Sexual propagation of *Abies religiosa* (Kunth) Cham, by immersion in water at different temperatures and periods

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ABSTRACT

Objective: To increase the germination percentage of *Abies religiosa* by immersion in water with different temperatures and periods.

Design/Methodology/Approach: A completely randomized factorial experiment was carried out with five temperatures (10, Room temperature (Rt), 30, 40, and 50 °C) and 13 periods expressed in seconds (10, 20, 40, 60, 600, 1200, 1800, 2400, 3000, 3600, 7200, 14400, and 21600), with a total of 65 treatments and 30 repetitions per treatment. The seeds were dipped in water with the temperatures and periods of each treatment to obtain the highest germination percentage.

Results: The best pre-germination treatment (73% germination) was obtained with the combination of 40 °C and 7,200 s. Meanwhile, the treatments with the lowest percentage (14%) were the combinations 10 °C×20 s and 10 °C×40 s. Twenty-six treatments recorded a >50% germination, reaching a peak value of 8.56 at 11.7 days.

Study Limitations/Implications: Keeping the water temperature and the immersion period constant during the experiment was more difficult in the longer treatments, with the lowest and highest temperatures.

Findings/Conclusions: *Abies religiosa* registers acceptable germination percentages with the highest temperature (50 °C).

Keywords: Germination, temperature, time, peak value.

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INTRODUCTION

Regeneration in conifers is a slow and difficult process that involves sexual reproduction as a propagation alternative under natural conditions. The oyamel (*Abies* sp.) genus reproduces with some ease in the forests, despite its low viability percentages and high percentage of vain seeds (Franklin, 1974). The sexual propagation of trees through seed germination is the main means of obtaining more vigorous, adaptable, and healthy plants

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(Briscoe, 1990; Trujillo-Navarrete, 1992). Seeds are considered the most important source of germplasm in forest species and they are the most frequently used source for the mass reproduction of plants (Meza, 2009). However, they are limited by the dormancy resulting from internal and external factors, which is only interrupted when adequate germination conditions are present. However, when this phenomenon does not occur or takes place gradually, the germination rate is very low (Rodríguez-Sánchez, 1995).

Several causes determine dormancy, including rudimentary or physiologically immature embryos, mechanically resistant or impermeable seed coats, germination inhibitors, and insufficient storage (Vindas, 2013). Varela and Arana (2011) and other authors consider this as an adaptation that contributes to survival, since it restricts germination in the face of unfavorable environmental factors (*i.e.*, temperature, humidity, and gaseous environment). Several pre-germination treatments can be used to remedy this situation and break seed dormancy. The most common methods are stratification, scarification, and leaching (Donoso, 1993; Franz-Eugen, 1996).

Consequently, several pre-germination treatments were evaluated to increase the germination percentage in *Abies religiosa* through their immersion in water with different temperatures and periods.

MATERIALS AND METHODS

The study was carried out in the Postgrado en Ciencias Forestales of the Colegio de Postgraduados - Campus Montecillo (19° 27' 38.95" N and 98° 54' 24.96" W), located at 2,246 m.a.s.l. The seeds used were donated by the San Luis Tlaxialtemalco nursery, located in Xochimilco, Mexico City. The seeds were collected in 2015 at the ejido San Andrés Hueyacatitlán, Puebla, Mexico (19° 15' 20" N and 99° 47' W). The experiment was carried out in thirty 20×27 cm plastic trays.

A 5×13 factorial experiment was established according to a completely randomized design, resulting in 65 combinations. The following factors were taken into consideration: temperature (10, room temperature (Rt), 30, 40, and 50 °C) and periods (10, 20, 40, 60, 600, 1200, 1800, 2400, 3000, 3600, 7200, 14400, and 21600 seconds). Each seed was considered as an experimental unit (EU). Thirty repetitions were performed for each combination, reaching a total of 1,950 EUs.

Once the 30 trays were ready, 200 mL of water were added to moisten their protective cloth. The seeds of Abies religiosa were placed in a beaker to which cold or hot drinking water was added, depending on the required temperature, which was measured with a digital thermometer.

Variables

Germination

The seed was considered to have already germinated when the radicle appeared.

Peak Value (PV)

PV is the maximum value obtained by dividing the germination percentage accumulated on each measurement date by the number of days elapsed to date.

Number of days to peak value (NDPV)

NDPV is the number of days necessary to reach a certain germination level, when the accumulated germination divided by the number of days of the test reached the maximum value (Czabator, 1962; Kolotelo *et al.*, 2001). The germinated seeds were evaluated daily during a 30 d period.

Data analysis

To determine which treatments recorded the highest germination percentages —taking into account the temperature and period that the seeds were exposed—, an analysis of variance (ANOVA) and the Tukey-Kramer significant difference test were used. The InfoStat software was also used (InfoStad, 2011).

The experimental design used was the following:

$$Y_{ijk}=\mu+T_i+S_j+TS_{ij}+B_k+\varepsilon_{ijk}$$

Y_{ijk}=observations of the response variable (RV) obtained from the i^{th} temperature (°C) with the j^{th} period, in the k^{th} block; μ=overall mean; T_i=effect of the i^{th} temperature (°C); S_j=effect of the j^{th} period; B_k=effect of the k^{th} block; TS_{ij}=effect of the interaction of the i^{th} temperature with the j^{th} time; ε_{ijk}=experimental error.

RESULTS AND DISCUSSION

Analysis of variance

Based on the ANOVA, significant effects were observed on temperature, as well as on its interaction with the periods applied (Table 1). Likewise, the combination which generated the highest germination percentage was determined.

Temperature effect

The different letters in the Tukey-Kramer test ($v-p$=0.0001) indicated significant differences between the temperatures used in the experiment (Figure 1). Three out of the five temperatures obtained similar means. The 30 °C temperature recorded the best germination percentages, followed by 40 °C (73% germination). The 50 °C temperature registered the lowest value range (23-66%).

Table 1. Significant differences between *Abies religiosa* treatments and interactions were determined with an analysis of variance.

Variable	P-valor		
	Temperature	Time	Temperature × time
Germination Capacity	0.0001	0.3471	0.0111
VP	0.9698	1.0000	1.0000
NDVP	0.9604	1.0000	1.0000

Alpha=0.05. PV (VP)=Peak value. NDPV (NDVP)=Number of days to peak value.

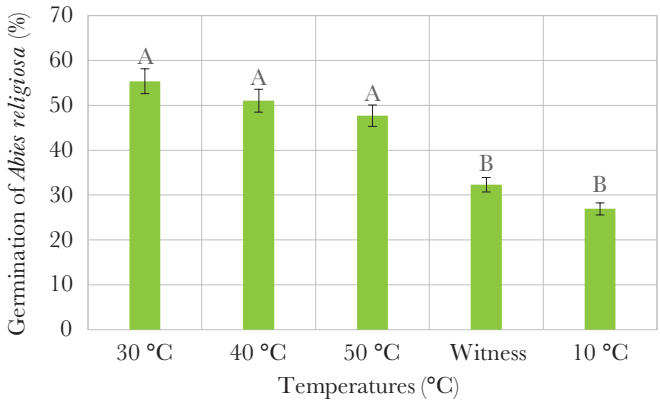

Figure 1. Application of five different temperatures for the reactivation of the dormancy state in *Abies religiosa* seeds.

Effect of the applied periods

According to Figure 2, the statistical analysis and the Tukey–Kramer test showed no significant difference between the periods used to increase the germination of Abies religiosa in this research, regardless of their length.

Effects of temperature × seconds interaction

The analysis determined that there is an interaction between the temperatures and the period that the seed was immersed in water (Table 2). A 73% germination was obtained with the best combination: a temperature of 40 °C and a period of 7,200 s. The lowest percentage (14%) was obtained with the combinations of 10 °C and 20 and 40 seconds. The best treatment must be determined based on the combination of both factors.

Peak value and number of days to reach peak value

The study lasted 30 d, whose peak value (PV) (8.57) was determined (Table 3). It represents the germination speed. For its part, NDPV was 11.7 (Table 4). In the first two weeks, the radicle was mostly observed in days 9, 10, 11, and 12.

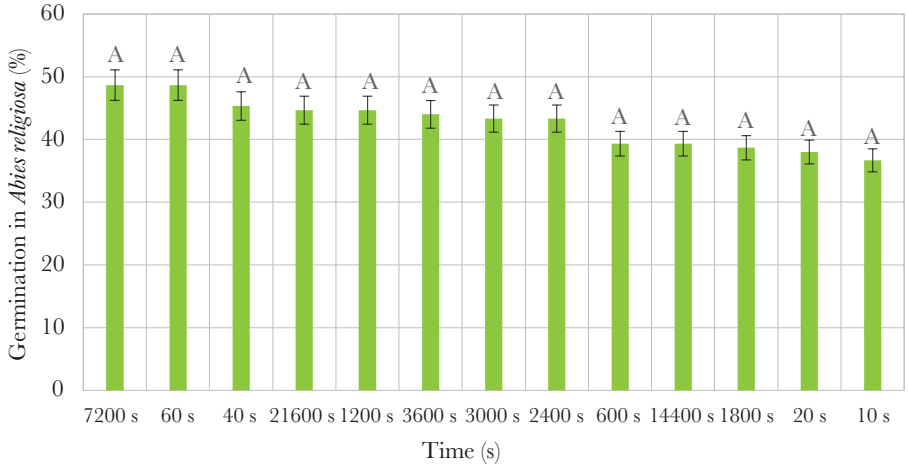

Figure 2. Exposure period of *Abies religiosa* seeds to water. Equal letters indicate equal means.

Table 2. Germination percentage of the different temperature × period combinations in *Abies religiosa* seed.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C
10	17 ab	23 ab	60 ab	48 ab	37 ab
20	19 ab	14 b	57 ab	48 ab	50 ab
40	40 ab	14 b	69 ab	48 ab	52 ab
60	33 ab	40 ab	57 ab	50 ab	66 ab
600	17 ab	17 ab	60 ab	53 ab	48 ab
1200	28 ab	23 ab	57 ab	57 ab	60 ab
1800	37 ab	20 ab	47 ab	43 ab	47 ab
2400	50 ab	23 ab	67 ab	40 ab	37 ab
3000	28 ab	27 ab	52 ab	52 ab	61 ab
3600	37 ab	41 ab	45 ab	60 ab	37 ab
7200	20 ab	37 ab	63 ab	73 a	50 ab
1400	47 ab	31 ab	30 ab	40 ab	53 ab
21600	53 ab	40 ab	57 ab	50 ab	23 ab

Rt=Room temperature.

The results showed different germination percentages for *Abies religiosa*. The highest value (73%) was higher than the percentage reported by Manzanilla (1974), who obtained a 45-49% germination with recently collected seeds without any kind of treatment. In contrast, a 96.8% germination was obtained when the seeds were soaked in coconut water for seven days. This has been the most successful pre-germination treatment for the *Abies religiosa* species (Mayen, 1987), followed by stratification at 1 to 5 °C for 14 to 28 d, which obtained a 70% germination (Patiño, P. Garza, Y. Villagomez, I. Talavera, and F.

Table 3. Peak germination value of *Abies religiosa* seeds.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C	Average
10	8.81 a	8.44 a	8.59 a	8.66 a	8.43 a	8.58 a
20	8.71 a	8.52 a	8.56 a	8.49 a	8.35 a	8.50 a
40	8. 70 a	8.81 a	8.59 a	8.39 a	8.48 a	8.56 a
60	8,76 a	8.53 a	8.51 a	8.50 a	8.43 a	8.53 a
600	8.78 a	9.04 a	8.51 a	8.58 a	8.42 a	8.57 a
1200	8.52 a	8.96 a	8.47 a	8.42 a	8.35 a	8.48 a
1800	8.47 a	8.86 a	8.33 a	8.47 a	8.35 a	8.45 a
2400	8.59 a	8.80 a	8.49 a	8.72 a	8.23 a	8.54 a
3000	8.68 a	8.33 a	8.59 a	8.57 a	8.86 a	8.65 a
3600	8.48 a	8.59 a	8.39 a	8.47 a	9.23 a	8.60 a
7200	8.79 a	8.53 a	8.45 a	8.41 a	8.54 a	8.50 a
1400	8.72 a	8.70 a	8.41 a	8.39 a	8.47 a	8.53 a
21600	8.62 a	8.80 a	8.38 a	8.42 a	8.67 a	8.55 a
Promedio	8.64 a	8.67 a	8.49 a	8.49 a	8.51 a	8.56 a

Rt = Room temperature.

Table 4. Number of days in which *Abies religiosa* seeds reached their peak germination value.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C	Average
10	11.40 a	11.85 a	11.66 a	11.57 a	11.90 a	11.69 a
20	11.50 a	11.75 a	11.70 a	11.80 a	12.00 a	11.78 a
40	11.53 a	11.40 a	11.66 a	11.92 a	11.80 a	11.70 a
60	11.50 a	11.75 a	11.76 a	11.80 a	11.89 a	11.76 a
600	11.40 a	11.20 a	11.77 a	11.68 a	11.93 a	11.71 a
1200	11.75 a	11.28 a	11.82 a	11.88 a	12.00 a	11.82 a
1800	11.81 a	11.33 a	12.00 a	11.84 a	12.00 a	11.86 a
2400	11.66 a	11.42 a	11.80 a	11.50 a	12.18 a	11.73 a
3000	11.62 a	12.00 a	11.66 a	11.68 a	11.47 a	11.64 a
3600	11.81 a	11.66 a	11.92 a	11.83 a	11.09 a	11.69 a
7200	11.50 a	11.72 a	11.84 a	11.90 a	11.80 a	11.80 a
1400	11.53 a	11.55 a	11.88 a	11.91 a	11.81 a	11.74 a
21600	11.62 a	11.41 a	11.94 a	11.93 a	11.57 a	11.73 a
Promedio	11.61 a	11.58 a	11.79 a	11.79 a	11.81 a	11.74 a

Rt=Room temperature.

Camacho, 1983; Willian, 1985). However, the most recent pre-germination treatments failed to exceed the 70% germination rate proposed by Patiño *et al*. (1983) and Willian (1985).

Authors such as Iglesias-Andreu *et al*. (2010) subjected *Abies religiosa* to ionizing radiation or gamma radiation, obtaining 62% germination with the application of a 5-Gy dose. Other authors, including Zulueta-Rodríguez, L.G. Hernández-Montiel, and Ruiz-Ramirez (2015) experimented with hydro and bio-conditioning to reactivate the embryo, through hydro-conditioning of the seeds in water, oxidation through bubble flow, and imbibition in a bacterial suspension, obtaining a 49-70% germination with a 12-h hydro-conditioning.

The germination rate of *Abies religiosa* is lower (15%) than the rate for the fresh seeds of *Abies guatemalensis*. It also decreases with storage: 2% reductions were recorded when the seeds were stored for one year at 3-5 °C temperatures (Donahue, Dvorak, Gutierrez, and Kane, 1985; Dvorak and Donahue, 1992). De Pascual-Pola, Musálem, and Ortega-Alcalá (2003) mention that large, heavy cones with abundant seeds have low viability and are equivalent to lighter, smaller cones, with fewer seeds but with a higher germination capacity.

Montserrat-Arista, Talavera, and Herrera (1992) mention that 33% of the larger seeds of *Abies pinsapo* Boiss germinate, in contrast with 8 to 26% of all the smaller seeds as a whole (Monserrat Arista, 1993).

Ortiz-Bibian *et al*. (2019) indicate that the variation between populations influences the germination of *Abies religiosa*. The seeds of populations that grow from 3,000 to 3,500 m.a.s.l. have the highest viability and germination values (48.7% and 19.6%, respectively), between 12 and 14 days. Likewise, viability and germination vary according to the area where the cones were collected; for example, Rio Frio, Ixtapaluca, Mexico registered a 37.33% viability and a 11.6 d germination for *Abies religiosa* seeds (de Pascual-Pola *et al*.,

2003). Pascual-Pola *et al.* (2003) likewise report 44.69 to 61.78% of non-germinated seeds. Franklin (1974) reported 35 to 93% non-viable seeds for *Abies amabilis* (Dougl.) Forb., *A. concolor* (Gord.) Engelm., and *A. magnifica*. A. Murr. The presence of non-viable seeds is considered a characteristic of the genus *Abies* and is associated with various causes, including an incorrect procedure for the collection and handling of cones and seeds, genetic irregularities, environmental adversities, pollen infertility, and entomological damage (Bramlett *et al.*, 1977; Franklin, 1974; Schopmeyer, 1974). Other causes include inbreeding and prezygotic events, including ovule abortion, parthenocarpy or the absence of pollination or fertilization. The resulting empty and wrinkled seeds have a thin or shrunken embryo, if any at all (Hartmann and Kester, 1988). This phenomenon is caused by incompatibility in the passage of pollen, the stunted growth of the pollen tube, or fertilization irregularities. This problem would be explained by postzygotic events, such as ovule abortion during postfertilization, the partial formation of embryos, or their early abortion, which is related to development problems (Baskin and Baskin, 2014).

Even under ideal conditions, storage also has negative effects on germination, as a result of aging and deterioration (Shaban, 2013). This could be one of the main causes of a low germination percentage, since the seed was stored for five years (2015-2020), before it was germinated.

CONCLUSIONS

The *Abies religiosa* seed was able to germinate and withstand a temperature of 50 °C in the longest periods. It was one of the three temperatures that generated the highest germination percentages (26 of the 65 treatments generated). Eight of the best germination percentages (50-73%) were obtained during the shortest period in which the seeds were immersed in water (10). These are the most accurate and their use is recommended, because the temperatures and periods in which the seeds should be removed and placed in the trays were controlled with greater efficiency.

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