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Biometric characterization and seed germination of giant mimosa (*Mimosa bimucronata* (DC) O. Kuntze)

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Abstract

This study aimed to characterize the biometric characteristics of *Mimosa bimucronata* (DC.) O. Kuntze seeds and to elucidate their physiological characteristics with respect to the effect of treatments and temperature on dormancy breaking. Treatments consisted of control (intact seeds), chemical scarification using sulfuric acid (density: 1.84 and purity: 98%) for 5 min, integument cutting at the region opposite the micropyle, soaking at 80°C followed by cooling for 24 h, and soaking in water at an ambient temperature for 24 h and 48 h. Subsequently, the effect of different temperatures (constant at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C and an alternating temperature of 20°C–30°C) on seed germination was assessed. The average dimensions of seeds with an initial moisture content of 16.25% were as follows: length: 4.17 mm, width: 2.95 mm, and thickness: 0.69 mm. Optimum seed germination and seedling development in *M. bimucronata* were obtained by seed integument cutting at 30°C.

Keywords: Dormancy, Mimosaceae, Reforestation, Temperature, Vigor.
Abbreviations: FGC_first germination count; GER_germination; GSI_germination speed index; ASG_average speed of germination; TM_average time, I_uncertainty; Z_germination synchrony; AS_abnormal seedlings; DS_dead seeds; CV_coefficient of variation; SK_Pearsons' coefficient of skewness; ATG_average time of germination; GU_germination uncertainty

Introduction

Giant mimosa [*Mimosa bimucronata* (DC.) O. Kuntze] is a medium-sized tree species belonging to the family Mimosaceae (Leguminosae: Mimosoideae), and it is naturally distributed in the northeastern, southern, and southeastern regions of Brazil; it is particularly frequent in the states of Pernambuco, Alagoas, and Paraná. These trees are semi-deciduous and deciduous species, which are characteristics of the initial stages of succession, and owing to their sturdiness and rapid growth, they play a crucial role in mixed reforestation programs that are involved in recovering permanent preservation areas, managing forest fragments, and landscaping projects (Carvalho, 2003).

Flowering occurs from December to March. The leaves of *M. bimucronata* are compound and paripinnate, with up to 10 pairs of leaflets. According to Carvalho (2004), the wood of *M. bimucronata* can be used in woodwork, shaped using a lathe, and used for internal purposes. *M. bimucronata* also exhibits phytotherapeutic properties; its sprouts are effective in treating asthma, bronchitis, and fever, and its leaves are used in emollients.

Despite these potential uses of *M. bimucronata*, few studies have been conducted to determine the ideal conditions for seed germination (Ribas et al., 1996; Fowler and Carpanezzi, 1998). *M. bimucronata* is not listed in the Rules for Testing Seeds (Brasil, 2009), which is an official guide for analyzing seed quality in Brazil. Dormancy breaking in the integument of *M. bimucronata* using methods such as hot water immersion (Fowler and Carpanezzi, 1996) and chemical scarification using sulfuric acid (Ribas et al., 1996) has been assessed. However, mechanical scarification was not used in these studies. Hot water immersion and chemical scarification using sulfuric acid are of limited use in nurseries and to seedlings producers; thus, safer alternative methods are required, which are widespread and ensure satisfactory seed germination.

Seed germination is a biological process that involves several chemical reactions in which organic compounds are broken down and reorganized to allow embryonic axis development (Marcos Filho, 2015), and its various stages occur under temperatures specific to metabolic and enzymatic processes. Thus, the effect of temperature on seed germination can be expressed in terms of cardinal temperatures, i.e., minimum, maximum, and optimum temperatures (Bewley and Black, 1994).

Only one study has assessed the effect of temperature on the germination of *M. bimucronata* seeds at 20°C, 25°C, and 30°C; these seeds were from southern Brazil, an area with an average annual temperature of 16.5°C (Fowler and Carpanezzi, 1998). Prevailing environmental conditions during seed formation affect germination (Bewley and Black, 1994; Carvalho and Nakagawa, 2012; Marcos Filho, 2015). Thus, studies are required to determine the effect of temperature on seed germination in *M. bimucronata* from warmer regions, such as northeastern Brazil.

Biometric characterization of seeds is a relevant tool to detect genetic variation within populations of the same species or to differentiate species within the same genus (Gusmão et al., 2006). In addition, it provides a substitute for characterizing ecological aspects, such as dispersion type, dispersion agents, and seedling establishment (Macedo et al., 2009), which makes biometric characterization essential in the present study.

Therefore, this study aimed to biometrically characterize seeds of *M. bimucronata* and to understand dormancy breaking and seed germination under different temperatures to standardize germination tests and seedling production for different purposes.

Results and Discussion

Biometric characterization

The moisture content of *M. bimucronata* seeds at harvest time was 16.25%. The seeds had an average length of 4.17 mm, width of 2.95 mm, and thickness of 0.69 mm, with an amplitude of variation of 0.71 mm, 1.50 mm, and 0.39 mm, respectively (Table 1).

The average mass of 1000 seeds was 8.28 g, which corresponded to approximately 120,000 seeds/kg. In this study, the coefficient of variation (CV) was 3.16%, which was within the limits defined in the Rules for Testing Seeds (Brasil, 2009) that allows a maximum of 4% (Table 2). However, these results differ from those found by Fowler and Carpanezzi (1998), who obtained approximately 91,575 units/kg. This difference can be attributed to moisture content and seed mass, which are influenced by the season and site of harvesting. According to Vaughton and Ramsey (1998), this variation in seed mass occurs in different plant species and frequently in the plant itself. Variations found in plants are not caused by genetic differences but rather by environmental effects during seed development (Leishman et al., 2000).

According to the Rules for Testing Seeds (Brasil, 2009), small seeds are defined as those with more than 5,000 units/kg, whereas large seeds have less than 5,000 units/kg; thus seeds of *M. bimucronata* can be classified as small. According to Braga et al. (2007), smaller seeds are produced in greater quantity and are easily dispersed, allowing dispersal in places that are unoccupied by larger seeds.

Biometric data of fruits and seeds are taxonomically questionable due to the strong influence of latitudinal, seasonal, and microclimatic variations. However, they have a great biological significance in relation to dispersal agents and dispersion syndromes (Rodrigues et al., 2006).

Frequency histograms for length, width, and thickness of *M. bimucronata* seeds (Figures 1A, B, and C) revealed that approximately 21% of the seeds were between 4.16 mm and 4.24 mm in length, approximately 57% were between 2.96 mm and 3.14 mm in width, and 27% were between 0.67 mm and 0.71 mm in thickness. An asymmetric coefficient was observed for the curves with a negative and low skewness (Figure 1A) or a moderate skewness (Figures 1B and C). Regardless of the skewness type (positive or negative), median values are usually the best measure of central tendency (Ferreira, 2003). The mean is sensitive to outliers and is pulled toward them; consequently, the mean could increase or decrease.

In addition to providing information on seed size, the thousand-seed weight assessment allows us to identify the state of maturity and health (Brasil, 2009) by comparing many samples from the same species. Pioneer species, such as *M. bimucronata*, usually produce numerous small-sized seeds that exhibit dormancy (Melo et al., 2004). Thus, the production of numerous seeds increases the possibility of some of them reaching an environment conducive for germination or remaining dormant in the soil until there is some natural or anthropogenic disturbance.

Pre-germination tests

The highest first germination count (FGC), germination (GER), germination speed index (GSI), and average speed of germination (ASG) values were obtained when the seed integuments were cut at the region opposite the micropyle (Table 3). Similar results were obtained by Pereira and Ferreira (2010), wherein seeds of *Parkia discolor* Spruce ex Benth were cut at the region opposite the micropyle. Cutting led to high germination percentages in seeds of *Senegalia tenuifolia* (L.) Britton & Rose (Araújo, 2014), *Macroptilium martii* Benth (Araújo et al., 2014), and *Piptadenia stipulacea* Benth (Farias et al., 2013). Ribas et al. (1996) used seeds of *M. bimucronata* and suggested that immersion in sulfuric acid for 5 min was the most effective treatment for dormancy breaking; however, they did not test seed cutting. Although chemical scarification using sulfuric acid was effective for different seed species whose dormancy is caused by integument impermeability, the corrosive effect of the acid can promote irreversible injuries to the embryo (Santos et al., 2014). This may explain the decrease in seed germination with this treatment in the present study (Table 3). Eisvand et al. (2006) reported that chemical scarification promoted a decrease in seed germination of *Astragalus siliquosus* Boiss. According to Rocha et al. (2011), another negative factor for acid scarification in seeds of forest species is the release of sugars resulting from cellulose degradation, increasing substrate availability for fungal colonization; however this was not observed in the present study.

Seed dormancy in *M. bimucronata* appears to be closely related to the cutting of its integument, which was the most efficient method to break dormancy. However, the physiological causes of seed dormancy in this species, as well as in most legumes, are unknown.

These results confirm that *M. bimucronata* seeds exhibit integumentary dormancy, in which the integument acts as an impediment to water and oxygen, owing to the presence of chemical inhibitors such as coumarin or parasorbic acid, or as a physical barrier to embryo development (Oliveira, 2012). Thus, all dormancy-breaking treatments studied have included integument breaking and seed soaking, consequently inducing germination.

The analysis of the average time of germination (ATG), germination uncertainty (GU), and germination synchrony (Z) in seeds of *M. bimucronata* (Table 4) confirmed that cutting was significantly superior to other treatments. Santana et al. (2010) studied seeds of *Kielmeyera coriacea* Mart. and found that they exhibited a high degree of uncertainty, low synchrony, and scattering in relation to the ATG, similar to that found in the present study. Increased synchrony expresses physiological seed homogeneity at the time of germination (Conserva, 2006).

Table 1. Descriptive analysis of the length, width and thickness of the seeds of *M. bimucronata.*

Statistical measures	Length (mm)	Width (mm)	Thickness (mm)
Average	4.17	2.95	0.69
Mode	4.05	3.00	0.59
Median	4.16	2.93	0.68
Minimum	3.84	2.48	0.49
Maximum	4.55	3.98	0.88
Standard deviation	0.15	0.14	0.09
CV (%)	8.67	4.88	13.20

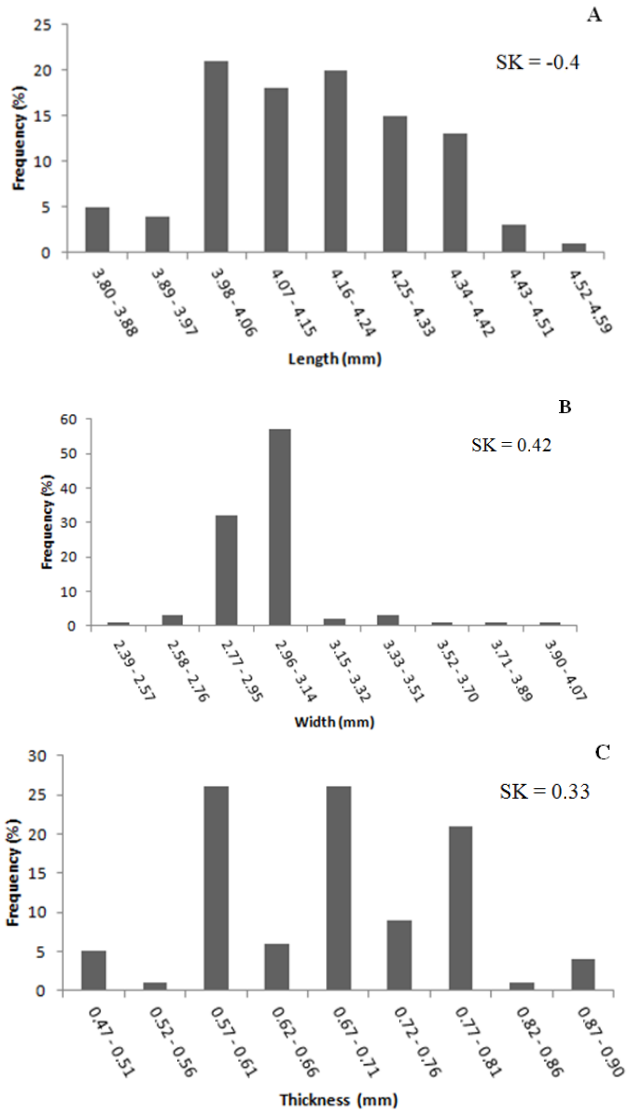

Fig 1. Distribution of the relative frequencies of the length (A), width (B) and thickness (C) *M. bimucronata* seeds.

Table 2. Descriptive statistics of weighings (100 seeds per replicate) obtained to calculate the weight of one thousand seeds of *M. bimucronata*.

Statistical measures	Weight of a thousand seeds
Average (g)	8.2800
Variance (s^2)	0.0006
Standard deviation (s)	0.0262
CV (%)	3.16

Table 3. First germination count (FGC), germination (GER), germination speed index (GSI) and average speed of germination (ASG) *M. bimucronata* seeds, subjected to treatments to overcome dormancy.

Treatments	FGC (%)	GER (%)	GSI	ASG
Witness	54 c	56 c	3.745 c	0.096 e
H_2SO_4/5 minutes	62 bc	68 bc	4.121 bc	0.166 b
Cutting	96 a	100 a	5.495 a	0.260 a
H_2O 80 ºC/24 hours	64 bc	70 b	4.250 bc	0.135 cd
H_2O/24 hours	72 b	74 b	4.577 b	0.156 bc
H_2O/48 hours	63 bc	67 bc	4.085 bc	0.123 de
Value de "F"	13.63**	28.16**	12.68**	67.46**
CV (%)	11.58	7.67	8.71	8.81

Means followed by the same lowercase letter in the column do not differ by 1% of probability by the Tukey test.

Table 4. Average time (TM), uncertainty (I) and germination synchrony (Z) *M. bimucronata* seeds, subjected to treatments to overcome dormancy.

Treatments	TM (days)	I (bit)	Z
Witness	12.4 a	1.341 a	0.377 b
H_2SO_4/5 minutes	5.9 bc	1.609 a	0.292 c
Cutting	3.5 c	0.246 b	0.996 a
H_2O 80 ºC/24 hours	6.4 b	2.020 a	0.251 c
H_2O/24 hours	6.6 b	2.075 a	0.198 d
H_2O/48 hours	7.8 b	1.632 a	0.328 b
Value de "F"	25.40**	16.68**	224.95**
CV (%)	16.39	21.96	9.66

Means followed by the same lowercase letter in the column do not differ by 1% of probability by the Tukey test.

Table 5. First germination count (FGC), germination (GER), abnormal seedlings (AS) and dead (DS) *M. bimucronata* seeds.

Temperature (ºC)	FGC (%)	GER (%)	AS (%)	DS
5	0 d	0 e	0 c	100 a
10	0 d	4 e	22 a	74 b
15	2 d	34 d	4 b	62 c
20	89 ab	94 b	0 c	6 e
25	90 ab	95 b	0 c	4 e
30	96 a	100 a	0 c	0 e
35	37 c	70 c	0 c	30 d
40	0 d	0 e	0 c	100 a
20-30	85 b	94 b	0 c	6 e
Value de "F"	348.57 **	411.75 **	385.50 **	410 **
CV (%)	10.14	8.08	26.65	9.77

Means followed by the same lowercase letter in the column do not differ by 1% of probability by the Tukey test.

Table 6. Speed Index (GSI), time (Tm), average speed (ASG) and uncertainty (I) germination *M. bimucronata* seeds, submitted to temperatures.

Temperature (ºC)	GSI	TM (days)	ASG	I (bit)
5	0.000 e	0.00 f	0.000 f	0.000 b
10	0.100 e	9.75 a	0.102 e	0.000 b
15	0.917 d	7.20 b	0.138 d	1.338 a
20	4.655 b	5.79 cd	0.172 bc	1.150 a
25	4.877 b	5.84 cd	0.171 bc	1.035 a
30	5.495 a	4.03 e	0.248 a	0.232 b
35	3.677 c	6.41 c	0.156 cd	0.977 a
40	0.000 e	0.00 f	0.000 f	0.000 b
20-30	5.121 ab	5.44 d	0.183 b	1.176 a
Value de "F"	470.00 **	339.20 **	406.08 **	25.28**
CV (%)	8.16	7.03	6.35	35.18

Means followed by the same lowercase letter in the column do not differ by 1% of probability by the Tukey test.

Germination at different temperatures

A temperature of 30°C produced the highest FGC and GER values (Table 5), which correspond to the results obtained by Ribas et al. (1996) but differ from those obtained by Fowler and Carpanezzi (1998), who indicated a temperature of 25°C for seed germination tests in *M. bimucronata*. According to Carvalho and Nakagawa (2012), this may be due to the predominant climate during seed maturation because it exerts a great influence on germination and the viability period.

The optimum temperature range for most species is between 20°C and 30°C (Marcos Filho, 2015) and extends to 35°C (Larcher, 2000). In seeds of *Peltophorum dubium* (Sprengel) Taubert, the highest number of germinated seeds at first counting was demonstrated at a temperature of 30°C (Oliveira et al., 2008). Bracalion et al. (2008) reported that a temperature of 30°C was the most favorable for seed germination of forest species, in addition to an association between optimum temperature and biome occurrence of the species. In the present study, among the different temperatures tested, no germination occurred at 40°C (Table 5). According to Bewley and Black (1994), temperature is important in germination and high temperatures can negatively affect germination by decreasing protein synthesis, anabolic reactions, and the free amino acid supply. Seeds of *M. bimucronata* at 5°C exhibited lower vigor (Table 5). Lower temperatures decrease seed metabolic activity, thus reducing germination (Sousa et al., 2008).

On comparing constant temperatures with alternating temperatures of 20°C–30°C (Table 5), the latter provided good results, with FGC and GER values of 85% and 94%, respectively; however, these values were statistically lower than those obtained at 30°C. Similar results were found by Oliveira et al. (2015), who observed that on comparing alternating temperatures with constant temperatures, there was a decrease in the percentage of seed germination in *Casearia gossypiosperma* Swartz.

The highest percentage of abnormal seedlings (AS) was observed at 10°C (Table 5), statistically differing from the percentages observed at the other temperatures. These results reveal that seeds subjected to below the optimum temperature cause a delay in germination and promote the formation of AS due to a reduction in enzyme activity related to respiration and cellular metabolism (Taiz and Zeiger, 2013). The highest number of dead seeds (DS) was observed at 5°C and 40°C (Table 5). This may have occurred because extreme temperatures hinder seed metabolic activity, leading to seed death (Melo, 2011). Null germination observed at these temperatures corresponds with the findings of Okusanya (1978, 1980) who showed that seeds of many tropical species are sensitive to extreme temperatures. The optimum temperature for seed germination is directly associated with the ecological characteristics of species (Oliveira et al., 2014a). Guedes et al. (2010) indicated a temperature of 35°C for germination and vigor tests in seeds of *Amburana cearensis* (Allemão) A.C. Smith, showing that seed response to temperature may also differ between species. In the present study, seeds of *M. bimucronata* germinated at different temperatures, which would allow colonization in a greater diversity of habitats, facilitating their dispersion. The thermal amplitude for seed germination of a species indicates the distance of a seed buried in relation to the soil surface because it tends to decrease as the depth increases (Melo, 2011). According to Ramos and Varela (2003), does the ideal germination temperature vary with emergence and seedling establishment. The results for seed vigor (Table 6), which were indirectly measured by the GSI, ATG, and ASG, were the best at a temperature of 30°C. Silva et al. (2014) studied seed germination of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. and stated that the germination speed is directly proportional to temperature. Germination percentage is a good index to assess the ability of a species to occupy a given environment as a rapid germination strategy permits establishment in the environment as quickly as possible, taking advantage of favorable environmental conditions. Oliveira et al. (2014a) also obtained a similar result in seeds of *C. gossypiosperma* Swartz, with the highest GSI and ASG values obtained at 30°C. Moreover, Oliveira et al. (2014b) observed that temperature is an important factor in seed germination of *Eriotheca gracilipes* (K. Schum.) A. Robyns, affecting total germination and seed vigor, which was evaluated using the germination speed. GSI and ASG were not obtained at temperatures of 5°C and 40°C as there was no germination (Table 6). These results correspond with those found by Oliveira et al. (2008), who observed that lower temperatures decrease seed metabolic activity, which reduces the GSI and slows the ASG, increasing the ATG. The temperature of 40°C favored greater evapotranspiration, which is responsible for substrate moisture decrease, and, consequently, a reduction in GSI and ASG in seeds of *M. bimucronata*, which have a small contact surface with the substrate used. In studies conducted by Silva et al. (2008) with *Erythroxylum ligustrinum* DC., Dias et al. (2011) with *Myrciaria cauliflora* Berg., and Araújo Neto et al. (2014) with *Caesalpinia pulcherrima* (L.) SW, a temperature of 30°C was considered ideal for germination, providing a higher GSI and confirming the results of Aguiar et al. (1993), who reported that seeds of several subtropical and tropical forest species have a higher germination potential at 30°C.

Seeds subjected to constant temperatures of 15°C, 20°C, 25°C, and 35°C and an alternating temperature of 20°C–30°C showed greater germination uncertainty, with no statistical difference between them. However, a temperature of 30°C provided the lowest uncertainty; this differed statistically from that of the other temperatures (Table 6). The lower the GU value, the more synchronized the germination, regardless of the total number of seeds germinating (Santana and Ranal, 2004). Labouriau and Agudo (1987) showed that seeds of *Salvia hispanica* L. maintained under stress temperatures show physiological heterogeneity by the loss of GU and quantified by values of synchronization indices.

Materials and Methods

Location

This study was conducted at the Laboratory of Plant Propagation of the Center for Agricultural Sciences, Federal University of Alagoas, Delza Gitaí Campus, Alagoas, Brazil. It is located at 9° 28′ 01″ S and 35° 49′ 32″ W, at an altitude of 141 m.

Harvesting and processing of seeds

Fruits of *M. bimucronata* were harvested from several trees located in Garanhuns, Pernambuco, Brazil from March to May 2015, using an aerial pruning shear with an extension cable. Between December and May, the local average temperature was recorded as 23.2°C, with the maximum and minimum temperatures not exceeding 28.9°C and 22.2°C, respectively, and a cumulative precipitation of 182.1 mm (INMET, 2015).

Biometric characterization

After harvesting, the initial seed moisture content was determined using an oven regulated at 105°C ± 3°C, according to the Rules for Testing Seeds (Brasil, 2009). For biometric characterization, eight replicates of 100 seeds were used. The length, width, and thickness were measured using a digital caliper, and the mass of 1000 seeds was measured (Brasil, 2009). For each variable, the mean, mode, median, standard deviation, CV, total amplitude, and relative frequency were calculated (Labouriau and Valadares, 1976). Pearson's coefficient of skewness (SK) was calculated as SK = 3 × (mean − median)/standard deviation, with results classified as having low skewness for $|SK| < 0.15$, moderate skewness for $0.15 < |SK| < 1$, and high skewness for $|SK| > 1$ (Lorentz and Nunes, 2013).

Overcoming dormancy and germination at different temperatures

Two germination tests were conducted to evaluate treatments for dormancy breaking (Experiment 1) and the effect of temperature on seed germination (Experiment 2). Prior to sowing, the seeds were sterilized by immersion in 70% alcohol for 1 min and were then washed in distilled water (Neves, 2013).

For dormancy breaking, the following treatments were tested: i) control (intact seeds); (ii) chemical scarification by immersing seeds in concentrated sulfuric acid for 5 min, followed by washing under running water; iii) cutting at the side opposite the micropyle; iv) immersion in hot water (80°C) and cooling for 24 h; v) immersion in distilled water (ambient temperature) for 24 h; and vi) immersion in distilled water (ambient temperature) for 48 h. In the present study, seed sterilization was conducted after dormancy breaking treatment.

Sowing was conducted on two sheets of Germitest paper (autoclaved) placed in transparent plastic boxes (Gerbox, 11.0 × 11.0 × 3.5 cm). Seeds were incubated at 30°C in a germinating biochemical oxygen demand (BOD) chamber.
Germination was tested at different temperatures. Cut seeds were conditioned on two sheets of Germitest paper and placed in transparent Gerbox containers. Seeds were incubated in BOD chambers at constant temperatures of 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C and an alternating temperature of 20°C–30°C. The photoperiod was set to 8 h under white light provided by four 20-W fluorescent lamps. At alternating temperatures, the light period coincided with the highest temperature.

For both experiments, germinated seed counting was performed daily over a 15-day period. The substrate was re-wetted when necessary. Physiological characteristics of seeds was assessed by measuring the FGC, GER, percentage of AS, number of DS, GSI, ASG, ATG, and GU. Only seeds with primary root length of ≥2 mm were considered as germinated (Giachini et al., 2010).

Statistical analysis

Statistical analysis was conducted by the SISVAR program, Federal University of Lavras (Ferreira, 2003). The design of the two experiments was fully randomized, with four replicates of 25 seeds per treatment, and the mean was compared using Tukey's test at 5% probability.

For biometric characterization, the mean, mode, median, amplitude of variation, variance, standard deviation, and CV were calculated according to Banzato & Kronka (1992).

Conclusion

Seeds of *M. bimucronata* are classified as small and exhibit little variation in their biometric characteristics. Cutting was the most efficient treatment for dormancy breaking in seeds of *M. bimucronata*, and this is recommended at a temperature of 30°C for germination and vigor tests in this species.

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