

GerminaQuant App

Flavio Lozano Isla

Omar Benites Alfaro

Marcelo Francisco Pompelli

17 Julio 2016

Contents

Introduction	5
1 Seed germination process	7
2 Germination variables	9
2.1 Germination (g)	9
2.2 Mean Germination Time (t)	9
2.3 Mean Germination Rate (v)	9
2.4 Uncertainty Index (U)	10
2.5 Synchrony Index (Z)	10
3 Germination Field book	11
3.1 Data Organization	11
3.2 Data Collection	11
4 Germination analysis	13
4.1 GerminaQuant Web Application	15
5 GerminaQuant data processing	17
5.1 Field Book	17
5.2 Import Data	17
5.3 Germination analysis	17
5.4 Statistical analysis	17
5.5 Germination in Time	18
5.6 Box Plot	18
6 Demonstrative Video	19

Introduction

GerminaQuant App allows make the calculation for the germination variables incredibly easy in a 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.

Features

- Allow calculate the principal Germination Variables.
- Statistical Analysis for Germination Variables.
- Easy way to plot the results.

Chapter 1

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 to 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, speed, homogeneity and synchrony are aspects that can be measured, and inform the dynamics of the germination process. These characteristics are interesting not only for physiologists and seed technologists, but also for ecologist, 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 the part of the environment formed seedlings (Ranal and de Santana, 2006).

Chapter 2

Germination variables

2.1 Germination (g)

According Gouvêa Labouriau (1983), the germinability of a sample of is the percentage of seeds in which the seed germination process comes to the end, in experimental conditions by the seminal intrauterine growth resulting protrusion (or emergence) of a living embryo. In general, it is presented as percentage, accompanied by some degree of dispersion, but it is possible to use proportions to one or more samples may be subjected to statistical tests (Carvalho and Santana, 2005).

$$g = \left(\frac{\sum_{i=1}^k n_i}{N} \right) 100$$

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)

It was proposed by Haberlandt in 1875. It is calculated as the weighted average germination time. The number of germinated seeds at the intervals established for the collection of data is used as weight. It is expressed in terms of the same units of time used in the germination count (Czabator, 1962).

$$t = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

Where t_i : Time of start of experiment tempo to observation (days or hours); n_i : number of seed germinated the i^{th} time (number corresponding to i^{th} observation); k : the last day of germination.

2.3 Mean Germination Rate (v)

The average speed of germination is defined as the reciprocal of the average time germination (Ranal and de Santana, 2006).

$$v = \frac{1}{\bar{t}}$$

Where t: mean germination time

2.4 Uncertainty Index (U)

The uncertainty index (U) is an adaptation of Shannon index measures the degree of uncertainty in predicting the informational entropy or uncertainty associated with the distribution of the relative frequency of germination (Gouvêa Labouriau, 1983; Labouriau and Valadares, 1983). Low values of U indicate frequencies with short peaks, i.e. the more concentrated the germination in time. Just a germinated seed changes the value of U. This means that u measures the degree of germination scattering.

$$-\sum_{i=1}^k f_i \log_2 f_i \Leftrightarrow f_i = \frac{n_i}{\sum_{i=1}^k n_i}$$

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

2.5 Synchrony Index (Z)

The Synchrony Index (Z) has been proposed to assess the degree of overlap between flowering individuals in a population. Adopting the idea expressed by Primack (1985), Synchrony index is the synchrony of germination of one seed with other seeds included in the same replication. When synchrony index = 1, germination of all the seeds occurs at the same time and when synchrony index = 0, at least two seeds can germinate one each time. Synchrony index produces a number if and only if there are two seeds finishing the seed germination process at the same time. Thus, the value of Z assessments is the grade of overlap between seed germination.

$$Z = \frac{\sum C_{n_i,2}}{N} \Leftrightarrow C_{n_i,2} = \frac{n_i(n_i - 1)}{2} \Leftrightarrow N = \frac{\sum n_i(\sum n_i - 1)}{2}$$

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

Chapter 3

Germination Field book

For correct analysis and fast data processing is important take account the data organization and the correct data collection of the germination process. This section we going to explain how you have to 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 3 essential parts; the factor columns (red). It will be use for the experiment treatment and statistical analysis and It will be according your experimental design; the seed number column (green) It will indicate the number of seed sown in each experimental units and the evaluation days column (blue), It will be fill with the germination values and It will be last according the experiment programmation and requirements. Figure ??

You can design your own field book with different names in the column, remember you need at least one column with factors or treatments, the column with the number of seed for each experimental unit and the evaluation days (with prefix) according to the time lapse of your experiment.

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 germination is complete and It can be when for 5 evaluation collection the values of germination are constant or according the experimental design.

Chapter 4

Germination analysis

After finish the data collection of the germination process these 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 ??) that allow to made the analysis very easy.

Chapter 5

GerminaQuant data processing

5.1 Field Book

For using the GerminaQuant app is necessary that you have a data with germination values. You can use a data sample for use GerminaQuant App. Open the link and download the data in csv format. The document should be 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 will be analysed. There are two case that will have default values if you use the . “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 Germination analysis

If the parameter in the “Import Data” tab are correct, in “Germination analysis” tab will be appear the values of the eleven germination variables for each experimental unit. Figure ??

GerminaQuant app allow to download the file in “csv” format with the calculation of the germination variables. Figure ??

5.4 Statistical analysis

In this tab, the app perform a unifactor variance analysis, calculate the statistical description of the factor, the mean differences through three mean test: Tukey, Student Newman Keuls and Duncan and made the

graphic 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 eleven of germination variables. Automatically the app will generate the graph 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 graphs represent the mean and central line the standard error.

5.5 Germination in Time

This Tab allows to visualise the germination process for each factor 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.

5.6 Box Plot

This section allows to plot the distribution of the germination observation through the box plot (outliers values and overlapping values) and allows to put label in the graph (Figure ??). In the section “Graphics variables”, “Axis X” and “Grouped” you should choose between the factor in the the field book, are recommended in the “Axis X” you should choose the factor with more level and “Grouped” the factor with less levels, and the “Axis Y” any of the eleven germination variables, in the case you have only one factor “Axis X” and “Grouped” should have the same factor. The axis label can be edited manually filling the case in the “Graphics labels” section.

Chapter 6

Demonstrative Video

- GerminaQuant with R console
- GerminaQuant web Application

Bibliography

- Carvalho, M. and Santana, D. (2005). Emergência de plântulas de *Anacardium humile* A. St.-Hil.(Anacardiaceae) avaliada por meio de amostras pequenas. *Revista Brasileira de*.
- Chang, W., Cheng, J., Allaire, J., Xie, Y., and McPherson, J. (2016). *shiny: Web Application Framework for R*. R package version 0.13.2.
- Czabator, F. J. (1962). Germination value: an index combining speed and completeness of pine seed germination. *Forest Science*, 8(4):386–396.
- Gouvêa Labouriau, L. L. G. L. (1983). *A germinação das sementes*. Washington.
- 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. G. and Valadares, M. E. B. (1983). The germination of seeds. *OEA, Washington, DC*.
- 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):3561–9.
- 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.