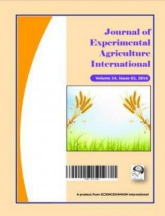

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Effect of Priming on Physiological Quality of *Handroanthus serratifolius* (Vahl.) Seeds

Girlânio H. Silva[1*], Anderson C. José[2], Fabrício P. Teixeira[1], Luciana M. Gonzaga[2], Raul R. Molina[2], Jordânia X. Medeiros[3], Maurício H. Okada[1] and Larissa Chamma[1]

[1]São Paulo State University (UNESP), Botucatu, SP, Brazil,
[2]Federal University of Lavras (UFLA), Lavras, MG, Brazil,
[3]Rural Federal University of Pernambuco (UFRPE), Recife, PE, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GHS, ACJ, FPT, LMG and RRM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JXM, MHO and LC managed the literature searches and revised the text. All authors read and approved the final manuscript.

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ABSTRACT

This work aimed to evaluate the effect of different priming treatments in the longevity of *H. serratifolius* seeds. Seeds were osmo-conditioned in PEG -1.0 MPa at 10, 15 and 20°C or hydro-primed at 5, 10 and 15°C. Final germination, speed and uniformity of germination were assessed. Priming did not affect the final percentage nor uniformity of germination; however, the germination speed was increased after hydropriming at 15°C and osmo-conditioning at 15°C compared to the control. Primed and not primed seeds were placed into an incubator (25°C, dark, 100% RH) until they reached 15% moisture content. Then, seeds were incubated in a container at 40°C for 0 to 144 hours, so, samples were taken in each period for determination of viability. The results suggest that priming increases longevity of *H. serratifolius* seeds.

Keywords: Seed longevity; controlled deterioration; hydro priming; forest seeds; germination.

**Corresponding author: E-mail: girlanio_holanda@hotmail.com;*

1. INTRODUCTION

The use of seeds with high physiological quality is one of the critical aspects to improve the plant performance in the field. Several factors can affect the quality of a seed lot, which range from the genetic characteristics of the specie to environmental factors. They affecting the development of seeds, methodology of collection and cleaning, storage and use of techniques such as priming [1].

Priming is a technique used for seed invigoration which aims the increase in germination rate and uniformity, especially in seed lots with low vigor. It was proposed by Heydecker et al. [2] and constitutes basically in a controlled hydration of the seeds, preventing the radicle protrusion. After the treatment, seeds can be dried back before use.

The main effects of priming are the increase in speed and uniformity of germination, and in some cases increases, tolerance to environmental stresses on the seeds and seedlings [3]. However, the priming effect cannot be positive, especially when it is followed by drying before germination. This inconsistent response is also observed in seed longevity. However, besides negative effects have been reported [4-6], increase in seed longevity has also been observed [7].

Considering the factors that determine the improvement of seed quality in relation to speed and uniformity of germination, it is expected an decrease in the longevity of the seed lots and, but in few cases are found an increased storage potential of the seeds after priming [7-8].

The *H. serratifolius* is an arboreal species of Bignoniaceae family. It is widely used in urban greening projects [9]. Some species of this genus show significant variation in seed quality during storage, which can hinder the conservation and propagation practices [10]. According to Souza et al. [11] the low longevity of seeds of *H. serratifolius* associated with the seasonality of production is a challenge for the production of seedlings of this specie. Thus, techniques that increase the storage potential of seeds of this species should be studied as a way to benefit the conservation and reforestation programs. Thus, the study aimed to evaluate the effect of different priming treatments in the longevity of *H. serratifolius* seeds.

2. MATERIALS AND METHODS

The experiment was conducted at the Forest Seed Laboratory (Department of Forest Science - Federal University of Lavras, Brazil).

Seeds of *Handroanthus serratifolius* were collected from trees located in Lavras - MG, in September 2013. After the removed of PEG, seeds were placed in a drying room (20°C/50% RH) for two weeks and stored in a cold chamber (5°C) in a semi-permeable container (plastic bag).

The seed coat was removed from the seeds in order to enable identification of damaged and deteriorated seeds and to reduce fungi infestation during the priming treatments and germination tests, and after this process, the seed were used in the tests.

2.1 Determination of Water Content

Water content of the seeds was assessed according to Brazil [12] using oven drying method (105 ± 3°C for 24 hours).

2.2 Priming Treatments

Seeds were first submitted to different priming methods in order to determine the best method of conditioning. Two priming methods were tested namely: Osmopriming using a polyethylene glycol (PEG) solution at -1.0 MPa, and hydro-priming (in distilled water). For each treatment, 100 seeds were soaked in 10 ml distilled water or 10 ml of PEG solution over filter paper in Petri dishes. Priming was conducted at three temperatures: 10, 15 and 20°C (osmopriming) and 5, 10 and 15°C (hydropriming). After priming, seeds were rinsed in tap water, blotted dry and placed in a dry room (20°C and 50% RH) for up to one week (until the equilibrium moisture content was reached).

After drying, seeds were germinated in Petri dishes at 25°C under constant light. In order to determine the best priming treatment, germination was scored daily for determination of final percentage of germination, germination rate (t50) and uniformity (u7525).

2.3 Germination Tests

Seeds were placed in 90 mm Petri dishes at 25°C under constant light over two moistened germination paper towels using four replicate samples of 25 seeds for each treatment. Before germination, seeds were surface-sterilized in 1%

sodium hypochlorite solution for 10 minutes and then rinsed for one minute with tap water. Germination was assessed daily by counting the numbers of seeds presenting radical protrusion of at least 2 mm.

2.4 Priming Effect on Seed Vigor

After determination of the best priming method (hydropriming at 15°C), seeds were primed as describe above and submitted to controlled deterioration. Dried seeds (not primed seeds) were used as control.

For controlled deterioration, primed and control seeds were placed in a moist chamber (25°C and 100%RH) for about five hours, when seed water content reached 15% (wet basis). Seeds (primed and control) were then transferred to a sealed container and incubated at 40°C for 0, 6, 12, 24, 36, 48, 60, 72, 96, 120 and 144 hours. After each period, a seed sample was taken for determination of viability by germination test.

2.5 Statistical Analysis

The effect of priming methods on the seed quality was analyzed using a completely randomized design with four replications of 25 seeds and six treatments.

The estimation of t50 and u7525 values was performed using GERMINATOR [13]. After the adjustment of curves and determination of the indices t50 (time required for 50% germination of seed germination, i.e. germination speed) and u7525 (time comprised between 25 and 75% of total germination, i.e. germination uniformity) mean values obtained were compared by the Studentized t test at 5% probability.

To analyze the effect of priming on seed germination, a completely randomized design with four replications of 25 seeds was used with treatments in a factorial scheme 2x11 (primed and not primed seeds x 11 periods of exposure to 40°C). Comparisons were performed by regression analysis using the software ASSISTAT v7.7 [14].

3. RESULTS AND DISCUSSION

3.1 Effect of Priming on Seed Germination

H. serratifolius seeds showed little variation in response to priming treatments in relation to maximum germination. In general, the osmo-priming and hydro-priming did not provide a statistically significant increase compared to the control during germination (Fig. 1).

It was observed that seeds submitted to hydro-priming showed 100% germination at temperatures of 10 to 15°C, however, low percentage of germination was observed when seeds were hydroprimed at 5°C. Osmoprimed seeds (PEG at -1.0 MPa at 10 and 15°C) presented 100% germination, with a slight reduction (not statistically significant) in germination (97%) when osmo-priming was performed at 20°C.

Regarding the speed of germination as measured by t50 (time required for 50% of the seeds germinate), there was a significant effect (Fig. 2) among the treatments. Osmo-primed and hydro-primed seeds at 15°C had lower t50 (higher speed of germination) when compared to the control.

Although *H. serratifolius* seeds showed a positive response to priming in terms of speed of germination (t50), there was no significant effect for most of the treatments in relation to the germination uniformity (u7525) (Fig. 3). The majority of the treatments did not differ in the uniformity of germination. Hydro-primed seeds at 5°C and 10°C showed a significant reduction in u7525, however, final percentage of germination of seeds hydro-primed at 5°C was very low. The best results were observed in the following treatments: HP15, OP10, OP15 and OP20. (Fig. 3).

3.2 Priming Effect on Seed Longevity

Once seeds did not present huge variations in response to priming, hydro-primed seeds at 15°C were selected because of high germination, low t50, u7525 and simplicity of the protocol. However, despite the low response of seeds to priming, probably due to high quality of the seed batch, hydroprimed seeds showed a different behavior when compared to the control (not primed) after controlled deterioration. There were significant differences ($P < .0001$) between treatments. Hydroprimed seeds showed higher tolerance to stress conditions during controlled deterioration (Fig. 4).

After incubation for up to 12 hours at 40°C, *H. serratifolius* seeds did not show changes in viability, which remained above 90%. However,

after 24 hours of controlled deterioration, non-primed seeds showed a reduction of 40% in germination, and the total mortality after 36 hours of controlled deterioration. Thus, primed seeds only showed significant reduction in viability after 60 hours (67%) of controlled deterioration.

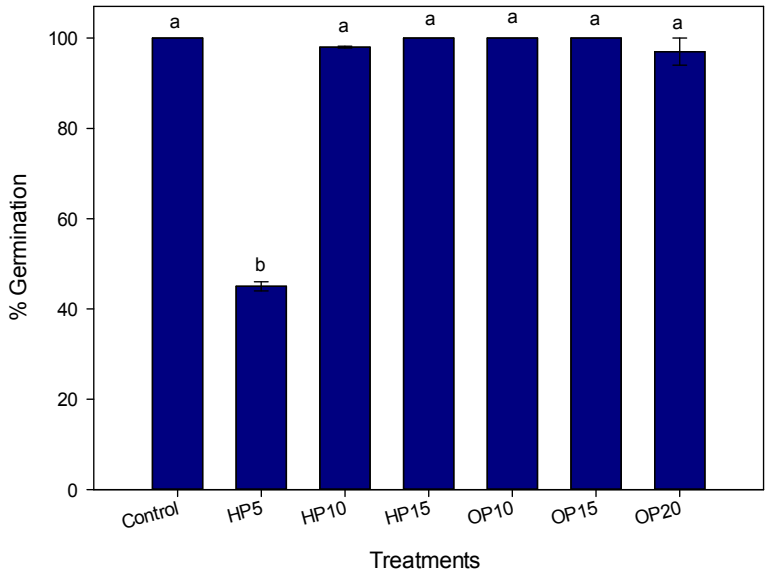

Fig. 1. Effect of priming on germination of *H. serratifolius* seeds. Control, HP (Hydropriming), OP (Osmopriming). Bars represent standard deviation

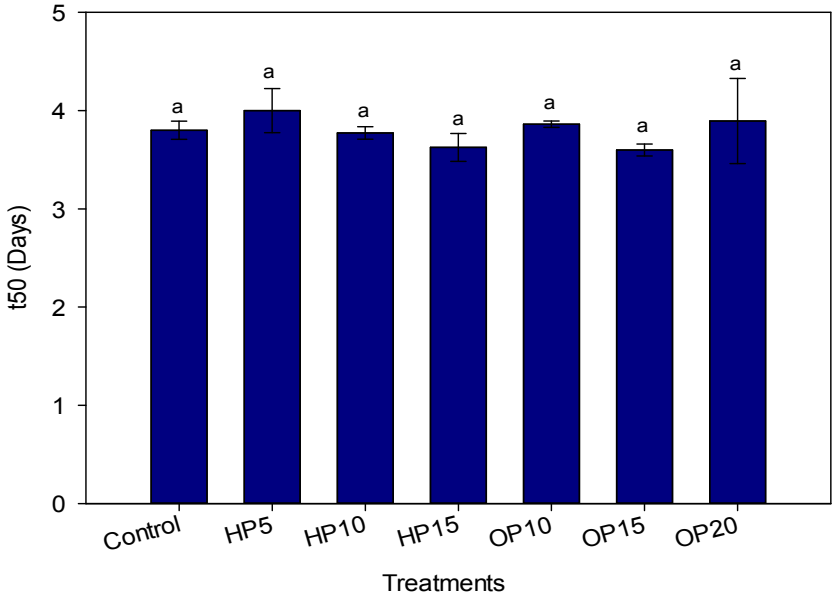

Fig. 2. Effect of priming on speed of germination (t50) of *H. serratifolius* seeds. control, HP (Hydropriming), OP (Osmopriming)

Mean ± S.E.M = Mean values ± Standard error of means of four replicates

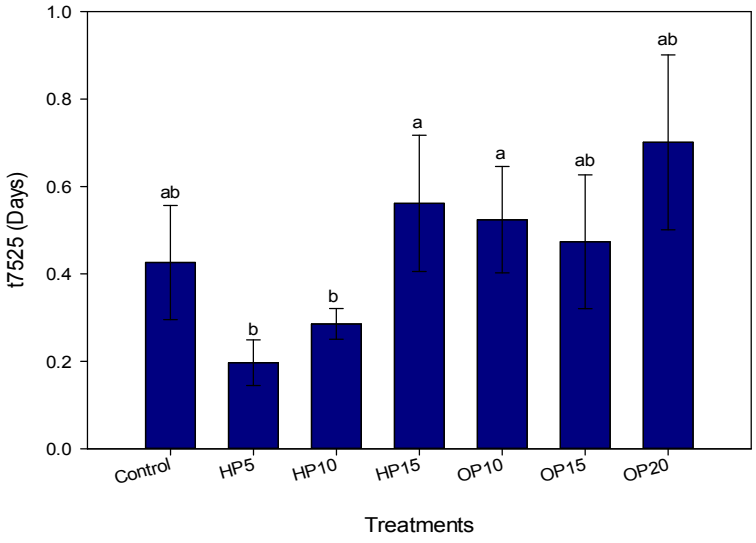

Fig. 3. Effect of priming on germination uniformity (u7525) of (*H. serratifolius*) seeds. control, HP (Hydropriming), OP (Osmopriming)

Mean ± S.E.M = Mean values ± Standard error of means of four replicates

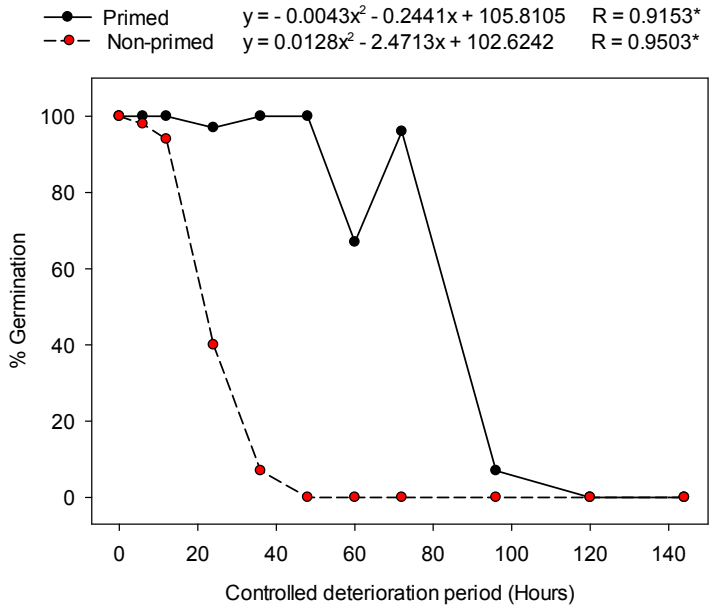

Fig. 4. Effect of priming on seed longevity after controlled deterioration on *H. serratifolius*)

3.3 Discussion

3.3.1 Effect of priming on seed germination

Some studies have found positive effects of priming on seed germination [15-17]. However, the effects of priming in other species has not shown significant changes in the final percentage of germination [18-21], as observed in this study.

One explanation for the absence of effect of treatments on the response of seeds in relation to germination is the initial quality of the seed batch used. seeds used in the control treatment

(non-primed), in this research, showed 100% germination, which removes the chances of improvement on seed quality. Only priming at 5°C adversely affect germination. This negative response may be related to the low temperature limits tolerated by this specie, indicating that temperatures around 5°C can cause damages to *H. serratifolius* during germination. Damages at low temperatures may also be an effect of freezing or chilling injuries at cellular level. According to Bewley et al. [22], low temperatures decrease metabolic activity, causing a reduction in the percentage of germination and therefore resulting in delayed germination process. You may also wish to discuss the role recalcitrance or orthodoxy nature of *H. serratifolius* with regards to low temperature damages to physiological responses.

Bearing in mind the high seed quality used in this research, was not observed positive effect on germination speed. These results are not similar to that found by Rodrigues et al. [23] and Balbinot and Lopes [24] that observed positive effects of hydro-priming in parsley and carrots seeds, which an increase in germination rate were found. Priming should normally improve seed germination in seed batches that originally have physiological quality concerns. The effect of priming was therefore seen on deteriorated lots of the seeds of *H. serratifolius* used in this study. I think that agrees with the previous works. This comment could be rephrased to discuss the results in a more robust way by reflecting on what happens in this work.

The germination uniformity, should be considered. In general, after priming there is an increase in the uniformity of germination of seeds compared to the control [25-27]. In this study, germination uniformity measured by u7525 was not affected by priming, as observed for *Senna spectabilis* seeds in [28].

3.3.2 Priming effect on seed longevity

According to Souza et al. [11] *H. serratifolius* presents problems during storage, so it is necessary studies to increase the longevity of seeds of this specie when stored.

It was observed that not primed *H. serratifolius* seeds were severely affected by controlled deterioration when compared to the primed ones. This result shows that hydro-priming probably induced physiological changes in the seeds leading to a higher tolerance to stress conditions (high humidity and temperature). After priming, sorghum seeds in PEG solution at -0.6 and -1.2 MPa, and distilled water (hydropriming), observed increase the germination percentage and longevity in hydro-primed sorghum seeds. Similarly, [24] and [29] found that the priming contributed to the increase of vigor in carrot and gherkin seeds. The authors discussed the importance of hydro-priming treatment for seeds that will be exposed to adverse environmental conditions after sowing.

The mechanisms associated with improvement in the quality of seeds after priming are not yet fully known [30]. Some authors attribute this effect to the increase in activity of the antioxidant system and membrane repair processes [31-35]. The greater tolerance of primed seeds under controlled deterioration can be correlated with the gain of longevity after priming. [7] analyzing seeds of *Digitalis purpurea* after priming found that the treatment increased the longevity of seeds. Priming treatments used in this study have been shown to increase the longevity of seeds *H. serratifolius*, however, more studies are needed using different priming conditions in order to optimize a protocol for this species, bearing in mind the potential of the priming technique in changing answers of *H. serratifolius* seeds for tolerance to stresses. In the same way, studies to determine the factors associated with better performance of *H. serratifolius* seeds after priming should be performed.

4. CONCLUSION

Hydropriming at 15°C increases germination speed and longevity of *H. serratifolius* seeds. However other studies should test this same treatment to evaluate the seed longevity along the storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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