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Germination of *Psidium friedrichsthalianum* (O. Berg) Nied. seeds under different temperature and storage conditions[1]

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ABSTRACT – *Psidium friedrichsthalianum* is a species whose fruit can be used to make juices, jellies/jams and sweets, and its seedlings serve as rootstocks with resistance to *Meloidogyne* spp. The aim of this study was to evaluate the performance of seeds of this species at different germination temperatures, and to verify the effects of different moisture levels on their storage in two experiments. In the first, six germination temperatures (15, 20, 25, 30, 35, and 40 ºC) were evaluated, and in the second, seeds with different moisture levels (15.4, 9.8, 9.0, 8.4, and 8.2%) were stored in sealed containers at 20 °C for 0, 1, 3, 6, 9, and 12 months. Seed germination was favored by temperatures of 20 ºC and 25 ºC, reaching 93% and 87%, respectively, along with the highest germination speed indexes (2.582% day^{1} and 2.568% day^{-1}) and shortest germination times (37.9 and 36.9 days). Temperatures of 30 °C and 35 °C maintained the seeds quiescent, while 40 °C was lethal. In storage, the seeds tolerated desiccation to 8.2% moisture content and could be stored in sealed containers at 20 ºC for 12 months, with germination higher than 70%.

Index terms: quiescence, desiccation tolerance, Myrtaceae.

Germinação de sementes de *Psidium friedrichsthalianum* (O. Berg) Nied. em função da temperatura e do armazenamento

RESUMO - *Psidium friedrichsthalianum* é uma espécie cujos frutos podem ser usados na preparação de sucos, geleias e doces, além das mudas servirem de porta-enxertos pela resistência a *Meloidogyne* spp. O objetivo deste trabalho foi avaliar o desempenho de sementes em diferentes temperaturas de germinação, bem como verificar os efeitos de diferentes graus de umidade no armazenamento de sementes dessa espécie, em dois ensaios. No primeiro, foram avaliadas seis temperaturas (15, 20, 25, 30, 35 e 40 ºC) e, no segundo, sementes com diferentes graus de umidade (15,4; 9,8; 9,0; 8,4 e 8,2%) foram armazenadas em recipientes herméticos a 20 ºC, durante 0, 1, 3, 6, 9 e 12 meses. A germinação de sementes foi favorecida pelas temperaturas de 20 e 25 ºC, alcançando 93 e 87%, onde também foram observados os mais altos índices de velocidade de germinação (2,582 e 2,568% dia^{-1}) e menores tempos de germinação (37,9 e 36,9 dias, respectivamente). As temperaturas de 30 e 35 ºC mantiveram as sementes quiescentes, enquanto a de 40 °C foi letal. No armazenamento, foi verificado que as sementes toleraram dessecação até 8,2% de água, podendo ser armazenadas em recipientes herméticos a 20 ºC, por 12 meses, com germinação superior a 70%.

Palavras-chave: quiescência, tolerância à dessecação, Myrtaceae.

Introduction

Psidium friedrichsthalianum – Myrtaceae, known as “cas”, in Costa Rica, or “Costa Rican guava”, is a fruit-bearing plant, originating from higher altitude forests and savannas from Honduras to Panama (Fouqué, 1972), which develops naturally from the south of Mexico to the north of South America (Lorenzi et al., 2006). The fruit has a rounded shape, slightly flattened at the poles, with a longitudinal diameter of 35.68 mm, transversal diameter of 42.90 mm, dry matter of 42.19 g, and pulp yield of 94% (Rebouças et al., 2008). It can be used to make juices, jellies/jams, and sweets (Lorenzi et al., 2006). The high number of seeds per fruit (on average, 73) represents a characteristic that facilitates seedling production

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for the species (Rebouças et al., 2008), including seedlings to be used as rootstocks for *Psidium guajava* L., providing resistance to *Meloidogyne enterolobii* Yang and Eisenback (Carneiro et al., 2007; Freitas et al., 2014), *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood (Aristizábal and Piedrahíta, 2013).

In seed management, the speed of seed deterioration must be minimized up to sowing time. Physiological quality of seeds declines at variable intensity after physiological maturity, depending on conditions to which they are exposed. A preponderant factor in conservation of seed viability and vigor is moisture content, which implies the need for drying for success in storage (Santos, 2015).

Seed tolerance to desiccation varies among species. Thus, Roberts (1973) classified seeds as orthodox and recalcitrant. Ellis et al. (1990) included the intermediate category in this classification, based on the response of *Coffea arabica* L seeds. It is understood that orthodox seeds tolerate desiccation to moisture content near 5%; intermediate seeds tolerate desiccation to moisture contents of around 10-12.5% and have reduced viability at lower moisture contents; and recalcitrant seeds lose viability when dried to 15-20% moisture (Hong and Ellis, 1996). A simple way of determining the nature of seeds in the laboratory consists of the germination test after desiccation to less than 10% moisture content: when the seeds germinate, they are considered tolerant to desiccation (orthodox) and when they do not germinate, they are probably recalcitrant (Silva and Ferraz, 2015). Among the native and exotic fruit-bearing Myrtaceae grown in Brazil whose seeds have been classified as recalcitrant are the following: *Eugenia brasiliensis* Lam. (Kohama et al., 2006; Delgado and Barbedo, 2007), *E. involucrata* DC., *E. uniflora* L. (Delgado and Barbedo, 2007), and *E. pyriformis* Camb. (Delgado and Barbedo, 2007; Scalon et al., 2012). Those with intermediate behavior include *Acca sellowiana* (O. Berg) Burret (Gomes et al., 2013). Those classified as orthodox seeds are *Campomanesia phaea* (Berg) Landr. (Maluf and Pisciottano-Ereio, 2005), *Psidium guajava* L. (Hong et al., 1996), *P. cattleianum* Afzel. ex Sabine (Silva et al., 2011), and *P. guineensis* Swartz (Masetto et al., 2014). Therefore, knowing the nature of seeds is essential for establishing storage conditions and, consequently, seed longevity (Silva and Ferraz, 2015).

In seed germination, temperature is an important environmental factor, which affects the germination percentage and germination rate of non-dormant seeds, the ability to overcome primary and/or secondary dormancy, and the induction of secondary dormancy of seeds (Bewley et al., 2013). Thus, germination occurs within certain temperature limits that are characteristic of each species; above upper limits and below lower limits, germination does not occur. Within these limits, there is a temperature or range of temperatures in which the process occurs most efficiently, i.e., when maximum germination is obtained in the shortest period of time (Carvalho and Nakagawa, 2012). Nevertheless, according to Lamarca et al. (2011), there may be variations even within the same species, probably due to the oscillation of environmental factors during seed development and maturation.

In evaluation of seed quality, the germination test is carried out under partially or totally controlled laboratory conditions for the purpose of determining the maximum germination potential of seed lots (Brasil, 2009). For that reason, environmental factors have been studied, such as temperature (Lamarca et al., 2011; Alves et al., 2015; Santos et al., 2015; Gomes et al., 2016), to bring about more regular, fast, and complete germination of samples of seeds from a determined species (Brasil, 2009). Furthermore, the temperature or range of temperatures most suitable for germination is likewise useful for obtaining uniform stands in commercial nurseries for production of plant seedlings.

Thus, knowledge regarding the physiological characteristics of seeds from fruit-bearing species that involves germination and storage is indispensable for generating technologies suitable for propagation and conservation of these genetic resources. In this context, the aim of this study was to evaluate the germination of seeds under different temperatures, as well as observe the effects of different moisture contents on storage of *P. friedrichsthalianum* seeds.

Material and Methods

The study was conducted in the seed laboratory and germination nursery of the Biodiversity Coordination of the National Amazon Research Institute (Instituto Nacional de Pesquisas da Amazônia - INPA), in Manaus, Amazonas, Brazil. Ripe fruit (epicarp of greenish-yellow color), originating from twelve plants on the INPA–V8 campus (3º5’32.64”S; 59º59’36.10”O), was collected after abscission.

The seeds were manually extracted from the fruit with the aid of a spoon. After remaining immersed in water for 24 hours, they were rubbed in a sieve and washed in running water. They were then immersed in 0.5% sodium hypochlorite for 15 minutes and washed in running water. After that, two independent trials were set up as described below.

Seed germination at different temperatures

The seeds were sown in *gerbox* type plastic boxes (11 x 11 x 3.5 cm) on two sheets of blotting paper moistened with 15 mL of water (equivalent to three times the weight of the non-hydrated substrate). The boxes were placed in transparent plastic bags to reduce loss of moisture and kept in germination chambers with a 12-hour photoperiod regulated at the following temperatures: 15, 20, 25, 30, 35, and 40 ºC.

Germination was evaluated every five days, considering the seed with primary root growth as germinated. From this data, germination percentage, germination speed index (GSI), and mean germination time (MGT) were calculated (Ranal and Santana, 2006). For statistical analysis, the germination percentages were transformed in arcsin $\sqrt{(x+0.5)/100}$, and the means of the original values were presented in the results. After concluding the germination test (90 days), the seeds of the treatments in which germination did not occur (30, 35, and 40 ºC) were transferred to the temperature of 20 ºC, which exhibited the best response, and they were evaluated for 50 days more.

A completely randomized experimental design was used, with six treatments (temperatures) and three replications, containing 25 seeds per plot. Data were subjected to analysis of variance and mean values were compared by the Tukey test at the level of 5% probability. Analyses were made in the Assistat 7.7 beta program (Silva and Azevedo, 2016).

Storage of seeds with different moisture contents

After processing, the seeds were exposed to environmental conditions (relative humidity of 60%, mean minimum temperature of 29 ºC, and mean maximum temperature of 32 ºC) for different periods (1, 3, 5, 7, and 9 days), which resulted in different moisture contents (15.4, 9.8, 9.0, 8.4, and 8.2%, respectively). Reaching each one of the moisture contents, the seeds were placed in six amber glass bottles, sealed with a stopper and plastic screw cap, and then stored in a chamber at a temperature of 20 ºC for 0, 1, 3, 6, 9, and 12 months. After each one of these periods, one bottle of seeds at each moisture content was removed from storage, and the seeds were sown in plastic boxes containing sand and sawdust substrate (1:1; v:v) and kept in a nursery (mean minimum temperature of 24 ºC and mean maximum temperature of 38 ºC).

Emergence evaluations were made every five days, and seedlings that exhibited hypocotyl growth above the level of the substrate and expanded cotyledonary leaves were considered as emerged. From the emergence data, the emergence speed index and the mean emergence time were calculated (Ranal and Santana, 2006). For statistical analysis, the emergence percentages were transformed into arcsin $\sqrt{(x+0.5)/100}$, and the means of the original values were presented in the results.

A completely randomized experimental design was adopted in split-plots in time, with four replications, each one containing 25 seeds. Five different seed moisture contents were considered in the plots, while six storage periods were considered in the split-plots. Data were subjected to analysis of variance and means were compared by the Tukey test at the level of 5% probability. Analyses were made in the Assistat 7.7 beta program (Silva and Azevedo, 2016).

Results and Discussion

Seed germination at different temperatures

Germination of *P. friedrichsthalianum* seeds was greater than 80% at the temperatures of 15, 20, and 25 ºC (Table 1). Santos et al. (2004), in a study with other Myrtaceae, found germination greater than 75% in *A. sellowiana*, *Campomanesia xanthocarpa* (Mart.) O. Berg, and *P. cattleianum* at constant temperatures of 15, 20, 25, and 30 °C. In *P. guajava*, germination exceeded 90% at temperatures of 20, 25, and 30 °C (Sugahara and Takaki, 2004).

The *P. friedrichsthalianum* seeds under the higher temperatures of 30, 35, and 40 ºC (Table 1) did not germinate in the period of 90 days (Figure 1). After transfer of the plots of these treatments to the temperature of 20 ºC, the seeds of the 30 and 35 ºC treatments achieved values near 70% germination, indicating that they were in a quiescent state (Carvalho and Nakagawa, 2012). Maeda et al. (1999) observed this phenomenon in germination of *P. guajava* under a temperature of 35 °C.

The *P. friedrichsthalianum* seeds of the 40 ºC treatment did not exhibit a germination response after transfer to the temperature of 20 ºC (Figure 1), showing that the temperature of 40 ºC is above the maximum germination temperature of seeds of this species. In *P. guajava*, Sugahara and Takaki (2004)

Table 1. Germination, germination speed index (GSI), and mean germination time (MGT) of *Psidium friedrichsthalianum* (O. Berg) Nied.– Myrtaceae seeds, under different germination temperatures.

Temperature (ºC)	Germination (%)	GSI (% day^{-1})	MGT (days)
15	84 a	1.617 b	54.1 b
20	93 a	2.582 a	37.9 a
25	87 a	2.568 a	36.9 a
30	0 b	-	-
35	0 b	-	-
40	0 b	-	-
F Test	73.07**	22.33**	87.33**
CV (%)	19.86	8.99	4.15

Mean values followed by the same letter in the column do not differ significantly by the Tukey test at the level of 5% probability. ** significant by the F test at the level of 1% probability.

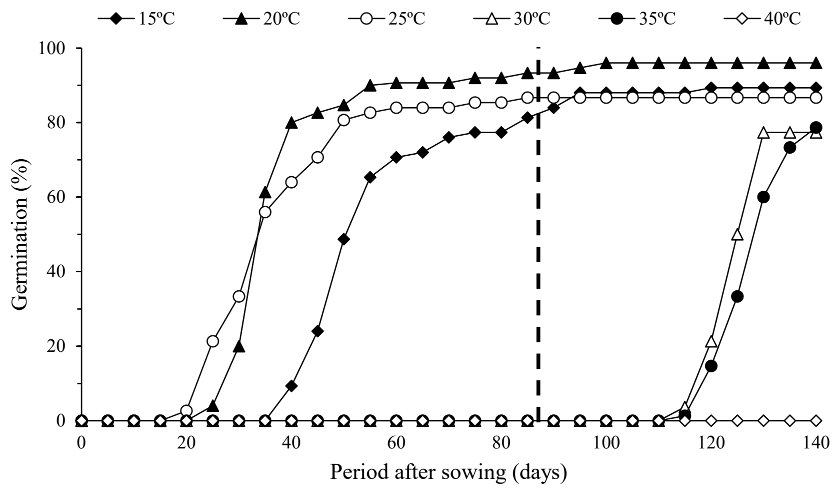

Figure 1. Accumulated germination of *Psidium friedrichsthalianum* (O. Berg) Nied.– Myrtaceae seeds, in relation to temperature; at ninety days after sowing, the seeds of the treatments in which there was no germination (30, 35, and 40 ºC) were transferred to the temperature of 20 ºC.

considered that the maximum seed germination temperature is situated between 35 and 40 ºC. According to Carvalho and Nakagawa (2012), exact determination of the cardinal temperatures of each species is impeded by a series of problems, among which are the seed vigor level and the adoption of an adequate methodology. In the case of *P. friedrichsthalianum*, it was not possible to establish the maximum temperature, but results indicated it to be between 25 and 30 ºC.

Germination began at 20, 25, and 40 days at the temperatures of 25, 20, and 15 ºC, respectively, with stabilization at 90 days (Figure 1). At the temperature of 15 ºC, the values of germination speed and mean germination time were significantly different than in the other treatments (Table 1) because decrease in temperature promoted reduction in germination speed and an increase in the mean time of the germination process, indicating it to be near the minimum temperature for germination of the species. For *A. sellowiana*, which occurs naturally at high altitudes, Gomes et al. (2016) expected that the temperature of 15 ºC would be a factor favorable to the germination process, which did not occur. In *P. guajava*, germination also did not occur under the temperature of 15 ºC, but exhibited a minimum temperature of germination between 15 and 20 ºC (Sugahara and Takaki, 2004).

The highest germination speed indexes (GSI), as well as the lowest mean germination times (MGT) were achieved under the temperatures of 20 and 25 ºC, which did not differ significantly from each other (Table 1). Thus, these temperatures can be suggested as suitable for germination of *P. friedrichsthalianum* seeds. In *P. guineense*, the temperatures of 20 and 25 °C were ideal for seed germination (Santos et al., 2015). In *P. guajava* (Maeda et al., 1999), *P. rufum* Mart. ex DC. (Soares et al., 2015), *A. sellowiana*, and *C. xanthocarpa* (Gomes et al., 2016), the temperature of 25 ºC allowed better germination performance of the seeds.

The temperatures that occur in the habitats of the species are important parameters for selection of the temperatures to be used in this type of experiment (Baskin and Baskin, 2014). Furthermore, a distinct germination response among populations of the same species, common among species of the same region, suggests that selection pressure of the environment is expressive over germination potential, adapting the requirements of germination to the thermal conditions of the place of origin (Lamarca et al., 2011). Thus, the fact of *P. friedrichsthalianum* originating in higher altitude forests and savannas of Central America (Fouqué, 1972) may be related to the germination response of seeds to the temperatures of 20 and 25 ºC.

The interaction between temperature and light merits attention in continuing research on germination of *P. friedrichsthalianum* seeds. In *P. guajava*, Sugahara and Takaki (2004) found that seeds can germinate in the dark at alternating temperatures and under lighting at constant temperatures, due to the participation of phytochromes (A and B, respectively) in control of germination. The amplitude between the highest and lowest temperatures can also affect the germination response of the seeds of this species (Bhanuprakash et al., 2008). At any rate, in the present study, the 12-hour photoperiod at constant temperatures seems not to have impaired the germination of *P. friedrichsthalianum* seeds.

Storage of seeds with different moisture contents

The variables observed showed a significant effect of interaction (moisture content x storage period). Therefore, it was decided to compare the means taking into account each one of the moisture contents in relation to the storage periods (Table 2).

At the beginning of storage, it was observed that the *P. friedrichsthalianum* seeds tolerated desiccation to the level of 8.2% moisture (Table 2), indicating that the species does not have recalcitrant behavior. To check if they are orthodox or intermediate seeds, it is still necessary to test viability after desiccation to 5% moisture content, as well as storage at -20 ºC for three months, according to the protocol suggested by Hong and Ellis (1996). In spite of that protocol, the seeds of *P. cattleianum* were considered orthodox by Silva et al. (2011) since they maintained viability for more than three years stored in different packages and environments at a moisture content of 7.1%. In the same way, Masetto et al. (2014) concluded that *P. guineensis* seeds had an orthodox response to desiccation, tolerating drying to 5% moisture content and storage for up to 90 days under a temperature of 5±1 °C.

In *P. friedrichsthalianum* seeds with lower moisture content (8.2%), emergence was initially lower (57%), exhibiting a rise after the first month (greater than 80%) and a small decrease at the end of storage (74%). In *P. guajaja* seeds, Maeda et al. (1999) also found an increase in germination at 30 days of storage in a sealed container at 20 °C.

The *P. friedrichsthalianum* seeds with moisture contents of 9.8, 9.0, and 8.4 % exhibited high emergence at the beginning and throughout storage (greater than 70%). In contrast, seeds at 15.4% moisture content had high emergence up to the third month (greater than 70%), but arriving at null values at six months of storage on.

With moisture contents of 15.4%, the emergence speed index did not significantly change in the first three months of storage, while the mean emergence time was greater in the first month (Table 2). As there was no emergence from six months on, these variables were no longer estimated, making visualization of data in reference to the degenerative process in progress impossible. According to Bewley et al. (2013), when damage to the key functional systems exceeds seed repair capabilities,

Table 2. Emergence, emergence speed index, and mean emergence time of *Psidium friedrichsthalianum* (O. Berg) Nied. – Myrtaceae seedlings, in relation to moisture content and storage period of the seeds.

Storage period (months)	Moisture content (%)				
	15.4	9.8	9.0	8.4	8.2
			Emergence (%)		
0	83 a	85 a	72 a	79 a	57 b
1	88 a	83 a	81 a	84 a	86 a
3	72 a	87 a	83 a	86 a	89 a
6	0 b	75 a	81 a	89 a	86 a
9	0 b	82 a	78 a	82 a	81 a
12	0 b	76 a	76 a	70 a	74 ab
F test interaction = 19.81**		CV plot (%) = 10.72		CV split-plot (%) = 11.69	
			Emergence speed index (% day^{-1})		
0	2.496 a	2.592 bc	2.369 ab	2.633 abc	1.774 c
1	2.243 a	2.259 c	2.173 b	2.359 bc	2.430 abc
3	2.288 a	3.695 a	3.043 a	3.119 a	3.098 a
6	-	2.891 bc	3.005 a	3.323 a	2.906 a
9	-	2.976 b	2.796 ab	2.917 ab	2.737 ab
12	-	2.209 c	2.145 b	1.925 c	2.094 bc
F test interaction = 14.23**		CV plot (%) = 12.35		CV split-plot (%) = 12.09	
			Mean emergence time (days)		
0	33.6 b	33.1 b	30.7 b	31.0 b	33.2 ab
1	40.1 a	37.4 a	37.9 a	36.2 a	35.8 a
3	33.3 b	24.3 d	28.1 b	28.2 bc	29.3 c
6	-	26.5 cd	27.7 b	27.2 c	30.4 bc
9	-	28.2 c	29.1 b	28.8 bc	31.8 bc
12	-	35.5 ab	36.4 a	37.8 a	36.2 a
F test interaction = 95.11**		CV plot (%) = 5.64		CV split-plot (%) = 6.09	

Mean values followed by the same letter in the column do not differ significantly by the Tukey test at the level of 5% probability. ** significant by the F test at the level of 1% probability.

the systems begin to fail and begin a trajectory toward death, resulting in loss of viability. Therefore, at some point beginning at three months of storage of *P. friedrichsthalianum* seeds with a moisture content of 15.4%, a decline in physiological quality began, which culminated in death at six months of storage. The impermeable packaging probably favored deterioration because, according to Carvalho and Nakagawa (2012), its use requires moisture contents ranging from 5% to 9%.

At all moisture contents of *P. friedrichsthalianum* seeds, except for 15.4%, the values of emergence speed index increased and the mean emergence times decreased at 3, 6, and 9 months of storage. This behavior was similar to that found by Maeda et al. (1999) in *P. guajava* seeds, suggesting that dormancy is overcome after six months of storage. The loss of dormancy that occurs during storage of dry seeds is called post-maturity (Bewley et al., 2013). Nevertheless, proof of the occurrence of dormancy in *P. friedrichsthalianum* seeds requires more specific studies.

The *P. friedrichsthalianum* seeds tolerate desiccation up to 8.2% moisture content and maintain high physiological quality for twelve months stored at 20 ºC in closed containers with germination greater than 70%. This information can subsidize actions related to seedling production, to maintenance of germplasm banks, and to restocking of areas of natural occurrence of the species.

Conclusions

The best temperatures for germination of *P. friedrichsthalianum* seeds were 20 and 25 °C. The temperatures of 30 and 35 ºC maintained seeds in quiescence, whereas the temperature of 40 °C was lethal.

Seeds of *P. friedrichsthalianum* can be desiccated up to the moisture content of 8.2% and maintain viability and vigor at high levels for twelve months.

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