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ARTICLE

Morphometric characterization and functional traits of fruits and seeds of *Neoglaziovia variegata* (Arruda) Mez.

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ABSTRACT: Global efforts to restore ecosystems have increased the demand for seeds of native species, both from tree-shrub and herbaceous strata. However, little is known about seed ecology or germination of many herbaceous species from the Brazilian Caatinga. *Neoglaziovia variegata* (*caroá*) is a bromeliad endemic to this biome and has great ornamental and fiber production potential. This study aimed to morphologically characterize *N. variegata* fruits and seeds and to evaluate its germination-related parameters. To do so, the morphometry of bunches, fruits, and seeds was investigated, and seeds were assessed for physiological quality at two maturation points and light response. The morphometric measurements showed normal distributions and a wide dispersion from the central values. Therefore, there is genetic variability among individuals of the same population. The seeds showed high germinability, and fruits had an average size of 8.5 x 9.4 cm, whose color (green or purple) did not influence their physiological quality. The seeds of *N. variegata* are positive photoblastic, and their germination reaches maximum values after two weeks in the presence of light.

Index terms: Bromeliaceae, Caatinga, forest seeds, photoblastism.

RESUMO: O esforço global para atingir as metas de restauração de ecossistemas resultou em um aumento da demanda por sementes nativas, não só dos estratos arbóreo-arbustivos, mas também dos estratos herbáceos. No entanto, pouco se conhece a respeito da ecologia das sementes e nem do processo germinativo de muitas das espécies herbáceas que compõem a Caatinga. *Neoglaziovia variegata* (caroá) é uma bromeliácea endêmica da Caatinga que possui grande potencial ornamental e de produção de fibras. O objetivo do trabalho foi caracterizar frutos e sementes de *N. variegata*, bem como diversos parâmetros relacionados à germinação desta espécie. Foram realizados estudos sobre morfometria de cachos, frutos e sementes; e de avaliação da qualidade fisiológica de sementes em dois pontos de maturação e resposta de sementes à luz. As medidas morfométricas realizadas demonstraram distribuições normais e com ampla dispersão dos valores centrais, indicando variabilidade genética entre indivíduos da mesma população de *N. variegata*. As sementes apresentam alta germinabilidade e a coloração de frutos (verdes ou roxos) de tamanho médio de 8,5 x 9,4 cm não influenciou sua qualidade fisiológica. As sementes de *N. variegata* são fotoblásticas positivas cuja germinação atinge valores máximos após duas semanas na presença de luz.

Termos para indexação: Bromeliaceae, Caatinga, sementes florestais, fotoblastismo.

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INTRODUCTION

The Caatinga is a seasonally dry tropical forest and is the dominant vegetation in northeastern Brazil. This vegetation can be found over an area of about 800 thousand km^2 and is highly rich in biodiversity and endemic flora. Plants of this ecosystem show modification that allow survival during long periods of drought (Fernandes et al., 2020). Among so many native plants of the Caatinga still little explored is "caroá", *Neoglaziovia variegata* (Arruda) Mez, which belongs to the Bromeliaceae family. It is an endemic plant of the lower stratum of the Caatinga and has great economic and conservation potential (Beckmann-Cavalcante et al., 2017a).

This bromeliad is an herbaceous, terrestrial, stoloniferous, xerophytic, succulent species, of about one meter in height. Its leaves are variegated and have involute and thorny margins. Its flowers are grouped in raceme-like inflorescences (Forzza et al., 2015). Flowering occurs mainly in the months of February and April, generally after the dry season, thus varying according to the region where the population occurs. Fruiting occurs between March and April, and fruit maturation can take about six months until the fruit is fully ripe (Pereira and Quirino, 2008). The floral axis, flowers and ripe fruits are reddish, conferring ornamental potential as cut flowers (Beckmann-Cavalcante et al., 2017b).

Its ornamental potential comes from its colorful flowers and fruits, which can be used in arrangements, vases, and ornamentation of squares and gardens. The species has high-strength fibers, which can be used to make strings, fishing lines, fabrics, baskets, hammocks, and hats, among other handicraft products. It also has medicinal properties that act against inflammation, pain, and gastric ulcers (Suárez et al., 2020).

In nurseries and restoration sites, fruit and seed biometrics are used for taxonomic identification of plant varieties and phenotypic changes (Vasconcelos et al., 2015), as well as for determining species response and adaptation to different ecosystems (Espitia-Camacho et al., 2020). Additionally, biometric and morphological traits, such as shape, size, and weight of fruits and seeds, may be indicative of physiological quality for many species (Santos et al., 2012).

Despite the numerous Caatinga plant species, little is known about the physiological maturation and ideal harvesting point of their fruits and seeds for maximum quality and vigor. Little is also known about their potential use for the recovery and conservation of ecosystems (Oliveira et al., 2020). Native species cultivation, whether for economic or conservation purposes, requires knowledge about ecophysiology and seed germination of species, so that ideal seedling production practices could be developed.

Seed germination can be inhibited or stimulated by light and/or light signals, which are captured by a system of receptor pigments, called phytochromes. This system is associated with triggering metabolic responses to light stimuli. Seeds are classified into three categories according to their sensitivity to light, which is called photoblastism. This phenomenon is associated with forms and mechanisms of action of phytochromes. The first category is called positive photoblastism, where seeds germinate only under white light. The second is negative photoblastism, where seeds germinate only in dark, and white light, otherwise, inhibits germination. Finally, the third category is light-insensitive seeds, where seeds germinate in both dark and white light (Takaki, 2001). It is known that light is an important factor for small seeds, such as those of Bromeliaceae and Cactaceae families (Meiado et al., 2008; Abud et al., 2010; Silveira et al., 2010; Coutinho and Silva, 2017; Nasser et al., 2019). Most plant species from Caatinga are classified as insensitive to light, which is not limited in most of this ecosystem, so it may influence the regeneration of seed bank species (Meiado et al., 2012). However, this functional characteristic has been little explored in studies on native seeds of the Caatinga.

Studies on *N. variegata* propagation are scarce (Silveira et al., 2009; 2011), and further clarification is needed on the morphological and functional traits of its seeds, in addition to its germination and factors that affect it. Therefore, the objective of this study was to evaluate the morphological traits of fruits and seeds at different stages of maturation, as well as some germination characteristics of *N. variegata*.

MATERIAL AND METHODS

Fruits of *Neoglaziovia variegata* were collected in the Experimental Field of Caatinga of Embrapa Semiárido (09° 04' 16.4" S, 40° 19' 5.37" W) in 2018. After collection, ripe bunches were separated to be characterized morphologically before seed processing. Afterwards, processed seeds were packed in cloth bags and kept in a cold chamber (T = 10 °C; RH = 60%) until testing.

Morphological characterization of bunches, fruits, and seeds of Neoglaziovia variegata

After collecting bunches, the experiment was conducted in the first half of 2018. First, the harvested bunches were separated into green and ripe (purple color). After selection, only 10 ripe bunches were morphologically characterized. They were evaluated for their weight (in g; with a 0.001-g precision scale), length from base to apex (in cm; with a graduated ruler), and the number of fruits. Then, we characterized fruits from three positions of the bunch (lower third [base], middle third [middle], and upper third [top]) for length, widest diameter, average weight, and the number of seeds in all fruits. For length and width, a 0.05-mm-precision digital caliper was used, and for average weight, a 0.001-g-precision scale was used.

Finally, seed biometrics was performed, evaluating: 100-seed weight, seed length, and seed width, using a millimeter sheet and ruler. To do so, a mixture of seeds from the upper, middle, and lower thirds of the bunch was used. 100-seed weight was measured using a 0.001 g precision scale, with eight replications of one hundred seeds separately (Brasil, 2009). For water content, two 50-seed replications were collected, placed in aluminum capsules, and taken to an oven at 105 °C for 24 hours for measurements (Brasil, 2009).

Data were subjected to the Kolmogorov-Smirnov normality test and descriptive analysis, with values expressed as means, standard deviation, mean standard error, variance, kurtosis coefficient, coefficient of variation, using Origin Pro software.

Germination of Neoglaziovia variegata (Arruda) Mez seeds from fruits with different stages of maturation

The experiment was conducted between February and April 2019. As previously described, seeds were from 2018 collections. The seeds were extracted from fruits at two maturation stages, green and ripe (purple). The experimental design was completely randomized. The physiological quality of each seed sample was evaluated by germination and seedling performance tests, using four 50-seed replications.

Before all germination tests, the seeds were subjected to asepsis with immersion in distilled water and neutral detergent (10 drops.L^{-1}) for five minutes to reduce the fungal incidence and affect results (Silveira et al., 2009). Then, seeds were placed in colorless transparent acrylic boxes - gerboxes (11 x 11 x 3.5 cm) distributed on two blotter paper sheets, moistened with distilled water at 2.5 times paper mass. These boxes were kept in a germination chamber set at 30 + -2 °C and a 12-hour photoperiod (Silveira et al., 2011). The number of germinated seeds (first primary root emitted) and normal seedlings formed were counted until 17 days after sowing when counts stabilized.

The first count of normal seedlings (FCNS) was performed after 12 days after the experiment started, and the last count (LCNS) was after 17 days. Seedlings were considered normal when they had the potential to continue their development and generate normal plants (Brasil, 2013), with well-developed radicle and primary leaves. In the final count, abnormal seedlings (AP%), those with damage and deformation in their vegetative structures, were also considered (Brasil, 2013).

From the data obtained, percentages of germinated seeds (G%), normal seedlings in both counts (FCNS and LCNS), average germination time (AGT) (Labouriau, 1983), germination speed (GS) (Labouriau, 1983), and germination speed index (GSI) (Maguire, 1962).

Averages were subjected to analysis of variance and compared by Student's t-test for independent samples at 5% probability, using AgroEstat® software (Barbosa and Maldonado-Júnior, 2012).

Effect of light on seed germination of Neoglaziovia variegata (Arruda) Mez

Photoblastism of *N. variegata* seeds was determined by a completely randomized design test with four treatments. The treatments comprised the number of days seeds were kept in total absence of light during 31 days of testing: environmental photoperiod conditions for 31 days (zero days in the dark); 10 days in the dark + 21 days in environmental conditions; 20 days in the dark + 11 days in environmental conditions; and 30 days in the dark + 1 day in environmental conditions.

Seeds used in this test came from the mixture of seeds extracted from green and mature fruits and stored in cotton bags at 10 °C and 40% relative humidity. The test was conducted between October and November 2019, under uncontrolled environmental conditions, with temperatures ranging between 34 and 24 °C and 12.5 h photoperiod.

Given the restriction in the number of seeds, four 25-seed replications were used. Seeds were sown on two blotting paper sheets moistened with 13 mL distilled water, which were placed in gerboxes. Light absence treatments were carried out in gerboxes wrapped with two layers of aluminum foil. After removing the aluminum foil, germinated seeds were counted daily, considering the radicle protrusion. Percentages of normal and abnormal seedlings, as well as seedling growth, were evaluated at the end of the experiment.

All data were subjected to analysis of variance using AGROESTAT® software. Accumulated data on germination and seedling formation percentages were fitted to Boltzmann's nonlinear sigmoidal regression model. The results obtained at the end of the experiment were subjected to polynomial regression, using Origin Pro software.

RESULTS AND DISCUSSION

Biometric characterization of bunches, fruits, and seeds of Neoglaziovia variegata (Arruda) Mez

All data on biometric traits of *N. variegata* bunches, fruits, and seeds showed a normal distribution. Most of them had symmetrical and platykurtic distribution (kurtosis < 0) except fruit mean weight from the upper part of bunches, which was symmetrical and leptokurtic (kurtosis > 0) (Table 1).

The species *N. variegata* has bunch-like racemose fruits weighing 69.4 ± 23 g, with an average length of floral scape of 35.1 ± 7.8 cm and the number of fruits per bunch of 73.3 ± 20.6 units (Table 1). Regarding the fruit position in the bunch, means and standard deviations showed that this parameter does not interfere with fruit size. However, the lower and middle thirds (base and middle, respectively) had heavier fruits (Table 1).

Many biological phenomena are complex that, often and under different situations, may require mathematical models to be detailed (Kato and Bellini, 2009). Thus, representing the morphometric characteristics of *N. variegata* fruits and seeds via mathematical variables and distribution curves can give a systematic idea of these biological systems. The term kurtosis indicates the number of results that are more concentrated (leptokurtic) or dispersed (platykurtic) around a mean value. Fruits and seeds of native species often have greater variability than cultivated species, which may be due to their genetic diversity (Araújo et al., 2015). Most morphological traits of *N. variegata* had a symmetrical and platykurtic normal distribution, demonstrating their genetic variability, even within the same population (Table 1).

The bunches evaluated in this study were highly fruitful, but the fruits were smaller than those from another area (Pereira and Quirino, 2008). Some authors have stated that once triggered, flowering phenophase and fruit ripening in *N. variegata* are not influenced by the weather. This is because they occur after the rainy season when there is minimal rainfall over the Caatinga; therefore, these processes are only influenced by internal factors (Pereira and Quirino, 2008). However, most of the rainfall events in arid regions and drier years of semi-arid regions are low (≤ 5 mm). Thus, these rains can play a crucial role in seed production and plant regeneration in such ecosystems (Sala and Lauenroth, 1982; Wang et al., 2019). In short, fruit and seed yield components are influenced by a combination of genetic and environmental factors (Pratap et al., 2018; Wang et al., 2019).

Germination of Neoglaziovia variegata (Arruda) Mez seeds from fruits at different maturation stages

Water contents were 10.9% and 8.2% in seeds from green and ripe fruits, respectively. Moreover, the 100-seed weight of unripe fruits was 1.0 g and 1.3 g for ripe ones. However, physiological quality (germination and vigor) was not different between green and ripe fruits (Table 2).

During maturation, the seed water content decreases concomitantly with an increase in weight (Nogueira et al., 2013). The same behavior was observed for *N. variegata* seeds from green and ripe fruits in our study. However, no significant difference was noticed in seed physiological quality, which had germination of around 96% (Table 2). In a study on in-vitro germination of *N. variegata* seeds, from freshly harvested fruits in a temperature-controlled growth room (27 ± 1 °C), germination was almost 100% in a 16-hour photoperiod (Silveira et al., 2009), as the findings of our study (Table 2).

Precise maturation indices improve seed harvesting, for cultivated or native species, avoiding collecting immature, low physiological quality, or even late seeds, with the risk of dispersion or predation of already mature fruits and seeds. There is a lack of scientific information about maturation indicators (size, color, dehiscence, opening) for forest species, whether arboreal-shrub or herbaceous (Schmidt et al., 2019). Some studies have shown that fruit color indicates the physiological maturity of seeds of arboreal-shrub species in the Caatinga, such as *Syagrus coronata* (Mart.) Becc., *Mimosa caesalpiniifolia* Benth. (Nogueira et al., 2013), *Cenostigma pyramidale* (Tul.) Gagnon & GP Lewis (Lima et al., 2012). Aiming for seed quality, it is empirically recommended that caroá fruits should be harvested purple, when they are fully ripe. However, in the field, there is a large amount of green and purple fruits of similar sizes (on average 8.5 x 9.5 mm and 0.8 g, Table 1), whose seeds have similar physiological quality (Table 2). In this study, we verified that the indicator of *N. variegata* seed maturation should not be based only on the color, but also on the size of its fruits.

Table 1. Descriptive statistics for morphometric characterization of *Neoglaziovia variegata* (Arruda) Mez bunches, fruits, and seeds.

	N	Mean	σ	MSE	Variance	Kurtosis	CV	Minimum	Median	Maximum	KS	(p)
						Bunch						
Weight (g)	10	69.39	22.8436	7.2253	522.0477	-4.23048	0.32927	46.30	63.1	120.2	0.28814	0.31459
Length (cm)	10	35.09	7.7989	2.4662	60.82322	-4.19726	0.22225	23.80	32.7	50.0	0.21057	0.7238
Fruit number	10	73.30	20.58074	6.5082	423.5667	-4.20052	0.28077	52.00	70.0	124.0	0.20582	0.75488
						Fruit						
						Length (mm)						
Upper third	30	7.42	1.6206	0.2959	2.62654	-2.86307	0.21833	4.99	7.16	11.6	0.12186	0.75921
Middle third	30	9.00	1.1328	0.2068	1.28335	-3.54503	0.12585	6.69	9.11	11.1	0.09407	1
Lower third	30	9.19	1.3443	0.2454	1.80734	-3.70086	0.14632	5.88	9.35	11.8	0.1251	0.72205
Mean	90	8.54	1.58011	2.4968	768.3722	-3.27699	0.18508	4.99	8.81	11.8	0.08654	0.49095
						Width (mm)						
Upper third	30	8.11	1.1672	0.2131	1.36231	-2.80983	0.14398	6.37	7.84	10.33	0.1469	0.49839
Middle third	30	9.91	1.0008	0.1827	1.00168	-4.00323	0.10096	7.82	10.19	11.23	0.16002	0.38764
Lower third	30	10.17	1.1960	0.2184	1.43042	-3.75657	0.11764	7.48	10.23	12.5	0.12495	0.72375
Mean	90	9.40	1.4447	0.1523	2.08715	-3.29944	0.15377	6.37	9.86	12.5	0.13583	0.06558
						Weight (g)						
Upper third	30	0.51	0.1699	0.0537	0.02887	2.78568	0.33548	0.28	0.40	0.84363	0.19578	0.82228
Middle third	30	0.91	0.1733	0.0548	0.03004	-8.41099	0.18989	0.59	0.94	1.15107	0.16823	1
Lower third	30	0.95	0.2301	0.0728	0.05292	-9.57908	0.24352	0.57	1.05	1.23663	0.27001	0.39112
Mean	90	0.79	0.2755	0.0503	0.0759	-4.19282	0.3496	0.29	0.81	1.237	0.15476	0.42982
						Seed number						
Upper third	30	12.27	4.0847	0.7458	16.68506	-3.21413	0.33299	6.0	11.0	21	0.15509	0.42708
Middle third	30	19.70	5.4719	0.9990	29.94138	-3.43727	0.27776	3.0	20.5	34	0.17752	0.26822
Lower third	30	16.67	5.3649	0.9799	28.78161	-3.56754	0.32189	3.0	17.5	25	0.19144	0.19475
Total	90	16.21	5.8299	0.6145	33.98864	-3.12321	0.35963	3.0	17.0	34	0.1316	0.08074

N: number of assessed plants; σ: standard deviation; MSE: mean standard error; CV: coefficient of variation; KS and (p): Kolmogorov-Smirnov test statistics.

Table 2. Germination and vigor of *Neoglaziovia variegata* (Rue) Mez seeds from fruits at two maturation stages, as a function of fruit color.

Fruit color	G (%)	AGT (days)	GS (days)	GSI (seedings.day^{-1})	NS (%)	AS (%)
Green	96.0 a	2.18 a	0.46 a	29.04 a	34.25 a	13.75 a
Purple	96.5 a	2.38 a	0.42 a	27.51 a	35.50 a	12.75 a
CV (%)	4.54	11.81	10.81	10.78	12.37	26.05

G: germination/radicle emission; AGT: average germination time; GS: germination speed; GSI: germination speed index, NS: normal seedlings; AS: abnormal seedlings.

Light effect on Neoglaziovia variegata (Rue) Mez seed germination

Sigmoidal curves of root emission and normal seedling formation accumulated over time (Figure 1) and regression curves of each variable as a function of incubation time in the dark (Figure 2) demonstrate how exposure to light influences *N. variegata* seed germination. Seeds in a 12.5-h photoperiod started germinating a few days after sowing and with t50 (time needed for 50% of seed population to germinate) of around 11 days. After 31 days, about 90% of seeds under light conditions germinated (Table 3; Figure 2). Otherwise, seeds incubated for 10 to 30 days in dark conditions germinated only after being exposed to the environmental conditions (Figure 1). Moreover, as seeds remained longer in the dark, germination, and hence seedling formation, decreased. At the end of the experiment, seeds incubated for 10 and 20 days in the dark showed about 84% and 73% germination, respectively. The t50 of these seeds was respectively 18 and 27 days (Table 3). Seeds kept in the dark during the entire experiment showed less than 10% germination (Figures 1 and 2).

Notably, the t50 of seeds kept for 10 and 20 days in the dark was calculated from the sowing day, while there were no light conditions for germination. Therefore, from the moment that adequate light conditions were re-established, seeds were already hydrated and could reach 50% germination in less time than those under light conditions as of the beginning of the experiment. In part, this process can be compared to hydropriming techniques, in which seeds are hydrated to the point of starting the first germination processes, but not to radicle emission. So, when the ideal conditions for germination (water availability, temperature, light) are re-established, germination occurs faster (Nascimento et al., 2021).

Likewise, the seedling formation had reductions in final percentages of t50 and seedling size (Figures 1 and 2; Table 3). Seeds exposed to light since the beginning of the experiment had a higher percentage of formed seedlings (86.75%) than the others, with 75%, 13%, and 2% of seedlings whose seeds were kept in the dark for 10, 20, and 30 days, respectively (Figures 1 and 2). The development of normal seedlings (shoot length and root length) followed the same trend, in which increasing incubation times in the dark decreased seedling growth (Figures 2c, d).

Regardless of the Brazilian ecosystem, like *N. variegata* from the Caatinga; *Bromelia antiacantha*, from the Atlantic Forest (Nasser et al., 2019); and *Ananas ananassoides* (Baker), from the Cerrado, also have seeds with a positive photoblastic response (Silveira et al., 2010). Moreover, some Caatinga native species also show the same response to light, such as *Bromelia laciniosa* Mart. ex Schult, and cacti such as *Pilosocereus catingicola* ssp. *salvadorensis* (Meiado et al., 2008), *Pilosocereus pachycladus* Ritter (Abud et al., 2010), *Diplopterys pubipetala*, and *Barnebya harleyi* (Coutinho and Silva, 2017).

For some small seeds, photoblastism is a mechanism for germination to occur close to the soil surface, benefiting from canopy openings and hence favoring seedling initial development (Nasser et al., 2019). Seed responses to light are an ecological factor and may be related to where germination takes place, as well as how seed phytochromes respond to the environment (Batlla and Benech-Arnold, 2014). A study on *Miconia chartacea* seeds (positive photoblastic) incubated in the dark showed that, when exposed to light, they have germination percentages higher than those seeds constantly exposed to light (Escobar and Cardoso, 2015), a result different from ours. In the case of the bromeliad *N. variegata*, it is not known whether continuous light is needed for a certain time or flashes of light after the start of the hydration process, but light indeed is essential for its seeds to fulfill its germination process (Figures 1 and 2).

Table 3. Parameters of germination (radicle protrusion and seedling formation) curves for *Neoglaziovia variegata* (Arruda) Mez seeds, accumulated in time and adjusted to the Boltzmann sigmoidal model.

Curve parameter	Biological meaning	Treatment			
		Light	10 d dark	20 d dark	30 d dark
		Radicle (R)			
A1	Initial R (%)	0	0	0	NA*
A2	Final R (%)	87.71331	83.93284	73.07266	
x0	t50 (days)	11.0137	17.95566	27.23054	
dx	Kinetic activation	2.16922	1.33497	0.94744	
R^2		0.99366	0.99481	0.97863	
		Seedling (S)			
A1	Initial S (%)	0	0	0	NA*
A2	Final S (%)	85.02338	76.58841	13.00694	
x0	t50 (days)	16.93056	23.72783	29.26649	
dx	Kinetic activation	2.26814	1.63778	0.10723	
R^2		0.98943	0.98968	0.9964	

Equation of the curve: $y = ((A1 - A2)/1 + e^{(x-xo)/dx}) + A2$
NA*: non-adjusted; t50: time needed for 50% of seeds to germinate.

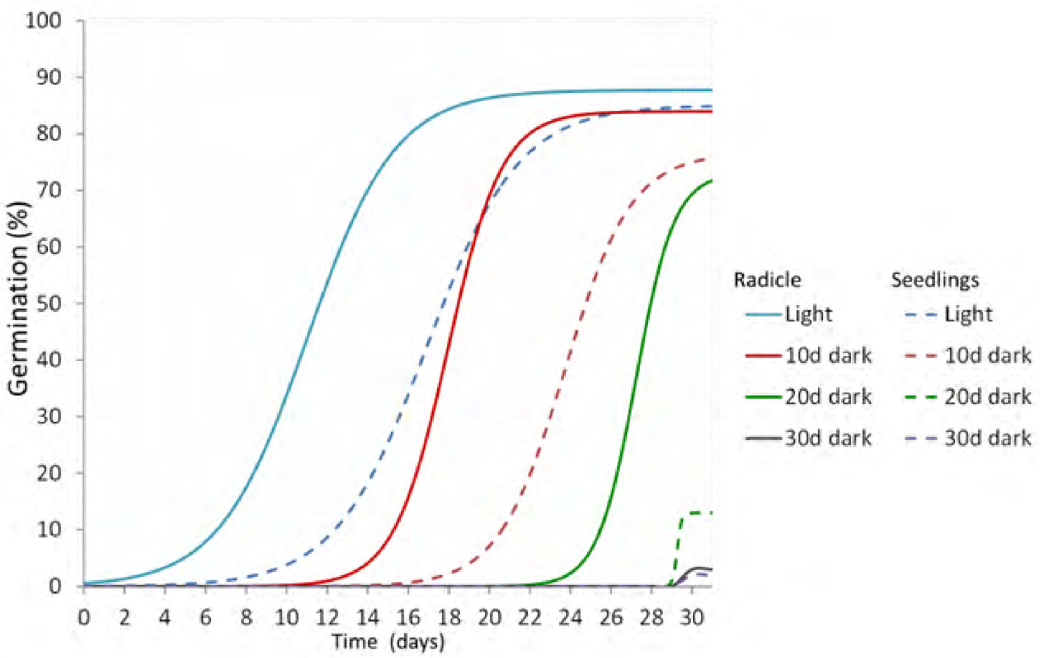

Figure 1. Germination curves (radicle emission or seedling formation) of *Neoglaziovia variegata* (Arruda) Mez seeds, accumulated in time in response to different periods of dark.

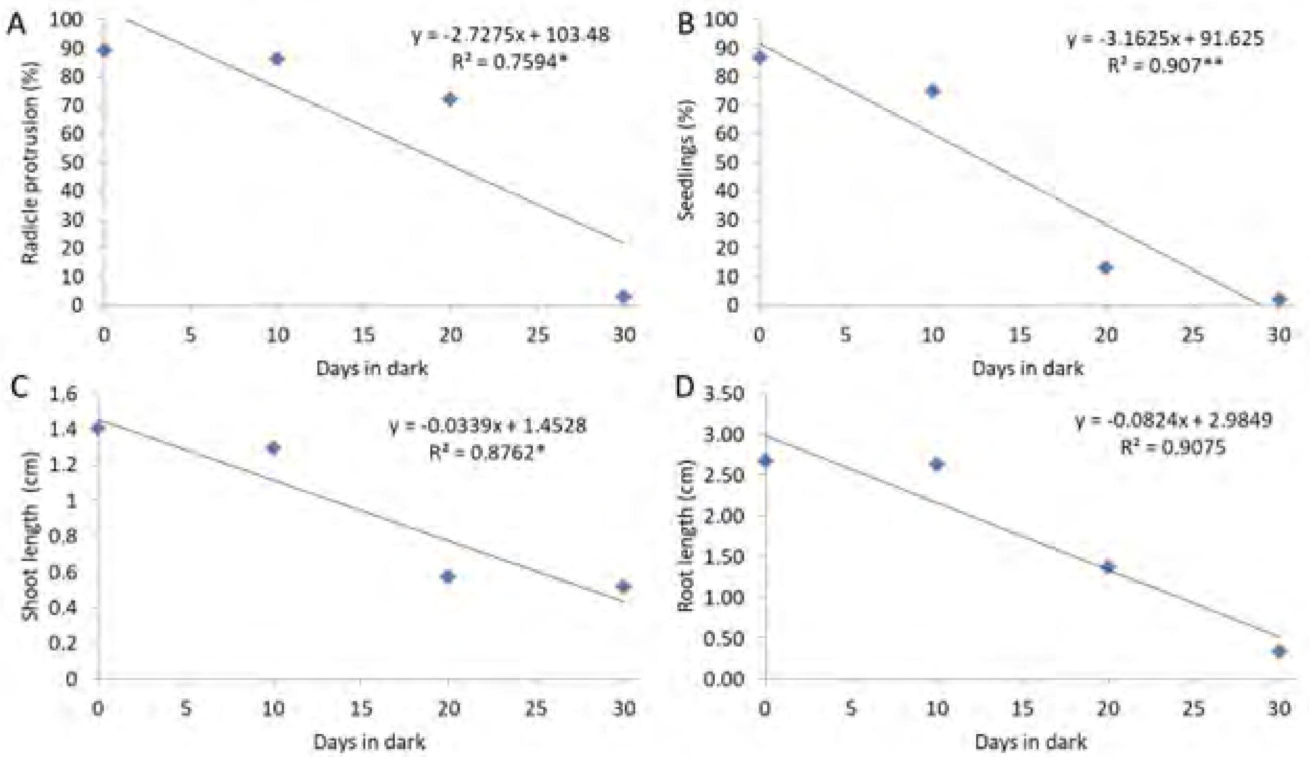

Figure 2. Radicle emission (A), seedling formation (B), shoot length (C), and primary root length (D) of *Neoglaziovia variegata* (Arruda) Mez seeds under different periods of the dark after sowing.

CONCLUSIONS

Neoglaziovia variegata fruits and seeds have great morphometric variability.

Color is not an efficient maturation index for similar-size fruits; further studies are still needed to define effective indicators for the harvesting of *Neoglaziovia variegata* seeds.

Neoglaziovia variegata seeds are positive photoblastic and do not germinate if kept in dark conditions.

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