GerminaQuant

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Introduction

GerminaQuant allows make the calculation of the germination variables incredibly easy in an interactive applications build in R (R Core Team, 2016), based in GerminaR and Shiny (Chang et al., 2016) package. GerminaQuant app is reactive!. Outputs change instantly as users modify inputs, without requiring a reload the app. The principal features of the application allow calculate the princiapal germination Variables, statistical analysis and easy way to plot the results.

Chapter 1

Evaluation of the seed germination process

The physiology and seed technology have provided valuable tools for the production of high quality seed and treatments and storage conditions (Marcos-Filho, 1998). In basic research, the seeds are studied exhaustively, and the approach of its biology is performed to fully exploit the dormancy and germination (Penfield and King, 2009). An important tool for indicate the performance of a seed lot is the precise quantification of germination through accurate analysis of the cumulative germination data (Joosen et al., 2010). Time, velocity, homogeneity, uncertainty and synchrony are measurements that inform the dynamics of the germination process. It is interesting not only for physiologists and seed technologists, but also for environmentalists, since it is possible to predict the degree of success of the species, based on the seed crop ability to redistribute germination over time, allowing the recruitment of part of the seedlings formed (Ranal and de Santana, 2006).

Chapter 2

Germination variables

2.1 Germinability (G)

According to Labouriau (1983a), germinability is the percentage of seeds in which the germination process comes to the end in the experimental conditions by the intraseminal growth, resulting in the protrusion (or emergence) of a living embryo.

$$g = \left(\frac{\sum_{i=1}^{k} n_1}{N} \right) 100 \quad (\textit{in percentage};\ \textit{expressed in the program as GRP})$$

Where: n_i: number of germinated seed in the i^{th} time; N: total number of seed in each experimental unit.

2.2 Mean Germination Time (t) and Standard Deviation (S_t)

The mean germination time was proposed by Haberlandt in 1875 (Labouriau, 1983a) and used by Czabator (1962) as mean length of incubation time (see Ranal and de Santana (2006) for other details). It is calculated as the weighted mean of the germination time (hour, day or other time unit). The number of germinated seeds at the intervals established for the data collection is used as weight (Ranal and de Santana, 2006).

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \ (\textit{in hour, day or other time unit};\ \textit{expressed in the program as MGT})$$

$$S_t = \sqrt{\left\{ \frac{\left\{ \sum_{i=1}^{k} n_i \left(t_i - \bar{t} \right)^2 \right\}}{\sum_{i=1}^{k} n_i - 1} \right\}} \ (\textit{in the same time unit of the mean germination time};\ \textit{expressed in the program as SDG})$$

Where $\bar{t}_i$: time from the start of the experiment to the i^{th} observation (hour, day or other time unit); n_i: number of germinated seeds in the i^{th} time (not the accumulated number) and k: the last time of observation.

2.3 Coefficient of Variation of the Germination Time

$CV_t = \frac{\{S_t\}}{\bar{t}} 100$ (*in the same tine unit of the mean ger* min *ation time*; exp *ressed in the program as SDG*)

where S_t: standard deviation of the germination time and $\bar{t}$: mean germination time.

2.4 Mean Germination Rate (v)

The mean germination rate is defined as the reciprocal of the mean germination time, since the mean germination rate increases and decreases with $\frac{1}{\bar{t}}$, not with $\bar{t}$ (Labouriau, 1983b).

$$\bar{v} = \frac{1}{\bar{t}} \ \left(in\ hours^{-1},\ day^{-1}\ or\ other\ reciprocal\ time\ unit,\ \exp ressed\ in\ the\ program\ as\ MGR\right)$$

2.5 Uncertainty Index (U)

This measurement is an adaptation of the Shannon index and measures the degree of uncertainty associated to the distribution of the relative frequency of germination (Labouriau and Valadares, 1976). Low values indicate more synchronized germination (Ranal and de Santana, 2006).

$$U \ = \ -\sum_{i=1}^{k} f_i \log_2 f_i \quad (in\ bit;\ \exp ressed\ in\ the\ program\ as\ UNC)\,,\ being\ f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

Where f_i: relative frequency of germination; n_i: number of seed germinated in the i^{th} time, and k: the last day of germination.

2.6 Synchronyzation Index (Z)

This index was proposed by Primack (1985) to assess the degree of overlapping of flowering among individuals in a population and Ranal and de Santana (2006) adopted it for seed germination. The synchrony of germination of one seed with another assumed $Z = 1$ when the germination of all seeds occur at the same time and $Z = 0$ when at least two seeds can germinate, one at each time.

$Z = \frac{\sum C_{n_1,2}}{N}$ (*a* dim *ensional measurement*; exp *ressed in the program as SYN*) ; *being* $C_{n_1,2} = \frac{n_i\,(n_i - 1)}{2}$; $N = \frac{\sum n_i\,($

Where $C_{n_i,2}$: combination of germinated seeds in i^{th} time, two together, and n_i: number of germinated seed in the i^{th}.

2.7 Limits and units of the germination variables in GerminaQuant

Table 2.1: Germination variables evaluated in GerminaQuant and limits according @Ranal2006 ; where: n_i, number of seed germinated in i^{nth} time ; K, the last day of the avaliation process for germination.

variables	abbreviation	limits	units
germinated seed number	GSN	\(0 \le n \le n_i\)	\(count\)
germinability	GNP	\(0 \le g \le 100\)	\(\%\)
germination asin	ASG	\(0 \le arsin \le 1\)	\(grade\)
mean germination time	MGT	\(0 \le t \le k\)	\(time\)
germination speed	SPG	\(0 < g_s \le 100\)	\(\%\)
mean germination rate	MGR	\(0 < v \le 1\)	\(time^{-1}\)
Synchronyzation Index	SYN	\(0 \le Z \le 1\)	\(-\)
Uncertainty Index	UNC	\(0 \le U \le log_2 n_i\)	\(bit\)
germination standard deviation	SDG	\(0 < s_t^2 \le \infty\)	\(time^2\)
germination variance	VGT	\(0 < s_t \le \infty\)	\(time\)
Coefficient of variation	CVG	\(0 < CV_t \le \infty\)	\(\%\)

Chapter 3

Germination field book

For correct analysis and fast data processing is important to take into account that the data organization and the correct data collection of the germination process is essential. In this section, we going to explain how can you collect and organize your data.

For data example and layout, you can access and download GerminaQuant spreadsheet.

3.1 Data Organization

The field book should have three essential parts. The treatment column (red), according to the experimental design (1); the seed number column (green) (2), and the observation moment column (blue) (3). In the green column you will indicate the number of seeds sown in each experimental unit and in the blue columns the germination values (Figure **??**). You can design your own field book with different names in the column.

3.2 Data Collection

The evaluation of the germination process is obtained of the count of the germination in each experimental unit and It can be evaluated in time lapse of hours, days or months in continuous interval of the same length always beginning with the time zero (ei. Ti00), until the end of the germination process or according to the researcher criteria

Chapter 4

Germination analysis

After the data collection, the information can be processed using GerminaQuant App. The web application can be used in any device, connected to the internet, in an interactive way. The application is compound in tabs (Table 4.1) that allow to make the analysis very easy.

Table 4.1: Name and description of each tab of GerminaQuant to evaluate and analyze the germination process

Tabs	Description
Introduction	Presentation of GerminaQuant and description of the germination variables
Data Import	Allow to upload the fieldbook, visualize all the data and chose the parameter for the analysis .
Germination Analysis	Calculate automatically the germination variables and export the data file.
Statisdtical Analysis	Allow to chose the variables according the experimental design for analysis of variance and summa
Multi Plot	Plot the mean comparison test for the variables selected in diffrente plots: bar, line and boxplot.
Germination in Time	Selecting the treatment, allows plotting the germination process.

Chapter 5

GerminaQuant data processing

5.1 Field Book

For using the GerminaQuant is necessary that you have a data with germination values. You can use a following data sample. Open the link and download the data in csv format.

Files >> Download >> Comma Separated Values (.csv, current sheet) >> "GerminaQuant - Sample.csv"

If you have a google account you can clone the document for you and edit it online and download for your own analysis.

5.2 Import Data

When you have your field book, you can go GerminaQuant and go "Data Import" tab. Figure **??**.

Choose the file in "csv" format it will be analysed. In "Column with seeds number" you have to write the name of the column containing the information of the number of seed sown in each experimental unit, "Prefix of evaluation days" you have to put the prefix of the name called for the day for evaluate the germination time lapse.

Below of the parameter for evaluation, you will find the option to select the parameter for the "csv" format file. In such way the file should have a table form. Figure **??**

5.3 Indices calculation

If the parameter in the "Import Data" tab are correct, in "Germination analysis" tab will be performed and the values of the germination variables for each experimental unit will be show. Table 2.1. GerminaQuant app allows downloading the file in "csv" format with the calculation of the germination variables. Figure **??**

5.4 Statistical analysis

In this tab, the app perform a factorial variance analysis, calculate the statistical description of the factor, the mean differences through Student Newman Keuls (de Mendiburu, 2016) and made the graphics (Wickham and Chang, 2016) for the chosen variable.

Remember, the independent variables will be the factor in your field book and the dependent variable will be any of the germination variables. Automatically the app will generate the graphs for the variable chosen and give the mean comparison test. The axis label can be edited manually filling the case in the “Graphics labels” section. The bar and line graphs are represented by the mean and central line is the standard error.

5.5 Germination in Time

This Tab allows to visualize the germination process included in the field book. Figure **??**

The app give two graphics, the first is the germination in percentage in time lapse and the second the relative germination that calculate the germination according the total number of seed germinated.

Bibliography

Chang, W., Cheng, J., Allaire, J., Xie, Y., and McPherson, J. (2016). *shiny: Web Application Framework for R*. R package version 0.14.1.

Czabator, F. J. (1962). Germination value: an index combining speed and completeness of pine seed germination. *Forest Science*, 8(4):386–396.

de Mendiburu, F. (2016). *agricolae: Statistical Procedures for Agricultural Research*. R package version 1.2-4.

Joosen, R. V. L., Kodde, J., Willems, L. A. J., Ligterink, W., van der Plas, L. H. W., and Hilhorst, H. W. (2010). germinator : a software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. *The Plant Journal*, 62(1):148–159.

Labouriau, L. and Valadares, M. (1976). On the physiology of seed of calotropis procera. anais da academia brasileira de ciência. *Rio de Janeiro*, 42(2):235–264.

Labouriau, L. G. (1983a). *A germinacâo das sementes*. Washington.

Labouriau, L. G. (1983b). Uma nova linha de pesquisa na fisiologia da germinacao das sementes. *In Anais do XXXIV Congresso Nacional de Botanica*.

Marcos-Filho, J. (1998). New approaches to seed vigor testing. *Scientia Agricola*, 55:27–33.

Penfield, S. and King, J. (2009). Towards a systems biology approach to understanding seed dormancy and germination. *Proceedings. Biological sciences / The Royal Society*, 276(1673).

Primack, R. B. (1985). Patterns of flowering phenology in communities, populations, individuals, and single flowers. In *The population structure of vegetation*, pages 571–593. Springer.

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ranal, M. A. and de Santana, D. G. (2006). How and why to measure the germination process? *Revista Brasileira de Botânica*, 29(1):1–11.

Wickham, H. and Chang, W. (2016). *ggplot2: An Implementation of the Grammar of Graphics*. R package version 2.1.0.