

Mathematical model GERMINAN for the analysis of germination test results

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ABSTRACT

The objective of this work was to propose a simple mathematical model, called GERMINAN, for the analysis of germination test results obtained through the application of different treatments. To verify the validity of the proposed model, the results of germination tests of *Vicia villosa* under water and salinity stress, and of *Panicum virgatum* under water stress, were analyzed and compared with the hydrottime model. Germination tests were performed with the following treatments: control and water potentials of -0.3, -0.6, -0.8, -1.0, and -1.2 MPa for *V. villosa*, and -0.8, -1.0, -1.2 MPa for *P. virgatum*. The osmolytes used were polyethylene glycol (PEG) to simulate water stress and sodium chloride (NaCl) for salinity stress. The overall regression coefficients (R^2) of the experimental and modeled results using the GERMINAN, Gummerson, and Bradford models for *V. villosa* under water stress were 0.981, 0.960, and 0.959, respectively; for salinity, 0.994, 0.963, and 0.962; while for *P. virgatum* under water stress, they were 0.949, 0.927, and 0.855, respectively. The GERMINAN model demonstrated greater alignment between the experimental and modeled values compared to those obtained with the Gummerson and Bradford models. Moreover, the proposed model can be easily applied using a scientific calculator or Excel program.

Keywords: germination, hydrottime, prediction

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RESUMEN

El objetivo de este trabajo fue proponer un modelo matemático simple, denominado GERMINAN, para el análisis de los resultados de ensayos de germinación obtenidos mediante la aplicación de diferentes tratamientos. Para verificar la validez del modelo propuesto, se analizaron los resultados de ensayos de germinación de *Vicia villosa* bajo estrés hídrico y salino, y de *Panicum virgatum* bajo estrés hídrico, y se compararon con el modelo de hydrottime. Las

pruebas de germinación se realizaron con los siguientes tratamientos: control y potenciales hídricos de -0,3, -0,6, -0,8, -1,0 y -1,2 MPa para *V. villosa*, y -0,8, -1,0, -1,2 MPa para *P. virgatum*. Los osmolitos fueron polietilenglicol (PEG) para simular estrés hídrico y cloruro sódico (NaCl) para salinidad. Los coeficientes de regresión globales (R^2) de los resultados experimentales y modelados con los modelos GERMINAN, Gummerson y Bradford para *V. villosa* bajo estrés hídrico fueron 0,981, 0,960 y 0,959, respectivamente; para salinidad fueron 0,994, 0,963 y 0,962; para *P. virgatum* bajo estrés hídrico fueron 0,949, 0,927 y 0,855, respectivamente. Consecuentemente, el modelo GERMINAN demostró mayor alineación entre los valores experimentales y modelados comparados con los modelos de Gummerson y Bradford. Además, el modelo propuesto puede aplicarse fácilmente utilizando una calculadora científica o Excel.

Palabras clave: germinación, tiempo hídrico, predicción

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INTRODUCTION

The hydrotime model (HT) is a unifying model to describe germination patterns that occur in response to water potential (Ψ), and the HT analysis provides several indices of seed quality in relation to water stress tolerance, speed, and uniformity of germination (Bradford and Still, 2004). Therefore, it allows the identification of tolerant species and cultivars to be included in breeding programs (Patanè et al., 2016; Tatari et al., 2020; Perissé et al., 2021). Previous studies on the response to water stress based on the HT model in different species, such as *Festuca pallescens* (López et al., 2021), *Phaseolus vulgaris* (Cardoso and Bianconi, 2013), *Peltophorum dubium* (Daibes and Cardozo, 2020), *Vicia angustifolia*, *V. unijuga*, *V. sativa* (Hu et al., 2015), *V. villosa* (Renzi et al., 2017; Perissé et al., 2021), *Ceratonia siliqua* (Cavallaro et al., 2016), and *Brassica napus* (Tatari et al., 2020), among others, provide relevant antecedents. The HT was first proposed by Gummerson (1986) and later modified by Bradford (1990, 1995, 1997) and Bradford and Steel (2004).

Although other germination models exist, such as hydrothermal time (HTT) (Bradford, 2002), halothermal time (HaloTT) (Bakhshandeh et al., 2020), generalized linear models (GLMs) (Carvalho

et al., 2018), among others, the HT model is currently the most widely used for germination analysis and therefore was chosen to compare the results in this study.

In Gummerson's model, the time required to obtain a given percentage of germination, the water potential ψ , and the base potential $\psi_B(p_g)$ for the germination percentage p_g , are related by the following equation:

$$t_g(p_g, \Psi) = \frac{\theta_H}{\Psi - \psi_B(p_g)} \quad (1)$$

where

$t_g(p_g, \Psi)$: time to reach the percentage p_g at water potential Ψ

θ_H : hydrotime constant

ψ : water potential

$\psi_B(p_g)$: base water potential for percentage p_g

The base water potential is the minimum potential that allows the stipulated germination percentage p_g to be obtained.

Bradford and Still (2004) applied a statistical model in which the germination percentages were assumed to have a cumulative normal distribution, and the base potentials have a Gaussian normal distribution with mean $\psi_B(50)$ and a standard deviation σ_{ψ_B} , according to:

$$\text{probit}(p_g) = \frac{\psi - \theta_H / t_g(p_g, \psi) - \psi_B(50)}{\sigma_{\psi_B}} \quad (2)$$

where σ_{ψ_B} is the standard deviation of the base potentials.

Estimations with both HT models (Gummerson and Bradford) are complex and usually require the use of specific computer code or software. Presently, there is not a simple model to analyze the results of germination tests under diverse treatments, such as: drought, salinity, temperature, or seed priming, among others. In this context, the main advantage of the proposed model (hereinafter referred to as GERMINAN) over GLMs, HT, TT, or HaloTT models is the fact that it requires less computational infrastructure, which enhances researchers' autonomy. Furthermore, this model enables the researcher or technician to analyze the results of germination trials without the necessity to learn any specific programming software. Therefore, the objective of this work was to propose a simple mathematical model for the analysis of the results of germination tests obtained through the application of different treatments.

MATERIALS AND METHODS

Germination trials

To exemplify the implementation and verification of the proposed model, the outcomes derived from the germination trials of *Vicia villosa* Roth. under water and salinity stress (Perissé et al., 2021), and of *Panicum virgatum* L. under water stress condition (Arias et al., 2018) were employed.

For *V. villosa* and *P. virgatum* the germination tests were carried out in plastic boxes and Petri dishes, respectively, on the base of which germination paper soaked in different solutions was placed, depending on the treatment: control (distilled water) and different water potentials (Ψ : -0.3, -0.6, -0.8, -1.0, and -1.2 MPa for *V. villosa*; and Ψ : -0.8, -1.0, -1.2 for *P. virgatum*), measured in megapascals (MPa). The osmolytes used were polyethylene glycol (PEG) to simulate water stress according to Michel (1983), and sodium chloride (NaCl) for salinity stress, according to Sosa et al. (2005).

Seeds of both species were moist pre-chilled for seven days at 5 °C. *Vicia villosa* was placed in a germination chamber at constant temperature of 20 ± 1 °C, and *P. virgatum* in alternating temperature of 30/15 °C; both species with an 8/16 h photoperiod, according to the International Seed Testing Association (ISTA, 2012).

The experimental units consisted of 25 seeds

with four replications per treatment, arranged in a completely randomized block design. The number of germinated seeds were recorded daily until the 15th and 28th days after sowing (DAS) for *V. villosa* and *P. virgatum*, respectively, time at which the trials were considered ended.

The measured variables were: cumulative germination percentage (GP), final germination percentage (FGP), and the number of days to reach 50 % germination (G_{50}).

Mathematical model

Preliminary analysis of germination trials results

This analysis is carried out to obtain relevant information about the results of germination trials in a simple and fast way. The potentials for the maximum germination percentages are considered as base potentials for these percentages, and both parameters are considered related according to a sigmoidal law, representative of the cumulative normal distribution. This is because, when the maximum germination percentages are plotted against each water potential, the resulting distribution fits well with a sigmoidal curve. Therefore, after testing different functions, the following one was adopted due to its simplicity:

$$\psi_B(p_{gmax}) = \alpha + \beta \ln \left[\frac{p_{gmax}}{100 - p_{gmax}} \right] \quad (3)$$

where $\psi_B(p_{gmax})$ is the minimum potential (base potential) to obtain the maximum germination percentage (p_{gmax}).

The inherent limitation of this function is that the logarithm approaches infinity when the percentage reaches 100 %, thereby precluding the possibility of solving Eq. (3) in this instance. However, the solution is to consider in this case a percentage of 99.99 % instead of 100 %, with a difference between the two values on the order of 10^{-4} , which is negligible for practical purposes.

The coefficients α and β are obtained from Eq. (3) by logarithmic regression, with the maximum germination percentage (MGP) corresponding to each water potential. Alternatively, they can be obtained by linear regression, with:

$$\begin{cases} x_i = \ln \frac{p_{gmax i}}{100 - p_{gmax i}} \\ y_i = \psi_B(p_{gmax i}) \end{cases} \quad (4)$$

Furthermore, these can be obtained by least squares with the following system of equations:

$$\begin{cases} n\alpha + \beta \sum_{i=1}^n x_i = \sum_{i=1}^n y_i \\ \alpha \sum_{i=1}^n x_i + \beta \sum_{i=1}^n x_i^2 = \sum_{i=1}^n x_i y_i \end{cases} \quad (5)$$

and the definitions of Eq. (4), which can be solved by any appropriate method, where n is the number of treatments.

The significance of the coefficients α and β , as derived from Eq. (3), is determined by the ability to calculate the following parameters:

a. The base potential for a given percentage of germination, directly with this equation.

b. The maximum germination percentages for a given potential. The potential considered for the calculation is the base potential, as it determines the maximum percentage of germination (MGP) for each treatment. Therefore, Eq. (3) is expressed as:

$$\ln \left[\frac{p_{gmax}}{100 - p_{gmax}} \right] = \frac{\psi - \alpha}{\beta} \quad (6)$$

from where

$$\frac{p_{gmax}}{100 - p_{gmax}} = \exp \left[\frac{\psi - \alpha}{\beta} \right] \quad (7)$$

and naming

$$z = \exp \left[\frac{\psi - \alpha}{\beta} \right] \quad (8)$$

Eq. (7) becomes

$$\frac{p_{gmax}}{100 - p_{gmax}} = z \quad (9)$$

and the maximum germination percentages for that potential is:

$$p_{gmax} = \frac{100z}{1+z} \quad (10)$$

c. The base potential for 50 % germination. For $p_g = 50$ the logarithm in Eq. (3) is zero and therefore:

$$\psi_B(50) = \alpha \quad (11)$$

d. The standard deviation of the base potential. It is calculated as:

$$\sigma_{\psi_B} = \sqrt{\frac{\sum_{i=1}^n [(\psi_{Bi} - \psi_B(50))^2]}{n}} \quad (12)$$

e. The parameter θ_H , as is explained in the section *Relation between GERMINAN and hydrotime models* (Eqs. 45 to 48).

Model for calculating cumulative germination percentages in function of time

The cumulative germination percentages are

obtained by means of a correlation function that must satisfy the following conditions for time t_g :

1. t_g equal to zero, the germination percentage must be zero.
2. t_g that tends to infinity, the percentage must tend to a limit value.

After examining different types of functions, and performing comparative analyses of the results obtained with them, the following simple exponential function was adopted:

$$\frac{p_g}{100 - p_g} = a_\psi \exp \left[\frac{b_\psi}{t_g} \right] \quad (13)$$

where for simplicity t_g is taken instead of $t_g(p_g, \psi)$.

Calculation of coefficients a_ψ and b_ψ

The values of the coefficients a_ψ and b_ψ depend on each water potential or treatment, and can be determined by exponential regression (or linear regression, taking logarithms on both sides) if working with a scientific calculator, or by the least squares method if a computer code is to be implemented. In this case, the coefficients for each treatment are obtained by applying logarithms in the Eq. (13):

$$\ln \frac{p_g}{100 - p_g} = \ln a_\psi + \frac{b_\psi}{t_g} \quad (14)$$

Doing

$$y_{i,j} = \ln \left[\frac{p_{g\ i,j}}{100 - p_{g\ i,j}} \right] \quad (15)$$

$$x_{i,j} = \frac{1}{t_{g\ i,j}} \quad (16)$$

$$\begin{cases} a_i = \ln a_\psi \\ b_i = b_\psi \end{cases} \quad (17)$$

where i indicates the potential or treatment, and j , the records of times and germination percentages in each water potential, Eq. (14) is expressed as:

$$y_{i,j} = a_i + b_i x_{i,j} \quad (18)$$

The coefficients a_i and b_i are obtained by linear regression, or by least squares of the following system of equations:

$$\begin{cases} n_i a_i + b_i \sum_{j=1}^{n_i} x_{i,j} = \sum_{j=1}^{n_i} y_{i,j} \\ a_i \sum_{j=1}^{n_i} x_{i,j} + b_i \sum_{j=1}^{n_i} x_{i,j}^2 = \sum_{j=1}^{n_i} x_{i,j} y_{i,j} \end{cases} \quad (19)$$

which can be solved by any appropriate

method, and where n is the number of treatments tested and n_i is the number of germination times and percentages corresponding to each treatment. The coefficients a_ψ result from:

$$a_\psi = \exp(a_i) \quad (20)$$

Once the coefficients a_ψ and b_ψ have been determined for each treatment, these can be plotted or tabulated to obtain by interpolation those corresponding to other untested treatments. Then, the germination percentages are calculated from Eq. (13) by means of:

$$p_g = \frac{100 \times a_\psi \exp\left[\frac{b_\psi}{t_g}\right]}{1 + a_\psi \exp\left[\frac{b_\psi}{t_g}\right]} \quad (21)$$

Conversely, the time corresponding to a given germination percentage is calculated as:

$$t_g = \frac{b_\psi}{\ln\left[\frac{p_g/(100-p_g)}{a_\psi}\right]} \quad (22)$$

This equation provides the times up to the maximum percentages obtained by means of Eq. (21) for each water potential. For untested potentials, the maximum percentages are estimated by linear interpolation between the maximum percentages corresponding to two adjacent potentials.

With this model, it is possible to achieve the following:

Estimated times for the 50 percent germination (G_{50}) at different water potentials

From Eq. (22) and for $p_g = 50$

$$t_{50} = \frac{b_\psi}{\ln\left[\frac{50/(100-50)}{a_\psi}\right]} = \frac{b_\psi}{\ln\left[\frac{1}{a_\psi}\right]} = \frac{b_\psi}{\ln(1) - \ln(a_\psi)} = -\frac{b_\psi}{\ln(a_\psi)} \quad (23)$$

Estimated germination onset times at different water potentials

If germination is considered to begin when a certain percentage (p_{go}) occurs (e.g., 1 or 2 percent, or a certain fraction of the maximum final percentage), from Eq. (22) the time of initiation is:

$$t_{go} = \frac{b_\psi}{\ln\left[\frac{p_{go}/(100-p_{go})}{a_\psi}\right]} \quad (24)$$

For example, if $p_{go} = 1\%$ is adopted for the onset of germination, the Eq. (24) results in:

$$t_{go} = \frac{b_\psi}{\ln\left[\frac{1/(100-1)}{a_\psi}\right]} = \frac{b_\psi}{\ln\left[\frac{1}{99a_\psi}\right]} = -\frac{b_\psi}{\ln(99a_\psi)} \quad (25)$$

Calculation of error and regression coefficients

Quadratic errors (ε)

For each treatment, the quadratic error between the modeled and experimental germination percentages is calculated as:

$$\varepsilon_i^2 = \sum_{j=1}^{n_i} [p_{gc}(i, j) - p_g(i, j)]^2 \quad (26)$$

where n_i is the number of registered percentages to be compared in treatment i , $p_{gc}(i, j)$ is the percentage j calculated in treatment i , and $p_g(i, j)$ is the corresponding experimental percentage. The average error for each treatment is given by:

$$\varepsilon_{im} = \sqrt{\frac{\varepsilon_i^2}{n_i}} \quad (27)$$

The total quadratic error for all treatments is:

$$\varepsilon^2 = \sum_{i=1}^n \varepsilon_i^2 = \sum_{i=1}^n \sum_{j=1}^{n_i} [p_{gc}(i, j) - p_g(i, j)]^2 \quad (28)$$

where n is the number of treatments.

The mean error for all treatments is calculated as:

$$\varepsilon_m = \sqrt{\frac{\varepsilon^2}{n_{tot}}} \quad (29)$$

Regression coefficients (R^2)

For each treatment, it is calculated as:

$$R_i^2 = \frac{S_{ti} - \varepsilon_i^2}{S_{ti}} \quad (30)$$

where ε_i^2 is given by Eq. (26), and the value of S_{ti} by:

$$S_{ti} = \sum_{j=1}^{n_i} [p_g(i, j) - p_{gmi}]^2 \quad (31)$$

where p_{gmi} is the average value of the germination percentage in treatment i , given by:

$$p_{gmi} = \frac{\sum_{j=1}^{n_i} p_g(i, j)}{n_i} \quad (32)$$

The mean regression coefficient for all treatments is calculated as:

$$R_m^2 = \frac{\sum_{i=1}^n R_i^2}{n} \quad (33)$$

Relation between GERMINAN and hydrotime models

Eq. (1) can be expressed as:

$$\psi - \psi_B(p_g) = \frac{\theta_H}{t_g} \quad (34)$$

from where

$$\psi_B(p_g) = \psi - \frac{\theta_H}{t_g} \quad (35)$$

and t_g is the time required for the percentage p_g at water potential ψ . If Eq. (3) is used to estimate the base water potentials, taking into account that the maximum p_{gmax} are those obtained at the maximum times t_{gmax} to each water potential, then:

$$\psi_B(p_{gmax}) = \alpha + \beta \ln \frac{p_{gmax}}{100 - p_{gmax}} = \psi - \frac{\theta_H}{t_{gmax}} \quad (36)$$

For times t_g less than t_{gmax} , the germination percentages p_g (taken as maximums for these times) are lower than p_{gmax} . Therefore, in general Eq. (36) can be put as:

$$\alpha + \beta \ln \frac{p_g}{100 - p_g} = \psi - \frac{\theta_H}{t_g} \quad (37)$$

from where

$$\ln \frac{p_g}{100 - p_g} = \frac{\psi - \frac{\theta_H}{t_g} - \alpha}{\beta} \quad (38)$$

and

$$\frac{p_g}{100 - p_g} = \exp \left[\frac{\psi - \frac{\theta_H}{t_g} - \alpha}{\beta} \right] \quad (39)$$

which can be expressed as

$$\frac{p_g}{100 - p_g} = \exp \left[\frac{\psi - \alpha}{\beta} \right] \exp \left[-\frac{\theta_H}{\beta} \frac{1}{t_g} \right] \quad (40)$$

With

$$a = \exp \left[\frac{\psi - \alpha}{\beta} \right] \quad (41)$$

$$b = -\frac{\theta_H}{\beta} \quad (42)$$

it results in

$$\frac{p_g}{100 - p_g} = a \exp \left[\frac{b}{t_g} \right] \quad (43)$$

which is formally identical to Eq. (13) of the GERMINAN model, but in the case of hydrotime model the coefficient b is constant, because θ_H is constant and with the same value for all treatments.

Therefore, for the GERMINAN model the coefficient b of Eq. (42) can be put as:

$$b_\psi = -\frac{\theta_H \psi}{\beta} \quad (44)$$

and then

$$\theta_H \psi = -\beta b_\psi \quad (45)$$

where now $\theta_{H\psi}$ is not constant since b_ψ depends on the treatment or water potential. To avoid confusion with θ_H of hydrotime and to maintain formal similarity, in the GERMINAN model it is named $\theta_{G\psi}$.

To obtain a unique value of θ_G for all the treatments, to be related with the constant value θ_H corresponding to hydrotime, the mean value of $\theta_{G\psi}$ is taken. A simple and approximate way to do this is to use the average value:

$$\theta_G = \frac{\sum_{i=1}^n \theta_{G\psi i}}{n} \quad (46)$$

where $\theta_{G\psi i}$ are the individual values of $\theta_{G\psi}$ for each treatment and n is the number of potentials or treatments. A more accurate approach is to apply the mathematical definition of mean value:

$$\theta_G = \frac{1}{\psi_n - \psi_1} \int_{\psi_1}^{\psi_n} \theta_{G\psi} d\psi \quad (47)$$

that for a discrete variable is calculated as:

$$\theta_G = \frac{1}{2(\psi_n - \psi_1)} \sum_{i=1}^{n-1} (\theta_{G\psi i} + \theta_{G\psi i+1})(\psi_{i+1} - \psi_i) \quad (48)$$

In general, GERMINAN θ_G values will differ from hydrotime θ_H values because these are calculated using different procedures. However, this is not crucial, as they simply indicate growth rates that are useful for comparison between different treatments or species. Their absolute numerical values are not relevant in themselves.

It is important to mention that all GERMINAN calculations can also be carried out with Microsoft Excel or similar programs (see Supplementary Material I).

RESULTS AND DISCUSSION

Preliminary analysis of germination test results

The maximum germination percentages (MGP) at different water potentials, experimental and calculated with the GERMINAN model, are presented in Figure 1, where it can be observed that in general, the GERMINAN results fit the experimental values.

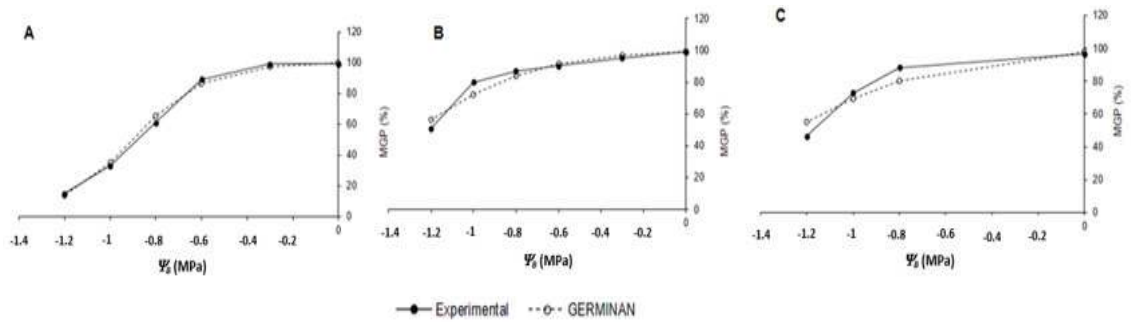


Figure 1. Comparison of maximum germination percentages (MGP) as a function of base water potentials (Ψ_b) between experimental results and those estimated with Eq. 10. A: *Vicia villosa* Roth. under polyethylene glycol (PEG), B: *Vicia villosa* under sodium chloride (NaCl), and C: *Panicum virgatum* under PEG.

Model for calculating cumulative germination percentages as a function of time

Calculation of coefficients a_ψ and b_ψ for Eq. (13)

Figure 2 shows the coefficients a_ψ and b_ψ for Eq. (13), calculated by exponential regression with experimental results, for *V. villosa* under water and salinity stress, and for *P. virgatum* under water stress.

Values of θ_H , $\psi_B(50)$, and $\sigma_{\psi B}$ estimated with Gummerson, Bradford and GERMINAN models

The values of $\psi_B(50)$ and $\sigma_{\psi B}$ from the preliminary analysis with Eqs. (11) and (12), and θ_G obtained with Eq. (48) are shown in Table 1. For the GERMINAN model the values of θ_G (equivalent to θ_H) are presented. Although the values were similar for the three models, GERMINAN provided a better

estimation of the $\psi_B(50)$, as can be seen in Figure 1. The values modeled using Gummerson and Bradford tended to overestimate the experimental ones. The θ_G values calculated by GERMINAN differed from Gummerson's but were similar to Bradford's. However, these values are not relevant in themselves, as they only serve as a reference for comparison purposes between different treatments. The $\sigma_{\psi B}$ values of GERMINAN were lower than those of the Gummerson and Bradford models for *V. villosa* and higher for *P. virgatum*. However, for the latter, Bradford's value almost doubled Gummerson's. As these values depend to some extent on the calculation procedure, it is not possible to determine which is the most accurate, at least in the context of the present work. In any case, the $\psi_B(50)$ value is the one of interest for practical purposes.

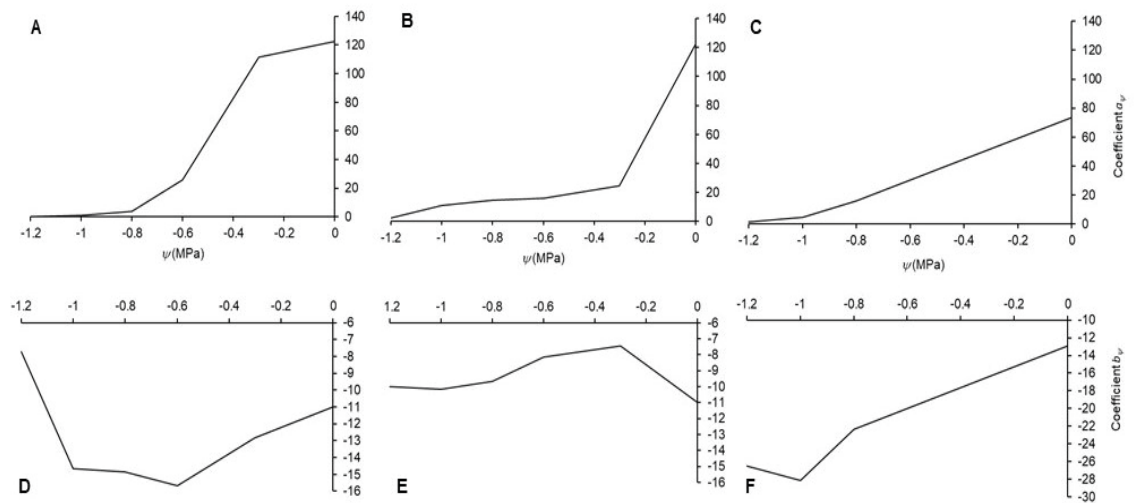


Figure 2. Coefficients a_ψ (A-C) and b_ψ (D-F). A, D: *Vicia villosa* under polyethylene glycol (PEG); B, E: *Vicia villosa* under sodium chloride (NaCl), and C, F: *Panicum virgatum* under PEG.

Table 1. Values of θ_H , $\psi_B(50)$, and $\sigma_{\psi B}$ with the Gummerson, Bradford and GERMINAN models for *Vicia villosa* under polyethylene glycol (PEG) and sodium chloride (NaCl), and for *Panicum virgatum* under PEG

Treatments		Gummerson			Bradford			GERMINAN		
Species		θ_H	$\psi_B(50)$	$\sigma_{\psi B}$	θ_H	$\psi_B(50)$	$\sigma_{\psi B}$	θ_G	$\psi_B(50)$	$\sigma_{\psi B}$
<i>V. villosa</i>	PEG	2.21	-1.01	0.22	2.74	-1.05	0.30	2.18	-0.90	0.21
<i>V. villosa</i>	NaCl	3.15	-1.56	0.43	3.02	-1.48	0.56	2.59	-1.27	0.37
<i>P. virgatum</i>	PEG	3.69	-1.22	0.15	5.76	-1.33	0.32	6.94	-1.27	0.44

Estimated times for the 50 percent germination (G_{50}) at different water potentials

The comparison between the times for G_{50} at different water potentials, experimental and estimated with Eq. (23), is shown in Tables 2a, b, and c. It is worth mentioning that germination percentages equal to or greater than 50 % were neither obtained for *V. villosa* under water potentials lower than -0.8 MPa (PEG-simulated) nor for *P. virgatum* under -1.2 MPa (NaCl-simulated).

A very good agreement was observed between the experimental and calculated values.

Table 2a. Experimental and calculated times provided by the GERMINAN model* for *Vicia villosa* under different water potentials simulated with polyethylene glycol (PEG)

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	2	2.3
-0.3	3	2.7
-0.6	6	4.8
-0.8	10	10.7

* G_{50} was not obtained experimentally for $\psi < -0.8$ MPa

Table 2b. Experimental and calculated times provided by the GERMINAN model for G_{50} in *Vicia villosa* under different water potentials simulated with sodium chloride (NaCl)

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	2	2.3
-0.3	2	2.3
-0.6	3	2.9
-0.8	3.5	3.6
-1.0	5	4.3
-1.2	12	12

Table 2c. Experimental and calculated times provided by the GERMINAN model* for G_{50} in *Panicum virgatum* under different simulated water potentials with polyethylene glycol (PEG)

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	3	3
-0.8	7.9	8
-1.0	19	18.5

* G_{50} was not obtained experimentally for $\psi = -1.2$ MPa

Estimated germination onset times at different water potentials

The comparison between times for the germination onset at different water potentials, experimental and estimated with Eq. (25), are shown in Tables 3a, b, and c. A very good agreement can be observed between the experimental and estimated values.

Table 3a. Germination onset times for *Vicia villosa* under different water potentials simulated with polyethylene glycol (PEG), experimental and estimated with GERMINAN

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	1	1.2
-0.3	1	1.4
-0.6	2	2
-0.8	2.5	2.5
-1.0	3	3.1
-1.2	2.5	2.3

Table 3b. Germination onset times for *Vicia villosa* under different water potentials simulated with sodium chloride (NaCl), experimental and estimated with GERMINAN

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	1	1.2
-0.3	1	0.93
-0.6	1.5	1.1
-0.8	1.2	1.3
-1.0	1.5	1.5
-1.2	2	1.8

Table 3c. Germination onset times for *Panicum virgatum* under different simulated water potentials with polyethylene glycol (PEG), experimental and estimated with GERMINAN

ψ (MPa)	Time (days)	
	Experimental	GERMINAN
0.0	1.8	1.4
-0.8	3	3
-1.0	5	4.6
-1.2	5.5	5.5

Cumulative germination percentages in function of time

Figure 3 shows the cumulative germination percentages as a function of time, both experimental and modeled with GERMINAN.

A very good fit can be observed between experimental and modeled results for *V. villosa* (Figures 3a and b). In the case of *P. virgatum* under PEG (Figure 3c), the GERMINAN model did not fit as well as it did in the case of *V. villosa*. This could be due to irregular germination behavior or to an anomalous factor at the lower potentials in the germination tests. However, the fits of both hydrotime models were even less accurate than the GERMINAN model, as shown in Tables 4c and 5.

Calculation of errors and regression coefficients

Quadratic errors and regression coefficients in function of water potentials

Mean errors (ϵ) and regression coefficients (R^2)

between experimental and modeled results as a function of water potentials for the Gummerson, Bradford, and GERMINAN models are shown in Tables 4a, b and c. In general, a better fit of the GERMINAN model can be observed with respect to the other two models.

Table 4a. Mean quadratic errors (ϵ) and regression coefficients (R^2) between the experimental and modeled results as a function of water potentials ψ (MPa) for the three models in *Vicia villosa* under polyethylene glycol (PEG)

ψ (MPa)	Gummerson		Bradford		GERMINAN	
	ϵ	R^2	ϵ	R^2	ϵ	R^2
0.0	4.73	0.986	4.45	0.988	2.41	0.996
-0.3	5.48	0.982	7.93	0.961	5.57	0.981
-0.6	6.45	0.971	6.81	0.968	3.80	0.990
-0.8	2.49	0.990	2.56	0.990	1.73	0.995
-1.0	2.09	0.969	3.45	0.915	1.54	0.983
-1.2	2.34	0.864	1.66	0.932	1.55	0.940

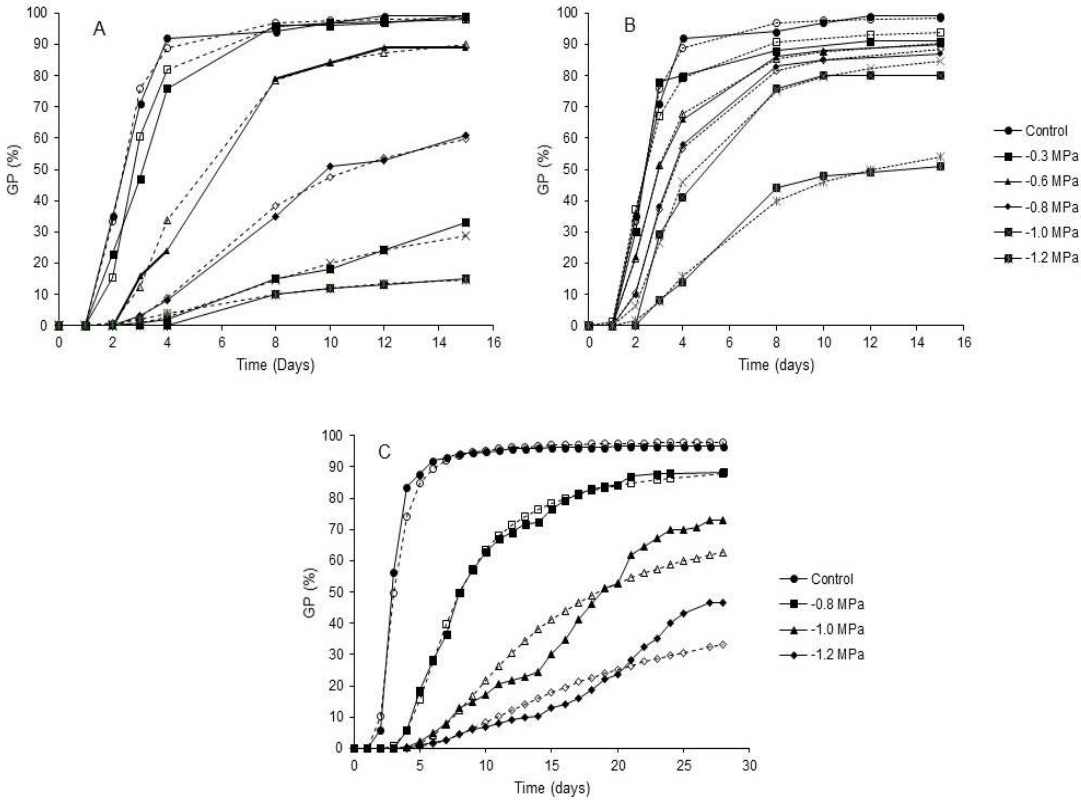


Figure 3. Experimental (solid line) and GERMINAN-modeled (dash line) cumulative germination percentages (GP %) as a function of time for *Vicia villosa* under different water potentials simulated with polyethylene glycol (PEG): (A) and sodium chloride (NaCl), (B), and for *Panicum virgatum* under different water potentials simulated with PEG (C)

Table 4b. Mean quadratic errors (ϵ) and regression coefficients (R^2) between the experimental and modeled results as a function of water potentials (ψ (MPa)) for the three models in *Vicia villosa* under sodium chloride (NaCl)

ψ (MPa)	Gummerson		Bradford		GERMINAN	
	ϵ	R^2	ϵ	R^2	ϵ	R^2
0.0	6.54	0.974	9.48	0.945	2.41	0.996
-0.3	5.08	0.982	4.86	0.984	4.79	0.984
-0.6	4.14	0.987	2.20	0.996	0.73	0.999
-0.8	5.80	0.974	6.11	0.971	0.95	0.999
-1.0	8.51	0.932	9.43	0.916	3.13	0.991
-1.2	5.81	0.931	4.49	0.959	2.10	0.991

Table 4c. Mean quadratic errors (ϵ) and regression coefficients (R^2) between the experimental and modeled results as a function of water potentials (ψ (MPa)) for the three models, for *Panicum virgatum* under polyethylene glycol (PEG)

ψ (MPa)	Gummerson		Bradford		GERMINAN	
	ϵ	R^2	ϵ	R^2	ϵ	R^2
0.0	5.55	0.976	18.5	0.734	3.24	0.992
-0.8	3.85	0.986	11.7	0.872	1.70	0.997
-1.0	7.53	0.916	6.76	0.932	6.56	0.936
-1.2	6.21	0.828	5.19	0.880	5.39	0.871

Mean quadratic errors and regression coefficients

As shown in Table 5, the global mean square errors (ϵ_m) and regression coefficients (R^2) between the experimental and modeled results for the three models were similar, although the results obtained with the GERMINAN model were slightly better.

The average global mean error values for all trials were 5.43 for Gummerson, 7.33 for Bradford, and 3.56 for GERMINAN. The respective average regression coefficients for the three models were 0.950, 0.925, and 0.975. These figures indicate that GERMINAN fitted better than the other two models, although the differences may not be significant in practice.

The enhanced outcomes observed in GERMINAN with respect to those provided by the hydrotime model (by Gummerson and Bradford) can be explained by the similarities and differences between the two models, as shown in the comparison between Eqs. (13) and (43).

A key distinction concerns the b coefficient, which is important to consider. While this coefficient

exhibits a constant value in the hydrotime model, it demonstrates variability in GERMINAN. This variation helps to align the model predictions more precisely with the experimental outcomes.

The proposed model is subject to two inherent limitations. First, it is only applicable when germination rates increase or decrease monotonically, that is, when there is no reversal in germination progression, as occurs, for example, in trials with different temperatures, as manifested in Eq. (13). This is also a characteristic of the hydrotime model, and therefore, when temperature-reversal occurs, the thermal-time model is applied. Second, it is only applicable when the germination process depends on a single variable such as water or saline stress, chemicals, etc. If a combination of two variables is considered, for example water stress and chemicals, the model cannot account for them simultaneously. The solution to these limitations is developed in the Supplementary Material II.

CONCLUSION

The most important advantage of the GERMINAN model is that, even though the results obtained are only marginally better than those of the hydrotime models, calculations can easily be performed by hand, using a scientific calculator, Excel or a similar program. However, simple computer software can be implemented using any programming language, if convenient. Hydrotime germination parameters θ_H , $\psi_B(50)$, and σ_{ψ_B} can also be calculated with the GERMINAN model in a simple way. The proposed model would also be applicable to discontinuous germination processes, as is the case, for example, with temperature, and when analyzing the results produced by two combined treatments. Moreover, this model allows researchers and technicians to analyze the results of germination trials without having to learn any specific programming software or seek a specialist for assistance. In some cases, it is possible that other models might fit the experimental data better than the proposed model. Therefore, additional studies are required to verify its validity. The next stage of the research program will involve performing a range of assays with different species and treatments to provide a more

Table 5. Global mean quadratic errors (ϵ_m) and regression coefficients (R^2) between experimental and modeled results for the three models

Species	Treatments	Gummerson		Bradford		GERMINAN	
		ϵ_m	R^2	ϵ_m	R^2	ϵ_m	R^2
<i>V. villosa</i>	PEG	4.19	0.960	4.90	0.959	3.14	0.981
<i>V. villosa</i>	NaCl	6.06	0.963	6.53	0.962	2.70	0.994
<i>P. virgatum</i>	PEG	6.05	0.927	10.56	0.855	4.85	0.949

exhaustive evaluation of the model's reliability.

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SUPPLEMENTARY MATERIAL I

The following example illustrates how the GERMINAN model can be applied using Excel or similar software.

Assume that a series of tests have been carried out with treatments of 0, -0.3, -0.6, -0.8, -1.0, and -1.2 MPa. The maximum germination percentages p_{gm} at each treatment ψ were

ψ	0	-0.3	-0.6	-0.8	-1.0	-1.2
p_{gm}	99	98	89	61	33	15

Also suppose that for a given treatment, for example control or 0 MPa, the following germination percentages p_g over time t_g were obtained:

t_g	0	1	2	3	4	8	10	12
p_g	0	0	35	71	92	94	97	99

Remarks:

Germination percentages equal to zero should not be included in the calculations. Otherwise, an error will occur in the regression calculation. For a better fit, it is convenient (but not necessary) that the germination percentages not be repeated at two or more successive times for each potential. For example, if the data were:

t_g	0	1	2	3	4	5	6	7	8	9	10
p_g	0	5	10	10	15	30	50	50	85	90	90

For calculations they should be replaced by

t_g	1	2	4	5	6	8	9
p_g	5	10	15	30	50	85	90

Calculation of coefficients α and β by logarithmic regression

They are obtained from Eq. (3):

$$\psi_B(p_{gmax}) = \alpha + \beta \ln \left[\frac{p_{gmax}}{100 - p_{gmax}} \right] \quad (3)$$

In an Excel sheet, the treatments or potentials ψ and maximum germination percentages p_{gm} are written in columns A and B, for example, from A2 to A7 and B2 to B7, respectively. In another column, for example D, the title of the operation is indicated in cell D1, and the formula of calculation in cell D2, as shown:

	A	B	C	D
1	ψ	pgm		$x = \text{pgm}/(100 - \text{pgm})$
2	0	99		$=b2/(100-b2)$
3	-0.3	98		
4	-0.6	89		
5	-0.8	61		
6	-1.0	33		
7	-1.2	15		

Click *Enter*, and this result will appear:

	A	B	C	D
1	ψ	pgm		$x = \text{pgm}/(100 - \text{pgm})$
2	0	99		99
3	-0.3	98		

Place the cursor in the result cell using the mouse or keyboard arrows, and then in the lower right corner of the cell:

	A	B	C	D
1	ψ	pgm		$x = \text{pgm}/(100 - \text{pgm})$
2	0	99		99
3	-0.3	98		

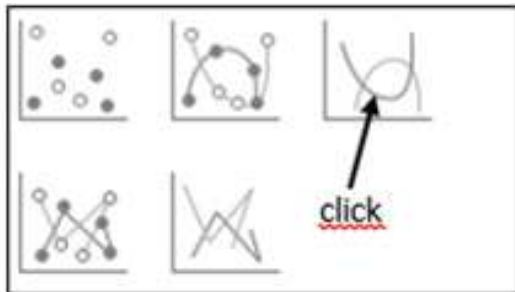
Holding down the left mouse button, drag down to the last row of data (in this case row 7, cell D7) for the remaining calculations:

	A	B	C	D
1	ψ	pgm		$x = \text{pgm}/(100 - \text{pgm})$
2	0	99		99
3	-0.3	98		49
4	-0.6	89		8.0909
5	-0.8	61		1.5641
6	-1.0	33		0.4925
7	-1.2	15		0.1765

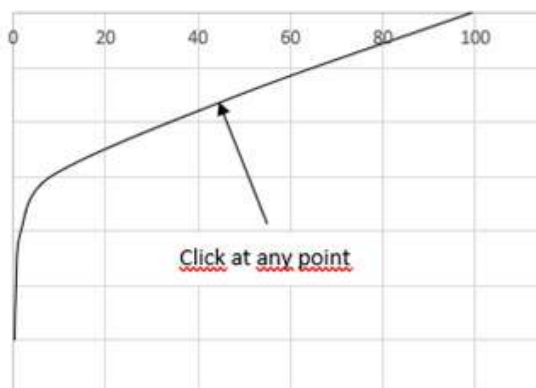
The same general procedure is followed for all other calculations. Copy in column E the treatments displayed in column A. Then select both columns:

	A	B	C	D	E
1	ψ	pgm		$x = \text{pgm}/(100 - \text{pgm})$	ψ
2	0	99		99	0
3	-0.3	98		49	-0.3
4	-0.6	89		8.0909	-0.6
5	-0.8	61		1.5641	-0.8
6	-1.0	33		0.4925	-1.0
7	-1.2	15		0.1765	-1.2

In the toolbar click *Insert*, then *Graphs*, and select *Dispersion*. Choose one of the types shown and click on it with the left mouse button. For example:

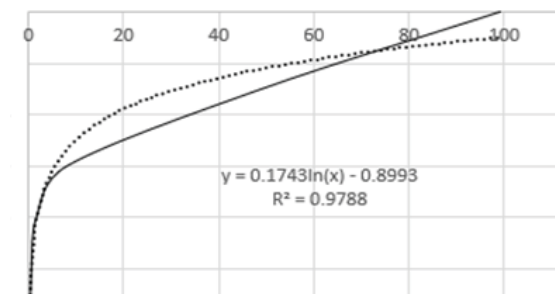
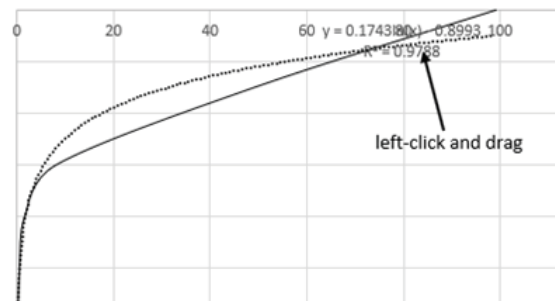


On the graph, click with the right button of the mouse on the curve, and select *Add trend line*:



Under *Trendline Options* select *Logarithmic*. At the bottom of the window select *Display Equation on chart* and *Display R-squared value on chart*. In the present example, the actual values are drawn as a continuous line and the trend line as dots.

Close the window. In the graph, click the left mouse button on the values displayed and, while holding down the mouse button, drag the legend to a convenient location:



To change the size of the legend, click it with the left mouse button, go to the toolbar, and select the letter type and size.

From the graph, the coefficients are:

$$\alpha = -0.8993$$

$$\beta = 0.1743$$

And from Eq. (11), it follows that

$$\psi_B(50) = \alpha = -0.8993$$

Calculation of coefficients α and β by linear regression

In an Excel sheet, the treatments or potentials ψ and maximum germination percentages are written in for example columns A and B, from A2 to A7 and B2 to B7, respectively. In another column, for example D, the title of the operation is indicated in cell D1, and the formula of calculation in cell D2, as indicated:

	A	B	C	D
1	ψ	pgm		$x=\ln(\text{pgm}/(100-\text{pgm}))$
2	0	99		$=\ln(b2/(100-b2))$
3	-0.3	98		
4	-0.6	89		
5	-0.8	61		
6	-1.0	33		
7	-1.2	15		

Click *Enter* and drag until D7, as explained before. Copy column A in column E, and select both columns:

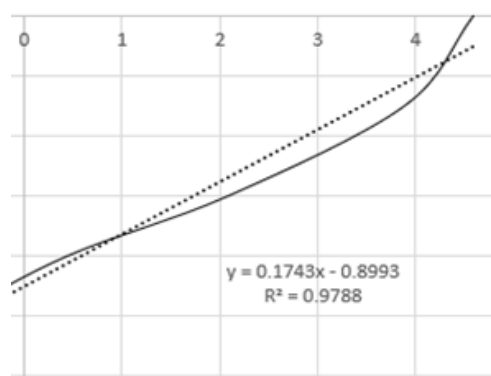
	A	B	C	D	E
1	ψ	pgm		$x=\ln(\text{pgm}/(100-\text{pgm}))$	ψ
2	0	99		4.5951	0
3	-0.3	98		3.8918	-0.3
4	-0.6	89		2.0907	-0.6
5	-0.8	61		0.4473	-0.8
6	-1.0	33		-0.7082	-1.0
7	-1.2	15		-1.7346	-1.2

From here the procedure is the same as before:

Insert a *Dispersion* graph, whatever straight or soft lines:

On the graph, add the trend line, in *Trendline options* select the *Linear*, and at the bottom of the window select *Display equation on chart* and *Display R-squared value on chart*.

Now the graph is



and the coefficients are:

$$\alpha = -0.8993$$

$$\beta = 0.1743$$

Coefficients a_ψ and b_ψ by exponential regression

They are obtained from Eq.(13):

$$\frac{p_g}{100-p_g} = a_\psi \exp \left[\frac{b_\psi}{t_g} \right]$$

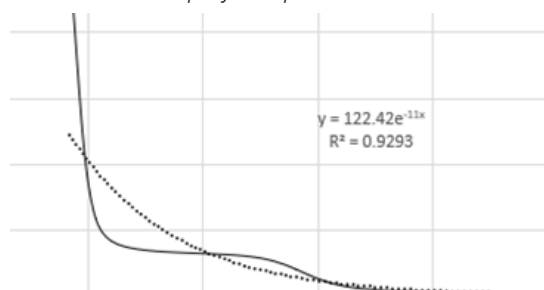
The data are written in columns A and B, for example, as shown. In column D the values $1/t_g$ are calculated and displayed as before. Now, in column E the following calculation is performed:

	A	B	C	D	E
1	t_g	p_g		$x=1/t_g$	$y=p_g/(100-p_g)$
2	2	35		0.5	$=b2/(100-b2)$
3	3	71		0.3333	
4	4	92		0.25	
5	8	94		1.125	
6	10	97		0.1	
7	12	99		0.08333	

With the values calculated in columns D and E:

	A	B	C	D	E
1	t_g	p_g		$x=1/t_g$	$y=p_g/(100-p_g)$
2	2	35		0.5	0.5385
3	3	71		0.3333	2.4483
4	4	92		0.25	11.5
5	8	94		0.125	15.6667
6	10	97		0.1	32.3333
7	12	99		0.08333	99

a *Dispersion* graph is inserted, the trendline of the type *Exponential*, and select *Display equation on chart* and *Display R squared value on chart*.



The coefficients are:

$$a_\psi = 122.43$$

$$b_\psi = -11$$

Coefficients a_ψ and b_ψ by linear regression

The germination times and percentages for each treatment or potential are indicated in columns A and B, for example, as shown. In column D, the $1/tg$ values are calculated. The title of the calculation is written in column E1 and the formula is set in cell E2:

	A	B	C	D	E
1	tg	pg		$x=1/tg$	$y=\ln(pg/(100-pg))$
2	2	35		0.5	$=\ln(b2/(100-b2))$
3	3	71		0.3333	
4	4	92		0.25	
5	8	94		0.125	
6	10	97		0.1	
7	12	99		0.08333	

Continue with the calculations in column E. The results are:

	A	B	C	D	E
1	tg	pg		$x=1/tg$	$y=\ln(pg/(100-pg))$
2	2	35		0,5	-0,6190
3	3	71		0,3333	0,8954
4	4	92		0,25	2,4423
5	8	94		0,125	2,7515
6	10	97		0,1	3,4761
7	12	99		0,08333	4,5951

From here the procedure is the same as before: Select cells D2–D7 to E2–E7.

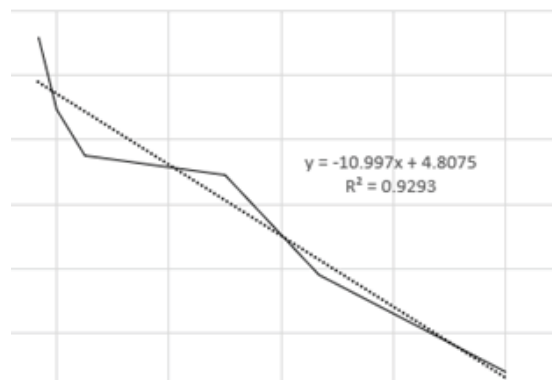
Click *Insert graph* in the task bar.

Select *Dispersion*, whatever straight or soft lines.

Click in the curve with the right button of the mouse, and select *Add trend line*.

In *Trendline Options* select *Linear*, select *Display Equation on chart* and *Display R-squared value on chart*.

The resulting graph is:



The values of the coefficients are:

$$a_\psi = \exp(4.8075) = 122.43$$

$$b_\psi = -10.997$$

Calculation of $\sigma_{\psi B}$

From Eq. (3) it is:

$$\psi_B(p_{gmax}) = \alpha + \beta \ln \left[\frac{p_{gmax}}{100 - p_{gmax}} \right]$$

In an Excel column (for example A), a set of germination percentages p_g is established (for example, from 10 to 90 %). In another column (for example B) the logarithms are calculated, and in a third column, for example C, the base potentials are calculated. The coefficients α and β obtained before are $\alpha = -0.8993$, $\beta = 0.1743$, and $\psi_B(50) = -0.8993$.

	A	B	C
1	pg	$y=\ln(pg/(100-pg))$	ψ_B
2	10	-2.1972	$=\ln(-0.8993+0.1743*b2)$
3	20	-1.3863	
4	30	-0.8473	
5	40	-0.4055	
6	50	0	
7	60	0.4055	
8	70	0.8473	
9	80	1.3863	
10	90	2.1972	

In column E (e.g.) the values of $[\psi_B - \psi_B(50)]^2$ are calculated:

	A	B	C	D	E
1	pg	$y=\ln(pg/(100-pg))$	ψ_B		$((\psi_B - \psi_B(50))^2)$
2	10	-2.1972	-1.2823		$=(-1.2823+0.8993)^2$
3	20	-1.3863	-1.1409		
4	30	-0.8473	-1.0469		
5	40	-0.4055	-0.9699		
6	50	0	-0.8993		

Click *Enter* and pull down until E10. The results are:

C	D	E
ψ_B		$((\psi_B - \psi_B(50))^2)$
-1.2823		0.1467
-1.1409		0.05839
-1.0469		0.02181
-0.9699		0.004994
-0.8993		0
-0.8286		0.004994
-0.7516		0.02181
-0.6577		0.05839
-0.5163		0.1467

Then in column G the summation of squares is performed:

C	D	E	F	G
ψ_B		$((\psi_B - \psi_B(50))^2)$		$\Sigma((\psi_B - \psi_B(50))^2)$
-1.2823		0.1467		=SUM(E2:E10)
-1.1409		0.05839		
-1.0469		0.02181		
-0.9699		0.004994		
-0.8993		0		
-0.8286		0.004994		
-0.7516		0.02181		
-0.6577		0.05839		
-0.5163		0.1467		

Click *Enter*. In column I the value of σ_{ψ_B} is calculated

$$\sigma_{\psi_B} = \sqrt{\frac{\Sigma(\psi_B - \psi_B(50))^2}{n}} \quad (n = 9):$$

G	H	I
$\Sigma((\psi_B - \psi_B(50))^2)$		σ_{ψ_B}
0.4637		=SQRT(g2/9)

Click *Enter*, and the result is given:

G	H	I
$\Sigma((\psi_B - \psi_B(50))^2)$		σ_{ψ_B}
0.4637		0.2270

Calculation of the germination percentages and errors

The procedure must be repeated for each treatment or potential. In the present case of the example, it is for *Control* or $\psi = 0$ MPa.

For this treatment the coefficients are:

$$a_{\psi} = 122.43$$

$$b_{\psi} = -10.997$$

The germination times t_g are displayed in column A (for example), and the experimental percentages p_g in column B. In column D the values of

$$z = \frac{p_g}{100 - p_g} = a_{\psi} \exp\left(\frac{b_{\psi}}{t_g}\right) \text{ are calculated.}$$

The value in column D2 must be zero, corresponding to $t_g = 0$. The calculation must be performed for times greater than zero. Otherwise, an error will occur.

	A	B	C	D
1	t_g	p_g		$z = a_{\psi} \exp(a_{\psi}/t_g)$
2	0	0		0
3	2	35		=122.43*exp (-10.997/a2)
4	3	71		
5	4	92		
6	8	94		
7	10	97		
8	12	99		

The results are:

	A	B	C	D
1	t_g	p_g		$z = a_{\psi} \exp(a_{\psi}/t_g)$
2	0	0		0
3	2	35		0.5011
4	3	71		3.1326
5	4	92		7.8325
6	8	94		30.9667
7	10	97		40.7656
8	12	99		48.9658

The values of germination percentages p_{gc} are calculated in column F with Eq.

$$p_g = \frac{100 \times a_{\psi} \exp\left[\frac{b_{\psi}}{t_g}\right]}{1 + a_{\psi} \exp\left[\frac{b_{\psi}}{t_g}\right]} \quad (21):$$

	A	B	C	D	E	F
1	t_g	p_g		$z = a_{\psi} \exp(a_{\psi}/t_g)$		$p_{gc} = 100 \cdot z / (1 + z)$
2	0	0		0		=100*d2/(1+d2)
3	2	35		0.5011		
4	3	71		3.1326		
5	4	92		7.8325		
6	8	94		30.9667		
7	10	97		40.7656		
8	12	99		48.9658		

The results are:

	A	B	C	D	E	F
1	t_g	p_g		$z = a_{\psi} \exp(a_{\psi}/t_g)$		$P_{gc} = 100 \cdot z / (1 + z)$
2	0	0		0		0
3	2	35		0.5011		33.3819
4	3	71		3.1326		75.8023
5	4	92		7.8325		88.6783
6	8	94		30.9667		98.8718
7	10	97		40.7656		97.6057

Calculation of quadratic errors and mean quadratic error

The quadratic errors are calculated by:

$$e_i^2 = (p_{gci} - p_{gi})^2$$

In column H the procedure is shown:

	A	B	C	D	E	F	G	H
1	tg	pg		$z=a_{\psi} \cdot \exp(a_{\psi}/tg)$		$Pgc=100 \cdot z/(1+z)$		$e2=(pgc-pg)^2$
2	0	0		0		0		$=(f2-b2)^2$
3	2	35		0.5011		33.3819		
4	3	71		3.1326		75.8023		
5	4	92		7.8325		88.6783		
6	8	94		30.9667		98.8718		
7	10	97		40.7656		97.6057		
8	12	99		48.9658		97.9986		

The results are:

	A	B	C	D	E	F	G	H
1	tg	pg		$z=a_{\psi} \cdot \exp(a_{\psi}/tg)$		$Pgc=100 \cdot z/(1+z)$		$e2=(pgc-pg)^2$
2	0	0		0		0		0
3	2	35		0.5011		33.3819		2.6181
4	3	71		3.1326		75.8023		23.0624
5	4	92		7.8325		88.6783		11.0340
6	8	94		30.9667		98.8718		8.2469
7	10	97		40.7656		97.6057		0.3669
8	12	99		48.9658		97.9986		1.002737

The quadratic mean error is calculated as:

$$e_m = \sqrt{\frac{\sum e_i^2}{n}}$$

The procedure is shown in column J:

H	I	J
$e2=(pgc-pg)^2$		Σe^2
0		$=SUM(h2:h8)$
2.6181		
23.0624		
11.0340		
8.2469		
0.3669		
1.002737		

The quadratic error is:

H	I	J
$e2=(pgc-pg)^2$		Σe^2
0		46.3310
2.6181		

The mean quadratic error for $n = 7$:

J	K	L
Σe^2		<u>em</u>
46.3310		$=SQRT(j2/7)$

and the result is:

J	K	L
Σe^2		<u>em</u>
46.3310		2.5727

Calculation of θ_{Gi} and θ_G

For this calculation, the values of the coefficients b_{ψ} for all treatments are required. For example, suppose that they are the following:

ψ	0	-0.3	-0.6	-0.8	-1.0	-1.2
b_{ψ}	-11	-12.8	-15.7	-14.9	-14.7	-7.7

In any column (for example A) the values of ψ are set, and in another (for example B), the values of b_{ψ} . In column C the values of θ_{Gi} from Eq. (45):

$$\theta_{H\psi} = -\beta b_{\psi}$$

are calculated, with $\beta = 0.1743$.

	A	B	C
1	ψ	b_{ψ}	$\theta_{G\psi}=-\beta \cdot b_{\psi}$
2	0	-11	$=-0.1743 \cdot b2$
3	-0.3	-12.8	
4	-0.6	-15.7	
5	-0.8	-14.9	
6	-1.0	-14.7	
7	-1.2	-7.7	

The results are:

	A	B	C
1	ψ	b_{ψ}	$\theta_{G\psi}=-\beta \cdot b_{\psi}$
2	0	-11	1.9173
3	-0.3	-12.8	2.2310
4	-0.6	-15.7	2.7365
5	-0.8	-14.9	2.5971
6	-1.0	-14.7	2.5622
7	-1.2	-7.7	1.3421

The mean mathematical value of θ_G with Eq. (48) is:

$$\theta_G = \frac{1}{2(\psi_n - \psi_1)} \sum_{i=1}^{n-1} (\theta_{G\psi_i} + \theta_{G\psi_{i+1}})(\psi_{i+1} - \psi_i)$$

In column D the values of $(\theta_{G\psi_i} + \theta_{G\psi_{i+1}})(\psi_{i+1} - \psi_i)$

are calculated:

	A	B	C	D
1	ψ	$b\psi$	$\theta_{G\psi} = -\beta * b\psi$	$x = (\theta_{G\psi} + \theta_{G\psi i+1}) * (\psi_{i+1} - \psi_i)$
2	0	-11	1.9173	$= (c2 + c3) * (a3 - a2)$
3	-0.3	-12.8	2.2310	

In column E the value of θ_G is calculated:

	D	E
1	$x = (\theta_{G\psi} + \theta_{G\psi i+1}) * (\psi_{i+1} - \psi_i)$	$\theta_G = \sum x / (2 * (\psi_7 - \psi_2))$
2	-1.2445	$= \text{SUM}(d2:d6) / (2 * (a7 - a2))$
3	-1.4903	
4	-1.06672	
5	-1.03186	
6	-0.7809	
Calculation up to row 6, because the sum is from 1 to n+1		

SUPPLEMENTARY MATERIAL II

The model is subject to two inherent limitations: 1) it is only applicable when germination rates increase or decrease monotonically, and 2) it is only applicable when the germination process depends on a single variable, e.g., water or saline stress, chemicals, etc. If two variables are considered, for example water stress and chemicals, the model cannot account for them simultaneously.

The solution for the first case is to split the germination process into two parts: one for the increase (or decrease) phase, and another for the decrease (or increase) phase. For each phase, the coefficients α and β are calculated with Eq. (3), resulting in the following:

$$\psi_{B1}(p_g) = \alpha_1 + \beta_1 \ln \left[\frac{p_g}{100 - p_g} \right] \text{ for phase 1} \quad (49)$$

$$\psi_{B2}(p_g) = \alpha_2 + \beta_2 \ln \left[\frac{p_g}{100 - p_g} \right] \text{ for phase 2} \quad (50)$$

In the intersection point

$$\psi_{B1}(p_{go}) = \psi_{B2}(p_{go}) \quad (51)$$

that is

$$\alpha_1 + \beta_1 \ln \left[\frac{p_{go}}{100 - p_{go}} \right] = \alpha_2 + \beta_2 \ln \left[\frac{p_{go}}{100 - p_{go}} \right] \quad (52)$$

From this

$$\alpha_1 - \alpha_2 = (\beta_2 - \beta_1) \ln \left[\frac{p_{go}}{100 - p_{go}} \right] \quad (53)$$

and

$$\ln \left[\frac{p_{go}}{100 - p_{go}} \right] = \frac{\alpha_1 - \alpha_2}{\beta_2 - \beta_1} = K_{12} \quad (54)$$

After some mathematical operations it finally results in

$$p_{go} = \frac{100 \exp(K_{12})}{1 + \exp(K_{12})} \quad (55)$$

The value of p_{go} is the limit or boundary of the two phases, and in each one the model applies with the respective coefficients. Once the value of p_{go} has been obtained, the corresponding potential or treatment is calculated using Eqs. (49) or (50). As expected, this is only an estimate for conducting further tests closer to the tentative potential. The procedure is schematically illustrated in Figure 1.

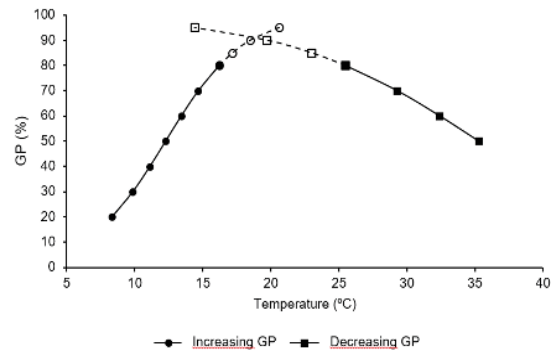


Figure 1. Estimated temperature and maximum germination percentage (GP %) when inversion occurs. Solid lines for experimental data, dashed lines for extrapolations. For illustrative purposes only.

In the second case, when two treatments are combined, such as water stress treatments at different temperatures, the solution is the following. For temperature T_1 , the germination percentages are obtained at various water potentials, and the coefficients a_1 , b_1 , a_{y1} , and b_{y1} are calculated. The tests are then repeated for temperature T_2 , and the coefficients a_2 , b_2 , a_{y2} , and b_{y2} are calculated. Tests are carried out in the same way for the other temperatures. Then the coefficients α , β , a_ψ , and b_ψ are presented graphically, as shown in Figure 2. In this way, the coefficients for any given combination of treatments are obtained, either directly from the graphs in the case of coefficients α and β , or by interpolation between two adjacent temperatures for the coefficients a_ψ and b_ψ .

Alternatively, tests can also be performed at different temperatures under a given water potential and repeated at other potentials. This will generate germination percentages based on temperature, using water potentials as parameters. In any case, the general procedure is the same as described above.

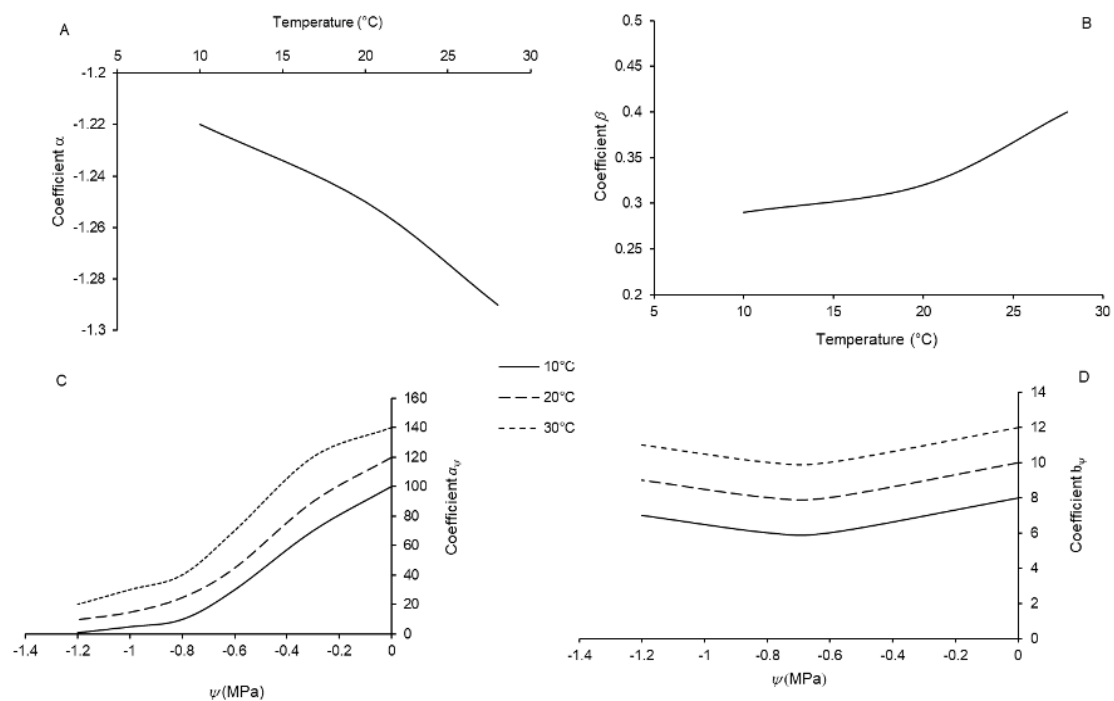


Figure 2. A-B) Coefficients α and β as a function of temperature, C-D) Coefficients a_{ψ} and b_{ψ} as a function of water stress and temperature, for illustrative purposes only