

Duration of germination test of *Parapiptadenia blanchetii* (Benth.) Vaz and M. P. Lima

Edimara Ribeiro de Souza^{1*}, Manuela Oliveira de Souza¹, Jiovana Pereira Amorim Santos¹,
Edvânia da Silva Carvalho¹, Andrea Vita Reis Mendonça¹

¹Federal University of Recôncavo of Bahia, Cruz das Almas, Brasil
*Corresponding author, e-mail: edimara21ribeiro@gmail.com

Abstract

Germination tests are essential tools in the characterization of seed lots. However, there is no recommendation for conducting the germination test for *Parapiptadenia blanchetii* (Benth.). The information about the species is limited to taxonomic studies and distribution area. The present study aimed to determine a criterion for the duration of the germination test of *P. blanchetii* seeds, with greater speed and efficiency, at different temperatures. The experiment was carried out at temperatures of 20, 25, 30 and 35°C, with four replications of 25 seeds. The germination curve was fitted by plateau-response regression models: segmented linear with plateau (LPM) and segmented quadratic with plateau (QPM). For germination, the QPM showed the best fit at 20°C and 25°C. However, at temperatures of 30°C and 35°C the performance of the two models was similar. For the formation of normal seedlings, the best fit of the data was obtained with QPM at temperatures of 25°C and 30°C, for the other temperatures the LPM showed better performance. The duration of germination tests depends on the germination criterion and on the temperature. For germination tests conducted at temperatures of 30°C or 35°C, for the criterion of root protrusion, a single count on the fourth day after sowing is recommended. To assess the formation of normal seedlings, performing first and second count, at six and nine days, is suggested.

Keywords: germination, regression models, plateau-response model, temperature

Introduction

Germination tests are indispensable tools in the characterization of seed lots, because they express the capacity of a given lot to produce viable seedlings (Brasil, 2013). Many aspects should be considered to ensure that germination tests conducted in laboratory are efficient in estimating performance under field conditions. Temperature, light and substrate are factors that exert a strong influence on germination and are also the most investigated in laboratory tests.

The germination test should be performed in sufficient time to achieve the proposed objectives. Shorter duration of the tests results in lower operational costs and lower risks of proliferation of pathogenic microorganisms (Caldeira et al., 2015; Michelon et al., 2021).

Temperature influences the time required for germination. Within the optimal temperature range for germination, the increase in temperature accelerates

the absorption of water by the seed, due to biochemical changes in membrane structure and increased activity of hydrolytic enzymes, which interferes in germination time (Bewley et al., 2013; Nikolić et al., 2021). For temperatures below optimal, delays in germination are expected. Temperatures above optimal can, up to a certain magnitude, accelerate germination, but usually there is a reduction in the amount of germinated seeds, to the point of making this process unfeasible (Tanveer et al., 2020).

Although studies on methods of determining the duration of germination tests are scarce, Tomaz et al. (2010, 2015, 2016) and Guimarães et al. (2013) conducted studies on this theme, successfully employing segmented plateau-response regression. The determination of the duration of germination tests, by fitting the germination curves, using segmented regression models, contributes to improving the techniques used in the area of seed

technology.

Despite the efforts of seed researchers and agricultural input regulatory agencies, there is a lack of definition of protocols for germination tests for native species in Brazil (Freitas et al., 2019; Carvalho et al., 2021). The Instructions for Analysis of Seeds of Forest Species (Brasil, 2013) do not describe protocols for conducting germination tests for *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima.

P. blanchetii is a native species of tree habit, belonging to the Fabaceae family, popularly known in Portuguese as 'fava', 'faveiro', 'fava-preta' and 'fava-cabocla', found in the states of Bahia and Pernambuco (Flora e Funga do Brasil, 2022; Ribeiro et al., 2016). Information about this species is limited to taxonomic studies and distribution area (Lima & Lima, 1984; Ribeiro et al., 2016).

The present study aimed to determine a criterion for the duration of the germination test of *P. blanchetii* seeds, with greater speed and efficiency, at different temperatures.

Material and Methods

The experiment was conducted at the Laboratory of Ecology and Forest Restoration of the Forest Engineering sector of the Federal University of Recôncavo da Bahia, at the campus of Cruz das Almas, Bahia, Brazil.

The seeds of *P. blanchetii* used in the study were obtained from fruits collected from two trees located in the municipality of Cruz das Almas-BA (12° 39' 21.1" S / 39° 04' 56.2" W) with an average altitude of 225 m. According to Köppen's classification, the climate of the region is of transition from Am to Aw (subhumid tropical to dry), the average annual temperature is 24.2°C, with annual relative humidity of 80% and average annual rainfall of 1200 mm.

The collected fruits were manually processed and the seeds were stored in a glass container under uncontrolled conditions, in a laboratory environment, until the germination tests were set up. Seed moisture content (11.30%) was determined by the oven method at 105 ± 3°C for 24 h (Brasil, 2009).

The experiment was conducted in a completely randomized design, with four treatments, corresponding to temperatures of 20, 25, 30 and 35°C, and four replicates of 25 seeds.

The seeds were arranged in a substrate consisting of a roll of germination paper, moistened with distilled water in the amount equivalent to 2.5 times the weight of the paper (Brasil, 2009), and placed in transparent plastic bags. The rolls were deposited in B.O.D (Biochemical

Oxygen Demand)-type germination chambers with temperature control and photoperiod of 12 h of light.

Daily counts of the following variables were performed: number of germinated seeds, according to the root protrusion criterion (Bewley et al., 2013), and numbers of normal seedlings, corresponding to those with all the essential structures well developed and proportional (Brasil, 2009). The counts were performed until 22 days after the beginning of the tests, when it was observed, in all treatments, that all viable seeds formed normal or abnormal seedlings. No hard seeds were observed in the experiment; the seeds germinated or deteriorated.

The germination curve was constructed, based on the criterion of radicle protrusion and formation of normal seedlings, for the temperatures of 20, 25, 30 and 35°C, by fitting the plateau-response regression models: segmented linear with plateau (LPM) and segmented quadratic with plateau (QPM). LPM assumes two segments; the first segment describes an increasing curve up to a certain point of the ordinate, corresponding to the plateau, and from this point on, the ordinate assumes a constant value, corresponding to the second segment (Guimarães et al., 2013). QPM is characterized by an increasing phase, described by a second-degree equation and, after stabilization, by a plateau (Malafaia et al., 2015).

The LPM model is given by: $a+bx_i+e_i$, if $x_i \leq x_c$; $P+e_i$, if $x_i > x_c$; where x_i : time in days; x_c = stabilization time at which the linear model reaches the plateau in relation to the abscissa; P = coefficient of variation at the plateau point; a = intercept; b = angular coefficient of the linear segment; e_i = random error. The QPM model is given by: $a+bx_i+cx_i^2+e_i$; if $x_i \leq x_c$, being described by the quadratic model, and if $x_i > x_c$, it is described by a plateau (constant). The interception point of the segments (quadratic or plateau) was considered the point of stabilization of germination or formation of normal seedlings.

The models were fitted by the least squares and Gauss-Newton method, through the nls function in R software version 4.0.2 (R Development Core Team, 2020). For the selection of the most appropriate model, the following parameters were used: deviance value, standard deviation, coefficient of determination (R^2), Akaike information criterion (AIC) (Akaike, 1974) and Bayesian information criterion (BIC) (Schwarz, 1978), for which the lowest value corresponds to the best fit for these criteria (Emiliano et al., 2014).

Results and Discussion

For each temperature, the performance of

quadratic and linear segmented plateau-response models was compared for germination, based on the protrusion of the primary root (Figure 1-A; Table 1), and for the formation of normal seedlings (Figure 1-B; Table 2).

For germination, according to the criterion of primary root protrusion, the quadratic plateau-response

model showed the best fit at the temperatures of 20°C (Table 1; Figure 2-A) and 25 °C (Table 1; Figure 2-B), with lower values of AIC, BIC and deviance. However, at temperatures of 30°C (Table 1; Figure 2-C) and 35°C (Table 1; Figure 2-D), the performance of the two models was similar.

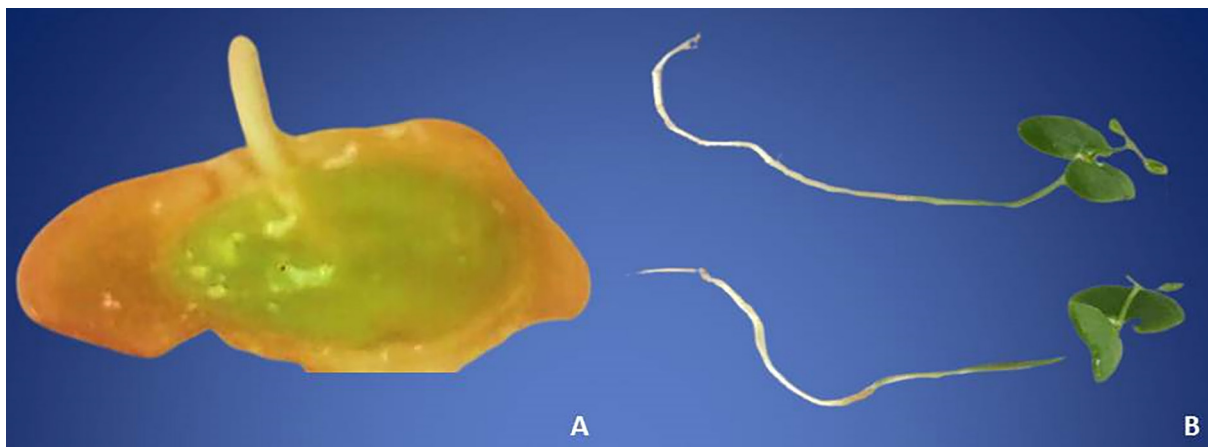
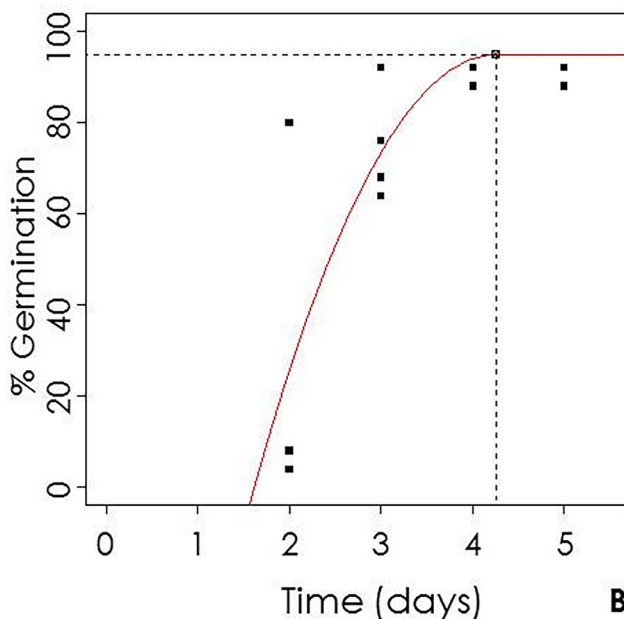
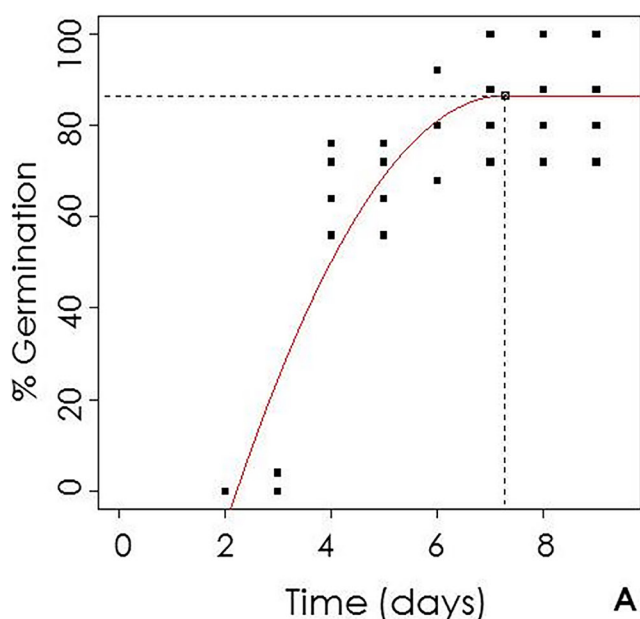


Figure 1. Primary root protrusion (A) and normal seedlings (B).

Table 1. Comparison of quadratic and linear plateau-response models for the germination curve (primary root protrusion) of seeds of *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima at temperatures of 20, 25, 30 and 35 °C

Temperature	20 °C		25 °C		30 °C		35 °C	
	Q	Linear	Q	Linear	Q	Linear	Q	Linear
TSG (days)	7.3	5.5	4.3	3.4	3.4	3.1	3.3	3
%G in TSG	86.49	86.06	94.9	94.79	93.37	93.37	92.68	92.68
%S	14.79	14.82	9.37	9.39	9.95	9.95	4.82	4.82
R ²	0.83	0.83	0.77	0.77	0.77	0.77	0.92	0.92
AIC	650.24	650.52	602.52	602.81	610.81	610.81	488.53	488.53
BIC	659.96	660.25	612.25	612.53	620.54	620.54	498.25	498.25
Deviance	10287.25	10321.76	5829.1	5848.63	6433.68	6433.68	1500.42	1500.42

Where: Q: quadratic; TSG: time required for stabilization of germination; %G: germination percentage; %S: standard deviation in percentage; R²: coefficient of determination; AIC: Akaike information criterion; BIC: Schwarz's Bayesian information criterion



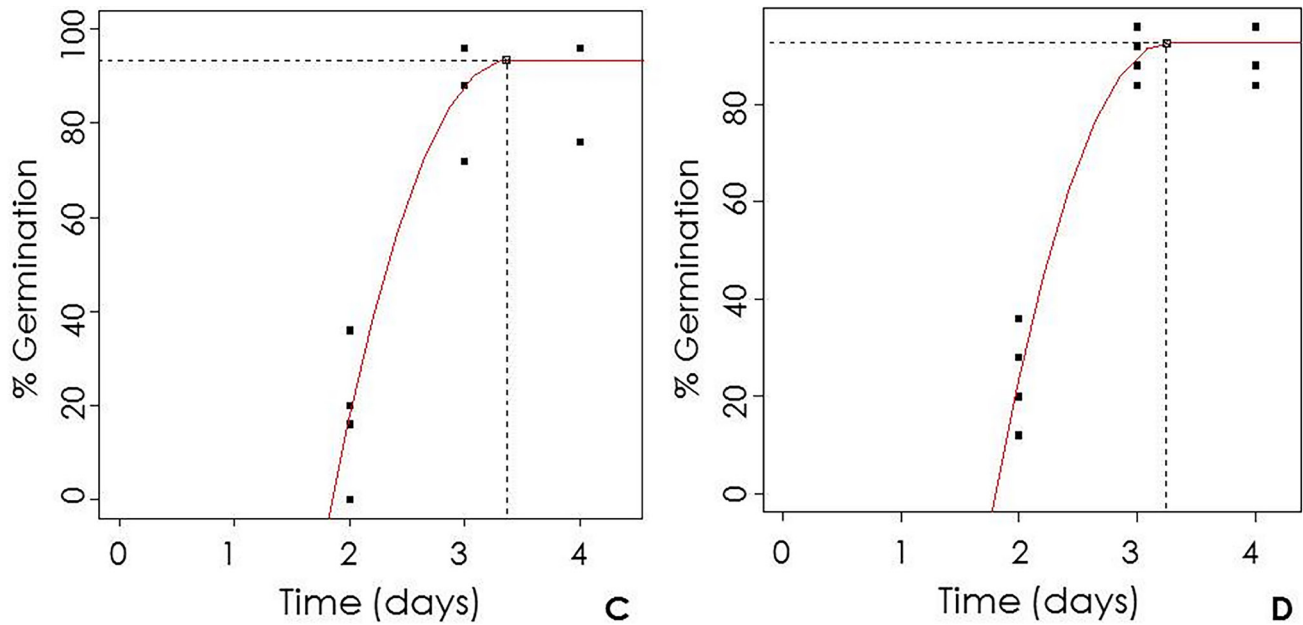
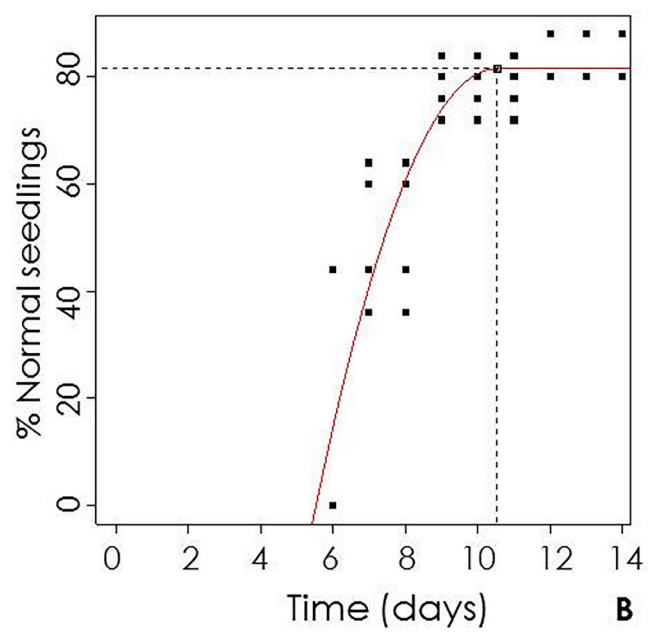
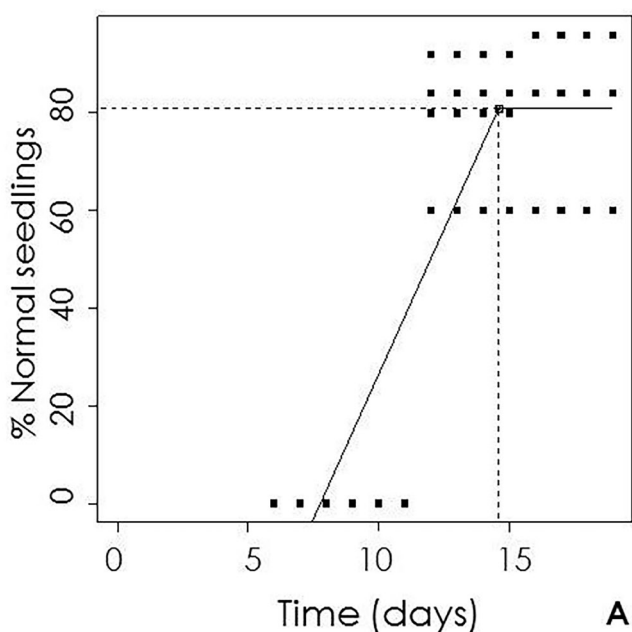


Figure 2. Stabilization time for germination of seeds of *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima in response to temperature. 20 °C (A), 25 °C (B), 30 °C (C) and 35 °C (D).

Table 2. Comparison of quadratic and linear plateau-response models for the curve of formation of normal seedlings of *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima at temperatures of 20, 25, 30 and 35°C

Temperature	20°C		25°C		30°C		35°C	
	Q	Linear	Q	Linear	Q	Linear	Q	Linear
TSN (days)	20	14.6	10.5	9.2	8.6	7	8.7	7.8
%G in TSN (%)	84.64	80.88	81.53	81.39	81.61	81.47	83.05	82.8
%S	36.25	39.42	11.01	11.25	14.03	14.03	11.18	11.07
R ²	0.78	0.75	0.84	0.83	0.56	0.56	0.86	0.87
AIC	608.67	597.29	482.26	485.19	522.35	522.36	488.53	487.1
BIC	617.55	606.17	491.14	494.07	531.23	531.24	497.41	495.98
Deviance	27310.65	23102.1	4255.86	4443.11	7674.57	7674.93	4666.74	4569.6

In which: Q: quadratic, TSN: time required for stabilization of formation of normal seedlings, %S: standard deviation in percentage, R²: coefficient of determination, AIC: Akaike information criterion, BIC: Schwarz's Bayesian information criterion, %G: germination percentage.



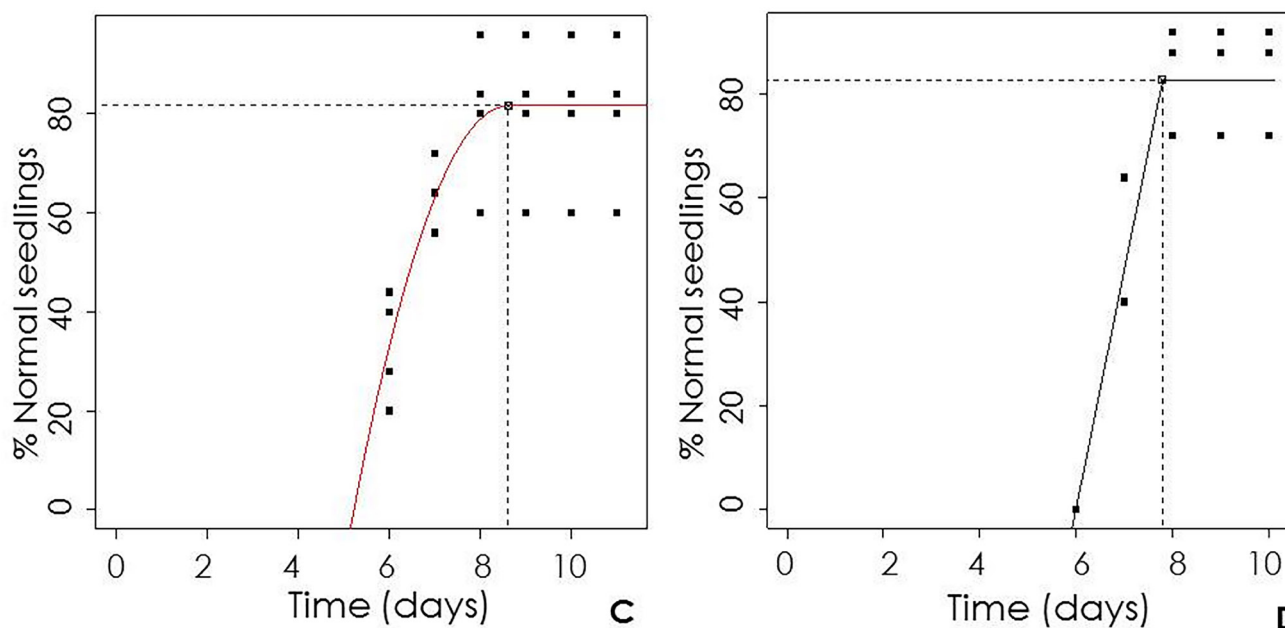


Figure 3. Stabilization time for the formation of normal seedlings of *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima in response to temperature. 20 °C (A), 25 °C (B), 30 °C (C) and 35 °C (D).

For the formation of normal seedlings (Table 2), a better fit was obtained with the quadratic plateau-response model at the temperatures of 25°C (Table 2; **Figure 3-B**) and 30°C (Table 2; **Figure 3-C**); however, for the other temperatures (Table 2; **Figures 3-A** and **3-D**), the linear plateau-response regression model showed better performance. At 30°C (Table 2), the differences between the model selection criteria were very small between the quadratic plateau and the linear plateau models.

Selecting regression models is a difficult step, and the AIC and BIC criteria are robust tools to assist in this step. Brewer et al. (2016) state that in the case of homogeneous variances the two criteria show similar performance, but in the presence of heteroscedasticity BIC is more indicated. Under the conditions of this study, both for radicle protrusion and for normal seedlings, the two criteria coincided in the indication of the best fit, which results in greater reliability in the choice of the equations obtained.

Based on the parameters of the fitted models and rounding the stabilization time up to the closest value, the duration of germination tests in a controlled environment for *P. blanchetii* seeds was defined (**Table 3**). The records of the first germination and first normal seedling (Table 3) were presented in the evaluation worksheets, without statistical treatment.

The germination tests, at different temperatures, performed for *P. blanchetii* revealed that germination (p -value = 0.4) and normal seedling formation (p -value = 0.7) were high (> 80%) and did not differ between the

temperatures evaluated. However, through the fits of the germination curves, using the segmented linear with plateau model (LPM) and the segmented quadratic with plateau model (QPM), it was possible to observe that the germination was late at the temperature of 20 °C, compared to the temperatures of 25, 30 and 35°C. The shortest time required to perform germination tests for *P. blanchetii* was observed at temperatures of 30 °C and 35 °C.

After water, temperature is the most important factor for germination. Very high or very low temperatures can influence the speed of imbibition, altering metabolic activities (Bewley et al., 2013). The increase in temperature favors the absorption of water by the seed as a result of the simplification of the water molecule (Khazaei & Mohammadi, 2009). After the minimum temperature, at which the seed germinates, temperature increments can accelerate germination, up to a point where the temperature increase begins to inhibit the germination process. The time required for activation of the germination process, which begins immediately after the water is imbibed by the seed, depends on the characteristics of the species, as well as on the temperature applied to it (Ataíde et al., 2016). Castilho et al. (2019) found that temperatures above 30°C can accelerate imbibition, due to changes in the cell membrane (Bewley et al., 2013).

The longer the period of conduction of germination tests, the higher the risks of proliferation of pathogenic microorganisms (Tomaz et al., 2010; Caldeira et al., 2015; Michelon et al., 2021), in addition to more

Table 3. Duration of germination test in controlled environment for seeds of *Parapiptadenia blanchetii* (Benth.) Vaz e M. P. Lima at temperatures of 20, 25, 30 and 35 °C.

Temperature	Time (Days)								
	BG	BN	TSG	TSN	DTG	DTN	ICG	ICN	ICGN
20 °C	3	12	8	15	8	15	3 and 8	12 and 15	3, 8, 12 and 15
25 °C	2	6	5	11	5	11	2 and 5	6 and 11	2, 6 and 11
30 °C	2	6	4	9	4	9	2 and 4	6 and 9	2, 6 and 9
35 °C	2	6	4	9	4	9	2 and 4	6 and 9	2, 6 and 9

BG: time required for the beginning of germination; BN: time required for the beginning of the formation of normal seedlings; TSG: time for stabilization of germination; TSN: time for stabilization of the formation of normal seedlings; DTG: duration of the germination test to evaluate primary root protrusion; DTN: duration of germination test to evaluate normal seedlings; ICG: interval for counts to evaluate primary root protrusion; ICN: interval for counts to evaluate normal seedlings; ICGN: intervals for counts to evaluate primary root protrusion and normal seedlings.

time and resources spent in the procedure (Guimarães et al., 2013; Tomaz et al., 2016; Michelon et al., 2021).

Considering germination based on the radicle protrusion criterion, a single count on the fourth day for temperatures of 30°C or 35°C and on the fifth day for temperature of 25°C is recommended for *P. blanchetii*. However, if the criterion evaluated is the formation of normal seedlings, two counts will be required, at 6 and 9 days after sowing for tests conducted at 30°C or 35°C and at 6 and 11 days for tests conducted at 25 °C (Table 3). According to the recommendations of Brasil (2013), the first count can be performed at times from three to ten days, and germination tests with less than 10 days do not require a first count.

In the Rules for Seed Analysis (Brasil, 2009), as well as in the Instructions for Analysis of Seeds of Forest species (Brasil, 2013), there are no recommendations on germination protocols for *P. blanchetii*. However, in Brasil (2013), there are recommendations of germination protocols for two species of the genus *Parapiptadenia*: initial and final counts at 7 and 16 days for *Parapiptadenia pterosperma* (Benth.), and initial and final counts at 7 and 14 days for *Parapiptadenia rigida* (Benth.).

The time for counting in germination tests is little discussed, but studying it allows identifying the condition, such as temperature, which promotes the shortest time to reach maximum germination, or it may also be an indication of vigor. Oliveira et al. (2014) used it as a criterion for classification of vigorous seedlings, those that were well-developed and normal on the first day of count, performed four days after sowing. Santos et al. (2020), when working with different cowpea cultivars, classified with greater vigor the cultivar that had the highest percentage of normal seedlings in the first count. Thus, the vigor of a seed lot can be determined from the germination development of the seed observed at the first count.

The fits of segmented plateau-response models contributed to the determination of the duration of germination tests and intervals between counts for *P. blanchetii*, reducing the subjectivity of the analysis. The

segmented plateau-response models have also been successfully used in studies conducted to improve germination tests of species of agricultural importance, in which the authors sought evidence to reduce the duration of the germination test for these species. Guimarães et al. (2013) used a segmented regression model to fit the germination curve of *Coffea arabica* L. seeds, in order to propose a modification in the duration of the standard germination test for the species. Tomaz et al. (2010), when fitting a regression model to the data, proposed a considerable reduction in the time of germination of Tanzania grass seeds compared to that recommended by Brasil (2009). Tomaz et al. (2016) evaluated the germination percentage of *Brachiaria humidicola* (Rendle) with regression model and found the need to reduce the time of the germination test from 21 to 10 days. Michelon et al. (2021) analyzed seeds of *Pinus taeda* L. with regression models, to define an efficient criterion that reduces the duration of germination tests.

Conclusions

The duration of germination tests depends on germination criterion and temperature. The evaluation of the root protrusion criterion can be performed with a single count on the fourth day after sowing, at temperature of 30°C or 35°C, making it possible to reduce the evaluation period.

The evaluation of normal seedlings at temperature of 30°C or 35°C reduced the evaluation period, with first and second count at six and nine days.

Acknowledgments

The authors thank the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), the Institute of the Environment and Water Resources (INEMA) and the Reference Center on Forest Restoration of the Atlantic Forest, CRRF/BA, Bahia.

References

- Akaike, H.A. 1974. New look at the statistic model identification. IEEE transactions on automatic control 9: 716-723.
- Ataíde, G.D.M., Borges, E.E.D.L., Gonçalves, J.F.D.C.,

- Guimarães, V.M., Flores, A.V. 2016. Alterações fisiológicas durante a hidratação de sementes de *Dalbergia nigra* (Vell.) Fr. All. ex Benth.). *Ciência Florestal* 26: 615-625.
- Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H. 2013. *Seeds: Physiology of Development, Germination and Dormancy*. Springer, New York, USA. p. 133-179.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS. 2009. 395 p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise_sementes.pdf <Acesso em 20 jul. 2021>
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. *Instruções para Análise de Sementes de Espécies Florestais*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2013. 98 p. https://www.gov.br/agricultura/pt-br/assuntos/laboratorios/arquivos-publicacoes-laboratorio/florestal_documento_pdf_iloivepdf_compressed.pdf <Acesso em 20 jul. 2021>
- Brewer, M.J., Butler, A., Cooksley, S. L. 2016. The relative performance of AIC, AICc and BIC in presence of unobserved heterogeneity. *Methods in Ecology and Evolution* 7:679-692.
- Caldeira, C.M., Carvalho, M.L.M.D., Oliveira, J.A., Kataoka, V.Y., Freire, A.I. 2015. Reduced time for evaluation of the germination test for sunflower seeds. *Journal of Seed Science* 37: 70-75.
- Carvalho, E.S., Souza, M.O., Silva, D.P., Souza, M.D.H. Mendonça, A.V.R. 2021. Thermal dependence in seed germination of *Diospyros inconstans* Jacq. (Ebenaceae). *Revista Brasileira de Ciências Agrárias* 16: 1-7.
- Castilho, I.M., Catão, H.C.R.M., Caixeta, F., Marinke, L.S., Martins, G.Z., Menezes, J. B. C. 2019. Teste de condutividade elétrica na avaliação do potencial fisiológico de sementes de grão de bico. *Revista de Ciências Agrárias* 42: 691-697.
- Emiliano, P.C., Vivanco, M.J., Menezes, F.S. 2014. Information criteria: How do they behave in different models? *Computational Statistics & Data Analysis* 69: 141-153.
- Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. Available at: < <http://floradobrasil.jbrj.gov.br/> >. Access on: Sep. 9, 2022.
- Freitas, T.A.S., Mendonça, A.V.R., Nascimento, K.F., Oliveira, L.F.B., Souza, L.S. 2019. Temperatura e fotoperíodo sobre a germinação de sementes de *Poincianella pyramidalis* (Tul.) LP Queiroz. *Magistra* 30: 94-103.
- Guimarães, G.C., Rosa, S.D.V.F., Coelho, L.F.S., Veiga, A.D., Clemente, A.C.S. 2013. Minimum period to assess the potential of germination of coffee seeds. *Journal of Seed Science* 35: 347-352.
- Khazaei, J., Mohammadi, N. 2009. Effect of temperature on hydration kinetics of sesame seeds (*Sesamum indicum* L.). *Journal of Food Engineering* 91: 542-552.
- Lima, M.P.M., Lima, H.C. 1984. *Parapiptadenia Brenan* (Leguminosae-Mimosoideae) - Estudo taxonômico das espécies brasileiras. *Rodriguésia* 30: 23-30.
- Malafaia, G., Rodrigues, A.S.D.L., Araújo, F.G.D., Leandro, W.M. 2015. Crescimento de plantas de milho em solo acrescido de vermicompostos de lodo de curtume e irrigado com água residuária de esgoto doméstico. *Revista Ambiente e Água* 10: 8474-862.
- Michelon, T.B., Santos, F.S.D., Belniaki, A.C., Vieira, E.S.N., Panobianco, M. 2021. Time reduction to evaluate *Pinus taeda* seeds germination by time-to-event analysis. *Scientia Agricola* 79: 1-5.
- Nikolić, Z., Miladinov, Z., Vasiljević, S., Katanski, S., Tamindžić, G., Milošević, D., Petrović, G. 2021. Legume vigour. *Acta agriculturae Serbica* 26: 19-26.
- Oliveira, S.S.C.D., Martins, C.C., Cruz, S.J.S., Silva, C.J.D. 2014. Seleção de progênies de nabo-forrageiro para germinação sob altas temperaturas. *Ciência Rural* 44: 217-222.
- R Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. 2020. <https://www.R-project.org/> <Acesso em 01 Mai. 2021>
- Ribeiro, P.G., Queiroz, L.P., Morim, M.P. 2016. Flora of Bahia: Leguminosae - *Parapiptadenia* (Mimosoideae: Mimoseae). *Sitientibus Série Ciências Biológicas* 16: 1-13.
- Santos, M.R., Teixeira, N.G.M., Junior, F.D.A.G., Farias, T.S.F., Leonardo, F.D.A.P. 2020. Germinação e vigor de sementes de feijão-caupi em condições de déficit hídrico. *Revista Cultura Agronômica*. 29: 389-399.
- Schwarz, G. 1978. Estimating the dimension of a model. *Annals of Statistics* 6: 461-464.
- Tanveer, A., Khan, M.A., Ali, H.H., Javaid, M.M., Raza, A., Chauhan, B.S. 2020. Influence of different environmental factors on the germination and seedling emergence of *Ipomoea eriocarpa* R. Br. *Crop Protection* 130: 105070.
- Tomaz, C.A., Martins, C.C., Carvalho, L.R.D., Nakagawa, J. 2010. Duração do teste de germinação do capim-tanzânia. *Revista Brasileira de Sementes* 32: 80-87.
- Tomaz, C.A., Martins, C.C., Sanches, M.F.G., Vieira, R.D. 2015. Time reduction for surinam grass seed germination test. *Ciência e Agrotecnologia* 39: 488-497.
- Tomaz, C.A., Martins, C.C., Silva, G.Z., Vieira, R.D. 2016. Period of time taken by *Brachiaria humidicola* (Rendle) Schweick seed to complete germination. *Semina: Ciências Agrárias* 37: 693-700.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

All the contents of this journal, except where otherwise noted, is licensed under a Creative Commons Attribution License attribution-type BY.