the test setup. These data indicate that *P. actinia* seeds exhibit both physical and physiological dormancies, but the utilization of proper treatments can improve the germination percentage and the speed at which this phenomenon happens.

Regarding the storage data (Table 3), the chosen environment did not statistically alter the germination potential

prior to the third month, even though the germination speed index started to decline in the same period. Such behavior was presumably influenced by the laboratory surroundings, as the higher conditions of temperature and relative air humidity might have favored the deterioration of seeds and the subsequent decrease of vigor.

Refrigeration (7.3-8.5 °C, 49-52% RH) kept both viability and vigor of yellow passion fruit seeds for nine months. By the end of this time, the germinative power was still

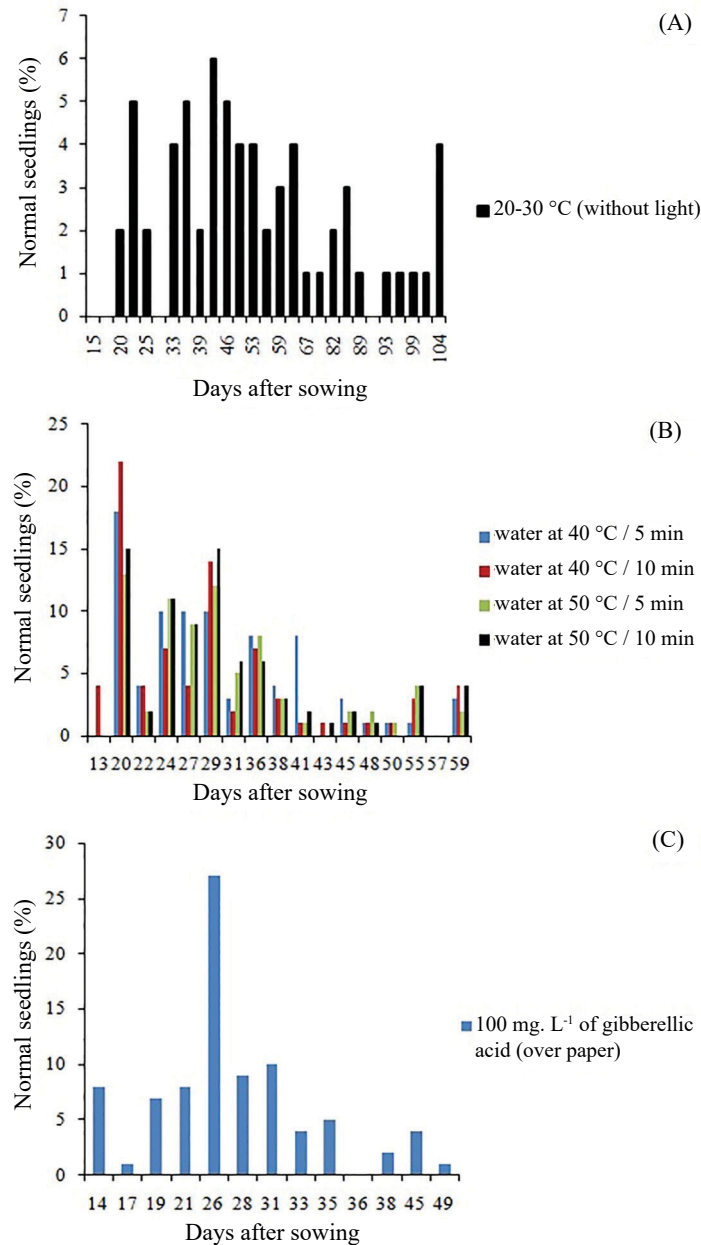


Figure 3. Mean percentages of normal seedlings emerged per day, in relation to the total amount of *P. actinia* seeds germinated at 20-30 °C, without light supply, in the control (A) and also in the best treatments for breaking physical (B) and physiological dormancies (C).

Table 3. Germination and vigor (appraised through the germination speed index – GSI) of *P. actinia* seeds, tested at 20-30 °C, without light supply, and stored in different environments, for different periods.

Period (months)	Storage conditions		CV (%)
	Laboratory ambient	Refrigeration	
	Germination (%)		
0	59 Aa	59 Aa	
3	60 Aa	53 Aa	23.1
9	24 Bb	60 Aa	
CV (%)	25.5		
	Vigor (GSI)		
0	0.3867 Aa	0.3867 Aa	
3	0.2100 Bb	0.4252 Aa	20.1
9	0.1187 Bc	0.4182 Aa	
CV (%)	21.7		

Means followed by the same letter (lowercase, in the column, and uppercase, in the line) do not differ, according to the Tukey's test ($p \leq 0.05$).

rounding 60%, which evidences that those are suitable storage conditions. This environment also proved its efficiency in preserving the viability of *P. edulis* seeds for up to ten months, regardless of the type of packaging used (Catunda et al., 2003). In other species, as *P. setacea* DC., for example, storing seeds in cold temperatures was also beneficial for maintaining their physiological quality (Pádua et al., 2011).

Conclusions

The germination test of yellow passion fruit seeds should be conducted over paper, at the alternating temperature of 20-30 °C, and without light.

Newly-harvested yellow passion fruit seeds exhibit both physical and physiological dormancies.

Treatments with immersion in water at 40 °C or 50 °C, for either 5 or 10 minutes, are indicated to break physical dormancy. Physiological dormancy, in turn, can be interrupted by applying 100 mg.L⁻¹ of gibberellic acid over the substrate paper.

Seeds stored inside hermetically sealed polyethylene packages, kept under refrigeration, retain their physiological quality for up to nine months.

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