New Forests (2015) 46:283–291
DOI 10.1007/s11056-014-9461-5

Loss of desiccation tolerance and storage behavior in germinating seeds of *Senna multijuga*: implications for seed germination and conservation

Ailton Gonçalves Rodrigues-Junior · José Marcio Rocha Faria · Tatiana Arantes Afonso Vaz · Anderson Cleiton José

Received: 24 January 2014 / Accepted: 14 October 2014 / Published online: 18 October 2014

Abstract Orthodox seeds lose desiccation tolerance (DT) and change their storage behavior with advancing germination and with different hydration conditions affect the extent of DT. The objectives of this study were to describe (1) the loss of DT in *Senna multijuga* seeds during hydration under different conditions and (2) the storage behavior of dry seeds and germinating seeds after desiccation. Seeds lose DT progressively as germination advances, and DT is totally lost before the radicle protrudes. Soaked seeds maintained DT for a longer period of time in comparison to imbibed seeds. Dry seeds tolerated storage even at sub-zero temperatures; however, when germinating seeds were dehydrated, the storability at sub-zero temperatures was reduced. Storage of dried germinating seeds at sub-zero temperature reduced germination and increased the proportion of abnormal seedlings. The hydration conditions changed seed response to desiccation, and this result may be related to differences in metabolism. Thus, the conditions in which seeds remain during hydration can affect the physiological response of seeds, and when studies are conducted to assess the DT, this fact should be taken into consideration. According to the results of this study, *S. multijuga* seeds can be classified as orthodox; however, storage at sub-zero temperatures is harmful to germinating seeds after drying. Thus, for successful long-term storage, although the current water content of the seeds is important, if the seeds are previously hydrated (and then dried), this will affect seed storability, with consequent implications for ex situ conservation.

Keywords Hydration · Drying · Longevity · Fabaceae

Introduction

Desiccation tolerance (DT) is defined as the ability of seeds to resume regular metabolic activity following rehydration after almost total water loss in the protoplasm (Oliver et al.

A. G. Rodrigues-Junior (✉) · J. M. R. Faria · T. A. A. Vaz · A. C. José
Laboratório de Sementes Florestais, Departamento de Ciências Florestais, Universidade Federal de Lavras, Caixa Postal 3037, Lavras, MG 37200-000, Brazil
e-mail: ailtongrodrigues@gmail.com

2000; Hoekstra et al. 2001). DT allows seeds to endure adverse environmental conditions and ensures that the plants life cycle is continued (Alpert 2000; Oliver et al. 2000). Not all seeds are able to tolerate desiccation and storage; therefore, seeds are classified into three groups. Seeds that tolerate drying down to 7 % of the water content (fresh weight) as well as long-term storage at sub-zero temperatures are classified as orthodox. Seeds that do not tolerate a reduction in water content and that lose viability when the moisture level falls to approximately 20 % are classified as recalcitrant. Additionally, recalcitrant seeds do not tolerate long-term storage or low temperatures (Roberts 1973). Some seeds, classified as the intermediate seed group, exhibit intermediate behaviors between the orthodox and recalcitrant seed groups, tolerating reductions in water content to levels below those of recalcitrant seeds, but not to the same extent as those in orthodox seeds. Nor can the intermediate seeds be stored at sub-zero temperatures (Ellis et al. 1990).

When mature orthodox seeds are hydrated, DT progressively decreases until it is totally lost (Dasgupta et al. 1982; Buitink et al. 2003; Faria et al. 2005) because the seed resumes its metabolic activities towards radicle protrusion and subsequent seedling formation (Bewley 1997). Furthermore, different hydration conditions may result in different seed responses to drying and may cause divergent results in experiments aimed at understanding the extent of DT in seeds. It also has been reported that orthodox seeds subjected to imbibition followed by drying have a shorter storability (Hong and Ellis 1992).

Senna multijuga (Rich.) H. S. Irwin & Barn. is widely distributed in Brazil and grows in the Atlantic Forest and Brazilian savanna within different vegetation types such as rainforest, semideciduous forest, deciduous forest and gallery forest (Oliveira-Filho 2006). *Senna multijuga* is an important tree species and is mainly used for landscaping and ecological restoration. The seeds of *S. multijuga* are physically dormant (Rodrigues-Junior et al. 2014), with the ability to form soil seed-banks (Ferreira et al. 2004). This kind of dormancy prevents water uptake; consequently, the onset of germination is also prevented. There is limited information available on the seed biology of the *S. multijuga* species; thus, not much is known about DT or the effects of germination on DT or about the seed behavior of *S. multijuga* during storage. Such data may be useful to develop seed storage protocols for the species, which may be particularly helpful in ex situ conservation. The objectives of this study were to understand (1) the loss of DT in *S. multijuga* seeds during hydration under different conditions and (2) the storage behavior of both dry and germinating *S. multijuga* seeds after desiccation.

Materials and methods

Seed collection

Dry pods with mature seeds were collected in September 2011 from *S. multijuga* trees during the time of natural dispersal (dry season) on the campus of the Federal University of Lavras, in Lavras city, MG, Brazil (21°14′S; 45°0′W) at 900 m of elevation. The vegetation of the Federal University of Lavras campus is characterized as a transition between the Atlantic Forest and Brazilian savanna. After collection, seeds were manually processed, separated by floating in water and dried in a climate-controlled room [20 °C; 60 % relative humidity (RH)] for 2 days. Seeds were then stored in a sealed semi-permeable plastic bag in a cold room (5 °C; 40 % RH) until the beginning of the experiments one week later.

Water content determination and dormancy release

Seeds were randomly sampled from the seedlot for water content determination by weighing four replications of 10 seeds each, placing them in an oven at 103 °C for 17 h and then weighing the seeds again. The data were expressed as percentage of water on a fresh basis (ISTA 2004). To characterize water absorption during germination, 20 replications of one non-dormant seed each (made non-dormant by immersion in hot water, as described below) were kept individually under germination conditions (moistened paper at 25 °C under constant light of approx. 27 μmol m^{-2} s^{-1}) and weighed several times until the protruded radicle reached 3 mm in length. Before each weighing, the superficial water was removed from the seeds using absorbent paper. After weighing, the seeds were returned to imbibition conditions and weighed regularly thereafter during this treatment. To release physical dormancy, seeds were immersed in hot water (initial temperature of 80 °C that reached room temperature within a few minutes) and kept for 12 h, as described by Rodrigues-Junior et al. (2014), and fresh mass was determined from the start of the hot water submersion.

Loss of DT during hydration

Intact seeds were subjected to dormancy breaking treatment (immersion in water at 80 °C initial temperature for 12 h); thereafter, the non-dormant seeds were placed in moistened paper at 25 °C under constant light (germination conditions) or kept immersed in water (30 mL, replaced daily) at 25 °C to assess the loss of DT. Loss of DT was evaluated from the start of the dormancy breaking treatment. One hundred twenty seeds of each condition were removed—after different time intervals—and subjected to drying in silica gel. Seeds were kept in a Gerbox® plastic container over a net, above a layer of active silica gel which kept the relative humidity of the air to approximately 8 %. The Gerbox® containers were sealed with plastic film. A moisture test was performed to check the seed water content (ISTA 2004) during drying (four replications of five seeds per treatment). After reaching the initial water content (approximately 8 %, after 1 day of drying), seeds were kept for three more days under the same drying conditions as described by Buitink et al. (2003). In preliminary studies, the seeds returned to the initial water content after 1 day in silica gel, and a moisture test was performed to confirm this. After drying the seeds, four replications of 25 seeds per treatment were pre-humidified in a water-saturated atmosphere (approximately 100 % RH) at 25 °C for 24 h. Imbibition in humid air normally is performed to avoid imbibition damage. Following this, seeds were allowed to germinate on moistened paper at 25 °C under constant lighting conditions. Germination percentage, germination speed index (GSI) (Maguire 1962) and percentage of normal seedlings were each assessed for a period of 9 days. Seedlings were considered to be normal when all of their parts were well developed and healthy, which was observed following a maximum of 9 days. The criterion for assessing germination was a protruded primary root of greater than or equal to 1 mm. To assess DT, the criterion was the formation of normal seedlings. The seeds were considered dead if disruption of the seed coat due to cotyledon expansion without radicle protrusion occurred, followed by deterioration of the seed.

Storage after imbibition and drying

Seeds were subjected to dormancy release treatment and then dried in silica gel for 4 days either (1) immediately after treatment or (2) following imbibition for 10 h under

germination conditions. The control treatment comprised dry seeds that were untreated by the aforementioned method to break dormancy. Fifteen samples containing 100 seeds for each treatment were placed in sealed plastic bags and stored in the dark at three temperatures: −18, 5 and 20 °C, for up to 240 days (0, 60, 90, 180 and 240 days). After storage, four replications of 25 seeds per treatment were pre-humidified (approximately 100 % RH) and placed at germination conditions, as described above. Dry seeds were subjected to the same breaking dormancy treatment described above and placed at germination conditions. Germination and formation of normal seedlings were both assessed for 9 days. The experiment was a full randomized factorial design with 45 treatment combinations (3 dormancy treatments × 3 storage temperatures × 5 storage durations).

Statistical analysis

The experimental design was completely randomized for all assays. A regression analysis was performed on the data of loss of DT. The model was tested at 5 % significance and evaluated by the coefficient of determination (R^2) using the software SigmaPlot® v. 11.0. The storage data were analyzed using a generalized linear model (GLM) followed by a Tukey test at 5 % significance, using the software R for Windows® 2.12.0 (R Development Core Team 2011). The model included the effects of dormancy treatments, storage temperatures and storage durations as well as the interaction between these factors.

Results

Water content determination and imbibition curve

Seeds having an initial water content of 8.4 % showed a triphasic pattern of imbibition. There was a rapid increase in the seed fresh mass that extended for approximately 18 h, characterizing phase I of imbibition. From this period onwards, seed fresh mass was maintained (phase II) and the water absorption remained stable for approximately 36 h. After this period, the radicle began to protrude and a further increase in the seed fresh mass occurred, characterizing phase III of imbibition. Radicle protrusion (visible germination) started after 32 h of imbibition. After 36 h of imbibition, 50 % of the seeds exhibited protruded radicles.

Loss of DT during hydration

Germination (radicle protrusion) of the seeds that were imbibed in paper and dried was high until 32 h of imbibition and exhibited reduction after this imbibition period (data not shown). Regarding the formation of normal seedlings (the criterion used to assess loss of DT), tolerance was maintained until 22 h of imbibition with a resultant normal seedlings amount of 86 %. After the 22 h imbibition period, the resultant normal seedlings amount dropped reaching 62 % after 27 h of imbibition and 13 % after 32 h. Normal seedlings did not form after 36 h of imbibition and desiccation (Fig. 1). The loss of DT was characterized by the high percentage of seedlings with damaged radicles (Fig. 2b). These seedlings showed necrosis in the radicle, which prevented radicle development. After 36 h of imbibition (and radicle emergence beginning after 32 h), seeds completely lost DT. The GSI ranged from 23.03 for dry seeds to 1.6 for seeds undergoing imbibition for 36 h (Fig. 2a).

Seeds that were soaked in water kept their DT for a longer time. Germination followed the same trend of the formation of normal seedlings with the percentage remaining above 70 % after 72 h of soaking. Soaking beyond the 72 h period (i.e., for 96 h) caused a decrease in normal seedling formation to 48 %, with no seedling survival occurring after 168 h of soaking (Figs. 1, 2b). Cotyledon expansion without radicle protrusion occurred in some cases and the germination speed also decreased as the soaking time advanced (Fig. 2a). No trend was observed between increasing formation of abnormal seedlings and longer soaking times ($P > 0.05$) (Fig. 2b). The reduction in the percentage of germination and formation of normal seedlings was thought to be due to seed mortality.

Storage after imbibition and drying

Dormancy treatment, storage temperature and storage duration treatments significantly (all $P \leq 0.05$) affected seed germination and the resulting proportion of normal seedlings. There were interactions between dormancy treatment and storage duration as well as between storage temperature and storage duration on seed germination. Interactions were also observed between storage temperature and dormancy treatment on normal seedling formation (all $P \leq 0.05$). Combining all storage temperatures, the germination of stored dry seeds and of those that were stored after dormancy breaking treatment and drying did not decrease during the 240 days of storage. However, seeds that were stored after a dormancy breaking treatment followed by 10 h of imbibition and drying showed decreased germination at 240 d of storage (Fig. 3A). Combining all dormancy treatments, no reduction in germination of seeds stored for 240 days at 5 and 20 °C was observed; however, seeds stored at −18 °C did exhibit reduced germination (Fig. 3b). The percentage of normal seedlings remained the same throughout the length of the storage of dry seeds; however, germinating seeds stored after drying exhibited an increase in the formation of normal seedlings (Fig. 3c). When all dormancy treatment results were combined, it was found that among the temperatures used for storage, −18 °C produced the lowest percentage of normal seedlings (Fig. 3d). Storage temperatures had no effect on the formation of normal seedlings of the seeds stored in the dry state; however, storage at −18 °C decreased the formation of normal seedlings of the germinating seeds stored after drying. In addition, the storage of dry seeds led to a high percentage of normal seedlings, irrespective of the storage temperature (Fig. 4).

Discussion

S. multijuga seedlings that originated from seeds that were imbibed, dried and rehydrated had damaged radicles, as necrosis and expansion of the cotyledons without elongation of the hypocotyl-radicle axis, suggesting that there are different DT responses among the embryo parts, with the radicle being the most sensitive part (Reisdorph and Koster 1999). In the *Tabebuia impetiginosa* seedlings, necrosis and subsequent death of radicles after treatment with polyethylene glycol and desiccation did not prevent seedling growth because adventitious roots that emerged from the hypocotyls allowed seedling survival (Vieira et al. 2010). However, adventitious roots were not observed in *S. multijuga* seedlings throughout the evaluation period. In addition to the visible damage (described above), there was a reduction in germination speed following longer imbibition, and this may be related to damage at the cellular and/or molecular level that must be repaired for germination to begin.

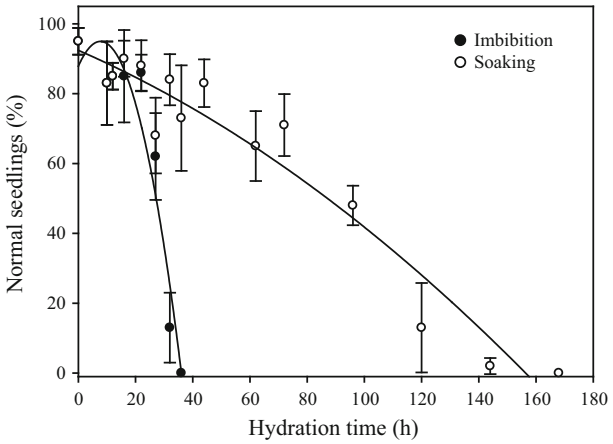

Fig. 1 Percentage of normal seedlings for *Senna multijuga* seeds in response to desiccation treatment following imbibition under normal germination (*filled circle*; $R^2 = 0.92$) or continuous soaking (*open circle*; $R^2 = 0.94$) conditions (mean ± standard deviation)

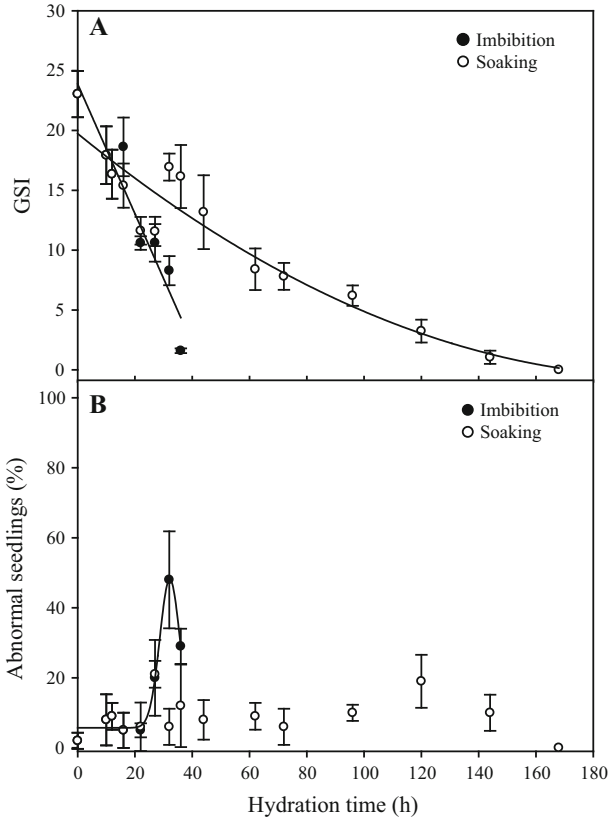

Fig. 2 Germination speed index (GSI) (**a**) and percentage of normal seedling formation (**b**) for *Senna multijuga* seeds in response to desiccation treatment following imbibition under normal germination (*filled circle*; $R^2 = 0.91$, **a**; $R^2 = 0.98$, **b**) or continuous soaking (*open circle*; $R^2 = 0.90$, **a**) conditions (mean ± standard deviation)

The hydration conditions assessed herein resulted in a changed seed response to desiccation, and this may be related to differences in metabolism. Conditions limiting metabolic activity in seeds may reduce the damage caused by desiccation (Leprince et al. 1995). The reduction of oxygen availability, which may have occurred during soaking of

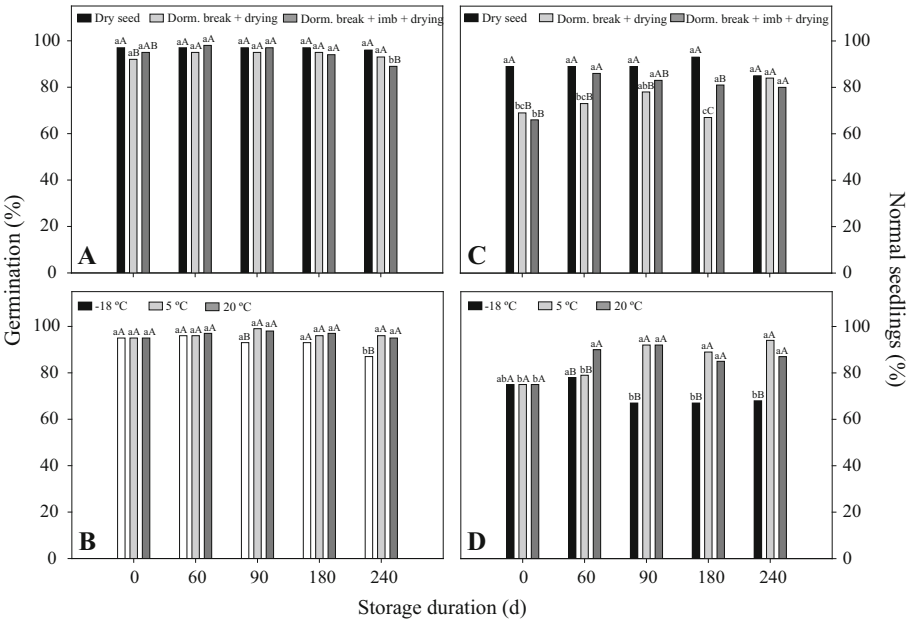

Fig. 3 Percentage of seed germination (**a**, **b**) and normal seedlings (**c**, **d**). Interaction effect between dormancy treatment and storage duration (**a**, **c**) and between storage temperature and storage duration (**b**, **d**). Means with the same letter are not significantly different ($P \leq 0.05$) among (*lower case letters*) and within each storage duration (*upper case letters*)

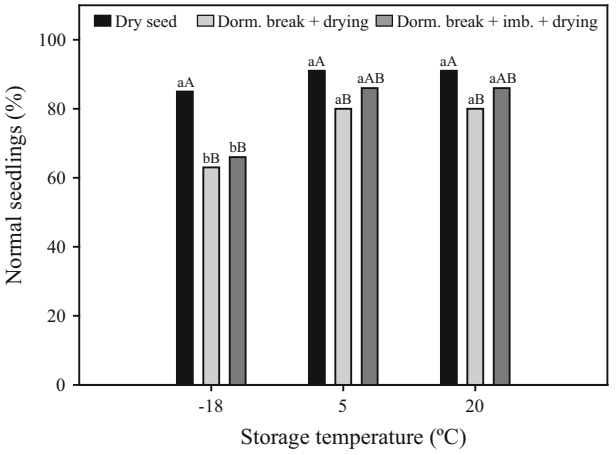

Fig. 4 Interaction effect between dormancy treatment and storage temperature on proportion of normal seedlings. Means with the same letter are not significantly different ($P \leq 0.05$) among (*lower case letters*) and within each storage temperature (*upper case letters*)

the seeds, reduces metabolic activity of plant tissues (Geigenberger 2003). The latter may then prolong the ability of cells to tolerate desiccation. Seed tolerance to hypoxic conditions varies among species, ranging from a few hours to days (Perata and Alpi 1993). A decrease in metabolism may have prevented cellular damage to *S. multijuga* seeds, allowing them to withstand drying after longer soaking times in comparison to seeds imbibed under germination conditions. During the soaking process, seeds do not reach phase III of imbibition; instead, the seeds remain at phase II. Thus, the response of seeds to

hydration conditions may differ, and this should be taken into consideration in studies performed to assess the DT.

Orthodox seeds with low water content are able to withstand storage over long periods (Roberts 1973). These seeds stored at −18 °C with a water content below 10 % are considered to be optimal for germplasm conservation (Genebank Standards 1994; Pritchard and Dickie 2003). Storing dry seeds (<10 % water content) of *S. multijuga* at −18 °C for up to 240 days had no harmful effects but caused damage to germinating seeds after drying. Interestingly, these seeds lost the characteristics that enabled them to tolerate extremely low temperatures, which decreased their storability at sub-zero temperatures. This finding supports previous studies showing a change in storage behavior of orthodox seeds after imbibition and drying by Hong and Ellis (1992). A similar behavior during drying and storage was found in *Coffea arabica*, *Carica papaya* and *Elaeis guineensis* seeds, which are classified as intermediate (Ellis et al. 1990, 1991a, b). Thus, it important that the seeds of *S. multijuga* should be stored at the correct water content level, but storability (at same seed water content level) will also be affected if the seeds have been previously hydrated.

It is concluded that the conditions in which seeds remain during hydration can affect the physiological response to DT as it relates to the seed metabolic state. *S. multijuga* seeds can be dried down to 10 % water content and stored at −18 °C for up to 240 days; therefore, seeds of this species can be considered to have orthodox seed characteristics. However, germinating seeds are harmed by sub-zero temperatures after they have been dried.

Acknowledgments Ailton G. Rodrigues-Junior thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship. Tatiana A. A. Vaz thanks the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the scholarship.

References

Alpert P (2000) The discovery, scope, and puzzle of desiccation tolerance in plants. Plant Ecol 151:5–17

Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055–1066

Buitink J, Vu BL, Satour P, Leprince O (2003) The re-establishment of desiccation tolerance in germinated radicles of *Medicago truncatula* Gaertn. seeds. Seed Sci Res 13:273–286

Dasgupta J, Bewley JD, Yeung EC (1982) Desiccation-tolerant and desiccation-intolerant stages during the development and germination of *Phaseolus vulgaris* seeds. J Exp Bot 33:1045–1057

Ellis RH, Hong TD, Roberts EH (1990) An intermediate category of seed storage behaviour? I. Coffee. J Exp Bot 41:1167–1174

Ellis RH, Hong TD, Roberts EH (1991a) Effect of storage temperature and moisture on the germination of papaya seeds. Seed Sci Res 1:69–72

Ellis RH, Hong TD, Roberts EH, Soetisna U (1991b) Seed storage behavior in *Elaeis guineensis*. Seed Sci Res 1:99–104

Faria JMR, Buitink J, van Lammeren AAM, Hilhorst WM (2005) Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. J Exp Bot 56:2119–2130

Ferreira RA, Davide AC, Motta MS (2004) Vigor e viabilidade de sementes de *Senna multijuga* (Rich.) Irwin et Barn. e *Senna macranthera* (Collad.) Irwin et Barn., num banco de sementes em solo de viveiro. Rev Bras Sementes 26:24–31

Geigenberger P (2003) Response of plant metabolism to too little oxygen. Curr Opin Plant Biol 6:247–256

Genebank Standards (1994) Food and agriculture organization of the United Nations. International Plant Genetic Resources Institute, Rome

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431–438

Hong TD, Ellis RH (1992) The survival of germinating orthodox seeds after desiccation and hermetic storage. J Exp Bot 43:239–247

International Seed Testing Association (2004) International rules for seed testing. Bassersdorf, Switzerland
Leprince O, Vertucci CW, Hendry GAF, Atherton NM (1995) The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature. Physiol Plant 94:233–240
Maguire JD (1962) Speed of germination—aid in selection and evaluation for seedling emergence and vigor. Crop Sci 2:176–177
Oliveira-Filho AT (2006) Catálogo das árvores nativas de Minas Gerais: mapeamento e inventário da flora nativa e dos reflorestamentos de Minas Gerais. Editora UFLA, Lavras
Oliver MJ, Tuba Z, Mishler BD (2000) The evolution of vegetative desiccation tolerance in land plants. Plant Ecol 151:85–100
Perata P, Alpi A (1993) Plant responses to anaerobiosis. Plant Sci 93:1–17
Pritchard HW, Dickie JB (2003) Predicting seed longevity: use and abuse of seed viability equations. In: Smith RD et al (eds) Seed conservation: turning science into practice. Royal Botanic Gardens, Kew, pp 653–722
Reisdorph NA, Koster KL (1999) Progressive loss of desiccation tolerance in germinating pea (*Pisum sativum*) seeds. Physiol Plant 105:266–271
Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Tech 1:499–514
Rodrigues-Junior AG, Faria JMR, Vaz TAA, Nakamura AT, José AC (2014) Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. Seed Sci Res 24:147–157
R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org
Vieira CV, da Silva EAA, de Alvarenga AA, de Castro EM, Toorop PE (2010) Stress-associated factors increase after desiccation of germinated seeds of *Tabebuia impetiginosa* Mart. Plant Growth Regul 62:257–263