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Germination Response of Ornamental Pine Seeds on a Two-Way Thermogradient Plate

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Abstract

The objective of this study was to evaluate the effect of temperature on germination of *Pinus tabuliformis* Carrière seed, *Pinus gerardiana* Wall. ex D. Don and *Pinus roxburghii* Sarg. Seeds of three species were distributed in Petri dishes with 1% agar solution; for the first step it was placed on a 2-way Grant's thermogradient plate (a bi-directional incubator) for a period of 28 days and during the second step in an incubator type B.O.D. also for 28 days with non-germinated seeds in the first step. This instrument allows an assessment of a wide regime of constant and alternating temperatures, resulting to 144 different combinations of temperature, scheduled to the temperatures 4˚C - 40˚C. The lighting regime was 12 hours photoperiod for the two stages of the experiment. The best temperature for the germination of *P. tabuliformis* was 26.2˚C (minimum of 7.9˚C and maximum 37.0˚C) and *P. roxburghii* was 15.6˚C (minimum 10, 4˚C and maximum 37.0˚C). The seeds are neutral photoblastic. *P. gerardiana* showed low germination percentage (below 10%). The temperature that provided the highest germination percentage for *P. tabuliformis* was 26.2˚C and 15.6˚C for *P. roxburghii*.

Keywords

Thermogradient Plate, Temperature Regime, Germination Percentage

1. Introduction

The study of temperature and light, suitable for the germination of forest species, has received attention in the scientific and academic environment, in order to use such information either for germination, for its conservation in seed

banks for the production of plants in nurseries, for recovery of degraded areas and for landscaping.

For germination, it is necessary that some intrinsic factors of the seed and related environmental act favorably. To this end, it is essential that the seed is alive, there is no numbness and has availability of water, temperature and oxygen [1].

Each species requires specific conditions for germination to happen, especially the quality of the presence of light and proper temperature [2]. These two environmental factors are of fundamental importance in controlling germination, and are considered to be the main extrinsic factors that influence germination [3] [4] [5].

Carvalho and Nakagawa [5] and Souza *et al*. [6] report that the effect of temperature on the germination can be described in terms of cardinal temperatures (minimum, optimum and maximum). The minimum and maximum temperatures are those in which, respectively above and below the seed does not germinate and the optimum temperatures for maximum germination percentage in the shortest period of time.

The effect of the presence of light favors somehow germination for some species, designating this effect as positive photoblastic. For other species the effect of the absence of light is considered better that the presence of light and this effect is called as the negative effect photoblastic. When they are indifferent to light this effect is called neutral photoblastism [7] [8] [9].

The thermogradient plate is a bi-directional incubator [10], and it permits for germination testing of seeds over a wide range of single temperature and alternating temperature regimes over a time continuum [11].

Pinaceae family species are among the most valuable and commercially important plants in the world. Most species are trees, and often are excellent sources of wood, wood products and resins; many are grown for reforestation and as ornamentals [12].

The pine, called *matsu* by the Japanese, is considered the "king" of plants in a garden and it is believed that this plant brings good fortune. In the floral art of ikebana, its branches and leaves are used in floral arrangements as a symbol of good luck [13].

In landscaping, many species of pine are used, such as *Pinus densiflora*, *Pinus parviflora*, *Pinus pentaphylla*, *Pinus pumila*, *Pinus thunbergii* [14].

Among the species of pine trees with high potential for landscaping, the main focus of this work are: *Pinus gerardiana*, *Pinus roxburghii* and *Pinus tabuliformis.*

P. tabuliformis, also known as Chinese pine, is an important ecologically and economically, a kind of native tree conifer in northern China, which naturally is distributed in 14 provinces and municipal regions, with a land cover about 3 million·km^2 [15].

Also known as Himalayan pine, *P. roxburghii*, is a native tree species in the

Midwest of the Himalayas, which is a third of the area of the Uttarakhand Himalaya Forest, between Siwaliks and Himalayan rivers, from Kashmir to Bhutan [16].

P. gerardiana is a species that grows in Pakistan, Afghanistan and India. This produces fruit species, called pinions, which are rich sources of unsaturated fatty acids and which, when ripe, has a good adhesive property anionic polysaccharide [17].

Although they have ornamental and industrial importance, there is still little information on literature on seed germination of these species. This work aimed to evaluate the effect of temperature on germination of *Pinus tabulaeformis*, *Pinus gerardiana* and *Pinus roxburghii* seeds.

2. Material and Methods

The work was carried out in the laboratories of the Millennium Seed Bank (MSB), Kew Gardens, Ardingly, England, with pine seeds (*P. tabuliformis*, *P. gerardiana* and *P. roxburghii*), acquired from a commercial company.

In the germination test, the seeds were rinsed in sodium hypochlorite at 2% for 10 minutes under gentle manual mixing. Then, the seeds were washed three times in distilled water. Only after this disinfecting process is that then the seeds were distributed in Petri dishes (5 cm diameter) with 1% agar solution.

To analyze the effects of temperature alternating light was used 12 hours photoperiod (light with a photon flux of 50 - 100 W/m) and alternating temperatures (day/night), which gave rise to a line of constant temperature from the bottom left of thermogradient table (always cold) to the upper right corner (always hot), as shown above (**Figure 1**).

The factorial scheme was 12 × 12 petri plates, which generated a diagonal line from the lower left corner to the upper right corner (bold) with alternating temperature fluctuations of 12 hours in one direction during the day and 12 hours in another direction at night, with temperatures from 4 to 40 degrees Celsius.

Two temperature gradients were programmed into the thermogradient plate

Hot / Day

Cold / Night												Hot / Night
35.1/6.6	35.3/9.4	35.5/12.1	35.6/14.9	35.8/17.6	36.0/20.4	36.2/23.1	36.4/25.9	36.6/28.6	36.7/31.4	36.9/34.1	**37.0**	
32.7/6.7	32.8/9.4	33.0/12.2	33.2/14.9	33.3/17.7	33.5/20.4	33.7/23.2	33.8/25.9	34.0/28.7	34.2/31.4	**34.3**	34.5/36.8	
30.3/6.7	30.4/9.5	30.5/12.2	30.7/15.0	30.9/17.7	31.0/20.5	31.2/23.2	31.3/26.0	31.5/28.7	**31.6**	31.8/34.3	31.9/36.7	
27.8/6.8	28.0/9.5	28.1/12.3	28.2/15.0	28.4/17.8	28.5/20.5	28.7/23.3	28.8/26.0	**28.9**	29.1/31.6	29.2/34.3	29.3/36.7	
25.4/6.8	25.5/9.6	25.7/12.3	25.8/15.1	25.9/17.8	26.0/20.6	26.1/23.3	**26.2**	26.4/28.9	26.5/31.6	26.6/34.4	26.7/36.6	
23.0/6.9	23.1/9.6	23.2/12.4	23.3/15.1	23.4/17.9	23.5/20.6	**23.5**	23.7/26.2	23.8/28.9	23.9/31.7	24.0/34.4	24.1/36.5	
20.6/6.9	20.7/9.7	20.8/12.4	20.9/15.2	20.9/17.9	**20.9**	21.1/23.5	21.2/26.2	21.3/29.0	21.4/31.7	21.5/34.5	21.6/36.4	
18.2/7.0	18.2/9.7	18.3/12.5	18.4/15.2	**18.2**	18.5/20.8	18.6/23.5	18.7/26.3	18.7/29.0	18.8/31.8	18.9/34.5	19.0/36.3	
15.8/7.0	15.8/9.8	15.9/12.5	**15.6**	16.0/18.1	16.0/20.8	16.1/23.6	16.1/26.3	16.2/29.1	16.3/31.8	16.3/34.6	16.4/36.2	
13.3/7.1	13.4/9.8	**13.0**	13.5/15.4	13.5/18.1	13.5/20.9	13.6/23.6	13.6/26.4	13.7/29.1	13.7/31.9	13.7/34.6	13.8/36.2	
10.9/7.1	**10.4**	11.0/12.7	11.0/15.4	11.0/18.2	11.0/20.9	11.1/23.7	11.1/26.4	11.1/29.2	11.1/31.9	11.2/34.7	11.2/36.1	
7.9	8.5/10.0	8.5/12.7	8.5/15.5	8.5/18.2	8.5/21.0	8.6/23.7	8.6/26.5	8.6/29.2	8.6/32.0	8.6/34.7	8.6/36.0	

Cold / Day

Figure 1. Distribution of temperatures in the thermogradient plate used to evaluate germination responses of *P. gerardiana*; *P. roxburghii* and *P. tabuliformis*.

in such a way as to vary from 4˚C to 40˚C. The first programmed temperature gradient started from bottom to top and, after 12 hours, alternated with another gradient from left to right.

This scheme allowed for 144 temperature regimes, of which 12 were constant and 132 were of alternating regimes.

The seeds were examined daily, and only when the radicle was two millimeters or more in length were considered in the evaluation.

The experiment was conducted for 28 days in the two-way thermogradient plate and, in addition to this step, the non-germinated seeds were conditioned in a germination chamber type BOD, regulated at a temperature of 25˚C ± 1.0˚C and photoperiod of 12 hours, to evaluate the viability of germination of the same.

The length, width and weight of 25 seeds were calculated. Each seed was evaluated individually for the determination of these measures.

To determine the length and width, a Zeiss Axiocam scale ruler coupled to a microscope (Zeiss Stemi SV11) was used. The data are presented as means, with their respective standard errors.

To determine the humidity, a portable digital hygrometer was used. The method measures the relative humidity balance, *i.e.* the relative humidity of the air in equilibrium with the seeds held in a closed chamber. It is fast, and non-destructive, making it ideal for small collections of rare and/or endangered species [18].

For the graphical presentation of the germination percentage, the SigmaPLot computer program was used.

3. Results and Discussion

The *P. gerardiana* species, probably due to the low seed quality, presented low germination (below 10%) for some temperature regimes at the thermogradiente plate and, therefore, was not evaluated for this experiment.

A large variation in length, width and weight was observed for the seeds of *P. tabuliformis*, *P. gerardiana* and *P. roxburghii*. For the species *P. tabuliformis* an average of 7.48 ± 0.92 mm for the length was found, 4.31 ± 0.51 mm for the width and 0.04 ± 0.01 mg for the weight. For *P. roxburghii*, the mean was 9.63 ± 1.11 mm for the length, 5.27 ± 0.61 mm for the width and 0.05 ± 0.02 mg for the weight.

The arrangement of the species in the plate of thermogradiente allowed 144 possible combinations, being 48 combinations for each of the three species. Germination occurred in 43 combinations for *P. tabuliformis*, 27 for *P. roxburghii* and 7 for *P. gerardiana.*

The germination rates, in percentage, and the seed distribution scheme in the thermogradient plate, during the initial 28 days are shown above (**Figure 2**).

The highest germination rates for *P. tabuliformis* occurred at the following temperature regimes: 8.6˚C/23.7˚C (100%); 11.1˚C/26.4˚C (100%); 16.1˚C/

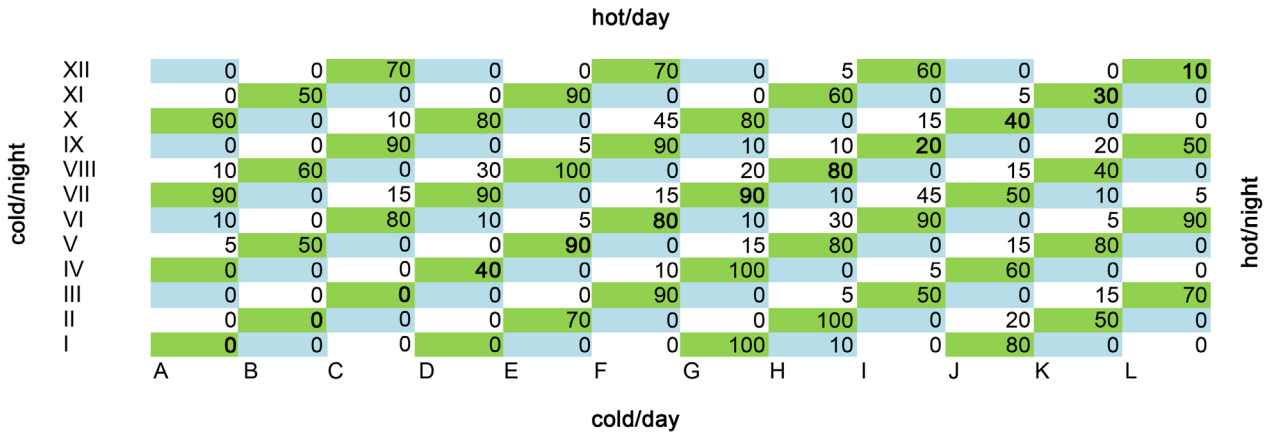

Figure 2. Scheme of distribution of the species and their germination points in the thermogradient plate used to evaluate germination responses of *Pinus gerardiana* (blue cells); *Pinus roxburghii* (white cells) and *Pinus tabuliformis* (green cells).

23.6˚C (100%); 25.9˚C/17.8˚C (100%); 13.5˚C/20.9˚C (90%); 18.2˚C (90%); 21.3˚C/29.0˚C (90%); 21.6/36.4 (90%); 23.0˚C/6.9˚C (90%); 23.3˚C/15.1˚C (90%); 23.5˚C (90%); 28.1˚C/12.3˚C (90%). *P. roxburghii* were: 23.8˚C/28.9˚C (45%); 31.0˚C/20.5˚C (45%); 21.2˚C/26.2˚C (30%) and 25.8/15.1 (30%).

It is noted that the seeds arranged in the low temperature regimes, ie the seeds that were placed near the lower left corner, with temperatures close to 7.9˚C did not germinate. The same observation occurs for the seeds placed near the upper right corner, that is, near the temperature of 37˚C, with no or low germination.

The same was observed for seeds of *Khayaanthotheca*, Meliaceae family, when submitted from 5˚C to 40˚C in a thermogradient dish with 100% germination at 5˚C/30˚C (day/night) and zero germination in regimes close to extreme temperatures [19].

The best constant temperature for germination of *Terminalia superba*, Combretaceae family, is 35˚C/35˚C and the best temperature combinations at alternating temperatures were 35˚C/40˚C (100% germination), 35˚C/15˚C (95%), 40˚C/25˚C (95%), 20˚C/40˚C and 40˚C/30˚C (92.5%) [20].

The optimal temperature zone for germination of cotton seed was determined to be 28˚C to 30˚C. As temperature decreased from the optimal zone, the germination rate decreased but percentage germination during the 10-day period was significantly lower only below 20˚C. As the temperature increased above the optimal zone, the germination rate decreased and the percentage germination sharply decreased above 32˚C - 34˚C [21].

Seed germination of *Moringaoleifera* and *M. peregrine*, Moringaceae family, in laboratory showed that 20˚C is the optimum temperature degree for both species. Good germination was also recorded at 15˚C and 25˚C for *M. oleifera*and *M. peregrina*, respectively. Nogermination was obtained from any speciesunder 10˚C and 40˚C [22].

It is observed, when comparing the germination above and below the constant temperature line, that is, when in the presence of light at low temperatures al-

ternating with the presence of light at high temperatures, that there was no influence of light in the germination process for the Species studied.

The period for the first germination was affected due to the temperature regime. For *P. tabuliformis* the time for the first germination was three days, under the temperature regime of 36.6˚C/28.6˚C, and for *P. roxburghii*, it was five days in the regime of 29.2˚C/34.3˚C.

The germination rate or germination speed, expressed as 1/T50, which is the time in days to reach 50% of the final germination percentage, was affected by the temperature regimes at which the seeds were arranged for germination in the dish of two-way thermogradient.

The results found for the germination speed were estimated for the constant temperature line of 7.9˚C to 37.0˚C. This model allowed the calculation of the cardinal temperatures (minimum, optimum and maximum), presented graphically as a line of descent and descent of temperatures, where the intersection of the straight lines represents the optimal temperature for the germination of each species.

According to these models, the best temperature for the germination of *P. tabuliformis* was 26.2˚C (minimum of 7.9˚C and maximum of 37.0˚C) and for *P. roxburghii* it was 15.6˚C (minimum of 10.4˚C and maximum of 37.0˚C).

The results of the germination, in percentage, obtained from the thermogradient plate, can also be shown in other types of graphs, where it is observed that the lower right (always cold) and upper right (always hot) corners exhibit low or no germination. (Figure 3 for *P. tabuliformis*, and Figure 4 for *P. roxburghii*).

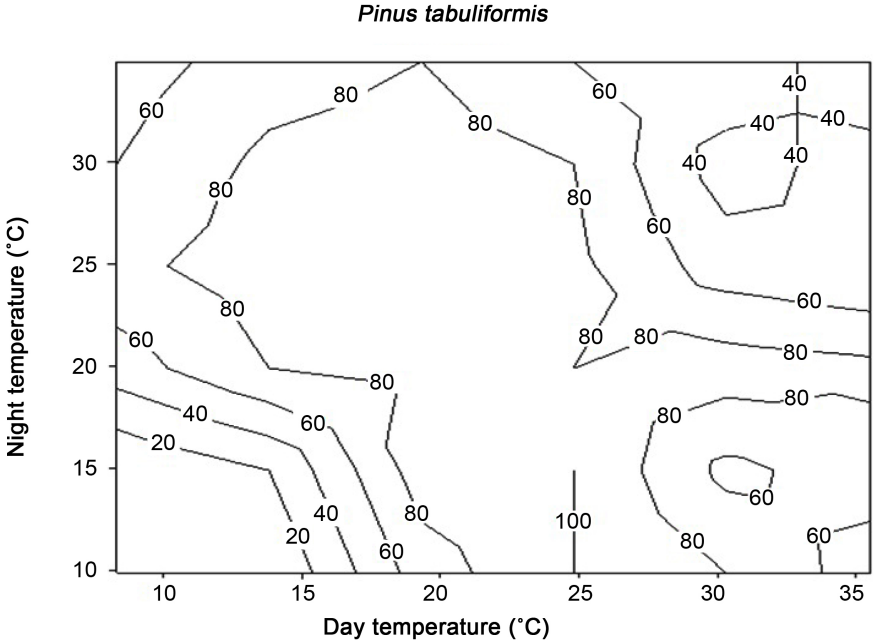

Figure 3. Percentage of germination of *Pinus tabuliformis* in two-way thermogradient plate in 12 hours of photoperiod. The numbers represent the percentage of germination and the curves show the limits between the results obtained.

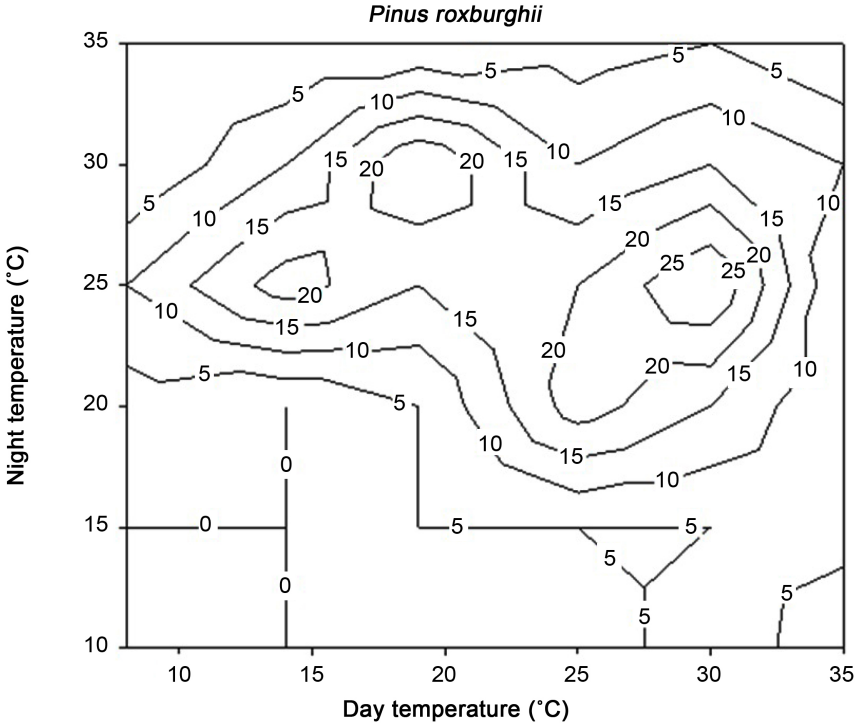

Figure 4. Percentage of germination of *Pinus roxburghii* in two-way thermogradient plate in 12 hours of photoperiod. The numbers represent the percentage of germination and the curves show the limits between the results obtained.

After incubation for 28 days in thermogradient plate, the non-germinated seeds were placed in incubator type B.O.D. At 25˚C for a further 28 days. The results are shown above (**Figure 5**).

The germination values obtained in this second stage of the study show that the seeds under extreme temperatures were not dead.

For *P. tabuliformis*, it is observed that the seeds that were under low or high temperature in the first stage of the experiment, in a plate of thermogradiente, when placed under a temperature of 25˚C (temperature close to the ideal of germination, which was 26.2˚C), germinated. In the temperature regime of 10.4˚C there was no germination and under temperature of 25˚C it presented 100% of germination. In the 37˚C regime, where there was 10% germination, under a temperature of 25˚C it presented 80% germination.

For *P. roxburghii* the same effect was observed and 40% germination was observed in a B.O.D. type incubator at 25˚C, where there had previously been no germination at the thermogradient table for the 11.0˚C/15.4˚C regime (day/night).

This is consistent with what Borges and Rena [23] and Bewley and Black [24] reported that in the viable seed at rest, by quiescence or dormancy, when they have met a number of exogenous and endogenous conditions, that is, extrinsic and intrinsic factors, there will be reactivation of the metabolism and, consequently, the growth of the embryo, which will lead to germination.

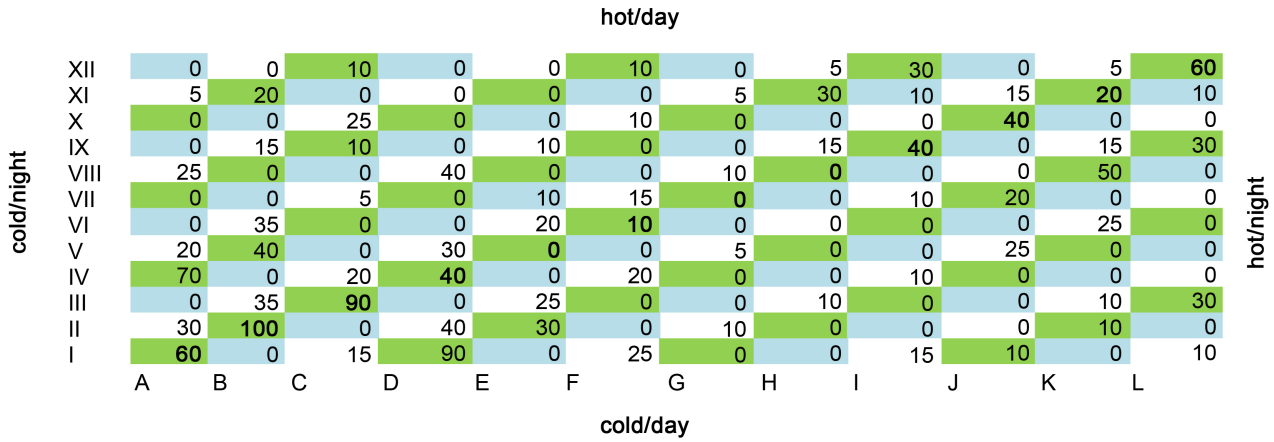

Figure 5. Germination, in percentage, of *Pinus gerardiana* (cells in blue color); *Pinus roxburghii* (white cells) and *Pinus tabuliformis* (cells in green color), during the second stage of the experiment, in incubator type B.O.D.

4. Conclusion

The optimum temperature for germination for *Pinus tabuliformis* is 26.2˚C and for *Pinus roxburghii* is 15.6˚C.

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