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Original Paper
Morpho-physiological classification of seeds and morphology of fruits and seedlings of *Richeria grandis*

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Abstract

The objective was to describe and characterize the fruit morphology, seeds, seedlings and germination of *Richeria grandis*. Mature fruits were collected from matrix trees located in gallery forests. After, morphological descriptions were performed. Biometric measurement of fruits and seeds were carried out. A total of 650 seeds were randomly separated, with 20% being used for germination and humidity testing on the same day of collection and the others stored for 5, 10, 15 and 20 days. The germination test was performed in a gerbox, conducted in a laboratory chamber of the type B.O.D (biochemical oxygen demand) with a photoperiod of 12h and a temperature of 25 ºC ± 1 ºC. Periodic observations were made to monitor the development of the seedlings until the first germinated seeds reached the full seedling stage. The fruit of *R. grandis* is a simple, dry, capsule type septifraga, dehiscent and schizocarpic, with average length of 16.24 mm and an average width of 8.57 mm. The seed is an ellipsoid shape, containing a sarcotesta and has an average length of 7.40 mm and an average width of 4.57 mm. *R. grandis* presents recalcitrant seeds, which emerge in a Phanero-epigene-foliaceous manner. The radicle breaks the integument on the second day after sowing, the collection is evidenced on the 10th day, separating the primary root from the hypocotyl. At day 16, the cotyledons expand from the forehead, positioned at a 90º angle to the hypocotyl. The protophyll and the epicotyl appear only after the 50th day, characterizing the complete seedling.

Key words: gallery forests, germination, *in situ* conservation, morphofunctional ecology, recalcitrant.

Resumo

Objetivou-se descrever e caracterizar a morfologia de frutos, sementes, plântulas, e germinação de *Richeria grandis*. Frutos maduros foram coletados de árvores-matrizes localizadas em matas de galeria e, posteriormente, foram realizadas descrições morfológicas. Procedeu-se a mensuração biométrica dos frutos e sementes. Ainda, após beneficiamento, foram separados de forma aleatória, 650 sementes, em que 20% foram utilizadas para germinação e teste de umidade no mesmo dia da coleta e as outras, armazenadas durante 5, 10, 15 e 20 dias. O teste de germinação foi realizado em gerbox, feito em câmeras do tipo B.O.D. com um fotoperíodo de 12 h e temperatura de 25 ºC ± 1 ºC. Realizou-se observações periódicas para o acompanhamento do desenvolvimento das plântulas até que as primeiras sementes germinadas atingissem o estádio de plântula completa. O fruto da *R. grandis* é simples, seco, do tipo cápsula septífraga, deiscentes e esquizocarpos, com comprimento médio de 16,24 mm e largura média de 8,57 mm. A semente é elipsoide com presença de sarcotesta, com comprimento médio de 7,40 mm e largura média de 4,57 mm. A *R. grandis* apresenta sementes recalcitrantes. A emergência é do tipo Fanero-epígeo-foliáceo. A radícula rompe o tegumento no segundo dia após a semeadura, o coleto é evidenciado ao 10º dia, separando a raiz primária do hipocótilo. A partir do 16º dia, os cotilédones expandem-se da testa, posicionados em um ângulo de 90º em relação ao hipocótilo. O protófilo e o epicótilo surgem somente após o 50º dia, caracterizando a plântula completa.

Palavras-chave: florestas de galeria, germinação, conservação *in situ*, ecologia morfofuncional, recalcitrante.

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Introduction

The Phyllanthaceae Martinov family was traditionally recognized among the Euphorbiaceae Juss. However, phylogeny (APG II 2003) revealed the need for its recognition as one independently (Souza & Lorenzi 2008). Phyllanthaceae Martinov is one of the most diversified families of Malpighiales order (clade Eurosidae I), which covers about 2,000 species and 60 genres, with pantropical distribution (Hoffmann *et al.* 2006; Souza & Lorenzi 2008). In Brazil, there are 14 genres and about 118 species, the majority belonging to *Phyllanthus* L. (BFG 2018; Flora of Brazil 2020, under construction).

Phyllanthaceae includes species of varied habits, ranging from herbs to trees, with alternating leaves, racemic inflorescences, axillaries, non-showy flowers, unisexed (monoic or dioecious plants), bifid stiletes and schizocarpic fruits (Souza & Lorenzi 2008). According to Judd *et al.* (2009), this family is presumably monophyletic. Among the main characteristics that differentiate them from the Euphorbiaceae are the absence of latex, the presence of two ova at each ovary loci (Secco & Rosario 2015), and the absence of aril (caruncle) in the seeds (Souza & Lorenzi 2008; Martins & Lima 2011).

The *Richeria grandis* Vahl belong to this family. It is a native shrub or tree that is not endemic to Brazil, although it has a confirmed occurrence from North to South of the country, in the geographical areas: Amazônia, Caatinga, Cerrado and Mata Atlântica (Kuhlmann 2012; Flora of Brazil 2020, under construction). The species is classified as very rare (Oliveira-Filho 2006), and is frequent only in humid lands (Silva-Júnior & Pereira 2009) with vegetation types of Campos de Várzea, Ambrófilas Forests, and, mainly, Ciliary and Gallery Forests (Kuhlmann 2012; Martins 2015; Freitas *et al.* 2016; Cerqueira *et al.* 2016; Gomes *et al.* 2016; Silva 2017).

However, the destruction, alteration and anthropogenic fragmentation in Ciliary and Gallery Forests entails an increasing state of degradation (Mallmann *et al.* 2016; Silva *et al.* 2017) and, in the long term, causes the extinction of arboreal species which are unique to these environments, such as *R. grandis*. Thus, the transfer of information necessary for the *ex situ* and *in situ* conservation of this species, besides promoting the restoration of these environments, is necessary.

Fruit and seed studies, responsible for the new generations (Oliveira 2012; Silva *et al.* 2015), as well as seedlings, allow conservation of biodiversity, provide subsidies for knowledge of seed storage and germination, seedling production (Oliveira & Conduro 2004; Davide & Silva 2008; Oliveira 2012; Mendonça *et al.* 2016; Bao *et al.* 2016), and broaden the knowledge about taxa with an ecological view (Oliveira & Paoli 2016). Furthermore, biometric characterization is also important to detect genetic variability within populations of the same species (Silva *et al.* 2008; Gonçalves *et al.* 2013; Ribeiro *et al.* 2016b).

However, Phyllanthaceae are still poorly studied (Secco & Silveira 2016), especially *R. grandis* species. The objective of this study was to describe the morphology and biometric aspects of the fruit and seed, the morphofunctional characteristics and seedling development, and to obtain the physiological classification of the storage capacity of the seeds of *Richeria grandis* Vahl.

Material and Methods

This study was carried out in the Laboratory of Forest Seeds and in Seedling Nursery belonging to the Centro Integrado de Propagação de Espécies Florestais of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (CIPEF - UFVJM) in Diamantina, MG, from January to March 2017.

Fruit and seed collection and processing

Mature fruits of *R. grandis* were collected at the beginning of dehiscence (January 2017), with the first cracks/crevices, but without seed dispersion, with the aid of a trimmer, of 18 matrix trees. The matrices, spaced at least 50 m apart, were selected from three well-preserved gallery forests in the Parque Estadual do Biribiri (PEBI), located between the coordinates 18º11’37.5”S, 43º34’41.2”W in Diamantina, Minas Gerais (altitude between 1,348 and 1,373 m). After collection, fruits were packed in polyethylene and transported to the CIPEF Seed Laboratory. Simultaneously to the fruit collection, the botanical material was collected and, after performing all the necessary procedures for its registration and deposit in the Herbário Dendrológico Jeanine Felfili (HDJF), presented the identification number HDJF1565.

The climatic regime of the Southern Espinhaço mountain range, Diamantina region, is typically subtropical at altitude, Cwb according to the global classification system of Köppen, characterized as humid temperate climate with dry winter and temperate summer (Alvares *et al.* 2013; EMBRAPA 2017). The average annual precipitation varies from 1,250 to 1,550 mm and the annual average temperature is in the range of 16º to 26 ºC, being predominantly mild throughout the year. Relative air humidity is almost always around 80% (INMET 2017).

After collection, the fruits were benefited manually, eliminating malformed fruits or mechanical and/or predatory injuries. Some seeds were discarded since they had small holes containing small larvae close to the embryonic axis after fruit processing. Three types of evaluations were carried out: physical characterization of fruits and seeds, physiological classification for seed storage, and morphological classification of germination and initial development of *R. grandis* seedlings.

Morphological aspects and biometric characters of fruits and seeds

Observations were made through a random sampling of 30 fruits, making descriptions associated with external structures, such as type, shape, coloring (following the Munsell Plant Tissue Color Book 2012), dehiscence and number of seeds per fruit. Seed characteristics observed were: seed form and its border, color (Munsell Plant Tissue Color Book 2012), texture and consistency of the integument. The terminology for fruits and seeds was adopted in Vidal & Vidal (2003) and Barroso *et al.* (1999).

The weight of one hundred seeds was determined according to the recommendations of the Rules for Seed Analysis (Brasil 2009) with three replicates (300 total seeds), using an accuracy scale of 0.001 g.

To evaluate the biometric data, another 50 fruits were measured (length and width) (5 replicates of 10 fruits) at random and 50 seeds resulting from the fruits measured. Seed removal with sarcotesta from within the fruits was done manually after biometric measurement of fruits. After sarcotesta removal from the seed, from macerations on sieve with steel meshes varying of 0.8 mm, under running water, the biometric measurement of seeds without sarcotesta was carried out. Biometric variables were in millimeters, determined using a universal pachymeter, with an accuracy of 0.01 mm, the measured length from the base to the apex and the width measured in the midline of the fruits and seeds with and without sarcotesta.

For each biometric characteristic, the mean, median, standard deviation and coefficient of variation were calculated as stated by Araújo Neto *et al.* (2002). Biometric data was classified by frequency distribution and plotted in frequency histograms (Oliveira *et al.* 2000), where the number of classes was determined by the Sturges rule, using the formula by Lana *et al.* (2013).

Seed germination and storage

For the germination study, after field collection and transfer to the Laboratory of Forest Seeds, other seeds of the same batch were immediately benefited by removing the sarcotesta by means of friction on a steel mesh sieve (0.8 mm) under running water, disinfected with 1% sodium hypochlorite for 3 minutes, and then washed in running water for 10 minutes (Brasil 2009).

After treatment, 650 seeds were randomly separated, from which 130 seeds were used for germination and humidity testing on the same day of collection and processing (time 0) while the others were stored for 5, 10, 15 and 20 days (treatments).

Moisture content (%) was determined by the greenhouse method with forced air circulation, at 105 ± 3 ºC during 24 h (Brasil 2009), containing three subsamples of 10 seeds (30 seeds). Moisture content of the seeds was determined on the date of collection and after the various storage periods.

The germination test was carried out in four replicates of 25 seeds (100 seeds), seeded in plastic boxes (gerbox) and the germ paper was autoclaved, distilled water (Brasil 2009), made in BOD type germinator (Biochemical Oxygen Demand) under fluorescent white light with controlled photoperiod of 12 h, set at a temperature of 25 ºC ± 1 ºC (Brancalion *et al.* 2010; Figliolia & Calvi 2011).

The asepsis of the boxes and tweezers was performed with alcohol at 70% and the germitest paper in a vertical autoclave at 121 ºC and a pressure of 1 atm for 15 minutes.

Germination assessment was carried out daily, starting the first day after the installation of the experiment and closed on the 30th day, adopting the root protrusion as germination criterion.

The other seeds were kept in a shaded environment, dried and stored in paper bags outdoors for the 4 periods of time. The moisture test and the germination were carried out in the same way according to the methodology detailed above.

Germination analysis was performed by calculating germination percentage (G%), Germination Speed Index (IVG) and Mean Time of Germination (TMG), in an experiment with a completely randomized design, consisting of 5 treatments (storage time) with four replicates. The IVG was calculated according to a formula by Maguire (1962), and the TMG, according to Labouriau (1983). The physiological classification of the seeds during storage was done according to the protocol proposed by Hong & Ellis (1996).

For statistical analysis, the assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test) were tested at a 5% level of significance and the data submitted to the F test for variance analysis at a significance level of 5%, and the means were compared by the Tukey test at a 5% error probability level. For all analyses, the free statistical software R Development Core Team R: a language and environment for statistical computing (2017) and the ExpDes package (Experimental Designs) were used (Ferreira et al. 2013).

Germination, morphology and seedling development

Seedling development was carried out in a BOD-type germinator (under fluorescent white light with controlled photoperiod of 12 h, adjusted at 25 ºC ± 1 ºC) and later in a greenhouse with irrigation in a controlled spray system (temperature around 28 ºC and relative air humidity above 80%), making observations every 2 days until the first seeds reached the stage of complete seedling (Brasil 2009).

For the visualization and description of the root protrusion, 30 seeds were sown in a plastic box (gerbox), using as a substrate the germ paper autoclaved, moistened with distilled water when necessary.

Simultaneously, another 30 seeds of the same batch were sown in a gerbox and, as substrate, the mixture of 70% vermiculite and 30% charcoal rice husk, also moistened with distilled water when necessary. The sowing was made so that seeds were not visible, and in this way, seedling emergence could be observed and described. As soon as this was visualized, seeds were transplanted into rigid plastic conical tubes of 180 cm^3 capacities with the same substrate for the continuation of the development in a greenhouse.

The classification of the morphofunctional characteristics was made from direct observations of the seedlings. The classification system used (Miquel 1987) comprises five morphofunctional types of seedlings: Fanero-Epígeo-Foliacea, Fanero-Epígeo-Armazenador, Fanero-Hipógeo-Armazenador, Crypto-Hylogeo-Armazenador, and Crypto-Epígeo-Armazenador (Garwood 1996). These morphofunctional types represented the dichotomous characteristics of exposition (phanerotic or cryptocotilar), position (epigial or hypogeal) and texture (folioceous or storage) described by Ressel *et al*. (2004).

Morphological descriptions were done based on sight. The most vigorous seedlings were used. They had their root systems exposed by washing them under running water, thus facilitating the visualization of structures under study. Germination was considered from radicle emission and the term "seedling" was used when the cotyledons and the first eophil (first-order prototype) were observed. Root description (primary and secondary), coleto, hypocotyl, cotyledons, epicotyl and the first prototyph were then performed.

Results

Morphological aspects and biometric characters of fruits and seeds

Richeria grandis fruits are simple, dry type and capsulid, have a shape of oblong capsule, with three septa isolated, slightly concave, colored green to dark green (5GY 5/8) when ripe (Fig. 1c,d,g). The fruits open when ripe, leading to rupture of the septa parallel to the axis of the fruits and seeds, classified as longitudinal dehiscents (Fig. 1b,f,g). They are unilocular dysrheric, two seeded. Out of the 30 evaluated fruits, a seed was aborted while 100% of seeds were characterized as healthy.

The coriaceous endocarp, perfectly distinct from the outside of the pericarp, separates into three biophyte portions at the apical side. The seminiferous central column, with membranous and aliform borders, remains intact after the dehiscence of the fruit, where the seed is trapped, classified as schizocarpic fruits (Fig. 1a).

The seed is ellipsoid of light yellow color (5Y 2/8) characterized by a smooth and polished integument (Fig. 1e). Its sarcotesta (fleshy tissue that recovers the seed) is of red orange coloration (10R 5/10) (Fig. 1d). The weight of 100 seeds without the sarcotesta soon after the processing was 5.674 ± 0.764 g.

The descriptive analysis of fruit and seed dimensions with and without sarcotesta is shown in Table 1 and, to better understand their frequencies in each size class (7 classes), results are arranged in histograms (Fig. 2).

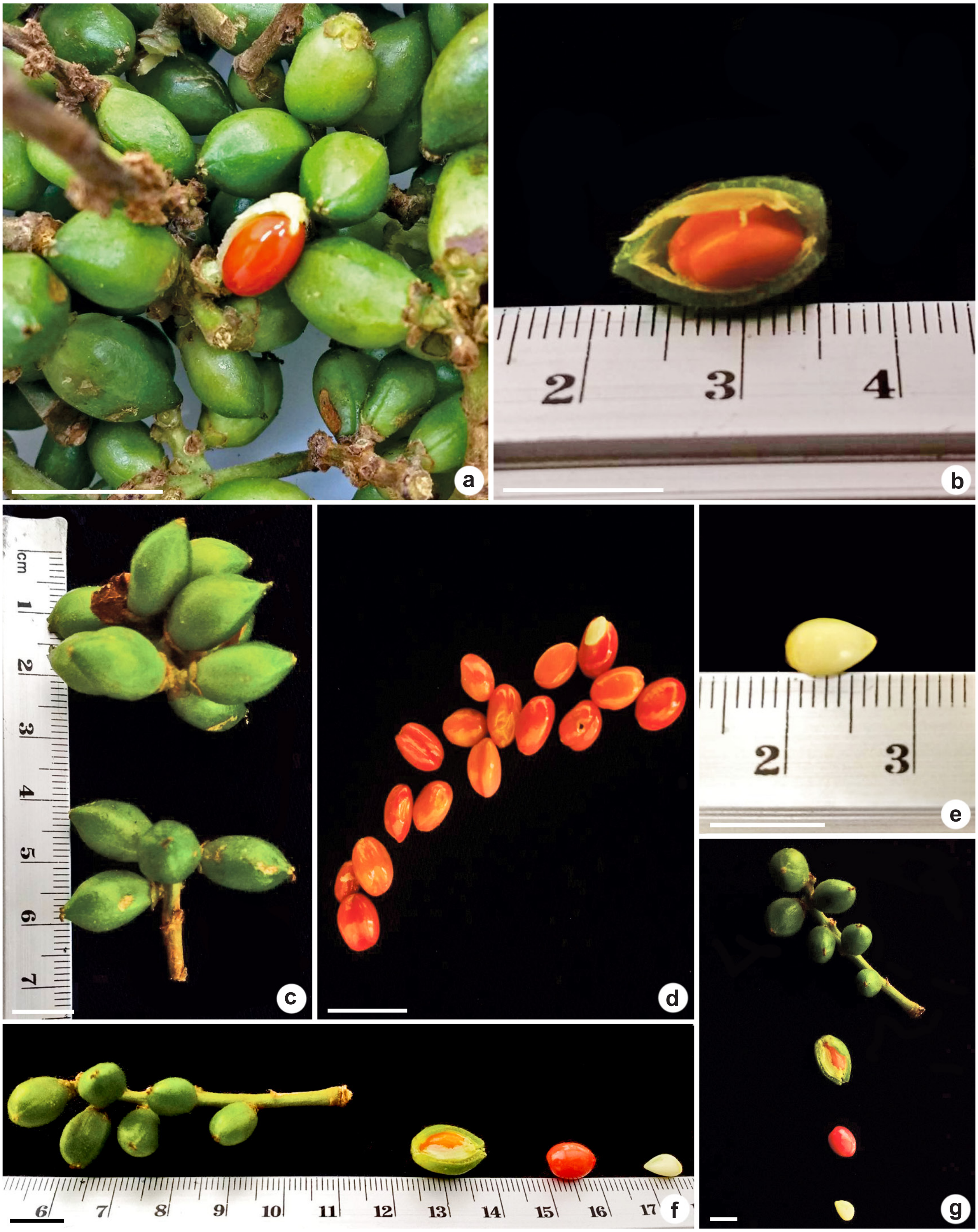

Figure 1 – a-g. Morphological characteristics of fruits and seeds of *Richeria grandis* (Phyllanthaceae) – a. presence of the central seminiferous column after dehiscence of the fruit; b. sarcotesta; c. simple, dry and capsulid type fruit; d. seed with sarcotesta; e. semen without seed; f-g. fruits, seed with sarcotesta and without sarcotesta. Scale bars: 1 cm.

Table 1 – Descriptive analysis of the dimensions (length and width) of fruits and seeds with and without sarcotesta of *Richeria grandis* (Phyllanthaceae).

Structure	Length			Width		
	Mean (mm)	**Medium (mm)**	**CV (%)**	**Mean (mm)**	**Medium (mm)**	**CV (%)**
Fruit	16.24 ± 1.16	16.20	7.12	8.57 ± 0.49	8.50	5.8
Seeds with sarcotesta	8.17 ± 0.47	8.20	5.35	5.62 ± 0.53	5.50	9.41
Seeds without sarcotesta	7.40 ± 0.51	7.50	6.85	4.57 ± 0.34	4.50	7.67

CV = Coefficient of variation

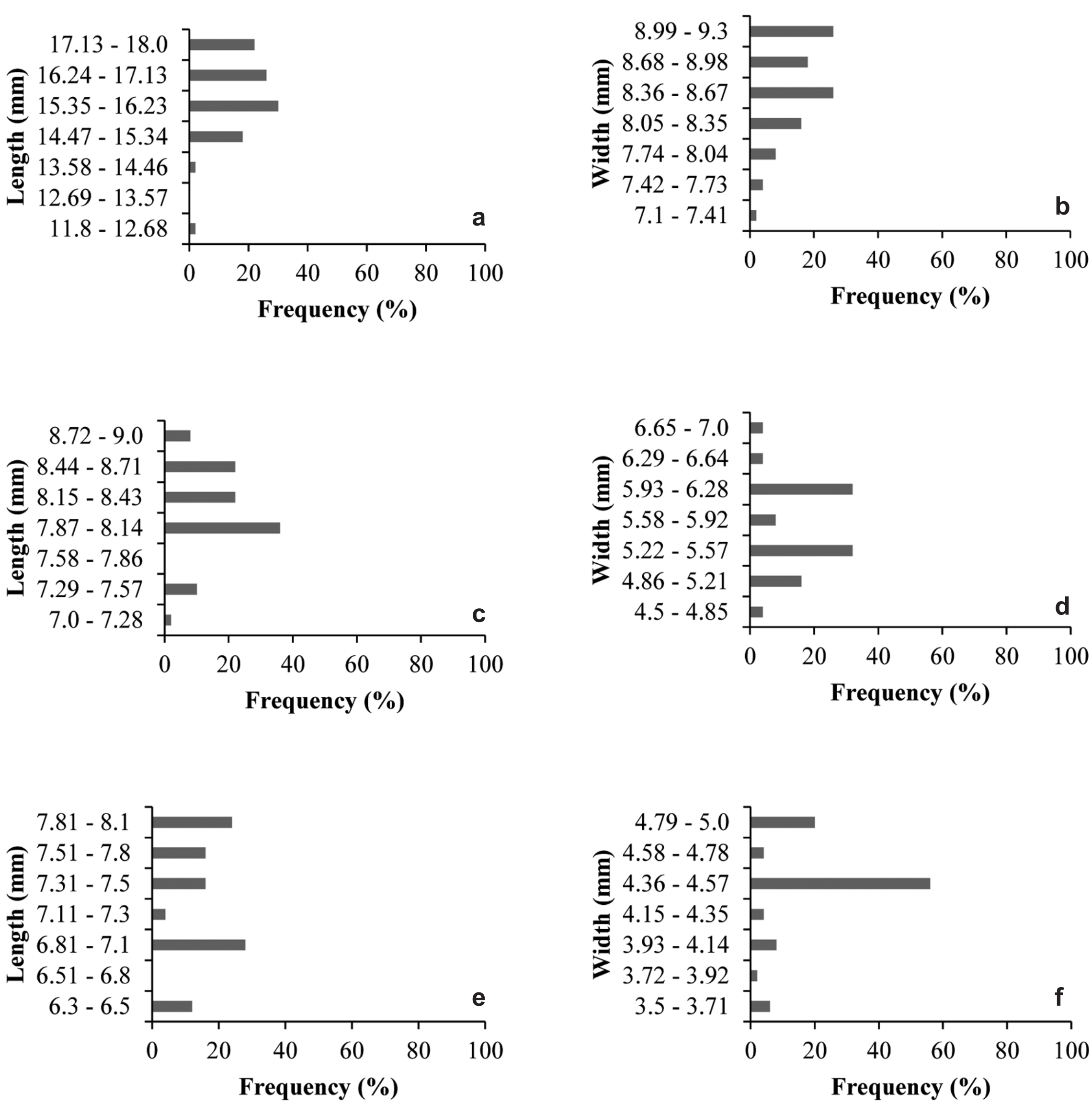

Figure 2 – a-f. *Richeria grandis* (Phyllanthaceae) in seven size classes – a-b. frequency of fruit size – a. length; b. width; c-d. seeds with sarcotesta (without benefit); e-f. seeds without sarcotesta (benefited).

Most fruits, seeds with sarcotesta and without sarcotesta have a length of about 14.47 to 18.0 mm, 7.87 to 8.71 mm and 6.82 to 8.1 mm, respectively (Fig. 2). Regarding the length for most seeds without the sarcotesta, higher frequency was observed in the third and seventh class, and lower frequency, in the fourth class of length (Fig. 2e).

Regarding width, fruits are mostly between 7.74 and 9.3 mm (Fig. 2b), seeds with sarcotesta, between 4.86 and 6.28 mm (classes 3 and 5) (Fig.2d), and seeds without the sarcotesta, between 4.36 and 4.57 mm (fifth class) (Fig. 2f).

Although biometric values found for *R. grandis* seeds and fruits were distributed in most classes, variation coefficients were relatively low (below 10%), indicating homogeneity of analyzed samples.

Seed germination and storage

To meet normality and homoscedasticity assumptions ($p > 0.05$), treatments without germination were discarded (G%), germination rate (IVG), and mean germination time (treatments 10, 15 and 20 days stored), in order to avoid loss of efficiency in estimating treatment effect and also loss of sensitivity of the test of significance. For statistical analysis of humidity percentage (%), all treatments were maintained, considering statistical assumptions ($p > 0.05$).

At the 30th day of analysis, average germination percentage was verified for newly harvested seeds (0 days) as 63% with an average IVG of 27.54 and TMG of 7 days with an average degree of humidity of 77.03%, whereas for the treatment in which *R. grandis* seeds were stored for 5 days there was a decrease in germination average (21%), mean IVG (7.85) and an increase in mean germination time to 9 days, a not statistically significant result, with an average degree of humidity of 52.03% (Tab. 2).

For treatments with 10, 15 and 20 days of storage, *R. grandis* seeds had already completely lost their viability, with mean moisture levels of 40.8, 28.2 and 11.8% each (Tab. 2). 10-days storage treatment did not germinate, even presenting a moisture level equal to the 5-days storage treatment (Tab. 2).

Table 2 – Response of moisture (%), germination (G) (%), germination velocity index (IVG) and average germination time (TMG) of *Richeria grandis* in different storage times.

Storage time (days)	Moisture (%)	G (%)	IVG	TMG (days)
Newly benefited	77.03a	63 *	27.54 *	$7^{n.s}$
5 days	52.03b	21 *	7.85 *	$9^{n.s}$
10 days	40.80bc	-	-	-
15 days	28.20cd	-	-	-
20 days	11.80d	-	-	-
Coefficient of variation (%)	15.04	33.33	35.79	17.04

Averages followed by the same letter do not differ from each other by the Tukey test at 5% probability. * = Significance in the test of analysis of variance F ($p < 0.05$). n.s. = Not significant in the analysis of variance analysis F ($p > 0.05$).

The average germination time for seeds with an initial moisture content (freshly processed) and seeds stored for 5 days were the same. However, for freshly processed seeds placed to germinate after collection, germination started on the second day of evaluation and stabilized on the 20th day. For seeds stored for 5 days, root protrusion began on the 5th day and stabilized on the 10th day (Fig. 3). After this period, they were not able to germinate since they had fungi and/or were rotted.

This study verifies that *R. grandis* seeds are sensitive to storage conditions, allowing humidity reduction. Seeds have been found to be dispersed with high-water content and lose their

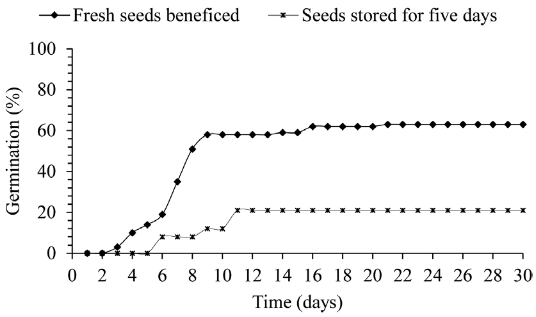

Figure 3 – Germination percentage along the assessment period (30 days) of *Richeria grandis* seeds placed to germinate at the time of collection and processing and after 5 days stored in paper bags outdoors.

viability when they are packaged in permeable packaging and dried at water contents below 40.8%. Therefore, according to the protocol used, they are classified as recalcitrant.

Germination, morphology and seedling development

The radicle breaks the integument on the second day after sowing (Fig. 4a,b), characterizing the visible beginning of germination. On the second day, the greenish-colored cylindrical radish presented a length varying from 0.8 to 2.0 mm.

On the eighth day the radicle was observed, already with a whitish yellowish color (aclorophyllate), thinned from the base to the apex, and the hypocotyl, light green in color, is noticeable (Fig. 4c,d). Ten days after sowing, the collection is evidenced by a small depression in the dark brown color (strangulation), separating the primary root, formed from radicle elongation, generating the main root, now with a yellow-brown coloration, of the hypocotyl (situated between insertion point of cotyledons and the one beginning at the root) of dark green color (Fig. 4e). At this stage, there is still no presence of secondary roots.

The emergence of *R. grandis* seeds is of epigeal type, characterized by foliaceous cotyledons positioned above the substrate by hypocotyl elongation (Fig. 4g). They emerge from seed integument, pluck out of the forehead, expand, and are classified as phaneroconuclear. Thus, the classification of the morphofunctional type of *R. grandis* according to Miquel (1987) is Fanero-epigene-foliaceus, dichotomous characteristics of cotyledons exposure, position and texture, respectively (Fig. 4g).

With regard to embryonic leaves (cotyledons), these are classified as dicotyledons, specialized for photosynthesis, have cotyledonary leaves betting, sessile, glabrous and consistency membranes, with smooth margin, dark green coloration, oblong shape with rounded apex, with well marked main vein and brochydroma (Fig. 4h). The time when the cotyledons were covered by the integument varied. In some samples that already had primary roots, collections and hypocotyls, it was still covered by the integument (Fig. 4e), already in other samples, it had expanded from the forehead only with the radicle and hypocotyl evidenced (Fig. 4f), but only from the 16th day, with periodic observations, that the cotyledons of all seeds germinated were fully expanded, which are positioned in search of light, and are arranged at a 90º angle to the hypocotyl (Fig. 4g,h).

The presence of secondary roots was observed only from the 22nd day (Fig. 5b). At this stage, the branching zone, which contains secondary roots which are thin, short (measuring about 0.5 to 1 cm long), tender, cylindrical and white (aclorophyllate), the growing zone, is also whitish, responsible for the growth of the main root whose root system is pivotal, with positive geotropism, and the coifa, also white (Fig. 5b). At this stage, the primary root developed rapidly, with a cylindrical and tender shape, brown and with length ranging from 5 to 9 cm (Fig. 5b).

Epicotyl develops more slowly than hypocotyl, and seedlings with about 30 days have not yet been present. It is only after 50 days of observations that the first eophilic (first-order prototype) appears measuring about 7 mm in length, characterized as a simple, symmetrical, broquidodromatic vein, with a very evident main vein elliptical (acuminated apex and wedged base), apparently sessile, with membranous consistency, glabrous and serrated margin (Fig. 5a,c,d). At this stage, the epicotyl growth was observed, very similar to hypocotyl: tender, cylindrical and dark green to brownish color, 2 mm in length and hairless (Fig. 5c,d). The hypocotyl in this phase has a length varying from 5 to 8 cm, light green color, primary root with a mean length of 11 cm, is brown in the base and yellow to white in the apex (forming the growth zone and hood), and white secondary roots, with a length of 1 to 4 cm, characterizing young seedlings (Fig. 5a).

Discussion

In order to certify the morphological classification of this study, Kuhlmann (2012) also

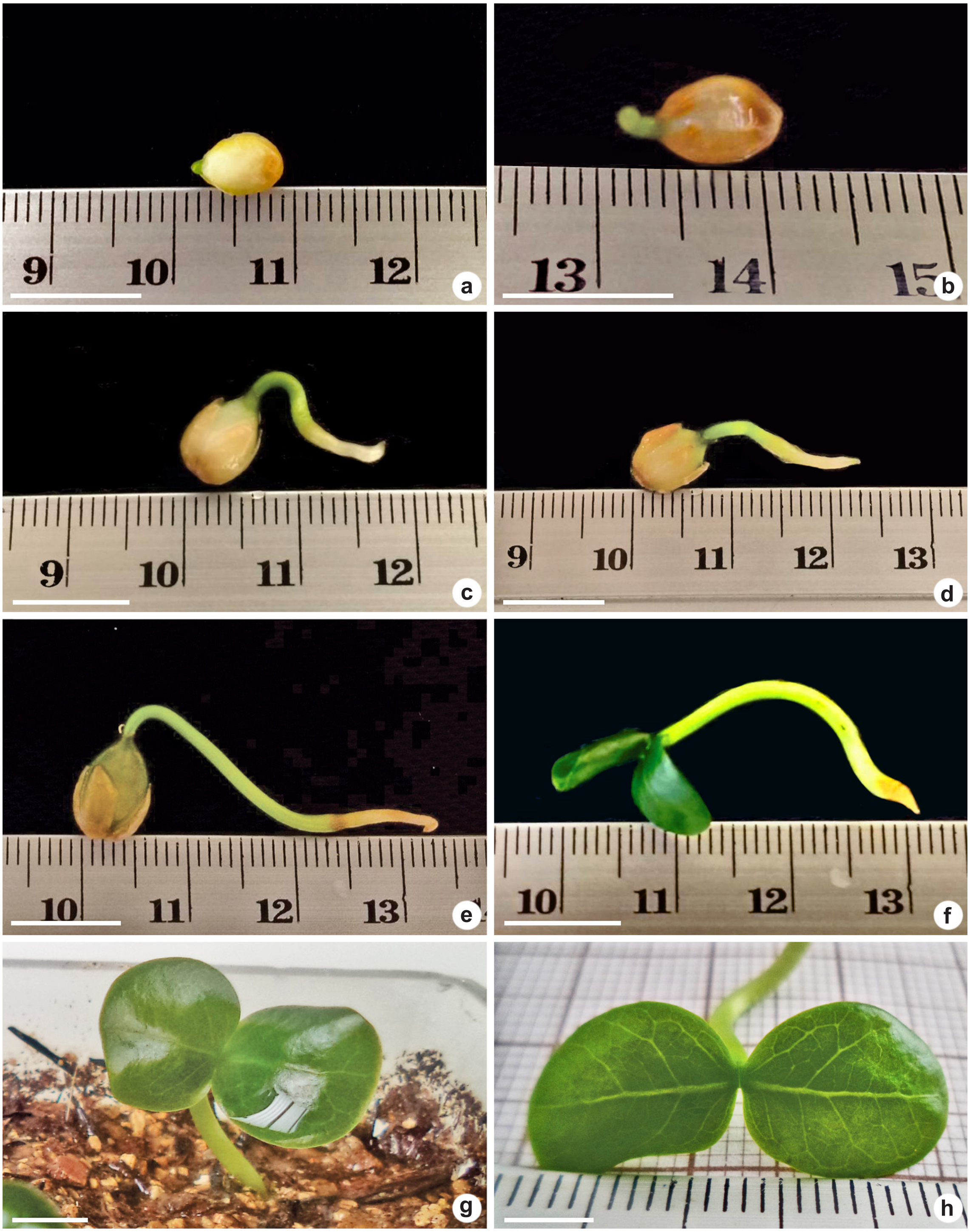

Figure 4 – a-h. Morphological characterization of germinative development up to the complete seedling stage of *Richeria grandis* (Phyllanthaceae) – a-b. radicle breaking the tegument; c-d. presence of radicle and appearance of hypocotyl; e. collection evidenced by dark brown color; f. embryonic leaves; g. morphofunctional type Phanero-epigene-foliaceous; h. embryonic leaves with brochidodroma nervation. Scale bars: 1 cm.

characterizes *R. grandis* as dry, dehiscent, simple fruit of the capusla type, and seeds with smooth, fragile tegument of cream color, being common seed viable fruit, as found in this experiment.

However, Kuhlmann (2012) and Silva-Júnior & Pereira (2009) point to *R. grandis* seed with presence of red juicy aryl, which contradicts these descriptive results referring to the presence of sarcotesta. Seeds devoid of aryl (caruncula) make up one of the main characteristics of the Phyllanthaceae family that differentiate them from Euphorbiaceae (Souza & Lorenzi 2008; Martins & Lima 2011; Secco & Rosario 2015), present species, since it belongs to the family Phyllanthaceae.

Barroso *et al.* (1999) argue that *R. grandis* seeds have red-colored sarcotesta, as those in the genus *Magnolia* L., *Michelia* Adans., *Talauma* A. Juss., besides having one of the aborted eggs, corroborating study result. Similarly, Cordeiro (1992, 2004) identifies *R. grandis* with oboval and globose capsule fruit, green even when ripe, with seeds with differentiated forehead smooth, bright and fleshy orange to coral, also defined as sarcotesta.

Some morphological descriptions of *R. grandis* found in scientific literature, contradicting the basic characteristics that differ the Phyllanthaceae from Euphorbiaceae, show the great variability within this family. As Oliveira & Paoli (2016) state, there is a need for new studies of the Euphorbiaceae family, studies with the Phyllanthaceae family, which address a larger number of species, establishing a greater knowledge about them and its relationships with other taxa. The need for better systematization also makes it important.

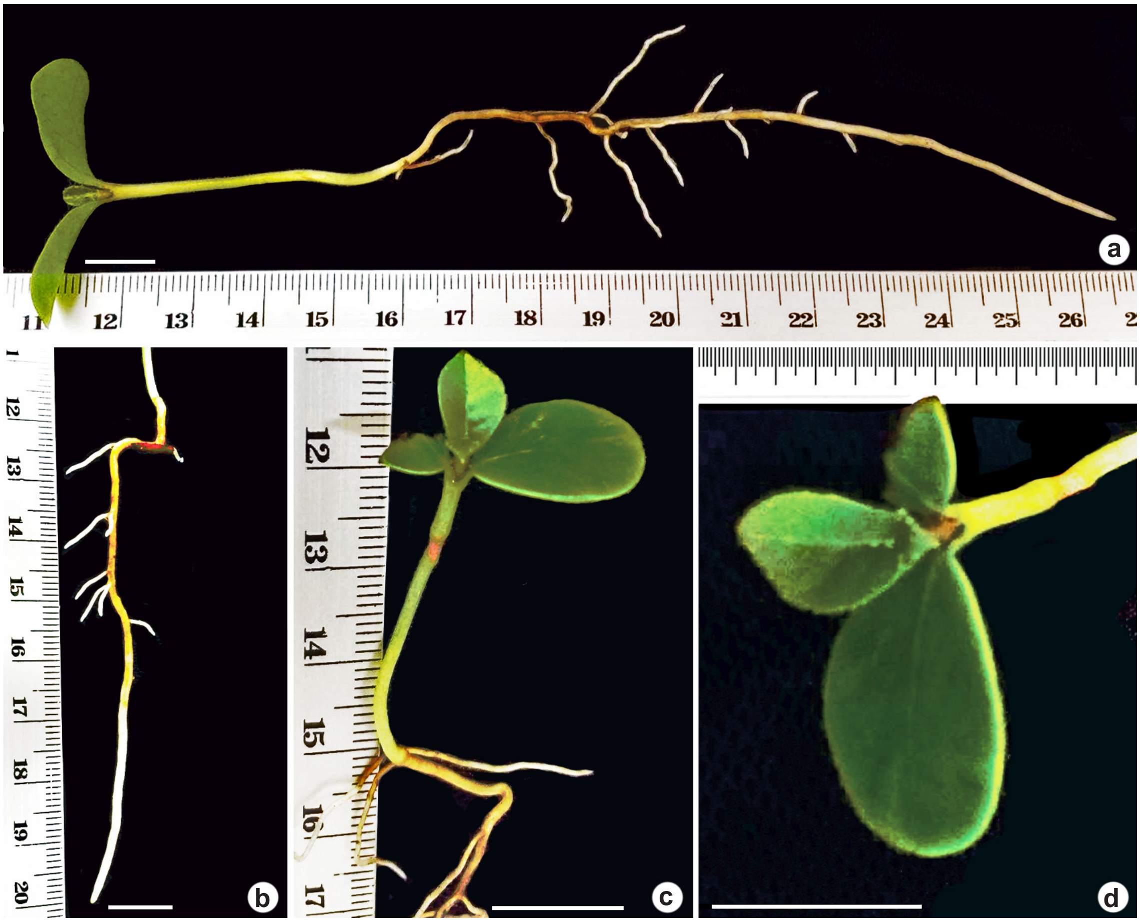

Figure 5 – a-d. Morphological characterization of germinative development up to young seedling stage of *Richeria grandis* (Phyllanthaceae) – a. young seedling with all the morphological characteristics; b. secondary roots (1), root growth zone (2), and coifa (3); c-d. the first prototyph and appearance of the epicotyl. Scale bars: 1 cm.

According to this study, Oliveira (2012) studying the species *Acalypha gracilis* Spreng., *Euphorbia cotinifolia* L. and *Jatropha gossypiifolia* L. (Euphorbiaceae) classified them as fruits in schizocarp capsules (segments elastically dehiscent from a column central), with elliptic or globose seeds. Differing from *R. grandis*, these species have latex since very young, except for *A. gracilis*, which is an herbaceous non-latex species (Oliveira 2012).

Species of the family Phyllanthaceae, also have capsulated fruits and two glabrous seeds by locules, as is the case of *Glochidion talakonense* sp. Nov., *Glochidion karnaticum* Chakrab. & M.Gangop. (Rao 2016), *Phyllanthus acuminatus* Vahl. (Martins & Lima 2011), and *Hyeronima alchorneoides* Allemão (Saueressig 2014).

Biometric description is an important technique to detect genetic variability within a population of the same species (Gusmão *et al.* 2006; Ribeiro *et al.* 2016b) and may provide important information for the distinction of species of the same genus in the future.

In relation to *R. grandis* fruits and seeds biometry, standard deviation values, when compared to average parameters, showed low variability. Low dispersion (Aoyama *et al.* 2015) of *R. grandis* fruits and seeds is concluded. Also confirming the low variability, data showed low correlation coefficients, giving an idea of greater precision in the measured dimensions (Banzatto & Kronka 2015).

Kuhlmann (2012), Cordeiro (1992, 2004), and Silva Júnior & Pereira (2009) reinforce the biometric results of this research, reporting that the fruits have up to 1.6 cm in length and seeds without the sarcotesta up to 0.75 cm in length.

The germination test, according to Oliveira & Paoli (2016), is one of the main means used to measure physiological quality of the seeds, learning about their germinative potential. The most important aspect with regard to seed quality is that it has uniform and rapid germination (Davide & Silva 2008). *R. grandis* seeds had a uniform and rapid germination, with 63% of germination percentage, and mean germination time of 7 days for newly benefited seeds. However, the species studied showed sensitivity to water loss and requires specific conditions for its conservation, since with only 5 days of storage in permeable packages, *R. grandis* already showed a significant decrease in its germinative capacity, with only 21 % of their germinated seeds. In this case, Davide & Silva (2008) recommend collection and immediate processing, followed by sowing.

Recalcitrant seeds, such as *R. grandis*, go straight from the maturation phase to the germination stage, and the drying phase is not as evident as the orthodox seeds (Silva *et al.* 2015). Recalcitrant seeds contain a high degree of moisture at dispersal time, such as species belonging to the Lauraceae family, such as *Ocotea corymbosa* (Meisn.) Mez, *Nectandra oppositifolia* Nees, *Persea pyrifolia* Nees & Mart., *Nectandra nitidula* Nees, *Nectandra lanceolate* Nees, *Nectandra grandiflora* Nees e *Ocotea pulchella* (Nees & Mart.) Mez with humidity varying from 38.3 a 50.4% (Carvalho 2006; Carvalho *et al.* 2008); and the last five species can be stored for a few months when packed in semipermeable packaging in a cold chamber (5 ^{o}C temperature) and 60% relative humidity (Carvalho 2006).

Thus, species with seeds intolerant to desiccation and storage require special conservation conditions, since conservation technique of *ex situ* seeds through seed banks is not viable (Carvalho *et al.* 2006). *In situ* conservation strategies, the most effective method to maintain the continuity of evolutionary processes that originate and maintain biodiversity through conservation units (Santos Filho 1995), should be taken into account for the genetic preservation of species with this behavior (José *et al.* 2007).

However, although this type of conservation is, to date, the most adequate form of gene preservation according to José *et al.* (2007), it has some disadvantages because it requires large areas that must be effectively protected from any disturbance, which demands a large allocation of financial resources (José *et al.* 2007), which generates the need for efficient conservation and the strengthening and monitoring of environmental policy (Carvalho *et al.* 2006).

There are alternative methods for *ex situ* storage of recalcitrant seeds, but specific studies for *R. grandis* are required for further conclusions. These methods must consider the reduction of metabolism, or induction of desiccation tolerance in sensitive seeds, with the use of PEG (Polyethyleneglycol) and ABA (exogenous abscisic acid) in osmotic solutions, partial dehydration (drying the seed to its lowest possible water amount), which may increase longevity, and also the excision of the embryonic axis followed by cryopreservation (Bonjovani 2011; Ribeiro *et al.* 2016a).

Silva-Júnior & Pereira (2009) and Lorenzi (2009) also describe *R. grandis* germination with rates around 50% when sown soon after harvest.

Oliveira & Paoli (2016), studying species of the family Euphorbiaceae, concluded that the viability of the three species (*Acalypha gracilis* (Spreng.) Mull. Arg, *Euphorbia cotinifolia* L. and *Jatropha gossypiifolia* L.) is low, remaining viable for only one month of storage in paper bags, with germination percentage of 80, 15 and 5% each.

Carvalho *et al.* (2006) studied 22 species in the cilliary forests of the Upper and Middle Rio Grande, Minas Gerais basin and observed that 22.7% had recalcitrant seeds. *Allophylus edulis* (A. St.-Hil. Cambes & A. Juss.) Radlk, *Ixora warmingii* Müll. Arg. and *Aulomyrcia venulosa* DC, *Calophyllum brasiliense* Cambess., *Calyptranthes lucida* Mart. *ex* DC., *Cupania vernalis* Cambess., *Eugenia handroana* D.Legrand, and *Talauma ovata* A.St.-Hil., forest species, were also classified according to Carvalho *et al.* (2006) and José *et al.* (2007), as recalcitrant, not tolerating drying to water contents below 12%, indicating that *in situ* conservation practices should be taken into account for the preservation of the genetic resources of these species.

Carvalho *et al.* (2006) state that there is an association between the successional stage to which the species belong and the physiological behavior regarding drying and storage. Some seed physiologists confirm that seeds of climax species have larger dimensions, and lack dormancy and recalcitrant behavior (Kageyama & Viana 1991), regenerating mainly by means of a seedling bank (Kageyama & Viana 1991), which can attribute to *R. grandis* as a typical species of communities with successional climax stage.

Research on seedling development is extremely important to understand the ecology and phenological characteristics of the species (Oliveira & Paoli 2016). Unfortunately, few studies on species of the Phyllanthaceae family have been found, and only one for species discussed here, where Silva-Júnior & Pereira (2009) confirm the elliptic leaves, broquidodroma nervation and protruding ribs of *R. grandis*.

Morphological characteristics of specie seedlings of the Phyllanthaceae family studied by Secco & Silveira (2016) confirms the profile found for *R. grandis*. *Phyllanthus carolinensis* Fl. Carol. and *Phyllanthus minutulus* Müll. Arg., *Fl. bras.* also have elliptic-oval, membranaceous, glabrous and well-evident veins (Secco & Silveira 2016). Oliveira & Paoli (2016) classify *Euphorbia cotinifolia* L. and *Jatropha gossypiifolia* L. (Euphorbiaceae) as phaneritic and epigene seedlings, with photosynthetic foliaceous cotyledons. In the 1970s, when the Phyllanthaceae were still contained in the Euphorbiaceae, Duke (1969) showed that phanerochaete seedlings are considered the standard model for the family, a characteristic found in *R. grandis* seedlings.

As justified by Oliveira & Paoli (2016), results should not be generalized, since there is a standard type of morphology and seedling development for species of the family Euphorbiaceae, because this is a very large and diverse family. The same can be valid for Phyllanthaceae, a scarcely studied family regarding their morphology and initial development.

Species that do not have commercial interests do not have an efficient methodology and procedures recommended in the literature (Oliveira & Paoli 2016). In general, there are no studies on the morphology of fruits, seeds and seedlings, as well as germination of *R. grandis* in the literature, making it difficult to confirm and analyze data from this study. However, these species should also be analyzed as they provide interesting data for ecology, silviculture for the production of seedlings for restoration of gallery forests and also for use in questions about family systematics.

Conclusion

Richeria grandis fruits are simple, dry, oblong capsule type, longitudinal dehiscents, unilocular dysrhythmic and schizocarps, with mean lengths of 16.24 mm, and mean widths of 8.57 mm.

The seed is ellipsoid with sarcotesta of red orange coloration, average length of 7.40 mm and average width of 4.57 mm without sarcotesta. The weight of 100 seeds without the sarcotesta soon after the processing was 5.67g.

Richeria grandis have recalcitrant seeds. After 10 days stored, the seeds completely lose viability.

The emergency has morphofunctional classification of phanerococcal exposure, epigeal position and foliaceus texture.

The radicle breaks the integument on the second day after sowing, the collection is evidenced on the 10th day, separating the primary root from the hypocotyl. From the 16th day, the specialized cotyledons for photosynthesis expand from the forehead, positioned at a 90º angle to the hypocotyl. The development of secondary roots is observed only from the 22nd day. The first protophyll and epicotyl appear only after the 50th day, characterizing a complete seedling.

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