Desiccation tolerance and longevity of germinated *Sesbania virgata* (Cav.) Pers. seeds[1]

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ABSTRACT- Seed desiccation tolerance (DT) and longevity are necessary for better dissemination of plant species and establishment of soil seed bank. They are acquired by orthodox seeds during the maturation phase of development and lost upon germination. DT can be re-induced in germinated seeds by an osmotic and/or abscisic acid treatment. However, there is no information on how these treatments affect seed longevity. Germinated *Sesbania virgata* seeds were used as a model system to investigate the effects of an osmotic treatment to re-establish DT on seed longevity. Longevity of germinated *S. virgata* seeds treated and non-treated by an osmoticum was analysed after storage or artificial ageing. The radicle is the most sensitive organ, the cotyledons are the most resistant, and the ability to produce lateral roots is the key for whole seed survival. Germinated *S. virgata* seeds with 1mm protruded radicle tolerate desiccation and storage for up to three months without significant losses in viability. An osmotic treatment can improve DT in these seeds, but not longevity. Germinated *S. virgata* seeds are a good model to study DT uncoupled from longevity. Further studies are necessary to unveil the molecular mechanisms involved in both DT and longevity.

Index terms: desiccation tolerance, storage, germination, osmotic stress.

Tolerância à dessecação e longevidade de sementes germinadas de *Sesbania virgata* (Cav.) Pers.

RESUMO - Em sementes, tolerância à dessecação (TD) e longevidade são necessárias para a melhor dispersão da espécie e para o estabelecimento de um banco de sementes no solo. Ambas são adquiridas durante fase de maturação do desenvolvimento das sementes e perdidas durante a germinação. A TD pode ser re-induzida em sementes germinadas por um tratamento osmótico e/ou com ácido abscísico. Entretanto, não há informações sobre como esses tratamentos afetam a longevidade das sementes. No presente estudo, utilizou-se sementes germinadas de *Sesbania virgata* como modelo experimental para investigar os efeitos na longevidade de um tratamento osmótico para re-induzir TD. A longevidade de sementes germinadas de *S. virgata* submetidas ou não a um tratamento osmótico foi analisada após armazenamento ou envelhecimento acelerado. A radícula é o órgão mais sensível e os cotilédones são o órgão mais resistente. A habilidade de produzir raízes laterais é imprescindível para a sobrevivência das sementes. Sementes germinadas de *S. virgata* com radícula de 1mm de comprimento toleram dessecação e armazenamento por até três meses sem redução significativa na longevidade. O tratamento osmótico melhora a TD nessas sementes, mas não a longevidade. Sementes germinadas de *S. virgata* são um bom modelo para estudar a TD desacoplada da longevidade.

Termos para indexação: tolerância à dessecação, armazenamento, germinação, estresse osmótico.

Introduction

Species from the genus *Sesbania* (Fabaceae) are distributed mainly in the African and American continents. Due to their fast growth, easy propagation, high biomass production, and potential to form symbiosis with nitrogen-fixing bacteria, they are used on a large scale in agroforestry and for ecological restorations (Florentino et al., 2009; Kwesiga et al., 1999;

Ståhl et al., 2005; Zanandrea et al., 2009). One species of this genus commonly used in agroforestry in Brazil is *Sesbania virgata*, a fast-growing pioneer species that tolerates long periods of flooding and has a highly branched root system that protects the soil against erosion (Florentino et al., 2009; Zanandrea et al., 2009).

Seeds of *S. virgata* are orthodox, meaning that they are able to tolerate desiccation and survive in the dehydrated state

for long periods of time (Pammenter and Berjak, 1999). In orthodox seeds, desiccation tolerance (DT) and longevity are considered necessary for the completion of their life cycle, permitting the plant to store seeds, and ensure better dissemination of the species (Ramanjulu and Bartels, 2002).

During the maturation phase, orthodox seeds acquire DT and longevity, enter a dormant or quiescent state and can remain apparently inactive for very long periods (Ooms et al., 1993; Toldi et al., 2009). During germination, upon imbibition, DT remains for some time but then starts to be lost when DNA synthesis and (somewhat later) cell division resume (Faria et al., 2005). After the loss of DT, the existence of a small developmental window during which DT can be re-established by treatment with an osmoticum and/or the plant hormone abscisic acid (ABA) was demonstrated in a number of species, including *Cucumis sativus*, *Impatiens walleriana* (Bruggink and Van der Toorn, 1995), *Medicago truncatula* (Buitink et al., 2003; 2006) and *Arabidopsis thaliana* (Maia et al., 2011; 2014). When DT is fully rescued, seeds seem to be in a stage resembling the developmental stage that they were in prior to germination (Buitink et al., 2006; Maia et al., 2011).

The ability of germinated seeds to re-acquire DT is thought to optimize successful seedling establishment under unpredictable environmental conditions (Dekkers et al., 2015). This ability, in conjunction with longevity, could represent an ecologically important stress tolerance mechanism that allows germinating/germinated seeds to remain viable in the dry state for a certain time. In the last few years, several studies have been carried out focusing on the acquisition, loss and re-induction of DT in seeds of model species (Dekkers et al., 2015). However, some diversity in stress-tolerance mechanisms is expected, raising the expectations that valuable information can be generated using non-model species that have to cope with such stress in their natural environments. In their natural habitat, *S. virgata* seedlings are subjected to irregular precipitation patterns at the start of the wet season. Despite the numerous studies on the re-induction of DT in germinated seeds, no information is available concerning the effects of treatments to re-induce DT on longevity. In the present study, the longevity of germinated *S. virgata* seeds was investigated. The results show that germinated *S. virgata* seeds with 1 mm protruded radicle can be dried back and be stored, and that an osmotic treatment can improve DT, but not longevity.

Material and Methods

Mature seeds of *S. virgata* were collected from 12 trees at Lavras (21˚22''S, 45˚1''W, Minas Gerais, Brazil) and stored in a cold room at 4 ℃. Prior to germination tests, seeds were immersed in concentrated sulphuric acid (H_2SO_4) for 30 min

and washed with abundant running water to remove physical dormancy. Germination assays were carried out in moist rolled paper, at 30 ℃, under constant light (30 W m^{-2}) for 36 h.

To determine the moment of loss of DT, three replicates of 20 seeds were selected according to their protruded radicle length (1 mm, 3 mm and 5 mm) and dried for three days. Throughout the study, drying treatments were performed by placing the seeds for three days at 40% relative humidity (RH) at 22 ℃, resulting in a water content as low as 0.14 g H_2O g^{-1} dry weight (Figure 1). Water content was assessed gravimetrically for triplicate samples of 10 seeds, by determination of the fresh weight and subsequent dry weight, after 18 h in an oven at 105 ℃. Water content is expressed on a dry weight basis. After drying, seeds were pre-humidified in air of 100% RH for 24 h at 22 ˚C in the dark to prevent possible imbibitional damage and subsequently rehydrated in water on a Copenhagen Table under a 12/12 h dark/light regime at 22 ˚C. Germinated seeds were evaluated according to the survival of their primary root, presence of green cotyledons (cotyledon survival) and growth resumption with both green cotyledons and development of a (secondary) root system (seedling survival) (Maia et al., 2011).
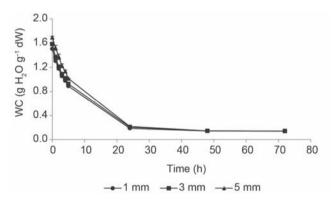

Figure 1. Changes in water content upon dehydration of germinated *S. virgata* seeds at 40% RH with 1 mm, 3 mm, and 5 mm protruded radicles. Each data point is the average of three replicates of 20 seeds. Bars represent standard error.

The re-establishment of DT in sensitive seeds was evaluated in three replicates of 20 germinated seeds selected according to their protruded radicle length (1 mm and 3 mm). These germinated seeds were dried (control) or treated with an osmoticum (incubation in 20 mL PEG solution with an osmotic potential ranging from -1.5 to -3.0 MPa at 4 ℃ or 22 ℃ (Villela and Beckert, 2001) in the dark, for 72 h) and dried. Possible hypoxic conditions were avoided by using an amount of PEG solution enough to cover the radicles, but not entire seeds. After incubation in PEG, seeds were rinsed thoroughly in distilled

water, dried, pre-humidified, rehydrated and evaluated as described above.

Longevity was evaluated in triplicates of 15 germinated seeds with 1mm protruded radicle and dried as described above. These seeds were stored in sealed plastic bags for three to eight months at 4 °C. Additionally, longevity was estimated based on survival after an accelerated aging assay consisting of storage for two to seven days at 80% RH and 40 °C in the dark. Survival was evaluated as described above and by a tetrazolium test. For the tetrazolium test, three replicates of six seeds were moistened on filter paper in Petri dishes for 24 h at 22 °C. After the removal of the seed coat, they were soaked in 0.5% (w/v) 2,3,5-triphenyltetrazolium chloride solution for 2 h in the dark at 30 ˚C and scored using location of staining as criteria (Camargos et al., 2008).

In order to evaluate the influence of a treatment to re-establish DT on longevity, germinated seeds with 1 mm protruded radicle incubated in -2.5 MPa PEG at 4 °C and dried were subjected to the same longevity tests as described for non-treated seeds.

Data were statistically analysed with SPSS 22.0 for Windows (IBM Corporation, Somer, NY, USA) using one-way ANOVA followed by Duncan post-hoc test ($P \leq 0.05$).

Results and Discussion

Seeds of more than 90% of angiosperm species for which data are available are orthodox (Royal Botanic Gardens Kew, 2008). During germination of these seeds, water is taken up and metabolic processes are resumed, leading to the progressive loss of DT (reviewed by Dekkers et al., 2015). The point during germination when DT is lost varies among species. For example, within legume species, *Copaifera langsdorfii* and *Pisum sativum* lose DT before radicle protrusion (Pereira et al., 2014; Reisdorph and Koster, 1999), while *Glycine max*, *Medicago truncatula* and *Peltophorum dubium* lose DT after radicle protrusion (Buitink et al., 2003; Guimarães et al., 2011; Senaratna and McKersie, 1983). Here it is shown that *S. virgata* (also a legume) seeds lose DT progressively after radicle protrusion (Figure 2), being almost completely sensitive when the protruded radicle length reaches 5 mm.

More than 50% of germinated *S. virgata* seeds with 1 mm protruded radicle are able to survive desiccation without any previous treatment. As the radicle grows to 3 mm, there is a considerable drop in seedling formation to around 10%. Germinated seeds with 5 mm protruded radicle are sensitive to desiccation. DT is first lost by the radicle, the most sensitive organ in seeds of *S. virgata* and in other species (Buitink et

al., 2003; Maia et al., 2011). The cotyledons are the most resistant organs, but the crucial point to determine if the germinated seed will survive is the ability to produce lateral roots (Bruggink and Van der Toorn, 1995; Maia et al., 2011). Germinated seeds are able to produce lateral roots when there are viable tissues that can differentiate to root primordia, as shown by the tetrazolium staining. When the non-stained areas included the hypocotyl, the germinated seeds did not produce root primordia and survival of the whole seed was compromised. On the other hand, when the hypocotyl was stained, lateral roots were produced to replace the main root and to enable the seedling to establish.
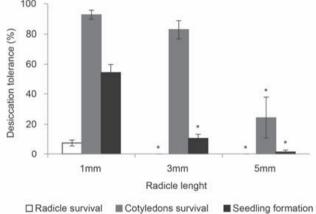

Figure 2. Desiccation tolerance in germinated *S. virgata* seeds with different protruded radicle lengths. Each data point is the average of three replicates of 20 seeds. Vertical bars represent standard errors. * indicate significant differences ($P \leq 0.05$) comparing to germinated seeds with 1 mm protruded radicle.

Previous studies have shown that after the loss of DT, a small developmental window is opened during which DT can be re-established by treatment with an osmoticum and/or ABA (Bruggink and Van der Toorn, 1995; Buitink et al., 2003; Maia et al., 2014). Attempts to re-induce DT in germinated *S. virgata* seeds by incubation in ABA failed, as ABA was not effective in arresting growth (data not shown). Figure 3 shows the percentage of DT obtained at different water potential/temperature combinations for germinated seeds with 1 mm and 3 mm protruded radicle. The osmotic treatment might inhibit radicle growth until enough ABA has accumulated and operates (Buitink et al., 2003). The water potential had considerably more impact on the re-establishment of DT than the temperature. For germinated seeds with 1 mm protruded radicle length, the water potential of -2.5 MPa led to the highest percentage of radicle survival. Still, even under these

conditions, DT could not be re-established in all radicles. At the lowest water potential tested (-3.0 MPa), DT was observed in the lowest percentages of seeds. For germinated seeds with 3 mm protruded radicle length, some of the osmotic treatments significantly improved DT compared to non-treated seeds with the same radicle length, but the maximal percentage of seedling formation was lower than 40% and radicle survival

was not improved. DT is a complex trait and it is possible that in *S. virgata*, as the radicle grows, the mechanisms needed for DT are progressively lost and cannot be fully re-activated anymore. Based on these results, the best radicle length and treatment to re-establish DT in germinated *S. virgata* seeds is 1 mm radicle length and incubation in an osmoticum of -2.5 MPa at 4 °C.
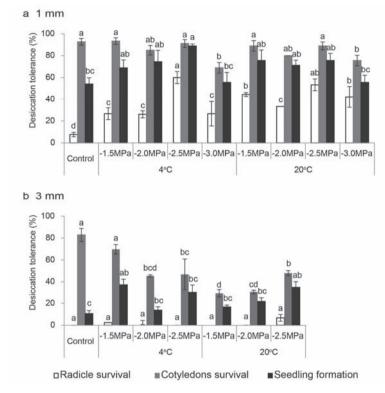

Figure 3. Osmotic potential and temperature effects on the re-establishment of desiccation tolerance of germinated *S. virgata* seeds. (a) Germinated seeds with 1mm protruded radicle. (b) Germinated seeds with 3 mm protruded radicle. Each data point is the average of three replicates of 20 seeds. Bars represent standard errors. Control = germinated seeds dried directly at 40% RH without previous osmotic treatment. Different letters above bars indicate significant differences ($P \leq 0.05$).

Seed DT and longevity are crucial for long-term survival of orthodox seeds after dispersal (Waterworth et al., 2015). As a result, both longevity and DT share several mechanisms. For example, mechanisms responsible for protection of cellular macromolecules and cellular structures in the dry state, damage repair during germination and the minimization of oxidative stress damage are well documented in relation to both DT and longevity (Rajjou and Debeaujon, 2008; Waterworth et al., 2015). Seed longevity can be analysed by storage and artificial aging. One of the methods to test artificial aging is the accelerated aging test, during which seeds are incubated at both high humidity and temperature. Cold storage (storage at 4 °C) and an accelerated aging test were used to determine

longevity of germinated seeds with 1 mm protruded radicle and of these seeds incubated in an osmoticum at -2.5 MPa at 4 °C (Figure 4). The radicle was the most sensitive organ with respect to aging and the osmotic treatment did not improve its survival. The cotyledons were the most tolerant organs. The osmotic treatment significantly improved cotyledon survival and seedling formation after accelerated aging for four days, but this positive effect was not observed in any of the other treatments. Overall, the osmotic treatment had a positive effect on seedling formation before aging, but was not able to improve longevity of the seeds.

The tetrazolium test (Figure S1) corroborates these results. In the treatments with high seedling survival, the staining was

homogeneous from the radicle tip to the cotyledons in most of the seeds. Low seedling survival was correlated with a bigger

extent of non-stained areas starting from the radicle tip and reaching the hypocotyl.
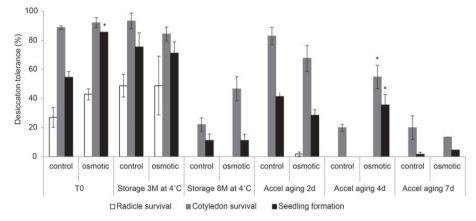

Figure 4.   Desiccation tolerance after storage or accelerated aging test (Accel aging) of germinated *S. virgata* seeds with 1 mm protruded radicle. Storage was performed at 4 ºC for three and eight months (M). Accelerated aging was performed for 2, 4 and 7 days (d). Each data point is the average of three replicates of 15 seeds. Bars represent standard error. T0 indicates germinated seeds dried directly at 40% RH without previous osmotic treatment. * indicate significant differences at $P \leq 0.05$ comparing control and seeds treated with an osmoticum (-2.5 MPa at 4 ºC) within the same aging treatment.
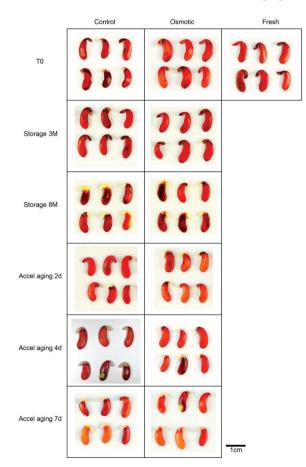

Figure S1. Tetrazolium staining of germinated *S. virgata* seeds with 1mm protruded radicle after storage or accelerated aging test (Accel aging). Storage was performed at 4 ºC for three and eight months (M). Accelerated aging was performed for 2, 4 and 7 days (d). Fresh seeds were imbibed, decoated and stained with tetrazolium. Two seeds per replicate are shown.

Conclusions

Germinated seeds could be stored for three months at 4 °C without significant losses in viability. Overall, longevity was not improved by the osmotic treatment.

Once DT is lost upon germination, an osmotic treatment can re-establish it up to a certain point during development. The same may not hold true for longevity. More studies are necessary to unveil the molecular similarities and differences between DT and longevity.

Acknowledgments

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