

Thermal-Biological Aspects of Seed Germination of *Colubrina glandulosa* Perkins Under Different Temperatures

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Abstract

The aim of this study was to determine the cardinal temperatures for germinating colubrina seeds, verify isothermal velocity variation based on the transition state model and calculate the ΔH^\ddagger variation as a function of temperature. Seeds were incubated at constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C and alternating temperature from 20-30 °C in an 8-hour photoperiod. The variables analyzed were: G, PC, IVG, TMG, VMG, Fi, U, Z, CR, CPA, MSR and MSP. Arrhenius equation was linearized by logarithmic transformation, producing the graph of $-R \ln v \times 1/T$ from the experimental values of velocity. A net enthalpy change (ΔH^\ddagger) in relation to temperature was represented by the expression: $\Delta H^\ddagger = [RT(\theta - T)(T_m + T_M)] / [(T - T_m)(T_M - T)]$. The logarithm regression of the reaction rate on the reciprocal of the temperature fit best to the quadratic model. The distribution of ΔH^\ddagger with asymptotes close to T_m and T_M indicated that the processes that occurred in the supra-optimal temperature range were of a different nature from those that occurred in the infra-optimal temperature range. The data showed $|\Delta H^\ddagger| < 12$ Kcal/mol in the optimal range and $|\Delta H^\ddagger| > 30$ Kcal/mol for temperatures of 10, 15 and 35 °C. The minimum and maximum temperature limits were 10 and 35 °C, respectively. Germination speed was related to temperature in a curvilinear manner. The germination process was endergonic and only occurred when energy was ≥ -38.35 Kcal/mol and ≤ 32.42 Kcal/mol.

Keywords: arrhenius equation, colubrina, net enthalpy change, transition state model

1. Introduction

Colubrina glandulosa Perkins, popularly known as colubrina, is a rare heliophyte and selective hygrophite tree species (family Rhamnaceae), which is native and distributed in South America, ranging from the coastal Brazil to Bolivia, Paraguay, and Peru. Its wood is suitable for civil and naval construction and external and hydraulic projects. The wood produces high quality charcoal and firewood. It also presents ornamental value, and is indicated for urban afforestation. Its flowers are honey-bearing and suppliers of nectar and pollen. This species has been recommended to help recover degraded ecosystems due to its rapid growth (Lorenzi, 2016). It has phytotherapeutic value, as the leaves and bark can be used as a fever reducer or for vitamin C deficiency. Carvalho (2005) classified this species in the initial secondary ecological group.

In view of the ancient logging and relictual situation of the colubrina populations, the seeds should receive special attention for conservation, and should be present in heterogeneous forests that are permanently preserved. A lack of specific information is available on the ecophysiology of seed germination of this species in the Rules for Seed Analysis (Brasil, 2009) and Instructions for Analysis of Seeds of Forest Species (Brasil, 2013). This species does not have established criteria for standardizing seedling production methods.

Temperature is one of the main environmental factors that govern seed germination, as it strongly influences both the rate of water imbibition by the seed and the biochemical reactions that determine the entire process (Oliveira, França, Torres, Nogueira, & Freitas, 2016). Consequently, temperature variations affect the speed, percentage, and uniformity of germination (Carvalho & Nakagawa, 2012). Each species has a temperature range where germination will occur and is considered optimal, where the efficiency of the process is total, and extreme limits of maximum and minimum tolerated by the seeds, above or below which, respectively, germinability cannot be measured (Bastos, Ferraz, Lima Junior, & Pritchard, 2017). Therefore, species with different

geographic and ecological distributions should produce seeds with variations regarding the thermal requirement for germination.

Based on these findings and considering that the study species is in the list of endangered Brazilian flora species, the present study was carried out with the objective of evaluating the ecological and applied aspects of temperature during germination of colubrina seeds, to verify the variations in germination speed along the entire thermal interval based on the transition state model, and estimate net variation for the activation of enthalpy for germination as a function of temperature.

2. Material and Methods

The experiment was conducted at the Laboratory of Seed Analysis, Agricultural Sciences Center, Federal University of Alagoas, Rio Largo, AL, Brazil.

2.1 Plant Material

The fruits were harvested from October to December 2015 to obtain the seeds from 10 trees belonging to forest fragments located in the municipality of Bom Conselho, PE, located at $09^{\circ}10'11''$ S, $36^{\circ}40'47''$ W and at 654 meters of altitude. According to the climatic classification of Köppen, the climate is BSh, semi-arid hot. The geographical location of this region is shown in Figure 1.

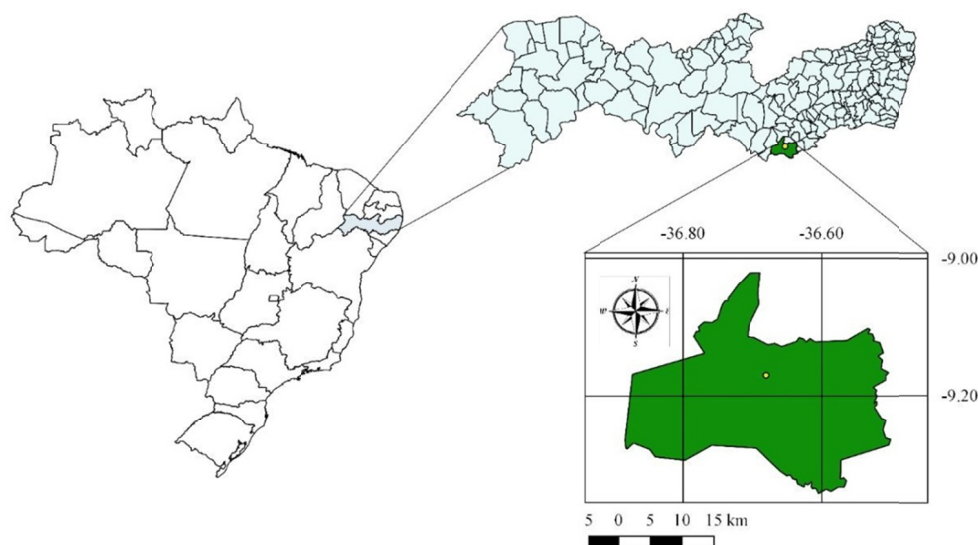


Figure 1. Geographic location of the seed collection region

The fruits were harvested with aerial scissors and an extensor cable at the end of the maturation period and held in the shade (shelter protected from sun and rain) for a few days to complete the drying process and facilitate dehiscence of fruits.

2.2 Isothermal Seed Germination

The isothermal incubation was carried out in a biological oxygen demand type germination chamber, at constant temperatures of 5, 10, 15, 20, 25, 30, 35, and 40 °C and alternating 20-30 °C, with a photoperiod of 8 hours light, simulated by four 20 W fluorescent daylight bulbs. The accuracy of the temperature control was within the range of ± 0.5 °C. Before the germination test, the seeds were cleaned in 70% alcohol for 1 min, followed by washes in running water (Rios, Araújo Neto, Ferreira, & Neves, 2016). Afterwards, the mechanical scarification treatment with scissors in the region opposite the hilum was used, and the seeds were placed to germinate on two sheets of paper towels moistened with distilled water volume equivalent to 2.5 times the dry paper weight (Brasil, 2009) and contained in clear plastic boxes ($11.0 \times 11.0 \times 3.5$ cm).

The seeds were considered germinated when they produced seedlings classified as normal (Brasil, 2009). The germinated seeds were counted daily for 19 days during the test. Water was added, when necessary, to ensure that the substrate remained sufficiently moist throughout the experiment. At constant temperatures of 5, 10, and

15 °C, the test was extended for another 7 days by transferring the seeds to the ideal temperature. The variables analyzed were:

- (a) Germinability: $gi = (\sum ki = 1ni/N) \times 100$ (Carvalho, Santana, & Ranal, 2005); where, ni the number of seeds germinated in time i and N the total number of seeds placed to germinate.
- (b) First germination count: It was performed together with the germination test by computing the percentage of normal seedlings obtained on the third day after the test started.
- (c) Speed index of germination: $IVG = G_1/N_1 + G_2/N_2 + \dots + G_n/N_n$ (Maguire, 1962); where, G_1 , G_2 , and G_n are the number of seeds germinated in the first, second, and last count; and N_1 , N_2 , and N_n are the number of sowed days at the first, second, and last count.
- (d) Mean germination time: $t = \sum ki = 1(niti)/\sum ki = 1ni$; where, ti is the time from the start of the experiment to the i nth observation (days or hours); ni is the number of seeds germinated at time i (corresponding number or i nth observation); and k is the last day of germination.
- (e) Average speed of germination: $v = 1/t$ (Ranal & Santana, 2006); where, t is average germination time.
- (f) Relative germination frequency: $Fi = ni/\sum ki = 1ni$; where, ni is the number of seeds germinated per day and $\sum ni$ is the total number of germinated seeds.
- (g) Uncertainty index: $U = -\sum ki = 1Filog_2Fi \approx Fi = ni/\sum ki = 1ni$; where, Fi is the relative frequency of germination; ni is the number of seeds germinated at time i (corresponding number or i nth observation); and k is the last day of germination.
- (h) Synchronicity index: $Z = \sum C_{n1,2}/N \approx C_{n1,2} = ni(ni - 1)/2$; $N = \sum ni(\sum ni - 1)/2$ (Primack, 1980); where, $C_{n1,2}$ is the combination of seeds germinated in the i th time, and ni is the number of seeds germinated at time i .
- (i) Length of aerial part and primary root: At the end of the germination test, the lengths of the primary root (from the base of the neck to the end of the primary root) and of the aerial part (from the collar to the apex of the seedling) of the normal seedlings in the experimental unit were measured using a graduated ruler.
- (j) Aerial part dry mass and primary root: After the measurements, the roots and aerial part of the normal seedlings of the experimental unit were conditioned in Kraft paper bags and placed in a forced air circulating oven, regulated at 80 °C, until the samples reached constant weight (24 h). Then, dry mass was determined on a precision analytical balance (0.0001 g).

Based on the activated complex model, the graph with the coordinates $y = -R \ln V$ (V = experimental values of velocity) = $A(1/T) \times 10^5$, with $R = 1,987 \text{ Kcal mol}^{-1}$ and T in Kelvin, was constructed to explain variations in germination velocity over the entire thermal range.

From the Arrhenius equation $\partial(-R \ln V)/\partial(1/T) = \Delta H^\ddagger + RT$, the net energy change (enthalpy) of germination activation was calculated for both the infra (V1) and the supra-optimal (V2), using the minimum (T_m) and maximum (T_M) germination temperatures as parameters (Labouriau & Osborn, 1984). Thus, in the range V1, $\Delta H^\ddagger_1 = RT_m \cdot [T/(T - T_m)]$, and in the range V2, $\Delta H^\ddagger_2 = -RT_M \cdot [T/(T_M - T)]$, the net change in enthalpy (ΔH^\ddagger) as a function of temperature was represented by the expression: $\Delta H^\ddagger = [RT \cdot (\theta - T) \cdot (T_m + T_M)] / [(T - T_m) \cdot (T_M - T)]$, where, θ (harmonic mean of minimum and maximum temperatures) = $[(2T_m \cdot T_M)/(T_m + T_M)]$, and T the experimental temperature, following the physiological interpretation of the opposite signs of ΔH^\ddagger in the infra and supra-optimal bands of germination.

The experimental design was completely randomized, with four replicates of 25 seeds per treatment. Data were submitted to analysis of variance and the means were compared by Tukey's test at a 5% probability. A polynomial regression analysis was performed to test the linear and quadratic models for quantitative effects, and the most significant R^2 was selected. The statistical program used was Sisvar version 5.6 (Ferreira, 2011).

The stochastic model is: $Y_{ij} = \mu_i + \varepsilon_{ij}$ ($i = 1, \dots, k$ and $j = 1, \dots, r$); where, i is the index referring to the treatment and j is the experimental unit.

3. Results and Discussion

The colubrina seeds germinated in the range $10 \text{ °C} \leq T \leq 35 \text{ °C}$, with the minimum cardinal point at $5 \text{ °C} < T < 10 \text{ °C}$, and the maximum at $35 \text{ °C} < T < 40 \text{ °C}$, *i.e.*, no germination occurred at 5 °C or 40 °C , resulting in physiological adaptation of the seeds to the environmental conditions for the species. The optimum temperature was $25 \text{ °C} < T < 30 \text{ °C}$, which allowed high germinability at a lower germination time (TMG) (Table 1). Nevertheless, the seeds present some plasticity regarding this adaptive character, as this species occurs over several Brazilian states, in regions of transition between the Cerrado or Atlantic Forest biomes for the Caatinga.

The temperature variation (20-30 °C) did not increase the percentage of germination (G) compared to the constant temperature of 20 °C (Table 1). F. N. Oliveira, J. R. Oliveira, Torres, Freitas, and Nogueira (2017) reported that the efficiency of germination can be quantified using the ideal temperature and maximum and minimum temperature limits. Other trees in the Caatinga biome present similar thermal requirements: *Amburana cearensis* (Allemão) A. C. Sm. (20 °C ≤ T ≤ 40 °C) (Almeida et al., 2017), *Mimosa ophthalmocentra* Mart. ex Benth. (25 °C ≤ T ≤ 35 °C) (Nogueira, Torres, Freitas, Castro, & Sá, 2017), *Mimosa tenuiflora* Willd. (20 °C ≤ T ≤ 40 °C) (Benedito, Ribeiro, Torres, Guimarães, & Oliveira, 2017), and *Senegalia bahiensis* (Benth.) Seigler & Ebinger (25 °C ≤ T ≤ 30 °C) (Lima, Mendonça, Paixão, Freitas, & Moreira, 2017).

Increasing the experimental temperature increased G, first count (PC) and germination speed index (IVG), within a certain limit, but temperatures > 30 °C caused a marked reduction in total germination until the maximum temperature was reached (35 °C) (Table 1). No seeds germinated at 35 °C, due to thermoinhibition, which can also cause thermal dormancy or loss of viability. On the other hand, a small number of seeds germinated (12%) at 10 °C during the 19 day incubation (Table 1), contributing to the proliferation of microorganisms harmful to the establishment of the seedlings. Low temperatures may have resulted in the gradual immobilization of seed reserves, gradually decreasing the percent germination (R. B. Silva, Matos, Farias, Sena, & D. Y. B. O. Silva, 2017).

Table 1. Germination (G), first count (PC), germination speed index (IVG) and mean germination time (TMG) of *C. glandulosa* seeds, submitted to different temperatures

Temperatures (°C)	G (%)	PC (%)	IVG	TMG (days)
10	12 e	0 f	0.331 g	12.0 f
15	32 d	2 ef	0.919 f	10.0 e
20	59 c	6 d	2.618 d	6.2 d
25	73 b	29 b	4.707 b	4.2 b
30	97 a	58 a	7.033 a	3.6 a
35	28 d	4 de	1.443 e	5.3 c
20-30	61 c	11 c	3.469 c	4.7 b
Value of "F"	495.74**	569.54**	933.61**	1160.59**
CV (%)	6.67	13.36	6.94	4.59

Note. Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test.

** Significant at the 1% probability level.

The Arrhenius curve is shown in Figure 2. The typical unimodal relationship between the logarithm of the reaction velocity and the reciprocal of the temperature better fit a quadratic regression model, where the decreasing part of the curve represented the supra-optimal thermal range, and the increasing part of the curve corresponded to the infra-optimal range. Based on the activated complex model and isothermal dependence of germination, the theoretical optimum temperature for velocity was 31.4 °C.

It should be emphasized that the energy barrier of activation encompasses both thermal and organizational transitions. The model assumes that an enzyme can exist in two states, such as active and inactive. According to Machado, Bortolin, Paranhos, and Silva (2016), products are formed when the enzyme is in its active state, which, in turn, is in equilibrium with the denatured or inactive form. As temperature increases, equilibrium shifts to the inactive enzymatic state.

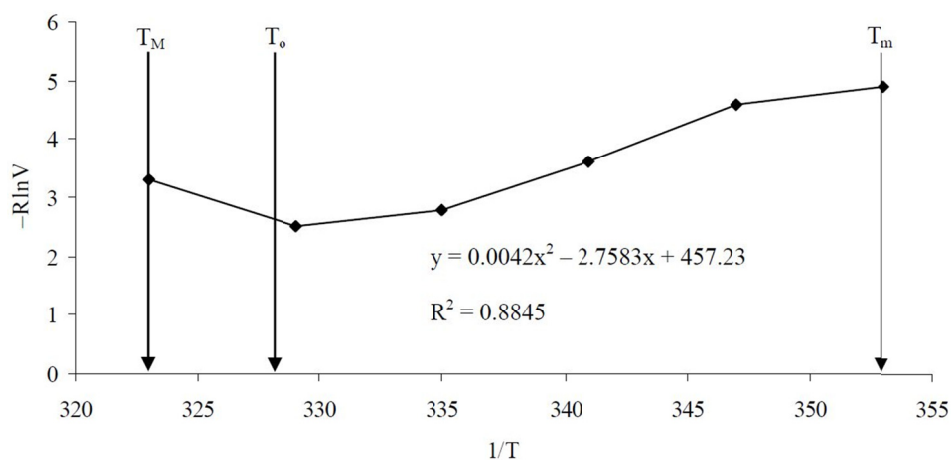


Figure 2. Arrhenius curve of the isothermal germination speed of *C. glandulosa* seeds. T_m = minimum temperature; T_M = maximum temperature; T_o = theoretical optimum temperature

The seeds showed germination capacity at well-defined temperature limits. This is an important feature; temperatures above the optimum for total germination accelerate the speed of the process, but disorganize it, so the number of seeds that can complete germination drops rapidly. However, temperatures below the optimum for germination tend to reduce the speed of the process, exposing the seedlings for a longer period of time to adverse environmental factors, which may lead to a reduction in total germination rate.

Albuquerque, Rodrigues, Minohara, Tebaldi, and Silva (1998) obtained low and slow germination with colubrina seeds held at constant temperatures of 25 and 30 °C and alternating between 20-30 °C. Considering these results, immersing the seeds in sulfuric acid for 25 min may not have been sufficient to overcome dormancy.

The results of uncertainty (U) (informational entropy) (Table 2) showed that seeds subjected to temperatures in the optimum range presented frequencies with a few peaks or a supposed unimodal frequency distribution; that is, they tended to concentrate germination within the shortest time possible. Above and below this range, the U increased significantly and germination involved a loss of order. In turn, the presence of the main mode would no longer occur and the germination of a few seeds would appear scattered over time, with a tendency for a polymodal distribution and dispersion of the relative frequency, indicating a compensation of the unfavorable temperature conditions by greater distribution of germination in time.

Buried seeds that leave the bank and are brought to the surface at the opening of clearings are exposed to better light and temperature conditions in closed tropical forest ecosystems, which promotes germination. The ability of this species to remain dormant in the bank is an important biological strategy for the dynamics of their populations, allowing them to utilize the opening of clearings in the forest or face drastic changes in their communities. In tropical forests, seed bank formation usually occurs in species located in initial succession or clearings.

Temperatures near the optimum provided the highest synchronization indices of germination (Z), confirming that this temperature range represents an environmental signal (communication of thermal energy between the external environment and the seed), which leads to a higher level of organization of the process, and reflecting higher G values and average germination velocity (VMG). While under lower temperatures, germination was more random and less synchronized (Tables 1 and 2). According to Medeiros, Souza, Santos, Azeredo, and Anjos (2017), this strategy provides greater efficiency to establish seedlings, as the seeds have the opportunity to produce seedlings that may find ideal conditions for development.

Table 2. Mean germination velocity (VMG), uncertainty index (U) and synchrony index (Z) of *C. glandulosa* seeds, submitted to different temperatures

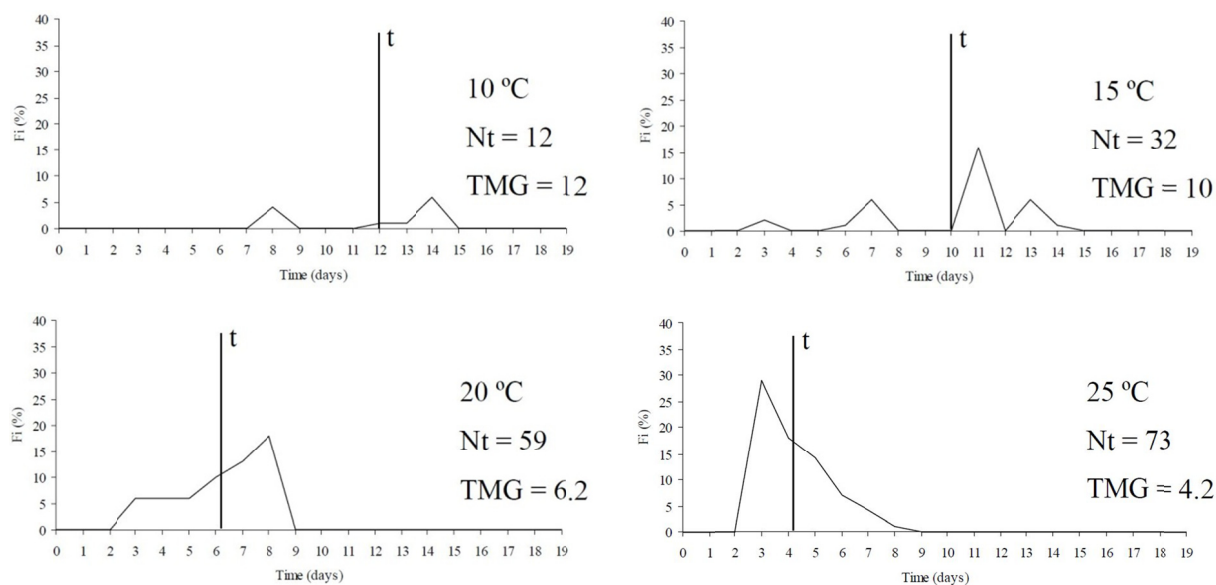
Temperatures (°C)	VMG (days ⁻¹)	U (bits)	Z
10	0.08 g	2.5216 f	0.0476 f
15	0.10 f	2.4089 f	0.1500 e
20	0.16 e	2.2258 e	0.1728 de
25	0.24 b	1.4688 b	0.3333 b
30	0.28 a	0.9183 a	0.4090 a
35	0.18 d	2.0844 d	0.2282 cd
20-30	0.21 c	1.6436 c	0.2787 bc
Value of "F"	3345.40**	1363.86**	145.83**
CV (%)	2.49	3.58	13.43

Note. Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test.

** Significant at the 1% probability level.

With the temperature dependence of the germination speed, the distribution of the isothermal germination frequencies can vary with temperature, and the analysis of these distributions suggests hypotheses about how the thermal signal is perceived by the seed (Zpevak, Perez, & Buckeridge, 2012).

The frequency polygons are shown in Figure 3. Depending on the temperature, the asymmetry of the distribution was due to the majority of seeds that germinated rapidly or to the minority of seeds that took extra time to germinate (or due to both cases). The graphs were unimodal at constant temperatures of 20, 25, 30 and 35 °C, and alternating between 20-30 °C, but polymodal at constant temperatures of 10 and 15 °C. A deviation in the germination time was observed with respect to the modal time; to the right ($t > tm_0$), from 25 to 35 °C and 20-30 °C, confirming and accelerated germination process, and to the left ($t < tm_0$), from 10 to 20 °C, reflecting a delay in this process. The ecological implication is that tropical and subtropical areas, especially in summer, can be an advantage for this species to establish. Sanhueza et al. (2017) reported that warmer environmental temperatures, such as those anticipated due to global warming, will increase reproduction by germination.



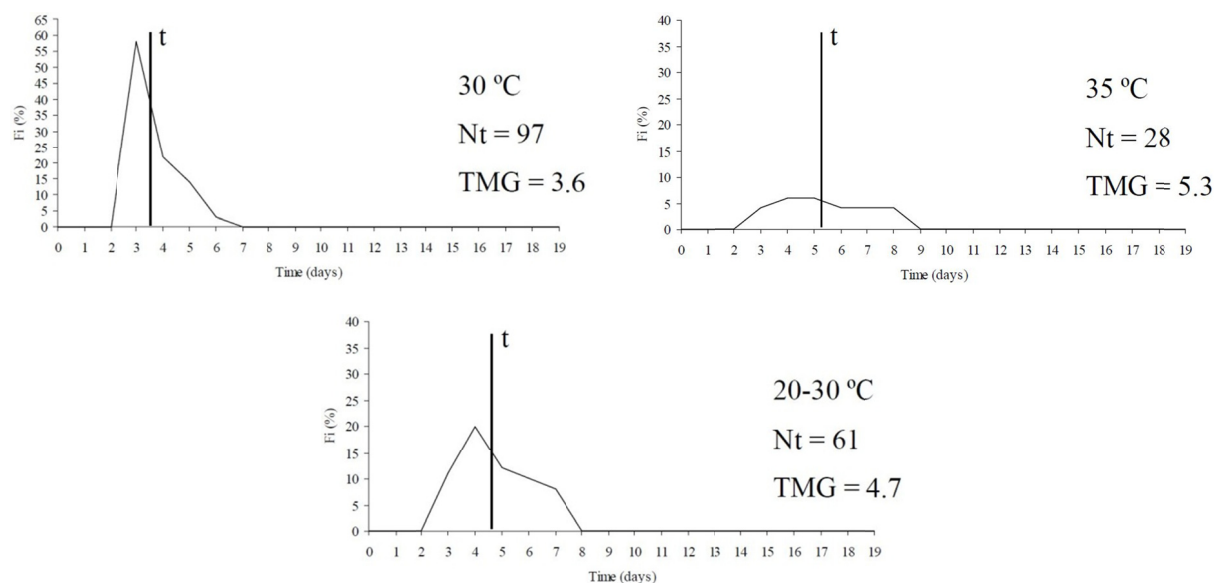


Figure 3. Relative frequencies of germination as a function of the isothermal incubation time of *C. glandulosa* seeds. Nt = total number of germinated seeds; t = time of germination; TMG = average germination time

The minimum and maximum temperatures were 10 and 35 °C, respectively, and the harmonic mean between them was 15.6 °C. Applying the laws of thermodynamics, the graph of the temperature-dependent variation in ΔH^\ddagger , whose curve has two asymptotes close to T_m and T_M (Figure 4), showed that activation energy (enthalpy) of germination was negative as temperature tended toward T_M , as the processes are occurring in the supra-optimal range, which was different from those occurring in the infra-optimal region. We verified $|\Delta H^\ddagger| < 12 \text{ Kcal mol}^{-1}$ in the optimum temperature range, and $|\Delta H^\ddagger| > 30 \text{ Kcal mol}^{-1}$ for 15 and 35 °C, where thermodesaturation processes should affect the perception and transduction capacity of the thermal signal in the seed. Activation of growth will be much more efficient the less energy consumed per unit of time.

The mean ΔH^\ddagger value, calculated for each experimental temperature range, was positive up to 30 °C ($\Delta H^\ddagger > 0$), with a signal inversion at 35 °C ($\Delta H^\ddagger < 0$) (Table 3), indicating that temperature affected the reaction rates and changed the physical state of the cellular components. The concept of energy barrier no longer makes sense at supra-optimal temperatures. The limitation of germination speed by T_m and T_M would be due to an energy demand above the capacity of energetic supply by the exergonic metabolic reactions.

The germination process in the optimum temperature range can be limited by diffusion phenomena during imbibition, the percentage of germination can be affected by thermal variations, with an exponential increase in tissue turgor. Mechanical scarification in the region opposite the hilum removes at least part of the physical barrier imposed by the integument. Notably, purely passive processes, such as imbibition, are less sensitive to temperature variations than metabolic processes.

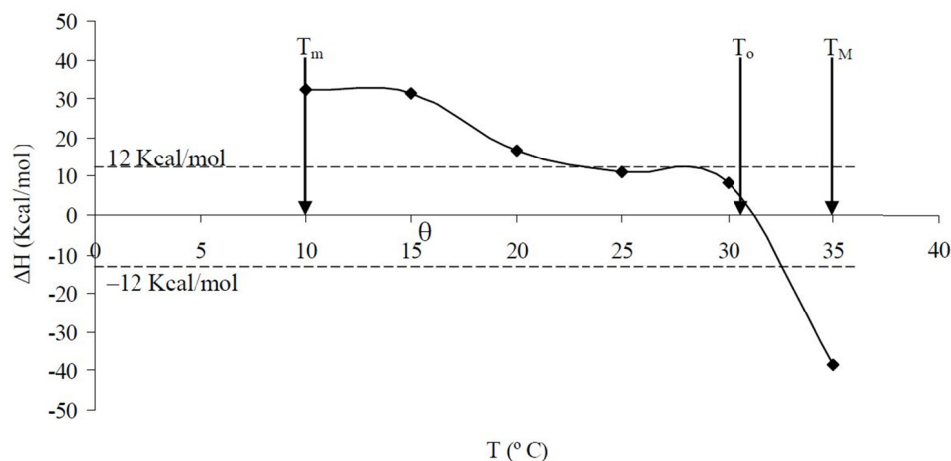


Figure 4. Net variation of the activation enthalpy of *C. glandulosa* seed germination. T_m = minimum temperature; T_M = maximum temperature; T_o = theoretical optimum temperature

Table 3. Temperature dependence of the net change in activation enthalpy (ΔH^\ddagger) in *C. glandulosa* seeds.

Temperatures		ΔT	ΔH^\ddagger (Kcal/mol)
$^\circ\text{C}$	K		
5	278.15	0-5	
10	283.15	5-10	32.42
15	288.15	10-15	31.30
20	293.15	15-20	16.49
25	298.15	20-25	11.18
30	303.15	25-30	8.53
35	308.15	30-35	-38.35
40	313.15	35-40	

Thus, at 15 and 35 $^\circ\text{C}$, high energy levels are required to trigger germination, indicating partitioning of the membrane phase/phospholipid phase changes, which are considered to be an important mechanism for limiting growth velocity, and thermo-transconformation protein, which can slow down the metabolic rate to the point where the pathways essential for germination stop (Ataíde, Borges, Picoli, Leite Filho, & Flores, 2017). Silva, Alves, Bruno, Santos-Moura, and Santos Neto (2016) reported that many enzymatic proteins may be overly folded at low temperatures, so their active centers are not reached by the respective substrates, which makes the reaction unfeasible. On the other hand, the limitation may occur under high temperatures due to adverse transconformations, also leading to loss of catalytic activity (Melo, Cavalcante, Alves, Martins, & Araújo, 2017).

Another process that can be affected by a decrease in temperature is the organization of tubulin in the microtubules, which are involved in synergistic partial processes acting in the infra-optimal range of germination (Bona, Karsburg, & Gallo, 2016).

Germination velocity (v) increases with temperature at infra-optimal temperatures, and the predominant reaction is endothermic (energy consuming), meaning that the overall velocity of the process is limited by partial processes whose effects increase with temperature, and that are synergistic; that is, aimed at growth of the embryo. v decreases with temperature in the supra-optimal range, and the reaction is exothermic (releases heat), indicating that the velocity is also limited by processes favored by a rise in temperature, but whose effects are antagonistic; that is, the energy is diverted to other processes, not linked to the embryonic growth.

Competitive thermodenaturation should occur beyond the optimum temperature, which is indicated by the appearance of heat shock proteins, to meet the extra demands imposed on cells under stress (Taiz, Zeiger, Møller, & Murphy, 2017).

Minimum temperatures, due to the effects on imbibition speed and mobilization of reserves, caused a decrease in root length (CR), length of the aerial part (CPA), root dry mass (MSR) and dry mass of the aerial part (MSP)

(Table 4), which may extend the problem to the rest of the plant cycle, with effects on speed of development and production by area (Missio et al., 2017). According to Marcos-Filho (2015), injuries due to cooling are probably related to damage to the membrane system, because embryonic axes subjected to these conditions lose organic substances. Temperature has its main effect on the physical state of the cell membrane, particularly on lipid fluidity (Lopes & Franke, 2011).

Some of the reactions that would generally culminate in the protrusion of the primary root proceeded normally at the maximum temperature, but subsequent normal seedling development did not occur (Table 4), possibly as a consequence of a lower rate of protein synthesis or other processes particularly sensitive to the temperature increase. This observation may be related to the loss of conformational structure of the enzymes at a given temperature, which also leads to loss of function or inactivation (Ataíde, Borges, & Leite Filho, 2016).

Temperature variations within the optimum range were the most adequate for seed germination and other aspects of initial development of the plant (Tables 1, 2, and 4), as there is a relationship between these temperatures and the biome where the seeds were produced.

Table 4. Root length (CR), length of the aereal part (CPA), root dry mass (MSR) and dry mass of the aereal part (MSP) of *C. glandulosa* seedlings, submitted to different temperatures

Temperatures (°C)	CR (mm)	CPA (mm)	MSR (mg)	MSP (mg)
10	3 f	36 f	2 f	26 e
15	9 e	56 e	4 e	28 e
20	19 d	59 e	9 d	38 d
25	28 b	97 b	19 b	85 b
30	34 a	109 a	23 a	93 a
35	7 e	76 d	3 ef	27 e
20-30	24 c	86 c	15 c	76 c
Value of "F"	236.00**	1462.59**	429.52**	920.69**
CV (%)	12.09	3.58	9.80	5.53

Note. Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test.

** Significant at the 1% probability level.

4. Conclusions

Colubrina seeds presented a wide range of tolerance to temperatures, with minimum and maximum limits of 10 and 35 °C, respectively.

The optimal temperature for colubrina seed germination was 30 °C.

Germination speed was in a curvilinear relationship with temperature.

The germination process was predominantly endergonic and occurred only when an energy ≥ -38.35 Kcal mol⁻¹ and ≤ 32.42 Kcal mol⁻¹ was reached.

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