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CLASSIFICAÇÃO FISIOLÓGICA DE SEMENTES FLORESTAIS QUANTO À TOLERÂNCIA À DESSECAÇÃO E AO COMPORTAMENTO NO ARMAZENAMENTO

Palavras chave:
Conservação *ex-situ*
Secagem
Ortodoxa
Recalcitrante

RESUMO: Este trabalho teve por objetivo classificar sementes de espécies florestais nativas da bacia do Alto Rio Grande quanto à tolerância à dessecação e ao comportamento no armazenamento. Testes de germinação e de umidade foram feitos para cada espécie: com as sementes recém-beneficiadas, imediatamente ao atingir 12% e 5% de umidade, e após três meses de armazenamento a -18°C, apenas para sementes com 5% de umidade. Com base nos testes de germinação e umidade, as sementes foram classificadas em recalcitrantes, intermediarias e ortodoxas. As sementes de *Brosimum gaudichaudii, Erythroxylum deciduum, Eugenia pleurantha, Myrcia venulosa, Nectandra megapotamica* foram classificadas como recalcitrantes (22.7% das espécies). Foram classificadas como intermediárias as sementes de *Aegiphila sellowiana, Aspidosperma parvifolium, Blepharocalyx salicifolius, Casearia lasiophylla, Cassia occidentalis, Dalbergia miscolobium, Diospyros brasiliensis, Diospyros hispida, Ilex brevicuspis, Ilex cerasifolia, Myrocarpus fastigiatus, Senna aversiflora* e *Senna splendida* (59.1% das espécies). *Miconia albicans, Platycyamus regnellii, Styrax camporum* e *Piptadenia gonoacantha* foram classificadas como ortodoxas (18.2% das espécies).

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PHYSIOLOGICAL CLASSIFICATION OF FOREST SEEDS REGARDING THE DESICCATION TOLERANCE AND STORAGE BEHAVIOUR

Keywords:
Ex-situ conservation
Desiccation
Orthodox
Recalcitrant

ABSTRACT: This work aims to classify forest seeds native to the Alto Rio Grande region regarding the desiccation tolerance and storage behaviour. Germination and water content tests were performed in seeds of different species. The tests were conducted immediately after seed processing, at 12% and 5% of water content, and at 5% after 3 months of storage in -18°C. Based on the results obtained, seeds were classified into recalcitrant, intermediate and orthodox class. Seeds of *Brosimum gaudichaudii, Erythroxylum deciduum, Eugenia pleurantha, Myrcia venulosa, Nectandra megapotamica* were classified as recalcitrant (22.7% of all species). Seeds of *Aegiphila sellowiana, Aspidosperma parvifolium, Blepharocalyx salicifolius, Casearia lasiophylla, Cassia occidentalis, Dalbergia miscolobium, Diospyros brasiliensis, Diospyros hispida, Ilex brevicuspis, Ilex cerasifolia, Myrocarpus fastigiatus, Senna aversiflora, Senna splendida* e *Blepharocalyx salicifolius* were classified as intermediate (59.1% of all species). Seeds of *Miconia albicans, Platycyamus regnellii, Styrax camporum* and *Piptadenia gonoacantha* were classified as orthodox (18.2% of all species).

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INTRODUCTION

Physiological classification regarding seed desiccation tolerance and storage behaviour is imperative to *ex situ* conservation since the knowledge of the water content and environmental temperature of storage are essential for seed viability maintenance (HONG;ELLIS, 1996). According to their ability to survive different water contents and storage temperature seeds are classified into recalcitrant orthodox (ROBERTS, 1973) and intermediate (ELLIS et al., 1990) classes.

Recalcitrant seeds are not able to tolerate water removal, losing its viability when reaching 12-31% of water content (Roberts, 1973). These seeds show intense metabolism during its developing and post harvesting stages (BARBEDO; MARCOS FILHO, 1998; CASTRO et al., 2004), and normally have large seeds and fruits that prevents them from dying by desiccation (FARNSWORTH, 2000; PRITCHARD et al., 2004; HONG; ELLIS, 1998). On the other hand, orthodox seeds can be dried to low moisture contents (2-5%) under diverse conditions without structural damage (Roberts, 1973); the ideal storage conditions are at or under -18°C. The longevity of these seeds increases with the decrease of temperature and relative humidity during storage, in a quantifiable and predictable way (HONG; ELLIS, 1996). Seeds with intermediate behaviour are those not included either in the orthodox or recalcitrant class (ELLIS et al., 1990; EIRA, 1996). Intermediate seeds are sensible to sub zero temperatures and tolerate partial desiccation (ELLIS et al., 1990).

The Alto Rio Grande region is very important for Brazil in economic and ecological terms. It covers 64 municipalities and an area of 15 thousand squared kilometers (SILVA et al. 2005). The main forest formations found in the region are semideciduous forests (VAN DEN BERG; OLIVEIRA-FILHO, 2000; SOUZA et al., 2003) and the main treats for the vegetation are agriculture (VAN DEN BERG; OLIVEIRA-FILHO, 2000), livestock and the expansion of the urban areas.

According to Tweddle et al. (2003), semi deciduous forests have about 75.0% of species producing orthodox seeds, 4.4% producing intermediate seeds and 20.6% producing recalcitrant seeds. So, due to the lack of information about seed behaviour regarding its desiccation tolerance and storage in the Alto Rio Grande region, the aim of this study was to classify the seed behaviour of 22 forest species native to forest fragments of the Alto Rio Grande region.

MATERIAL AND METHODS

Fruit collection and processing

The fruits were collected from January 2011 to December 2012 in forest fragments of the Alto Rio Grande region, Lavras municipality, Minas Gerais, Brazil [(21°14'30"S and 45°00'10" W and 919 m a. s. l. (BRASIL, 1992)]. According to Köppen's climatic classification, the regional weather is Cwa with attributes of Cwb, presenting two well-defined seasons: dry, from April to September, and rainy, from October to March (KÖPPEN, 1936). The region is characterized as a transition between Mata Atlântica and Cerrado vegetation, with a predominance of a semi deciduous seasonal rainforests (VAN DEN BERG; OLIVEIRA-FILHO, 2000).

Observing such traits as color change, texture and early dehiscence, mature fruits were collected and transported to the laboratory immediately after collection. Processing was made manually, without sun exposition and following recommendations found in Davide et al. (1995).

Determination of seed water content

Shortly after processing, seeds were bottled, dried with paper towels and the water content was determined using four replicates varying from 0.5 to 6 grams of seeds, depending on the species, using the oven method at 103°C for 17 hours. To help the water loss process, species with large seeds were cut into small parts. The results were expressed as percentage of the fresh weigh (ISTA, 2004).

Drying curve and storage

Seeds were submitted to artificial drying using silica gel placed in boxes of the type "higrostat" (5 kg) with forced ventilation or boxes of the type "gerbox" (90 g) for small-sized seeds (up to 1 cm in major axis), with an equilibrium of the relative humidity around 5%, and placed in a acclimatized room at 20 °C. Silica gel was replaced as soon as it showed color changes. Seed water content was monitored daily using the target weights to 12% and 5% of moisture content, determined by the Cromarty et al. (1982) equation.

Fresh seeds and those at 12 and 5% of water content were submitted to water content (described above) and germination tests. Seeds at 5% of water content were divided in two smaller samples, one of these samples was taken immediately to germination test and the other sample to storage at -18 °C for three months, undergoing water content and germination tests after that.

For the germination test four replicates of 25 seeds each were used, incubated in a germination chamber (Magelsdorfi) at 25 °C and constant light, following the instructions of Brancalion et al. (2010), that recommended 25 °C for germination tests of Brazilian tree species native to Cerrado and Atlantic Forest. Autoclaved sand was used as substrate, which was pre-moistened and autoclaved, and placed in plastic trays for large seeds, or three pre-moistened paper sheets in petri dishes for small-sized seeds (up to 1 cm in the major axis). All the samples were kept moistened during the test period and the germination tests were conducted until all the seeds germinated or died (rotted).

Seed classification

The methodology proposed by Hong and Ellis (1996) was used to classify the seeds into one of three classes of seed behaviour, described shortly in the Figure 1.

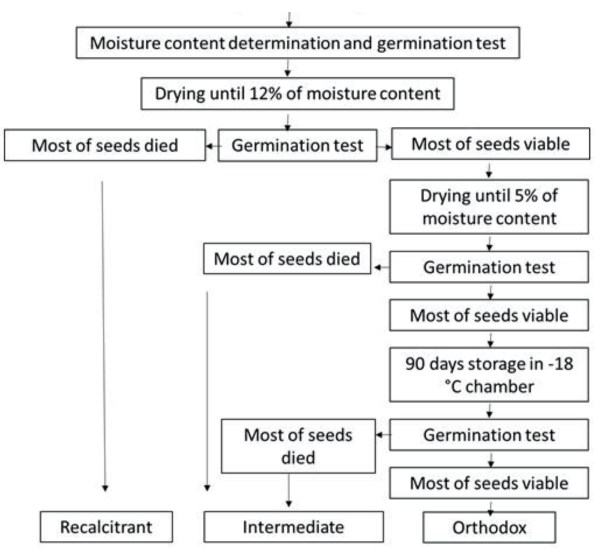

FIGURE 1 Simplified protocol for seed storage behaviour determination adapted from Hong and Ellis (1996).

FIGURA 1 Protocolo simplificado para classificação de sementes de acordo com o comportamento no armazenamento adaptado de Hong e Ellis (1996).

Statistical Analysis

All All the data were submitted to normality test (Shapiro Wilk). Normally distributed data were submitted to an analysis of variance (ANOVA) followed by a Tukey test to compare the means of germination in each point of drying (fresh, 12%, 5%, and 5% of water content + storage). Non-normally distributed data were submitted to Kruskal Wallis analysis followed by Tukey test. All the tests were conducted using 5% probability.

RESULTS AND DISCUSSION

Among all species studied, 22.7% were classified as recalcitrant *(Brosimum gaudichaudii, Erythroxylum deciduum, Eugenia pleurantha, Myrcia venulosa and Nectandra megapotamica*); 59.1% were classified as intermediate (*Aegiphila sellowiana, Aspidosperma parvifolium, Casearia lasiophylla, Cassia occidentalis, Dalbergia miscolobium, Diospyros brasiliensis, Diospyros hispida, Ilex brevicuspis, Ilex cerasifolia, Myrocarpus fastigiatus, Senna aversiflora, Senna splendida* and *Blepharocalyx salicifolius)*; and 18.2% were classified as orthodox *(Miconia albicans, Platycyamus regnellii, Styrax camporum* and *Piptadenia gonoacantha)* (Table 1). Theseis percentages did not agree with those found by Tweddle et al. (2003), for semideciduos forest species.

No previous studies about seed storage behavior for *Aegiphila sellowiana*, *Myrocarpus fastiggiatus* and *Myrcia venulosa* seeds were found. *Aegiphila sellowiana* and *Myrocarpus fastiggiatus* were classified as intermediate while *Myrcia venulosa* was recalcitrant. Similarly, *Aspidosperma parvifolium* was classified as intermediate. However, Carvalho et al. (2006) classified others Aspidosperma species (*Aspidosperma cylindrocarpon* and *Aspidosperma polyneuron)* as orthodox. *Piptadenia gonoacantha* was classified as orthodox in the present study but Carvalho (2004) found recalcitrant behaviour for these seeds. *Blepharocalyx salicifolius* was classified as intermediate in this study and Rego (2012) classified it as recalcitrant.

Nectandra megapotamica was classified as recalcitrant. Similarly, Carvalho et al (2008) classified 3 other Nectandra species (*Nectandra lanceolata, Nectandra oppositifolia* and *Nectandra grandiflora*) as recalcitrant as well. *Dalbergia miscolobium* showed intermediate behaviour. According to Nogueira et al (2010), other Dalbergia species show medium germination at 10% of moisture, which makes sense for intermediate species.

Erythroxylum deciduum was classified as recalcitrant. Similarly, Da Silva et al (2014) classified *Erythroxylum squamatum* as recalcitrant. In previous studies, Wetzel (1997) and Carvalho (2000) classified *Brosimum gaudichaudii* and *Eugenia pleurantha* as recalcitrant, the same classication found in this study. *Diospyro hispida* was classified as intermediate in this study and also by Salomão et al (1997). *Diospyro brasiliense*, which belongs to the same genus of *Diospyro hispida* was classified as intermediate as well. *Platycyamus regnelli* was considered orthodox in the present study, likewise by Salomão et al., (1997). Seeds of Casearia lasiophylla were classified as intermediate in this work, however, Carvalho et al. (2006) could not classify this species in their work due to the presence of dormancy (hard tegument, possible physical dormancy), which can explain why the germination of these seeds was low in the present work. Probably, if dormancy breaking treatments were applied, the germination percentages may differ from the values found in the present study.

Fresh seeds of *Illex brevicuspis* had 80% germination and at 5% of moisture content the germination dropped to 59%. When subjected to 3 months storage in -18°C, the germination was 31%, thus, *Illex brevicuspis* was classified as intermediate. *Ilex cerasifolia* followed a similar pattern, with initial germination of 91%, at 5% water content germination was 72% and after 3 months of storage germination was only 5%, being also classified as intermediate. Fresh seeds of *Miconia albicans* had 90% germination and 77% germination after 3 months storage at -18°C, being safely classified as orthodox.

Fresh seeds of *Styrax camporum* presented low germination percentage, however, presented 82% of germination after 3 months storage at -18°C, hence, the specie was classified as orthodox. According to Kermode and Bewley (1985), mature fresh seeds of *Ricinus communis* presented 30% of normal seedlings, but using seeds after drying and rehydration, the germination test presented 100% of normal seedlings. The authors state that drying process collaborates to finish the developing process and stimulate germination in orthodox seeds. Seeds of *S. camporum* also could present some kind of dormancy overcoming by storage in low temperature as happens with other seeds as related by Souza et al.

TABLE 1 Classification of forest seeds collected at Alto Rio Grande basin, at Lavras region, MG, regarding its desiccation tolerance and storage behaviour.

TABELA 1 Classificação de sementes de espécies florestais coletadas na bacia do Alto Rio Grande, na região de Lavras, MG, em relação a tolerância a dessecação e comportamento no armazenamento.

Species	P value	DMC	GI	G12	G5	G-18	Classification
Aegiphila sellowiana	0.004	30.5	100a	100a	94a	57b	Intermediate
Aspidosperma parvifolium	0.010	22.4	82a	71a	75a	11b	Intermediate
Blepharocalyx salicifolius	0.002	50.4	89a	88b	60b	60b	Intermediate*
Brosimum gaudichaudii	0.002	62.0	67a	0b	0b	0b	Recalcitrant
Casearia lasiophylla	0.016	50.6	92a	20b	18b	13b	Intermediate*
Cassia occidentalis	0.006	13.2	82a	82a	27b	15b	Intermediate
Dalbergia miscolobium	0.008	15.9	100a	90ab	90ab	57.5b	Intermediate
Diospyros brasiliensis	0.012	43.3	67.5a	67.5a	60a	0b	Intermediate
Diospyros hispida	0.003	49.5	86a	56b	63b	51c	Intermediate
Erythroxylum deciduum	0.002	53.9	94.4a	0b	0b	0b	Recalcitrant
Eugenia pleurantha	0.002	58.9	95a	3b	0b	0b	Recalcitrant
Ilex brevicuspis	<0.001	12.2	90a	80a	59b	32c	Intermediate
Ilex cerasifolia	0.008	16.0	91a	85a	72b	5c	Intermediate
Miconia albicans	<0.001	9.1	90a	NP	69b	77b	Orthodox
Myrcia venulosa	0.002	60.1	96a	0b	0b	0b	Recalcitrant
Myrocarpus fastigiatus	0.070	21.0	16a	16a	30a	9a	Intermediate
Nectandra megapotamica	0.002	47.2	85a	0b	0b	0b	Recalcitrant
Piptadenia gonoacantha	0.002	13.1	97a	72b	56b	67b	Orthodox*
Platycyamus regnellii	<0.001	14.4	85b	100a	100a	90a	Orthodox
Senna aversiflora	0.094	18.0	26a	21a	21a	5.25b	Intermediate*
Senna splendida	0.020	26.6	60a	66.2a	67.5a	0b	Intermediate*
Styrax camporum	<0.001	27.5	39b	49b	54b	82a	Orthodox

NP: test not performed; DMC: Dispersal moisture content; IG: Initial germination; G12: Germination at 12% of moisture content; G5: Germination at 5% of moisture content; G-18: Germination at 5% of moisture content stored for 3 months at -18 °C. Means followed by the same letter do not differ among other means (p>0.05). *result obtained differ from results found in literature.

NP: Não realizado; DMC: Umidade de dispersão; IG: Germinação inicial; G12: Germinação aos 12% de umidade; G5: Germinação aos 12% de umidade; G-18: Germinação aos 5% de umidade após 3 meses de armazenamento a -18°C. Médias seguidas por letras iguais não diferem entre si (p>0.05). *resultados obtidos diferem daqueles encontrados na literatura.

(2007) and Zaidan e Barbedo (2004). Further studies about its germination process should be conducted.

Seven of the all studied species belongs to Fabaceae family, of which five were classified as intermediate and two as orthodox. Seeds of *Senna aversiflora* and *Senna splendida* had low germination in all water contents tested, and were classified as intermediate. However, the low germination percentage can be attributed to a possible physical dormancy. Several papers have been reported physical dormancy in seeds of the *Senna* genus, for example, *Senna macranthera* (POZITANO e ROCHA, 2011), *Senna Silvestris* (MARANHO and PAIVA, 2012), *Senna multijuga* (RODRIGUES-JUNIOR et al., 2014), among others. Jayasuriya et al. (2013) studying 100 Fabaceae species from Sri Lanka, found 94 presenting orthodox behaviour, of those 91 presented some kind of dormancy and 86 presented physical dormancy. Although there are exceptions, most of Fabaceae species produce dormant (DICKIE and PRITCHARD, 2004; SAUTU et al. 2006) and orthodox (CARVALHO et al., 2006; JAYASURIYA et al. 2013) seeds. Thus, a possible presence of physical dormancy could have led to a wrong classification in these species, and more studies are necessary to properly classify the behaviour of the Senna species of this study.

Two species from Aquifoliaceae and Ebenaceae family were studied in this work and all of them were classified as intermediate, however, Ellis et al. (1987) pointed out that Aquifoliaceaes species normally have orthodox seeds. Some studies show the drying rate influencing the desiccation tolerance of some species, as shown by Jose et al. (2011) and Magistralli et al. (2015), who found greater desiccation tolerance in *Magnolia ovata* and *Genipa americana*, , respectively, when submitted to slow drying rather than fast drying. The drying rate of Aquifoliaceae seeds should be tested in further investigations. From three Myrtaceae species studied, two were classified as recalcitrant and one as intermediate. Carvalho et al (2006), studying seed storage behaviour, found two Myrtaceae species with recalcitrant behaviour.

Swaine and Whitmore (1988) proposed a forest species classification based on ecological classes, as pioneer and non-pioneer, which were divided in climax shade tolerant and climax light demanding. Pioneer species normally produce orthodox and dormant seeds (KAGEYAMA and VIANA, 1991) while non-pioneer species normally produce recalcitrant seeds (KAGEYAMA and VIANA, 1991; PAMMENTER and BERJAK, 2000). From all of recalcitrant species, three were pioneer and two non-pioneer. From intermediate species, there were six pioneer species and three non-pioneer species. Two intermediatte species were not classified by previous authors regarding its ecological classification. For orthodox species, there were three pioneer and one non-pioneer species. Jose et al. (2007), studying species from riparian forest in Minas Gerais, did not find the classical relationship among ecological groups and seed storage behaviour. They classified *Metrodorea stipularis* and *Miconia argyrophylla* as orthodox, which are climax species. Similarly, Carvalho et al. (2006), studying seed storage behaviour and its relation to ecological groups, found orthodox seeds in the pioneer, shade tolerant and climax light demanding groups.

The highest water content found in recalcitrant seeds was 62% (*Brosimum gaudichaudii)* and the minimum was 47.2%, (*Nectandra megapotamica*). For orthodox seeds, the highest value found was 27.5% (*Styrax camporum)* and the lowest was 9.1% (*Miconia albicans)*. The water content found in intermediate seeds was somewhere between the values of recalcitrant and orthodox seeds (Figure 2). Similarly, Hong and Ellis (1996) affirmed that eeds with moisture content lower than 25% tend to behave in an orthodox way. A variety of studies support the fact that recalcitrant seeds show higher moisture content than intermediate seeds, and intermediate seeds show higher water content than orthodox seeds (BERJAK and PAMMENTER, 1984; HONG and ELLIS, 1998; DAWS et al, 2006; HAMILTON et al., 2013).

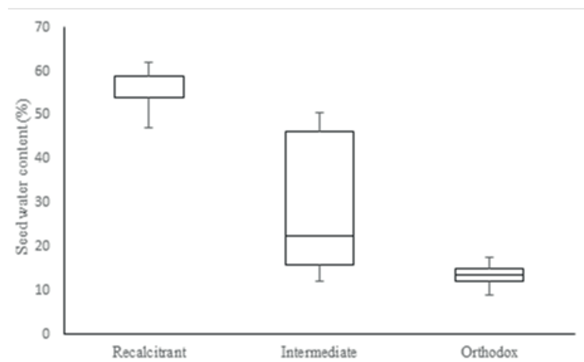

FIGURE 2 Box plot analysis of the dispersal water content of 22 seeds of species from the Alto Rio Grande region.

FIGURA 2 Análise de box plot do conteúdo de água de sementes durante a dispersão de 22 espécies da região do Alto Rio Grande.

CONCLUSION

In conclusion, seeds of *Brosimum gaudichaudii, Erythroxylum deciduum, Eugenia pleurantha, Myrcia venulosa* and *Nectandra megapotamica* were classified as recalcitrants, which is equivalent to 22.7% of the studied species. Seeds of *Aegiphila sellowiana,* *Aspidosperma parvifolium, Blepharocalyx salicifolius, Casearia lasiophylla, Cassia occidentalis, Dalbergia miscolobium, Diospyros brasiliensis, Diospyros hispida, Ilex brevicuspis, Ilex cerasifolia, Myrocarpus fastigiatus, Senna aversiflora and Senna splendida* were classified as intermediate, 59.1% of all the species. Seeds of *Miconia albicans, Platycyamus regnellii, Styrax camporum* and *Piptadenia gonoacantha were classified as orthodox*, which is equivalent of 18.2% of the studied species.

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