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Seed Technology of *Myrcianthes pungens* (Berg) Legr: An Approach to Biometry and Germination

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Abstract

Myrcianthes pungens, native fruit with medicinal, ornamental and ecological potential, lacks information on biometry and technology for seed germination. Thus, the aim of this study was to characterize the fruits and seeds of *M. pungens*, determining the appropriate substrate for laboratory tests, as well as to evaluate the effect of different concentrations of gibberellic acid (GA_3) on the germination of the species. In the biometry, the mensuration of the fruits and seeds was made with the aid of a digital caliper. In the laboratory, three substrates (between filter paper, between vermiculite and between sand) were used, with five concentrations of GA_3 (0; 125; 250; 375 and 500 mg L^{-1}) and the tests were conducted in a germination chamber. The percentage of germination, hard seeds, germination speed index and seedling length root and shoot were evaluated. The average fruit length and width were 17.38 and 16.05 mm, respectively. The fruits presented one or two seeds with 8.10; 9.56 and 6.49 mm in length, width and thickness. The germination test is more efficient between fine sand, and vermiculite may also be used. The wetting of the substrate with gibberellin in the concentration of 125 to 274 mg L^{-1} optimizes the percentage and speed of germination.

Keywords: native fruit, guabijú, gibberellin, substrate

1. Introduction

Myrcianthes pungens (BERG) LEGR, which belongs to the family Myrtaceae, is popularly known as guabijú, guabijuzeiro, among others. It occurs naturally in South of Brazil (Backes & Irgang, 2009) and also in Argentina, Uruguay, Paraguay and Bolivia (Tropicos, 2019). Its fruits are edible globose type berry and the seeds are reniform, measuring from 6 to 7 mm (Lorenzi, 2002).

The species, which is characterized as late secondary (Vaccaro, Longhi, & Brena, 1999), stands out among the native fruit of Brazil, and it is indicated for ornamental purposes, honey producer, as well as for the production of fruits for consumption in natura or in industry (Sarmento & Villela, 2010). The guabijú also presents a medicinal potential, with analgesic and antiseptic properties (Nessello, Campos, Capistrano, Buzzi, & Cechinel Filho, 2016), antioxidant activity (Dalla Nora et al., 2014), and also presents positive effects in Alzheimer's treatment (Silveira et al., 2011). Besides those indications, it presents a great environmental value, being used for reforestation of degraded areas and permanent preservation because its flowers are nectariferous and its fruits are attractive to bird fauna (Backes & Irgang, 2009).

In Brazil, studies in the field of forest seed technology follow the recommendations of Seed Testing Rules (RAS) (Brasil, 2009), and the Instructions for Testing of Forest Seed Specimens (Brasil, 2013). However, given the great diversity of species, there is still missing information about the vast number of native species, especially those that belong to Myrtaceae family, which have seeds whose tolerance to dissection and the behavior in storage characterize themselves as recalcitrant or intermediate (Mayrinck, Vaz, & Davide, 2016). Thus, studying

procedures for conducting trials with species that are not yet in official records is of great importance for enabling the certification and commercialization of seed lots (Oliveira, D. C. F. Dias, Hilst, Silva, & L. A. Dias, 2014), as in the case of *M. pungens* (Fior et al., 2010).

Plants produce fruits and seeds of uneven sizes due to genetic, nutritional and environmental reasons, which can influence seed physiological quality. Thus, the biometric characterization of fruit and seeds is a main tool that can be employed in conservation and genetic improvement studies of populations, identification and differentiation of species of the same genus, as well as laboratorial testing standardization (Silva, Santos, Lima, & Morais, 2014).

Besides the biometric characteristics, germination of seeds can also supply important information about the quality of the analyzed seed lot, being a complex process, because it involves several factors, among them we highlight quantity of water, lighting, temperature, oxygen, substrates, presence or not of dormancy and pathogen incidence. One of the basic components used on germination tests is the substrate (Sorana et al., 2019), whose choice must consider the size of the seed, its water demand, photoplasty and facility for the development and evaluation of seedlings (Figliolia, Oliveira, & Piña-Rodrigues, 1993; Brasil, 2009) besides material availability.

Additionally, to support fast germination, with homogenous seedlings development, phytoregulators promoting growth such as the gibberellic acid (GA_3) are being used in fructiferous seed species technology (Lata, Sharma, Garg, & Joshi, 2018) and more recently in forest species (Cabello, Espinoza, Espinoza, Cabrera, & Santelices, 2019). When it comes to seeds, the exogenous application of GA_3 can increase the potential growth of the embryo (Pipinis et al., 2015), promoting the cellular stretching that induces the radical development, increasing the germination percentage (Campos, Abreu, Guimarães, & Seleguini, 2015).

Because of the great importance of *M. pungens* and the lack of information about the species seed technology, the objectives of this present work was: a) characterize biometrically fruits and seeds of *M. pungens*; b) determinate the proper substrate for species germination; and c) evaluate the effect of using different concentrations of GA_3 on *M. pungens* seed germination.

2. Material and Methods

2.1 Collection of Fruits and Seeds Lot Formation

M. pungens fruits where collected when they showed a dark-purpled coloring, in five remaining trees located in remnants of Subtropical Seasonal Forest, in the Central region of Rio Grande do Sul State (29°38′14.79″ S and 53°27′44.11″ W) in Southern Brazil. The local weather, accordingly to Köppen classification, is humid subtropical, with average annual precipitation between 1400 and 1760 mm, with fair distribution along the year with matching temperatures between -3 ºC and 30 ºC (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). According to the Brazilian System of Soil Classification, the soil of the region is Lithic Neosol type, physically flat and undeveloped (Embrapa, 2013).

After being collected, the fruits were taken to Forestry and Forest Nursery Laboratory (Laboratório de Silvicultura e Viveiro Florestal) belonging to Forest Science Department (Departamento de Ciências Florestais) from Universidade Federal de Santa Maria (UFSM), in Santa Maria, RS. The fruits were submerged in water for 24 hours to ease the extraction process. Then, pulping was made in streaming water, extracting the seeds, which were dried with shady environment and ventilated for two days, manually homogenized, forming the lot under studying.

2.2 Biometry of Fruits and Seeds

Initially the length and width of the fruits were determined, and also the length, width and thickness of the seeds, based on a sample of 100 units for each attribute. A digital pachymeter was used to determine dimensions (0.001 mm), being subsequently calculated the maximum, minimum, average and standard deviation, for each biometric characteristic.

Besides that, it was determined the number of seeds for each fruit, the weight of thousand seeds by using eight samples of one hundred seeds, and the degree of humidity by greenhouse method for 24 hours at 105±3 °C (Brasil, 2009).

2.3 Germination Test

The germination test was performed, right after the lot formation. Prior to the test installment, the seeds were disinfested, being plunged in distilled water solution with 2% of sodium hypochlorite for two minutes and after washed with distilled water.

The testing was performed with "gerbox" transparent containers by using a fully casual design, with factorial scheme (5 × 3). The treatment consisted in five concentrations of GA_3 (0; 125; 250; 375 and 500 mg L^{-1} of distilled water) and three substrates (BFP: between filter paper, BV: between vermiculite and BS: between sand), totaling 15 treatments with four repetitions of 25 seeds each. The dilution of GA_3 concentrations in distilled water followed the recommendations of Brasil (2009).

In BFP substrate two "Germitest" paper sheets were used, one in base and one at the top, dampened with the solution (represented by the different GA_3 concentrations), knowing that the utilized volume matched 2,5 times the paper weight. In relation with the other substrates, sand (0.84 mm of sifting mesh) and vermiculite (thin granulometry), both were dampened at 60% capacity of the solution retaining capacity (Brasil, 2009).

The test was conducted in the germination chamber, Mangelsdorf type, with constant lighting and 25±2 °C of temperature. Counts were made every three days, in order to obtain normal seedlings percentages (NS), according to technological criterion (Gui-Ferreira & Borghetti, 2004), and the number of firm seeds (hard) (HS) in accordance with Brasil (2009). In each evaluation, the values evaluated were the length of shoot (LS) and length of root (LR) of the NS, with graduated millimeter ruler aid. According to the observations, it was possible to determine the germination percentage (G%) and germination speed index (GSI) (Maguire, 1962).

The data were submitted to normality residual presumptions by Shapiro-Wilk and variances homogeneity by Barlett, by Action software means (Team Estatcamp, 2014). When it was necessary, the data transformation was proceeded with Box-Cox, for further variances analysis, Tukey average comparison (0.05) and regression analysis with SISVAR software aid (Ferreira, 2011).

3. Results and Discussion

3.1 Biometry of the Fruits and Seeds

The fruits of *M. pungens* had an average length of 17.38 mm and a width of 16.05 mm, and one to three seeds, and 77% of the fruits had one seed and 20% two units. The seeds presented on average 8.10; 9.56 and 6.49 mm in length, width and thickness, respectively. In general, a small variation in the fruit and seed dimensions of *M. pungens* was observed, whose coefficient of variation was below 10% for most of the observed variables.

The results obtained in the biometric characterization of the fruits and seeds of *M. pungens* in the present study, differ from the values evidenced by Assumpção, Dalmaso, and Bragança (2017) evaluating the same species. The variation in the dimensions of the fruits and seeds between individuals of the same species can be influenced by environmental factors as rainfall, temperature and photoperiod, during the flourishing and development, as well as for the genetic variability and age among the main trees (Macedo et al., 2009; Pereira et al., 2017).

Table 1. Values (maximum, minimum, average, standard deviation and variation coefficient) regarding the biometric characterization of fruits and seeds of *M. pungens*, Santa Maria, RS

	Determinations	Maximum	Minimum	Average	Standard Deviation	CV (%)
Fruit	Length (mm)	20.70	14.45	17.38	1.31	7.50
	Width (mm)	18.91	13.48	16.05	1.34	8.30
Seed	Length (mm)	9.80	4.55	8.10	0.75	9.30
	Width (mm)	11.23	7.65	9.56	0.73	7.60
	Thickness (mm)	8.42	4.24	6.49	0.92	14.2

Note. CV: variation coefficient.

The weight of a thousand seeds was 344.11 grams, totaling 2.906 seeds kg^{-1}. These results are in agreement with Fior et al. (2010) who obtained an average of 2.576 and 5.429 seeds kg^{-1} from seeds collected in six trees in the municipality of Cachoeira do Sul and Encruzilhada do Sul, respectively. However, they differ from those described by Lorenzi (2002), who observed 4.000 seeds kg^{-1}. That difference among results confirmthe morphologic variation among the seeds of that species, that can be attributed to the climate and soil conditions, genetic factors, position of the fruit in the plant mother (Fenner & Thompson, 2005) and anthropic modifications in the area where the seed trees are established (Mendonça, Ramos, & Paula, 2001).

The seeds of *M. pungens* presented high humidity degree (38.2%), characteristic of the intolerant species to the dissection. Wielewicki, Leonhardt, Schlindwein, and Medeiros (2006) found average values of 39.6% and proposed a 34.8% humidity degree for seeds of *M. pungens*. L. dos S. de Souza, Fior, P. V. D. de Souza, and Schwarz (2011), by studying this same species, obtained degree of humidity of 38.7% and Fior et al. (2010)

between 41.4% and 43.6%. According to the autors, the variation in the number of seeds and humidity degree verified in the different studies can be related to the time, collection place and degree of maturation of the fruits, as well as to the climatic conditions of the area, and procedures before the performance of the analysis.

3.2 Germination Test

The germination of the seeds of *M. pungens* was slow and lasted longer. The germination was first attained on the 35th day and lasted for 71 days from first day of experiment. Interaction was evinced among the factors (substrate x concentrations of GA_3) just for the length of the shoot (LS). For the other attributes, effect was just verified for the isolated factors ($p < 0.05$).

Maximum seed germination (87%) was attained when the substrates were moistened with 125 mg L^{-1}solution of GA_3. However, favourable germination was observed to be higher up to concentrations of 274 mg L^{-1}, suggesting a positive effect in the germination at doses within the 125 to 274 mg L^{-1} interval. Minimum germination was observed seeds without pre-germination treatment (79.67%) (Figure 1A).

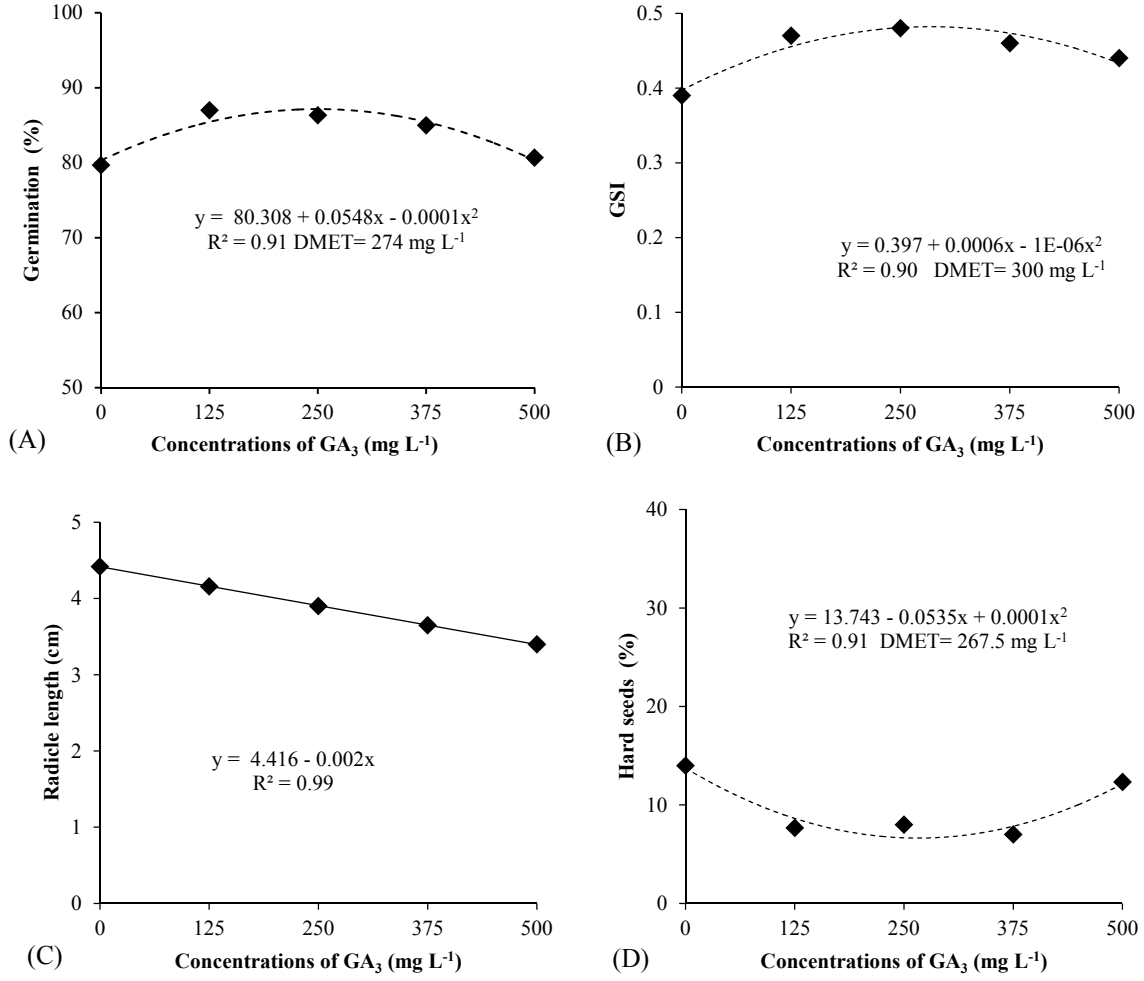

Figure 1. Effect of different concentrations of gibberellic acid about the germination (A), germination speed index (GSI) (B), radicle length (C) and hard seeds (D) of *M. pungens*

Similarly, a higher index of germination speed (GSI) was obtained in the same foregoing interval, with 0.47 (Figure 1B). GSI is calculated by the amount of germinated seeds divided by the number of days resulting from the germination test, and the higher the GSI, the greater the batch vigor (Nakagawa, 1994).

The beneficial effect of GA_3 in the germination of seeds of forest species was also observed by Saldias and Velozo (2014) in seeds of *Myrcianthes coquimbensis* (Barneoud) Lnadrum and Grifo and by Cabello et al. (2019), in seed of *Nothofagus* glauca (Phil.) Krasser. The positive influence of GA_3 on the germination of seeds

is related with the capacity of the phytoregulator to act on the cellular elongation, generating a larger metabolic incentive and larger mobilization of nutritious and energy reservations that are supplied for the development of the embryo (Taiz et al., 2017). Besides, GA_3 stimulates the synthesis and translation of the specific mRNA for the enzyme α-amilase (Muralikrishna & Nirmala, 2005), causing the degradation of the starch and weakness of the layer of the endosperm that involves the embryo, promoting thus, a faster germination and the reduction of the number of hard seeds (Figure 1D).

The use of GA_3 caused linear reduction in the development of the root system of *M. pungens* (Figure 1C), resulting on the contrary to what was observed by Campos et al. (2015), which showed a linear increase in the length of *Rollinia mucosa* (Jacq.) Baill radicle in increasing concentrations of gibberellic acid (0, 125, 250, 500 and 1000 mg L^{-1}). According to Macedo et al. (2009), application of GA_3 can effect germination among species. There is a probability that the concentrations of GA_3 promoted an increase of the auxins synthesis in the developing radicle (Fang et al., 1960; Michelwolwbrtz & Sjronval, 1963), provoking inhibitory effect in the elongation of the root system.

In relation to the tested substrate, treatments between vermiculite (BV) and between sand (BS) presented the largest germination averages (86.4 and 88.2%), and a smaller number of HS (7.4 and 4.6, respectively) (Table 2). In that sense, those substrates were the most appropriate to express the vigor of *M. pungens* seeds.

Wielewicki et al. (2006) supported use of paper roll as a substrate for *M. pungens* seed germination. Although in the literature there are no reports about the use of the substrates BS and BV in the test of germination of *M. pungens* seeds, however, use of BS and BV in this study was favourable for germination. This observation is in agreement with the Instruction for Analysis of Seeds of Forest Species. The substrates BS and BV are recommended for the species belonging to the same botanical family of *M. pungens* (Brasil, 2013).

Table 1. Effect of different substrates in germination (G), germination speed index (GSI), radicle length (RL) and number of hard seeds (HS)

Parameters	Substrates			CV (%)
	BFP	BV	BS	
G (%)	77 B*	86 A	88 A	7.23
GSI	0.4 B	0.4 B	0.5 A	15.31
RL	4.5 A	3.4 B	3.8 B	17.25
HS (%)	17.4 B	7.4 A	4.6A	42.48

Note. BFP (between filter paper); BV (between vermiculite) and BS (between sand). Averages followed by the same letter in the line do not differ among them by the Tukey test at 5% of error probability.

The substrate sand is indicated for every seed type, besides the ones of the most sensitive species to the drying and that demand a prolonged period for completing the germination (Abreu et al., 2005). However, according to Gasparin, Araujo, Tolfo, Foltz, and Magistrali (2013) the substrate BS presents disadvantages due to the largest weight, being necessary additional cares with the sowing depth, for not harming the germination. Additionally, the substratum vermiculite, according to Figliolia et al. (1993), is used for germination of seeds of forest species due to good absorption capacity and retention of water, being also indicated for seeds with slow germination and emergency.

The largest values of GSI were observed in the substrate BS, possibly due to larger contact area that it offers to the seeds, favoring the absorption of water and GA_3, corroborating with what was evinced by Gasparin et al. (2013) for the species *Parapiptadenia rigida* (Benth.) Brenan. Additionally, according to Flores et al. (2014) the contact area of the substrate moistened with the seed is very important, because in spite of not being limiting to germination, it influences the germination speed.

Through the analysis of length of shoot (LS) of the seedlings, it was verified that for the substrate BS and BFP the maximum efficiency was reached when the concentrations of 225 mg L^{-1} and 270 mg L^{-1} were used, respectively. However, seedlings that grew in the substrate BV presented larger LS when this was only moistened with water (Figure 2).

In the conditions of BS or BV and about 274 mg L^{-1} of gibberellins, the first counting can be accomplished at the 35 days and the last with 50, being necessary intermediate counting, avoiding compromising of the results by the pathogens presence.

It was verified that more vigorous seedlings are produced when the seeds of *M. pungens* are submitted to the action of GA_3, generating earnings of time in the formation of seedlings, for the fastest and uniform emergency. In spite of the obtained verifications, other studies related to the species should be accomplished seeking to accelerate the speed of the germination, reducing thus the pathogens presence. Studies are suggested to evaluate the possible presence of a non deep or combined physiologic dormancy of *M. pungens* seeds (Baskin & Baskin, 2004), bearing in mind that the same presents appropriate soak, however with non-uniformity and expressive time for germination.

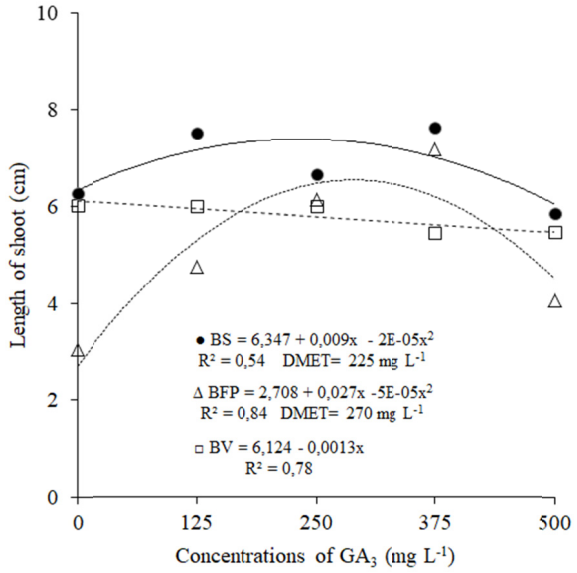

Figure 2. Effect of five different concentrations of gibberellic acid in three types of substrate, in the length of shoot of seedlings (LS) of *M. pungens*

Note. BS = between sand, BFP = between filter paper, and BV: between vermiculite.

4. Conclusions

The length and the width of the *Myrcianthes pungens* fruits are uniform (17.38 and 16.05 mm, respectively), usually presenting, one or two seeds, with 8.10, 9.56 and 6.49 mm length, width and thickness.

The germination test is more efficient among sand, and vermiculite can also been used. The first germination counting should be accomplished at the 35th day, closing up the test on the 71st day after the sowing.

The moistening of the substrate with gibberellins is recommended in the concentration of between 125 and 274 mg L^{-1} for obtaining larger percentage and speed of germination of *M. pungens*.

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