http://journal.unoeste.br/index.php/ca/index
DOI: 10.5747/ca.2020.v16.n4.a387
ISSN on-line 1809-8215

Colloquium Agrariae

Submetido: 17/04/2020 Revisado: 25/05/2020 Aceito:27/05/2020

Pre-germinative treatments and seed storage of *Psidium cattleyanum* Sabine morphotypes

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Abstract
This study aimed to evaluate the germination behavior of two morphotypes of *P. cattleyanum* submitted to storage and pre-germinative treatments. The experiment was designed in a factorial scheme 2 x 3 x 3 (morphotypes x storage periods x pre-germinative treatments) completely randomized, using for each treatment 5 replicates with 30 seeds. The seeds were stored in semipermeable packages in a cold chamber at 5 ± 2 °C, for 14, 45, and 90 days. After each storage period, the seeds were submitted to treatments to overcome dormancy: immersion in water at 80 °C, kept in imbibition for 24 and 48 h and control, without any treatment. The seeds were arranged under moistened substrate in transparent plastic boxes and allocated in a germination chamber at 25 °C and photoperiod of 8 h for evaluation of germination percentage (G), germination speed index (GSI) and mean germination time (MGT). The red morphotype seeds showed higher germination percentages than the yellow morphotype and did not have a reduction in these values after 90 days of storage. MGT for yellow morphotype was lower in all pre-germinative treatments. The GSI had better results at 45 days in the control treatments and 24 h in immersion in water and 90 days for the three pre-germinative treatments. The red morphotype does not require pre-germinative treatments, while the yellow morphotype presents better germination when the seeds are stored for 45 days and immersed in water at 80 °C for 48 h.
Keywords: Germination potential; red araçá; tegumentar dormancy; yellow araçá.

Tratamentos pré-germinativos e armazenamento de sementes de morfotipos de *Psidium cattleyanum* Sabine

Resumo
Objetivou-se avaliar o comportamento germinativo de dois morfotipos de *P. cattleyanum* submetidos a acondicionamento e tratamentos pré-germinativos. O experimento foi delineado em esquema fatorial 2 x 3 x 3 (morfotipos x períodos de armazenamento x tratamentos de superação de dormência) inteiramente casualizado, utilizando-se para cada tratamento 5 repetições com 30 sementes. As sementes foram armazenadas em embalagens semipermeáveis em câmara fria a 5 ± 2 °C, durante 14, 45 e 90 dias. Posteriormente a cada período de armazenamento as sementes foram submetidas a tratamentos para superação de dormência: imersão em água a 80 °C mantidas em embebição por 24 e 48 horas e testemunha, sem tratamento. As sementes foram dispostas sob substrato umedecido em caixas plásticas transparentes e alocadas em câmara de germinação a 25 °C e fotoperíodo de 8 h para avaliação da porcentagem de germinação (G), índice de velocidade de germinação (IVG) e tempo médio de germinação (TMG). As sementes do morfotipo vermelho apresentaram maior porcentagem de germinação em relação ao morfotipo amarelo e não tiveram redução nesses valores após 90 dias de armazenamento. O TMG para o morfotipo amarelo foi menor em todos os tratamentos pré-germinativos. O IVG teve melhores resultados aos 45 dias, nos tratamentos controle e 24h em imersão em água, e aos 90 dias, para os três tratamentos pré-germinativos. O morfotipo vermelho não necessita de tratamentos pré-germinativos, enquanto o morfotipo amarelo apresenta melhor germinação quando as sementes são armazenadas por 45 dias e imersas em água a 80 °C por 48 h.
Palavras-chave: araçá amarelo; araçá vermelho; dormência tegumentar; potencial germinativo.

Introduction

Popularly known as araçá, *Psidium cattleyanum* Sabine is a species native to Brazil (PATEL, 2012; REFLORA, 2020), and can also be found in Uruguay (FRANZON *et al.*, 2009). There are two morphotypes of this species, differentiated by the color of the fruit in yellow (Ya-cy) or red (Irapuã) (ROCHA *et al.*, 2020; PEREIRA *et al.*, 2018). The species has a fleshy fruit, widely consumed by the avifauna (SILVA *et al.*, 2011), and is recommended for areas in the process of restoration. In addition to their ecological importance, araçá fruits have the potential for commercialization, to supply the demand for their consumption *in natura* or in the form of agro-industry derivatives, such as jams (SANTOS *et al.*, 2007). The fruits have a high concentration of phenolic compounds and secondary metabolites that act as antioxidants, also enabling their use in the pharmaceutical industry (PATEL, 2012; PEREIRA *et al.*, 2018). Given its ecological and economic importance, the species has great potential for economic exploitation in small farms, being incorporated into preservation or agroforestry areas (HOSSEL *et al.*, 2017).

Plants develop several strategies, according to their natural environment, so that germination occurs satisfactorily, resulting in radicle emission and development of normal seedlings (SHU *et al.*, 2016; NONOGAKI, 2019). The fleshy fruit indicates that the dispersion of the species is barochoric and zoochoric. These types of dispersion increase the seed bank and, consequently, promote the formation of the seedling bank (OLIVEIRA, 2012). However, evaluating the seed bank of *P. cattleyanum*, Uowolo and Denslow (2008) stated that the species does not form a persistent seed bank, losing viability after 6.5 months.

Some species require pre-germinative treatments to reduce time and increase homogeneity in germination. Kumar (2016) indicates the use of pre-germinative treatments to increase the results of this species germination, such as immersion in water or sulfuric acid, at different times and concentrations, or mechanical scarification. It is known that araçá seeds have dormancy; however, there is no consensus on the best method for overcoming it, being suggested cold stratification for yellow araçá (TREVISAN *et al.*, 2004) and the need for light presence for the red araçá (SANTOS *et al.*, 2004).

Given the economic potential of the species and its ecological importance, studies are needed on conservation, adequate storage (SILVA *et al.*, 2011), and overcoming dormancy, thus enabling their availability and use throughout the year. Therefore, this study aimed to evaluate the germination behavior of two morphotypes of *P. cattleyanum* submitted to different storage times and pre-germinative treatments.

Material and Methods

The seeds of *P. cattleyanum* were donated by the Chauá Society, located in Campo Largo/PR. They collected the seeds from ten parents trees in the second half of February 2018. According to Köppen (ALVARES *et al.*, 2013), the climate of the region is Cfb, a temperate climate with uniformly distributed rains, mild summer, and severe and frequent frosts. The storage period and evaluations were carried out at the Laboratório de Sementes Florestais of UFPR, Campus Botânico, Curitiba/PR, in February 2018.

The seeds were processed in the Forest Seed Laboratory. For this, we manually macerated the fruits in a fine-mesh sieve with running water to expel the seeds. After the washing process, the seeds were arranged to dry outdoors for 24 h and then packed in semipermeable packaging in the cold chamber.

Initially, we determined the weight of 1,000 seeds, the number of seeds per kilo, and seed moisture content, following the methodologies described in the Rules for Seed Analysis - RAS (BRASIL, 2009). We evaluated the seed moisture content following the drying method in an oven at 105 °C for 24h. For the determination of biometrics characteristics, we measured the length, width, and thickness of 50 seeds of each morphotype with a digital caliper with an accuracy of 0.05 mm. We considered the length as the extent of the hilum up to the base of the seed, in the longitudinal direction; the width in the transverse direction of the seed, in the area of smaller seed diameter; and the thickness also in the transverse direction, in the area with the largest diameter of the seed.

The seeds were submitted to three storage times (14, 45, and 90 days) in semipermeable packages kept in a cold chamber at 5 ± 2 °C. After each storage period, the seeds were submitted to three treatments to overcome

dormancy. The seeds were placed in containers with water initially at 80 °C, without the maintenance of this temperature, for 24 and 48 h. The seeds of the control treatment did not undergo pre-germination treatments.

For germination evaluation, after pre-germinative treatments, the seeds were placed to germinate in transparent acrylic boxes (gerbox®), using germitest® paper towels as substrate. The boxes with seeds were kept in BOD germination chambers (Biochemical Oxygen Demand), at 25 °C and photoperiod of 8 h. As response variables, we calculated germination percentage (G), germination speed index (GSI) (MAGUIRE, 1962), and mean germination time (MGT) (LABOURIAU; AGUDO, 1987).

The experiment was designed in a factorial scheme 2 x 3 x 3 (morphotypes x storage periods x dormancy overcoming treatments) completely randomized, using for each treatment 5 replicates with 30 seeds. The data were submitted to analysis of Bartlett's homogeneity and Shapiro-Wilk's normality. If normality and homogeneity of the data were confirmed, they were submitted to variance analysis (ANOVA), followed by the Tukey test ($P < 0.05$) (TUKEY, 1977). The analyses were performed with the aid of the Rbio software (BHERING, 2017).

Results and Discussion

The seed lot used had 12.99 g/thousand seeds, accounting for 76,982 seeds/kg. The initial seed moisture content was 13.95 % for the red araçá and 12.55 % for the yellow araçá. After 45 and 90 days of cold chamber storage, the humidity of the red and yellow araçá were 13.34 and 12.01 % for 45 days and 15.46 and 14.03 % for 90 days, respectively. Both values are higher than those observed by Silva *et al.* (2011) for seeds of the same species (7.1 %) at the time of packaging, indicating that the species can tolerate lower levels of desiccation. The same authors also observed a subtle increase in the moisture content of the seeds when stored in semipermeable packages in a cold chamber, due to the exchange of water between the seeds and the environment (MASETTO *et al.*, 2014). The maintenance of the adequate degree of moisture during seed storage is important for its viability (CARVALHO *et al.*, 2006), this characteristic is intrinsic to each species, and its knowledge is determinant for the establishment of sexual propagation protocols at the commercial level.

The red araçá seeds have a length of 4.10 mm (± 0.45 mm), a width of 3.21 mm (± 0.36 mm) and thickness of 1.93 mm (± 0.30 mm). The yellow morphotype has a length of 3.67 mm (± 0.36 mm), a width of 2.81 mm (± 0.30 mm), and a thickness of 2.03 mm (± 0.27 mm). These values indicate that the seeds of *P. cattleyanum* are smaller than the seeds of other species of the same genus, such as *P. sobralianum* (length of 5.0 - 7.0 mm) (FREITAS *et al.*, 2018) and *P. guajava* (length of 2.70 - 5.25 mm) (MISHRA *et al.*, 2018). The size and shape of seeds are related to their dispersal strategy and are determinant for the establishment of seed or seedling banks. This information is relevant for species differentiation, as well as for understanding dispersal strategies and physiological mechanisms (SILVA; MÔRO, 2008).

All factors influenced the germination percentage of the seeds, but there was no interaction between the three factors simultaneously (Table 1). There was a significant interaction between the three factors analyzed; However, only for the GSI, there were no significant interactions between morphotype x dormancy and morphotype x storage.

Table 1. Summary for the variance analysis for germination (G), mean germination time (MGT), and germination speed index (GSI) of *Psidium cattleyanum* seeds.

	Medium Square (MS)			
	GL	G	MGT	GSI
Morphotypes	1	2740.86**	1366.53**	4.58**
Dormancy	2	591.11**	114,04**	0.54**
Storage	2	1033.70**	55.28**	0.10**
Morphotypes x Dormancy	2	640.49**	8.15**	0.01^{ns}
Morphotypes x Storage	2	674.19**	64.94**	0.05^{ns}
Dormancy x Storage	4	158.70^{ns}	20.20**	0.07**
Morphotypes x Dormancy x Storage	4	181.04^{ns}	1.68^{ns}	0.04*
Residues	72	85.30	1.23	0.01
Total	89			
Coefficient of variation (%)		12.57	4.47	14.49

* significant at $p < 0.05$; ** significant at $p < 0.01$; ^{ns} non significant.

The red araçá presents a higher percentage of germination than the yellow araçá, and it requires 48 h immersed in water to equal the values observed for the other morphotype. Regarding storage time, we observed higher sensitivity of yellow araçá seeds, which showed a decrease in germination percentage when maintained for 90 days in a cold chamber (Table 2).

Table 2. Germination percentage of *Psidium cattleyanum* seeds, red and yellow morphotypes, submitted to different storage times and dormancy overcoming treatments.

Germination (%)				
Dormancy overcoming (h)	Morphotypes			
	Red		Yellow	
0	79.33	aA	57.78	bB
24	78.22	aA	70.89	bA
48	79.33	aA	75.11	aA

Storage times (days)	Morphotypes			
	Red		Yellow	
14	82.89	aA	70.89	bA
45	77.33	aA	76.22	aA
90	76.67	aA	56.67	bB

Means followed by the same lowercase letters in rows and uppercase letters in columns do not differ by the Tukey test ($p < 0.05$).

In this study we observed that yellow-araçá seeds require a longer time submerged in water to achieve the same germination values as the other morphotype. This is due to the thicker integument, which promotes physical dormancy and prevents the entry of water (TUAN *et al.*, 2019), which hinders the soaking process for the resumption of embryonic growth. However, different values were observed by Tomaz *et al.* (2011), in which yellow-araçá seeds presented better germination performance than those of red-araçá. On the other hand, the results obtained for the red araçá are consistent with that observed by Santos *et al.* (2004), in which seeds of the red morphotype reached 82% germination at 90 days after sowing.

The increase in seed storage time reduced the germination percentage, although it was significant only for the yellow morphotype. This behavior may be related to environmental conditions at the place where they were collected since environmental variations can influence the

viability of seeds (SANTOS *et al.*, 2015). Basic studies on the limit moisture levels supported by the species is an important tool to adapt the best drying and storage method of seeds, to maintain their viability (CARVALHO *et al.*, 2006).

Tomaz *et al.* (2011) obtained 90 % germination in *P. cattleyanum* seeds stored in a refrigerator for 48 h and immersed in water at 80 °C for 25 seconds. Silva *et al.* (2011), on the other hand, obtained initial germination percentage below 75 % and significant decrease over time when evaluating seeds of the same species stored under different conditions for up to 1,107 days. As in the present study, for the red morphotype, Dresch *et al.* (2014) affirmed the application of pre-germinative treatments in seeds of *P. guinean* does not influence the germination of the species. The association of genetic characteristics and biochemical events is related to the vital processes of the seed, determining the maintenance or not of its viability (MARCOS FILHO, 2005; CARVALHO *et al.*, 2006; TUAN *et al.*, 2019). It is important to note that all seeds, regardless of storage method and period, are subject to reduced viability, which can be overcome by keeping seeds stored properly (CARVALHO *et al.*, 2006; MASETTO *et al.*, 2014).

The mean germination time (MGT) was higher for yellow-araçá seeds regardless of treatment to overcome dormancy or storage time (Table 3). For both morphotypes of araçá, the MGT reduced as the seeds were submitted to increasing time of pre-germinative treatment and, for the yellow-araçá, the MGT also decreased with the increase of the seed storage time.

Table 3. Mean germination time of *Psidium catleyanum*, red and yellow morphotypes, submitted to different storage times and dormancy overcoming treatments.

Mean germination time				
Dormancy overcoming (h)	Morphotype			
	Red		Yellow	
0	23.40	bA	30.79	aA
24	20.39	bB	27.41	aB
48	19.07	bC	28.05	aB

Storage times (days)	Morphotype			
	Red		Yellow	
14	21.10	bA	31.32	aA
45	20.55	bA	29.18	aB
90	21.23	bA	25.75	aC

Storage times (days)	Dormancy overcome		
	0	24	48
14	29.14 aA	27.88 aB	24.26 cA
45	24.39 aB	22.99 bB	24.30 aA
90	25.07 aB	23.72 bB	21.90 cB

Means followed by equal lowercase letters in rows and uppercase letters in columns do not differ by Tukey test ($p < 0.05$).

The MGTs observed in this study are lower than those observed by Gentil *et al.* (2018) for *P. friedrichsthalianum* seeds and those observed by Santos *et al.* (2004) for *P. cattleyanum* seeds submitted to different temperatures. The germination of the species is slow probably due to the mechanical barrier of the tegument, as observed by Freitas *et al.* (2018) for *P. sobralianum*, which may have been altered with the increase of imbibition time. According to Masetto *et al.* (2014), the soaking of *P. guinean* seeds is effective for increasing germination, with a TMG higher than that found in the present study. The homogeneous germination, with the reduction of the average germination time, is another indication of the need to use pre-germination treatments to obtain better results in germination, positively influencing the production of seedlings of the species.

The germination speed index (GSI) shows that the pre-germination treatments were more efficient at 45 and 90 days of storage (Table 4). For this variable, there was no influence of morphotypes.

Table 4. Germination speed index of *Psidium catleyanum* seeds, red and yellow morphotypes, submitted to different storage times, and dormancy overcoming treatments.

Germination speed index						
Dormancy overcoming (h)	Storage time (days)					
	14		45		90	
0	0.80	bA	1.09	aA	1.07	aA
24	0.83	bA	1.08	aA	1.12	aA
48	0.82	bA	0.82	bB	1.05	aA

Means followed by equal lowercase letters in rows and uppercase letters in columns do not differ by Tukey test ($p < 0.05$).

The GSI observed in this study are within the range obtained by Hossel *et al.* (2017) for *P. cattleyanum* seeds kept at different temperatures and are higher than that observed by Silva *et al.* (2011) for seeds of the same species stored in different ways. Porto *et al.* (2018), state that yellow araçá seeds maintain their viability for up to 330 days in Eppendorf® or TetraPak® packages, but the authors did not report the moisture content of the seeds. The GSI values are important because they express the germination viability of the seeds submitted to different treatments, even if there are no differences between germination percentages (RANAL; SANTANA, 2006).

The results obtained in this study indicate the differences between the red and yellow morphotypes of *P. cattleyanum*. We demonstrated that each morphotype has intrinsic physiological differences that can influence the storage, germination, and even seedling formation. This information is important to support projects involving the use of seedlings of the species, ensuring greater efficiency in the storage and proper germination of seeds.

Conclusion

The red morphotype of araçá does not require pre-germination treatment, as it presents high germination potential even when stored for 90 days. The yellow morphotype of araçá has higher germination when immersed in water at 80 °C for 48 h. Immersion in water for 48 h reduces the average germination time of the two morphotypes.

Acknowledgments

Non-governmental organization - Chauá Society for seeds donation.

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