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ARTICLE

Reduction of desiccation sensitivity in seeds of tree species

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ABSTRACT: Desiccation sensitivity is a condition present in seeds of several tree species of economic and ecological importance, which makes such seeds unable to tolerate drying and storage. Thus, studies that seek strategies to maintain the viability of such seeds after being subjected to drying are necessary. An alternative for this purpose is the use of priming in solutions of known osmotic potential. Solutions of polyethylene glycol (PEG), glycerol, as well as phytohormones such as abscisic acid (ABA) and salicylic acid (SA) are related to stress tolerance in seeds. The objective of this work was to study desiccation sensitive seeds of *Inga vera*, *Eugenia uniflora* and *Tapirira guianensis*, using solutions of PEG, SA, sucrose, glycerol and combinations of SA+PEG in an attempt of reducing seed desiccation sensitivity. The responses varied according to the species, but in general, successful results in maintaining the viability of the seeds after drying were obtained from the treatmen of seed with sucrose, glycerol and combinations of PEG and SA. Treatment of seeds with these compounds is promising in reducing the desiccation sensitivity of seeds of tree species.

Index terms: desiccation tolerance, osmoconditioning, seed storage.

RESUMO: A sensibilidade à dessecação é uma condição presente em sementes de diversas espécies arbóreas de importância econômica e ecológica, o que torna tais sementes incapazes de tolerar a secagem e o armazenamento. Assim, são necessários estudos que busquem estratégias para manter a viabilidade dessas sementes após serem submetidas à secagem. Uma alternativa para esse fim é a utilização de priming em soluções de potencial osmótico conhecido. Soluções de polietilenoglicol, glicerol, bem como fitohormônios como ácido abscísico e ácido salicílico estão relacionados à tolerância ao estresse em sementes. O objetivo deste trabalho foi estudar sementes sensíveis à dessecação de *Inga vera*, *Eugenia uniflora* e *Tapirira guianensis*, utilizando soluções de PEG, SA, sacarose, glicerol e combinações de SA+PEG na tentativa de reduzir a sensibilidade à dessecação das sementes. As respostas variaram de acordo com a espécie, mas em geral resultados bem-sucedidos na manutenção da viabilidade das sementes após a secagem foram obtidos a partir do tratamento das sementes com sacarose, glicerol e combinações de PEG e SA. O tratamento de sementes com estes compostos é promissor na redução da sensibilidade à dessecação de sementes de espécies arbóreas.

Termos para indexação: tolerância à dessecação, osmocondicionamento, armazenamento de sementes.

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INTRODUCTION

Seeds of tree species are known to behave differently in terms of their tolerance to drying and storage and are therefore classified as orthodox, recalcitrant or intermediate. Currently, the biggest challenge in seed conservation is to develop a technology that allows the storage of recalcitrant seeds for periods that allow their conservation in germplasm banks, reducing the risk of extinction of these species (Barbedo, 2018; Barbedo et al., 2018).

Desiccation tolerance involves a series of structural and metabolic mechanisms that include hormonal changes, quantitative and qualitative changes in proteins and sugars, which allow cells to tolerate water loss (Bonjovani and Barbedo, 2020). Since the 1990s, studies have been intensified in an attempt to understand how these mechanisms work at the physiological and molecular levels, but due to the large number species, conclusive information is still scarce.

In recalcitrant seeds, the control of water mobilization through the use of osmotic solutions seeks to reduce their metabolism and has shown satisfactory results, especially for legumes. Seed priming has been used as a tool to understand desiccation tolerance mechanisms (Pelissari et al., 2022).

There are already results in the literature indicating that the use of polietilenoglicol (PEG) has a positive effect on *Inga vera* Willd seeds, allowing the embryos of this species to remain viable for longer periods during storage (Pereira et al., 2020). Similarly, the use of salicylic acid (SA) has been highlighted as a strategy to reduce the effects of stress in plants (Khan et al., 2015), as well as seed germination, membrane permeability, stomatal closure, photosynthesis and plant growth (Hussein, 2015) and increased seed desiccation tolerance (Sahu and Naithani, 2023).

Thus, this study aimed to reduce the desiccation sensitivity of recalcitrant seeds, contributing to the storage of species that produce short lived seeds.

MATERIAL AND METHODS

The fruits of *I. vera*, *Eugenia uniflora* L. and *Tapirira guianensis* Aubl. were collected in the municipality of Lavras, MG and processed at the Tree Seed Laboratory of the Federal University of Lavras. After cleaning, the seeds were placed in a ventilated, shaded area to remove surface water and used immediately in the experiments.

Immediately after collection and processing, the initial characterization of the batches was carried out by germination and moisture content tests, in which the initial water content of the seeds (fresh basis) was assessed using four replications of 8 seeds, dried in an oven at 105 ± 2 °C for 24 hours as recommended by the Rules for Seed Testing (Brasil, 2009).

The germination tests were carried out with freshly harvested seeds and then after they had reached the target water content for each species (Table 1) after drying. The tests with *I. vera* were carried out in 4 replications of 25 seeds for each treatment, on a roll moistened germitest paper, kept at 30 °C in constant light. As *I. vera* seed is polyembryonic, embryos from the same seed were kept together during all counts. Seed was considered germinated when at least one of its embryos showed root elongation (2 mm).

The germination tests with *E. uniflora* seeds were carried out in gerboxes containing sand, in four replications of 20 seeds for each treatment at 25 °C with a 12-hour photoperiod.

Table 1. Target water contents for each species.

Species	Target water contents
Inga vera	45%, 40% and 30%
Eugenia uniflora	40%, 30% and 20%
Tapirira guianensis	10% and 5%

The germination of the T. guianensis seeds was carried out in gerboxes, on paper, in 4 replications of 25 seeds, at 25 °C with a 12-hour photoperiod.

After the initial assessment, the seeds were incubated in solutions described in table 2 for 72 hours at 10 °C in the dark to avoid oxidative damage. The conditions of treatments (time, temperature and light) were chosen based on previous assays (data not shown). Treated seeds were washed in running water and dried at 20 °C for 1 hour, in order to remove surface water. The treatments were carried out in solutions with the following concentrations (Table 2).

After the initial assessment, the seeds were incubated in the solutions described in table 2 for 72 hours at 10 °C in the dark to avoid oxidative damage.

Based on the initial water content and the target water content, the weight was calculated according to Eq. (1) (Hong and Ellis, 1996).

$$Target\ water\ content = \frac{(100 - Initial\ water\ content)}{(100 - Target\ water\ content)} \times Initial\ weight \quad (1)$$

Seeds treated with different solutions were dried at room temperature (20 °C and 50 ± 10% RH), laid out in a single layer on trays. The seeds were weighted at regular intervals until they achieved the desired weight (Eq. 2) and, when this was reached, the water content was assessed in an oven as previously described (Brasil, 2009).

Statistical analyses were carried out using the R for Windows statistical software (R Core Team, 2023). The data was analyzed using Generalized Linear Models (GLM), following the Binomial family and, when a significant treatment effect was observed using the Chisq test, the means were compared using the LSD test at 5% probability.

RESULTS

The water content of the seeds at the time of collection and after the treatments is shown below (Table 3).

Immediately after processing, the *I. vera* embryos had 55% water content and 98% germination. Drying to 45% water content did not compromise seed germination, even after treatment with PEG, which showed the lowest averages, with germination remaining at around 88%. At this water content, it is worth noting that although the seeds from the control treatment attained high germination values, they did not develop into normal seedlings, showing only the root system, without shoot emission (Figure 1).

When dried to 40% water content, the untreated seeds died, whereas those treated with sucrose and salicylic acid maintained germination at levels close to those of freshly harvested seeds (Figure 2). The treatment in which the seeds were treated with 0.5mM salicylic acid exceeded the germination percentage of the non-dried seeds. After

Table 2. Solutions used to treat the seeds to reduce their sensitivity to desiccation.

Treatment	Concentration
Control	*
Sucrose	0.75M
Glycerol	10% (v/v)
Salicylic acid (SA)	0.1 mM and 0.5 mM
Polyethyleneglycol (PEG)	-1.6 MPa and -2.1 MPa
PEG + SA	PEG -1.6 MPa + SA 0.1 mM
	PEG -1.6 MPa + SA 0.5 mM
	PEG -2.1 MPa + SA 0.1 mM
	PEG -2.1 MPa + SA 0.5 mM

* Seeds kept under the same conditions (72 hours at 10 °C in the dark), stored in a plastic bag, were used as a control.

Table 3. Seed water content before (fresh seeds) and after treatment in the different solutions.

Treatments	*I. vera*	*T. guianensis*	*E. uniflora*
	Water content (% fw)		
Fresh seeds (untreated)	55.3	40.0	50.0
Salicylic acid (SA)	52.6	43.6	54.5
PEG	51.5	39.4	56.9
PEG+SA	53.1	39.0	54.5
Glycerol	49.9	41.4	54.6
Sucrose	50.3	42.0	54.7

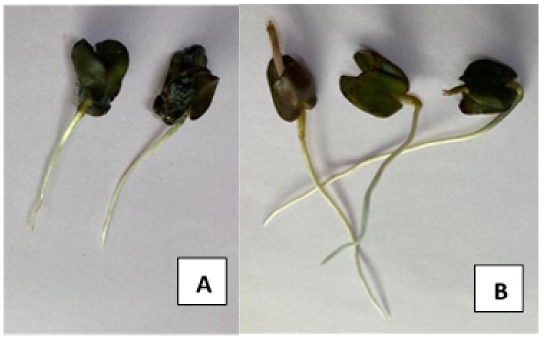

Figure 1. Germinated *Inga vera* embryos after drying to 45% water content. A- Untreated embryos (Control): protruded root and no shoot growth. B- Embryos treated with salicylic acid before drying: formation of normal seedlings.

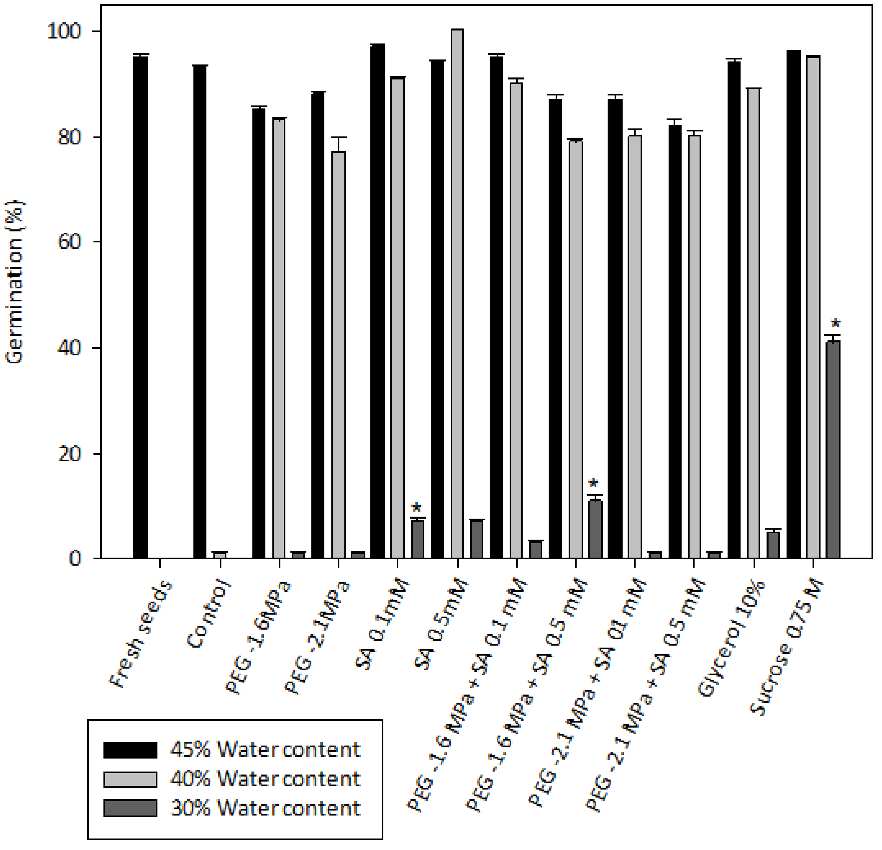

Figure 2. Germination percentage of *Inga vera* embryos after treatment in different solutions. The statistical diferences (LSD test at the 5%) betwen the treatments and control at 30% water content are indicated by asterisk.

drying to 30% water content, the best treatment for maintaining the viability of seeds of this species was sucrose, with germination remaining at around 34% (Figure 2).

Fresh *T. guianensis* seeds had 40% water content and 100% germination at the time of collection (Figure 3). When drying was carried out to 10% water content, the best treatments for maintaining seed viability were PEG -1.6 MPa + SA 0.1 mM and PEG -2.1 MPa + SA 0.5 mM (24% germination), followed by SA 0.1 mM, PEG -2.1 MPa + SA 0.1 mM, sucrose and PEG -1.6 MPa + SA 0.5 mM (18% germination). When the seeds were dried to 5% water content, none of the treatments reached a positive result in maintaining seed viability.

E. uniflora seeds dispersed at 50% water content had 99% germination at the time of collection (Figure 4). After drying to 40% water content, seeds treated with PEG -1.6 MPa + SA 0.5 mM and glycerol, followed by PEG -1.6 MPa + SA 0.1 mM and PEG -2.1 MPa + SA 0.1mM showed the best results in maintaining viability. For seeds dried to 30% water content, treatment in PEG -2.1 MPa + SA 0.1mM was the most efficient, maintaining germination at 23%. At 20% water content, no treatment was able to maintain seed viability. At this point the seeds from all treatments were dead.

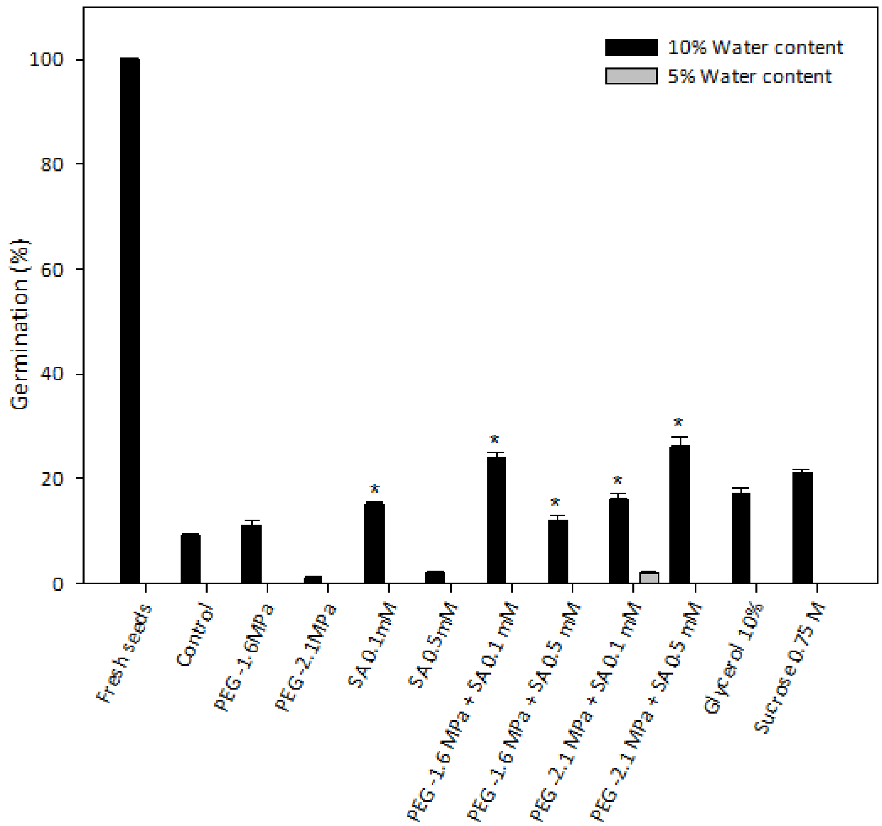

Figure 3. Germination percentage of *T. guianensis* seeds submitted to incubation in different solutions. The statistical diferences (LSD test at the 5%) betwen the treatments and control are indicated by asterisk.

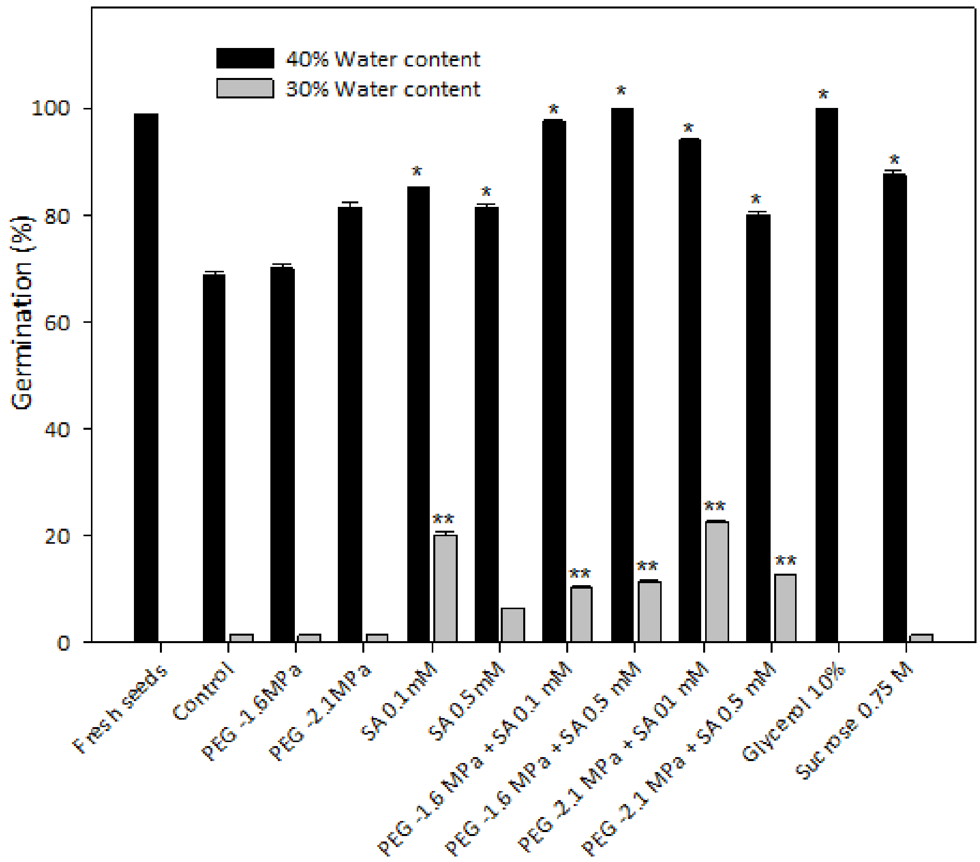

Figure 4. Germination percentage of *Eugenia uniflora* seeds subjected to treatment in different solutions. The statistical diferences (LSD test at the 5%) betwen the treatments and control at 40% (*) and 30% (**) water content are indicated.

DISCUSSION

The reduction in seedling length is mainly due to changes in cell turgidity as a result of the reduction in protein synthesis when there is a water deficit (Zhang et al., 2018). It is also worth noting that both the cell elongation process and cell wall synthesis are very sensitive to restricted water availability, and the reduction in growth in these situations would be due to a decrease in the turgidity of these cells. In addition, the greater development of the root system of seedlings under water stress is a way for them to absorb water at greater depths (Ahmad et al., 2018).

Desiccation tolerance occurs at varying levels in different parts of the seeds and may be a reason for the formation of abnormal seedlings after drying. Pelissari et al. (2022) obtained the same result after priming of *Magnolia ovata* seeds, in which only the root system was formed after drying at low water contents. In this case, when we look at the seeds that had not undergone any treatment and had been dried (Figure 1), and compare them with the seeds that had been treated with salicylic acid and dried, we observe that drying caused damage to the untreated seeds, confirming what has been described in various studies on the action of salicylic acid in promoting tolerance to water stress in plants (Khan and Khan, 2014). Studies show that salicylic acid acts by inducing genes encoding, for example, heat shock proteins (HSPs) and antioxidants, suggesting that application of this regulator can improve stress tolerance in plants (Khan et al., 2015).

Sucrose is known to protect seeds from damage caused by desiccation and also acts as a source of carbohydrates and increases the permeability of the cell wall, allowing the seeds to absorb more water and nutrients, resulting in faster embryo growth (Rodriguez-Enriquez et al., 2013; Pereira et al., 2021).

The beneficial effect of osmotic conditioning is the result of mechanisms to repair damage to the membrane system and the respiratory apparatus, as well as other biochemical events initiated during priming (Zhang et al., 2018).

McCue et al. (2000) reported that salicylic acid treatment mobilises endogenous auxin, which stimulates seed development. In addition, its exogenous application stimulates cell wall lignification by directing the pentose phosphate and shikimate pathways to produce lignin, which increases seed vigour (Pacheco et al., 2007), resulting in enhancement of physiological traits, fruit yield, and quality parameters of cantaloupe under water-deficit stress (Alam et al., 2023). Silveira et al. (2000), treating rice seeds with salicylic acid, observed that a concentration of 0.1 mM had a positive effect on the electrical conductivity of the seeds, preventing the leaching of solutes, although no differences were observed in the percentage of emergence, the speed of emergence and the growth of the seedlings at this concentration. The authors also observed that after treatment with SA, the amount of soluble sugars decreased and the amount of starch and protein increased when salicylic acid was used.

Several studies have reported that treatments such as priming improve germination, uniformity and speed of germination, as well as seedling vigour (Lopes et al., 2019; Espíndola et al., 2020) for different species (Alves et al., 2020; Bisognin et al., 2016; Thakur et al., 2019; Muhie, 2018; Rhaman et al., 2021). Rifna et al. (2019) also state that seeds that are soaked and then dried show greater vigour after storage than those that are not treated.

Thus, although most studies involving treatments such as seed priming are aimed at improving the germination of orthodox seeds (Li et al., 2023) and not at maintaining the viability of desiccation sensitive seeds, this study shows that the treatment of seed with salicylic acid, glycerol, sucrose and PEG can reduce the damage caused by the drying process in seeds with non-orthodox behaviour, highlighting that the best treatment to use, its concentrations and potential vary from species to species.

CONCLUSIONS

Positive results were obtained with treatments using PEG, SA, PEG+SA, glycerol and sucrose, promoting a reduction in desiccation sensitivity of forest tree seeds, with the best treatment varying between the species studied, indicating that these treatments can be effective reducing seed desiccation sensitivity and should be investigated in further studies.

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