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Performance of seed germination of six different varieties of basil (*Ocimum basilicum spp.*)

Desempenho da germinação de sementes de seis diferentes variedades de manjericão (*Ocimum basilicum spp.*)

Rendimiento de la germinación de semillas de seis variedades diferentes de albahaca (*Ocimum basilicum spp.*)

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Abstract
Basil is an aromatic and medicinal plant that has a wide variety of species due to cross-pollination processes that influence germination and properties. In this work, germination parameters of six different basil species were evaluated in order to describe which ones have the highest germination potential. For this, the basil varieties were grown in a greenhouse at 20-25 ºC, and the germination percentage was monitored for 14 days. The Weibull modeling allowed us to evaluate that the species *O. basilicum var. basilicum*, *O. Basilicum var. purpurascens* and *O. basilicum minimum* are those that germinate in large proportions and that *O. basilicum L. cv. Genovese* has an intense dormancy process. Considering the germination time, the species *O. basilicum var. basilicum* is the variety with the highest germination potential.
Keywords: Basil; *Ocimum basilicum* L.; Germination; Dormancy; Natural products.

Resumo

O manjericão é uma planta aromática e medicinal que apresenta grande variedade de espécies devido a processos de polinização cruzada e que influenciam a germinação e propriedades. Neste trabalho, foram avaliados parâmetros de germinação de seis espécies diferentes de manjericão visando descrever quais apresentam maior potencialidade de germinação. Para isso, as variedades de manjericão foram cultivadas em estufa entre 20-25 ºC, e a porcentagem de germinação monitorada por 14 dias. A modelagem de Weibull permitiu avaliar que as espécies *O. basilicum var. basilicum*, *O. Basilicum var. purpurascens* and *O. basilicum minimum* são aquelas que germinam em grande proporção e que a *O. basilicum L. cv. Genovese* apresenta intenso processo de dormência. Considerando o tempo de germinação, a espécie *O. basilicum var. basilicum* é a varidade com maior potencial de germinação

Palavras-chave: Manjericão; *Ocimum basilicum L.;* Germinação; Dormência; Produtos naturais.

Resumen

La albahaca es una planta aromática y medicinal que presenta una gran variedad de especies debido a procesos de polinización cruzada que influyen en la germinación y propiedades. En este trabajo se evaluaron los parámetros de germinación de seis especies diferentes de albahaca con el fin de describir cuáles tienen el mayor potencial de germinación. Para ello, las variedades de albahaca se cultivaron en invernadero a 20-25 ºC, y se monitoreó el porcentaje de germinación durante 14 días. El modelado de Weibull nos permitió evaluar que la especie *O. basilicum var. basilicum*, *O. Basilicum var. purpurascens* y *O. basilicum minimum* son las que germinan en mayor proporción y que *O. basilicum L. cv. Genovese* tiene un intenso proceso de latencia. Considerando el tiempo de germinación, la especie *O. basilicum var. basilicum* es la variedad con mayor potencial de germinación.

Palabras clave: Albahaca; *Ocimum basilicum L.;* Germinación; Latencia; Productos naturales.

1. Introduction

Basil (*Ocimum basilicum* L.) belongs to the Lamiaceae family and it is an annual or perennial plant, originating from Southeast Asia and Central Africa (Srivastava et al., 2018). It is an aromatic sub-shrub and inserted in Brazil by Italian immigrants. Used as medicinal and aromatic plant, it contains substances of interest to the food, pharmaceutical and cosmetic industries worldwide (Zagoto et al., 2021; Zagoto et al., 2022; Shahrajabian et al., 2020). Because these varied possibilities of use, industries have been using basil as a raw material for valuable products development.

Reports recorded about 150 species of Ocimum in the world, among these 60 species of basil are in Brazil. However, the botanical identity of all these varieties of *Ocimum basilicum* is a difficult task due to cross-pollination, which favors hybridizations and results in a large number of varieties and subspecies (Gurav et al., 2020). As a consequence, the size of plant constituents, leaf colors, chemical composition and yield of essential oils present great diversity (Costa et al., 2014; Varga et al., 2017).

For seed quality control it is very important to check seed germination frequently. Germination tests aim to determine the maximum germination potential of a seed lot, which can be used to compare the quality of different lots and also to estimate the value for sowing in the field (Ranal & Santana, 2006). The germination test results are used to compare the physical quality of lots, determine a seeding rate and serve as a parameter of seed sale (Scott, Jones & Williams 1984).

In basil seeds, the dormancy mechanism is identified, which usually occurs after they reach physiological maturity, resulting from the adaptation of the species to the environmental conditions of reproduction (Kildisheva et al., 2020; Koornneef et al., 2002; Nonogaki, 2019). In this way, it is necessary understand how species overcome the dormant state, developing treatments for rapid and homogeneous germination for agronomic applications (Thongtip et al., 2022; Singh et al., 2019; Singh et al., 2022).

In this perspective, we intend to evaluate the germination of six varieties of commercial basil seeds common in Brazil. The germination was monitored by 14 days under controlled conditions and the results were statistically evaluated by the Weibull accumulative mathematical distribution model (Weibull, 1951; Carneiro & Guedes, 1992; Takahashi et al., 1996; Guedes et al., 2014). This three-parameter model has allowed a complete and concise description of the performance of seed lots (Carneiro & Guedes, 1992). Thus, the percentage of germinated seedling (%G), average germination time and its variability can provide more efficient seedling production on a commercial scale.

2. Methodology

The experiments were carried in the laboratory at temperatures between 20 and 25°C. For the development of the present work, seeds of six genotypes of *Ocimum spp* were selected (Table 1). The germination percentual (% G) and % purity were obtained from each seed producer.

Table 1 - Nomenclature and germination parameters of the six varieties of *O. basilicum.*

Variety	Common name	Scientific name	% G	% purity
V1	Basilicão Alfavaca	*O. basilicum var. basilicum*	98	99.9
V2	Red Basilicão Alfavaca	*O. basilicum var. thyrsiflora*	98	100.0
V3	Red Ruby Basilicão Alfavaca	*O. basilicum var. purpurascens*	98	100.0
V4	Fine Leaf	*O. basilicum var. anisatum*	85	99
V5	Genovese	*O. basilicum L. cv. genovese*	85	99
V6	Grecco Palla	*O. basilicum var. minimum*	94	100.0

Source: Authors (2022).

Germination percentage of seeds

To carry out the test, the germination method on paper in a plastic box (Gerbox) was adopted with eight repetitions with 50 seeds per box, with a depth of 3 cm, distributed seeds on a sheet of paper Germitest®, DLabor, moistened with 5 mL of a 0.2% solution of potassium nitrate (KNO_3), following the recommendations of the Rules for Seed Analysis (BRASIL, 2009), in an amount equivalent to 2.5 times the weight of the paper. Seeds were kept in a germination chamber previously set at a temperature of between 20 ºC and 25 ºC.

The evaluations were carried out from the second day after the test was set up and evaluated every 2 days resulting in 7 counts. The results were expressed in % G. The first count test computed the number of germinated seedlings, determined at the time of the first count of the germination test, on the second day after setting up the test (BRASIL, 2009), where they were considered germinated when the cotyledon leaves were exposed above the surface of the seed.

Statistical analysis of germination

In the evaluation of germination performance, the accumulated frequencies were combined with the distribution function model of Weibull used according to the following reparameterization:

$$Y = M\left(1 - e^{-\left(\frac{t}{b}\right)^{C}}\right) + \varepsilon \qquad \text{Eq. 1}$$

In which:

- Y the percentual accumulated germination observed over time t;
- M the maximum germination percentage for the variety (the asymptotic value of the germination percentage);
- b the estimated time to obtain approximately 63.21% of the germinated seeds expected by M, it means $0{,}6321M$;
- C the germination progression rate as a function of time b (percentage of germination in the day b);
- t the time (day) at which the count is performed (days after being placed to germinate);
- ε the random error assumed to be independent and identically distributed with a normal distribution of zero mean and constant variance, $\varepsilon \overset{iid}{\sim} N(0, \sigma^2)$.

The time required for the seeds to reach p% of total germination, considering the estimate obtained for M (Ávila et al., 2019) is given by Eq. 2:

$$Dia_p = b[-\log(1-\frac{p}{M})]^{1/c} \quad \text{Eq. 2}$$

with estimated variance given by (Ho & Silva, 2005):

$$\bar{var}(\bar{Dia_p}) \approx \bar{var}(\bar{b}) + [\log(-\log(1-p))]^2 + \bar{var}(\bar{c}) + 2[\log(-\log(1-p))]\bar{cov}(\bar{b},\bar{c}) \quad \text{Eq. 3}$$

Analyzes were performed in the static environment R (R Core Team, 2021). To adjust the model, the `nls` function from the `stats` package was used. The discrimination between the varieties was performed by contrasts the estimates of the model parameters using the Bonferroni test of the `pairComp` function of the `aomisc` package (Onofri, 2020) with 5% significance.

3. Results and Discussion

The accumulative germination percentage presents an exponential growth at the beginning of the observation period and it stabilizes at a maximum value. This behavior indicates the existence of an asymptote (maximum germination percentage), so that the model of Weibull assumed for the percentage of germinated seeds is plausible. From the mathematical modelling, the statistical parameters are presented in Table 2.

Table 2 - Germination performance of Basil seeds, evaluated by estimates of the parameters of the Weibull distribution function and Bonferroni test.

Variety	M (%)	b (days)	C
V1	97.9^{a}±1.4	1.93^{ac}±0.13	1.17^{a}±0.20
V2	83.3^{b}±1.3	2.50^{d}±0.12	1.77^{a} ±0.25
V3	89.8^{ab}±2.7	0.47^{a}±0.50	0.53^{a} ±0.40
V4	75.5^{c}±1.8	2.55^{cd}±0.20	1.10^{a} ±0.18
V5	45.7^{d}±1.2	2.48^{cd}±0.23	2.71^{a} ±0.98
V6	91.7^{ab}±4.7	5.26^{b}±0.52	1.22^{a} ±0.14

Lowercase letters differentiate significant differences ($p<0.05$) between varieties. Source: Authors (2022).

According to the parameter estimates, Table 2, variety V1 has the maximum germination power (M) of 97.9 %, followed by varieties V6 and V3, 91.7% and 89.8 % respectively. However, this numerical difference is not significant at 5 % by the Bonferroni test. The variety V5 has the lowest value of M, 45.7 %, about 2 times smaller than the values for V1, V3 and V6.

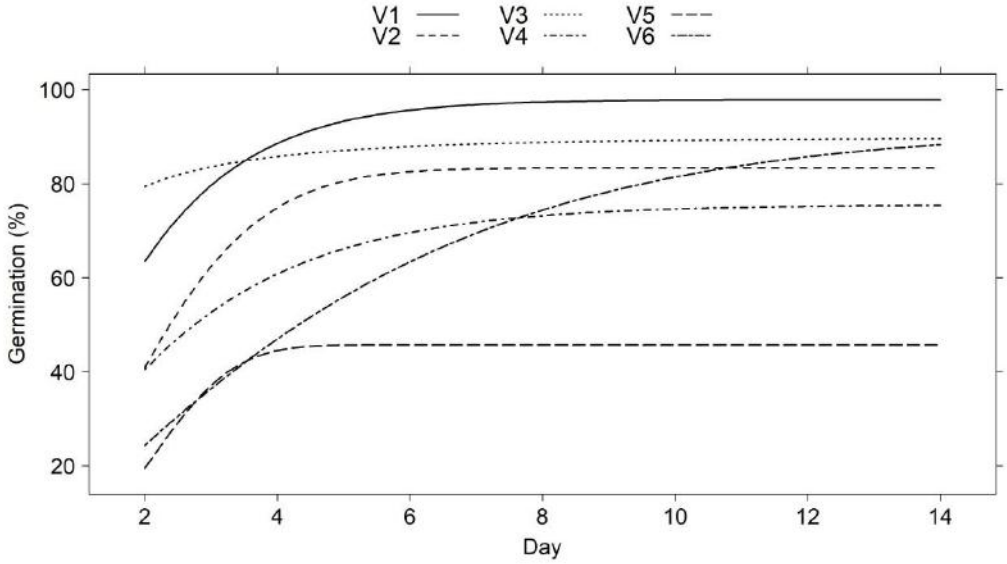
In addition to showing a higher percentage of germination, variety V1 has a lower standard error (SE) value of parameter M (1.4 %) compared to varieties V3 and V6. This result indicates that the eight samples analyzed for the V1 variety are more similar to each other than the others, it means less variability.

The maximum germination values predicted by the Weibull model were similar to the values provided by the manufacturer only for the V1 and V6 varieties. For varieties V2, V3, V4 and V5, the predicted values were much lower, which may indicate that germination did not occur correctly. This difference may be due, for example, to commercial seed storage conditions with consequent loss of viability. Thus, it appears that the proposed methodology works in the experimental quality control of seed germination. A previous studies presented that cultivar ‘Grecco a Palla’ (V6) presented superior seed germination percentage and seedling development when compared to ‘Thai Basil’ and ‘Alfavaca Basilicão Vermelho’ (V2)

(França et al., 2017), in agreement with our results. Low %G values are quite worrying in terms of costs. As an example, in the case of V5, there is a need to buy 2x more seeds to obtain the expected amount if the %G was close to the total.

The analysis of parameter b reveals that variety V3 was the faster to reach 63.21 % of germination of the seeds (0.47 days). however, the time to reach this germination percentage did not differ significantly from the V1 variety. The variety V6, despite present high values of M, was the slowest in the germination process (b = 5.26 days). this result is very important because it is an indicative that cultures of V3 and V1 tend to be the fastest crops to be harvested. As for the rate of germination progression as a function of time, parameter C, we highlight that despite the numerical differences, none of the varieties stood out significantly in relation to the others. From the parameters of Table 2, it was possible describe a single curve for the germination of each one of the varieties, which are represented in Figure 1.

Figure 1 - Variety-adjusted Weibull model of percentage of germination for the six species of basil.

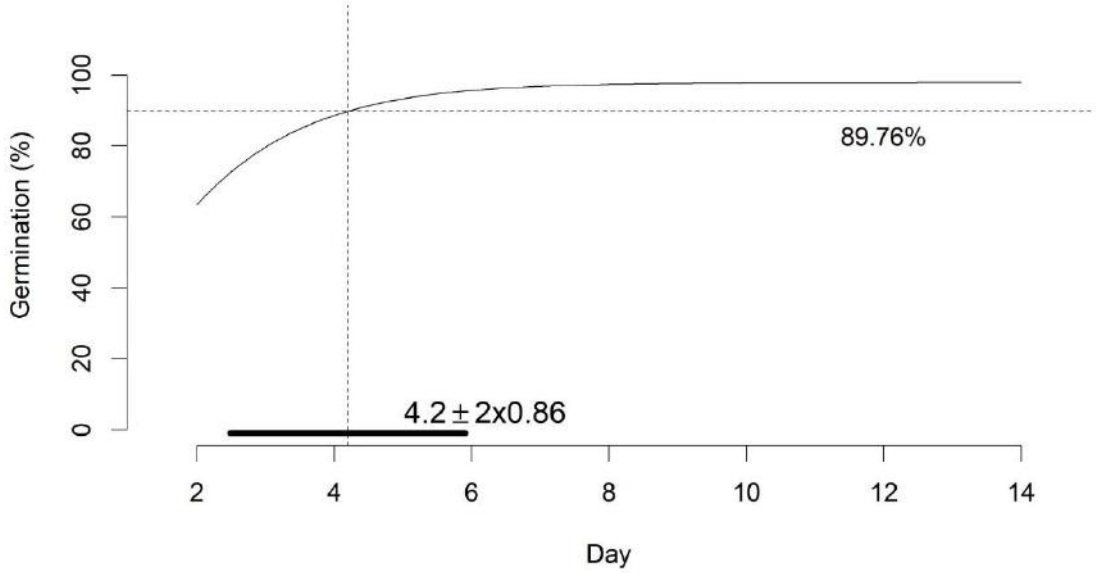


Source: Authors (2022).

It is observed from the curves of the adjusted model, Figure 1, that, except for variety V6, the other varieties reach their maximum germination around the eighth day. The curves also suggest (there is still an indication of) that the V1 and V3 varieties have the best germination.

In the Figure 2, the Weibull model was adjusted for the germination of seed of the V1 variety, which presented the best performance of germination (higher values of M).

Figure 2 - Weibull model adjusted for the germination of seeds of the V1 variety, and prediction of the time required for germination of 89.76% (dashed lines) with prediction interval.



Source: Authors (2022).

Figure 2 makes it possible to graphically visualize the time when the V1 variety reaches the maximum percentage of germination reached by the V3 variety, that is, the V1 variety reaches 89.78% between the fourth and fifth day after planting. This result permit infers that in 4.2 days the percentual germination of V1 is the same the maximum of the V3.

4. Conclusion

The quantitative control of germination is very important to provide seed quality. From this study, *O. basilicum var. basilicum* (V1), *O. Basilicum var. purpurascens* (V3) and *O. basilicum minimum* (V6) are those varieties that present the highest germination while *O. basilicum L. cv. Genovese* (V5) were the less germinative. The variety V1 presented the highest value of 97.9±1.4 % do germination and it was considered the standard for comparisons. The mathematical modeling of the germination allows the rational planning of the production of basil cultures aiming to optimize the agricultural productivity. For future studies, one can continue the studies with other basil varieties, compare the Weibull model with other mathematical models and also carry out large-scale studies.

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