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RESEARCH NOTE

SeedCalc, a new automated R software tool for germination and seedling length data processing[1]

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ABSTRACT – The need to optimize seed quality assessment using new, more accessible, and modern computational resources has led to the emergence of new tools. In this paper, we introduce SeedCalc, a new R software package developed to process germination and seedling length data. The functions included in SeedCalc allow fast and efficient data processing, offering greater reliability to the variables generated and facilitating statistical analysis itself since the data are already processed with the appropriate structure to be statistically analyzed in the R software. SeedCalc is available free of charge at https://CRAN.R-project.org/package=SeedCalc.

Index terms: R package, germination indexes, seedling uniformity, seed vigor, data analysis.

SeedCalc, uma nova ferramenta automatizada do software R para processamento de dados de germinação e comprimento de plântula

RESUMO – A necessidade de otimizar a avaliação da qualidade de sementes utilizando novos recursos computacionais mais acessíveis e modernos levou ao surgimento de novas ferramentas. Neste artigo, introduzimos o SeedCalc, um novo pacote do software R desenvolvido para processar dados de germinação e comprimento de plântulas. As funções incluídas no SeedCalc permitem um processamento de dados rápido e eficiente, oferecendo maior confiabilidade às variáveis geradas e facilitando a própria análise estatística, uma vez que os dados já são processados com a estrutura apropriada para serem analisados estatisticamente no software R. O SeedCalc está disponível gratuitamente em https://CRAN.R-project.org/package=SeedCalc.

Termos para indexação: Pacote do R, índices de germinação, uniformidade de plântulas, vigor de sementes, análise de dados.

Introduction

Electronic spreadsheets are used in a generalized manner to tabulate data, perform the most diverse experimental calculations, and process information. Nevertheless, errors in formulas or in handling of spreadsheets can significantly compromise the information generated (Powell et al., 2009). Thus, development of tools that minimize these risks and can increase the reliability of results is important.

Tests for evaluation of seed quality are routinely used in experimentation. One of the most prominent of these routine tests is the germination test, nearly always associated with some vigor test, such as a seedling growth test (Nakagawa et al., 1999). In the germination test, daily counting of the number of seeds germinated is common, and these data are frequently used for calculation of the Germination Speed Index (Maguire, 1962) and Germination Speed (Edmond and Drapala, 1958). This same procedure is applied to the seedling emergence data. Nevertheless, various other indexes can be calculated based on these data, which may be useful for making inferences regarding different aspects of seed germination response, such as uniformity, coefficient of

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variation, synchrony, among others.

Regarding seedling growth data, variables such as shoot, root, and total length are those most used, when image analysis software that contains other indexes that are automatically calculated based on these parameters is not used. Thus, several indexes may be calculated from these data, which could be a complicated task in dealing with a large number of lots/treatments, as occurs, for example, in phenotyping studies (Joosen et al., 2010), when a large number of seed lots or seeds from different cultivars are analyzed.

Among the tools that are able to serve the above purposes is SeedCalc, a new R package. R (R Core Team, 2019) is a powerful software used for statistical data analysis that has grown in importance in seed research. In this respect, several packages are available for this platform, which increases its applicability for analysis of the most varied types of data. SeedCalc was developed from the need to optimize acquisition of data from seed quality indicators. The package is available free of charge through CRAN (https://CRAN.R-project.org/package=SeedCalc), and it can be used on various platforms, such as Windows, MacOS, and Linux.

For calculations, SeedCalc uses data from daily counting of germination/emergence and seedling length measurements to automatically generate a series of variables related to seed physiological quality. It includes calculations of germination/emergence percentage, time required for germination or emergence (T10, T50, T90, and mean germination time), speed (germination speed index, mean germination rate), variability or heterogeneity (coefficient of variation of germination time, variance of germination), uncertainty, and synchrony. In addition, through seedling measurements, some indexes can be generated, such as growth, uniformity, and vigor indexes.

Thus, considering the importance of developing systems that allow calculation of seed quality indicators in a simple and automatic way, the aim of this study was to present the SeedCalc package and its applicability to analysis of data obtained from germination and seedling growth tests.

Materials and Methods

All the functions developed were written in R programming language and, therefore, can be carried out in the R environment. R can be installed in the Windows, Linux, or Mac systems. Thus, this package can be freely used by the scientific community, regardless of the operating platform used.

The SeedCalc package can be installed simply by inserting the following command in the R software:

```
> install.packages("SeedCalc")
```

The following command must be typed in to load the package:

```
> library(SeedCalc)
```

The functions inserted in the package and the respective equations used, which were obtained from the scientific literature in the seed area, are described as follows.

`GermCalc`: applies all the functions related to data of seed germination and seedling emergence (Table 1).

`PlantCalc`: applies all the functions related to seedling analysis (Table 2).

To use these functions, the original data files can be saved in text files or electronic spreadsheets. However, they must meet some requirements. For the `GermCalc` function, the file must contain the first column with the time (any unit of time - days, hours, etc.), and the rest of the columns, identified with the treatment/lot, the number of seeds germinated in a cumulative manner (see Figure 1). For the `PlantCalc` function, the file must contain four columns in the following order: identification of the lot/treatment, seedling (identified from 1 to *n*, with *n* being the total number of seedlings), shoot length, and root length. The title of the columns, contained in the first line of the file, can receive any name, as long as the order of the columns respects the sequence of reference (see Figure 2A).

For generation of the corrected vigor index (Medeiros and Pereira, 2018), an additional file is necessary, composed of two columns: the first identifies the treatments/lots and the second, the germination percentages (see Figure 2B). The identifications of the treatments/lots must be identical in both files.

As an example of practical application of the SeedCalc package, real data were used from an experiment conducted with five commercial lots of soybean seeds. The seeds were tested for germination in accordance with the Rules for Seed Testing (Brasil, 2009). Four replications of 50 seeds each were placed on paper toweling (Germitest®) moistened with distilled water at the rate of 2.5 times the weight of the paper. Then rolls were made, and they were kept in BOD at 25 ºC in a program of 8 hours of light and 16 hours in the dark. Daily counts of the number of normal seedlings were made. For the seedling growth test, the seeds were placed in a linear arrangement on the upper third of the paper toweling (Germitest®) and were maintained under the same conditions described for the germination test. At three days after the beginning of the test, the shoot and root length were measured using a millimeter ruler. The data obtained in the germination and seedling growth tests were processed through the SeedCalc package.

Table 1. Functions contained in the SeedCalc package for calculation of indexes, using data from daily counting of seedling on germination/emergence test.

Function	Measure	Formula	Reference
FGP	Final germination percentage	FGP=(n/N)×100 *n* is the number of seeds germinated, and *N* is the total number of seeds.	ISTA (2015)
GI	Germination speed index	$GI=\sum_{i=1}^{k}(n_i/t_i)$ *n* is the number of seeds germinated on each day of daily counting up to the last count, and *t* is the number of days after the beginning of the test in each count.	Maguire (1962)
T10	Time required for germination of 10% of the seeds	$T_{10}=\frac{ti+\left\{\left[\frac{N}{\left(\frac{100}{10}\right)}\right]-ni\right\}(tj-ti)}{(nj-ni)}$ N is the final number of seeds germinated, and n_i and n_j are the total number of seeds germinated in adjacent counts in time t_i and t_j, respectively, when $ni<\frac{N+1}{2}<nj$.	Adapted from Farooq et al. (2005)
T50	Time required for germination of 50% of the seeds	$T_{50}=\frac{ti+\left\{\left[\frac{N}{\left(\frac{100}{50}\right)}\right]-ni\right\}(tj-ti)}{(nj-ni)}$ Same codification as for T10.	Farooq et al. (2005)
T90	Time required for germination of 90% of the seeds	$T_{90}=\frac{ti+\left\{\left[\frac{N}{\left(\frac{100}{90}\right)}\right]-ni\right\}(tj-ti)}{(nj-ni)}$ Same codification as for T10.	Adapted from Farooq et al. (2005)
MGT	Mean germination time	$MGT=\sum_{i=1}^{k}n_it_i/\sum_{i=1}^{k}n_i$ n_i is the number of seeds germinated per day (not the accumulated number, but the number corresponding to the *i-th* observation), and t_i is the time since the beginning of the germination test up to the *i-th* observation.	Labouriau (1983)
MGR	Mean germination rate	$\bar{v}=CoVg/100=1/\bar{t}$ $\bar{t}$ is the mean germination time, and *CoVg* is the germination speed coefficient.	Labouriau (1983)
VarGer	Variance of germination time	$s_t^2=\sum_{i=1}^{k}n_i\,(t_i-\bar{t})^2/(\sum_{i=1}^{k}n_i-1)$ *t* is the mean germination time, t_i is the time between the beginning of the experiment and the *i-th* observation (day or hour), n_i is the number of seeds germinated in time *i*, and *k* is the last count of the germination test.	Labouriau (1983)
CVt	Coefficient of variation of germination time	$CV_t=(S_t/\bar{t})100$ *St* is standard deviation of the germination time, and $\bar{t}$ is mean germination time.	Carvalho et al. (2005)
Sinc	Germination synchrony	$Z=\sum Cni,2\,/\,N$ Cni, 2 = ni (ni-1)/2 and $N=\sum ni(\sum ni-1)/2$ *Cni* is the combination of the seeds germinated in time *i*, two by two, and *ni* is the number of seeds germinated in time *i*.	Primack (1980)

Table 1. Continuation.

Function	Measure	Formula	Reference
`Unc`	Uncertainty	$\bar{E}=-\sum_{i=1}^{k} f_i \log 2\, f_i$ with f_i given by, $f_i = n_i / \sum_{i=1}^{k} n_i$ f_i is the relative frequency of germination, and n_i is the number of seeds germinated on day i.	Labouriau and Valadares (1976)
`CVG`	Germination speed coefficient	$CVG=(\sum_{i=1}^{k} f_i / \sum_{i=1}^{k} f_i x_i)\, 100$ f_i is the number of newly germinated seeds on day i, and x_i is the number of days from sowing.	Nichols and Heydecker (1968)
`UnifG`	Uniformity of germination	UnifG=(T90-T10) T90 is the time required for germination of 90% of the seeds, and T10 is the time required for germination of 10% of the seeds.	Demilly et al. (2014)

Table 2. Functions contained in the SeedCalc package for obtaining indexes using seedling length measurement data.

Function	Measurement	Formula	Reference
`mean_pa`	Mean shoot length	$Mean_{pa}=\frac{\sum_{i=1}^{k} HL}{n}$ HL is the length of the shoot of each seedling, and n is the total number of seedlings evaluated.	Nakagawa et al. (1999)
`mean_raiz`	Mean root length	$Mean_{raiz}=\frac{\sum_{i=1}^{k} RL}{n}$ RL is the length of the root of each seedling, and n is the total number of seedlings evaluated.	Nakagawa et al. (1999)
`mean_total`	Mean total length	$Mean_{total}=\frac{\sum_{i=1}^{k} SL}{n}$ SL is the total length of each seedling, and n is the total number of seedlings evaluated.	Nakagawa et al. (1999)
`mean_razao`	Mean of the root/shoot ratio	$Mean_{razao}=\frac{\sum_{i=1}^{k} RRA}{n}$ RRA is the ratio between the root and the shoot of each seedling, and n is the total number of seedlings evaluated.	Benincasa (2003)
`Unif_1`	Uniformity index	$Uniformity\_1=\left[1-\frac{\sum_{i=1}^{n}\lvert Xi-\bar{X}\rvert}{n \times X}\right]\times 1000-\left[n_{dead}\times\left(\frac{50}{n_{total}}\right)\right]$ Xi is the length of the seedling analyzed, X is the mean length of seedlings of the seed lot analyzed, n is the variable of total number of seedlings evaluated, n_{dead} is the number of ungerminated or dead seeds present, and n_{total} is the total number of seedlings.	Christiansen (1942), adapted by Castan et al. (2018)
`Unif_2`	Uniformity index	$Uniformity\_2=\max[1000-(0.75\times sh+0.5\times sr+2.5\times s_{total}+50\times srh)-0\times ndead]$ sh, sr, and srh are the standard deviations of the length of the shoot, primary root, and root/shoot ratio, respectively.	Sako et al. (2001)
`Growth`	Growth index	$Growth=[(mean(h)\times wh)+(mean(r)\times wr)]$ mean(h) and mean(r) are the arithmetic means of shoot length and root length, respectively. wh and wr are adjustable weights in the formula for shoot and root, however, with reference values of 10 and 90, respectively.	Sako et al. (2001)

Table 2. Continuation.

Function	Measurement	Formula	Reference
`Vigor`	Vigor index	Vigor=(Growth×wg)+(Uniformity×wu) Growth is the growth index, and Uniformity is the uniformity index chosen by the user. wg and wu are adjustable weights in the formula for growth and uniformity, however, with standard values of 70 and 30, respectively.	Sako et al. (2001)
`Vigor_corr`	Corrected vigor index	$\text{Vigor\_Corr}=[(\text{Growth}\times\text{wg})+(\text{Uniformity}\times\text{wu})]\times\left(\frac{G}{100}\right)$ G is the percentage of germination of the seed lot.	Medeiros and Pereira (2018)

dados_ger.txt

Arquivo Editar Formatar Exibir Ajuda

Days	L1R1	L1R2	L1R3	L1R4	L2R1	L2R2	L2R3	L2R4	L3R1	L3R2
1	37	35	40	42	25	18	34	23	41	41
2	49	50	50	50	50	49	50	50	50	50
3	50	50	50	50	50	50	50	50	50	50
4	50	50	50	50	50	50	50	50	50	50
5	50	50	50	50	50	50	50	50	50	50
6	50	50	50	50	50	50	50	50	50	50
7	50	50	50	50	50	50	50	50	50	50
8	50	50	50	50	50	50	50	50	50	50

Figure 1. Presentation of the germination count data organized for processing by SeedCalc.

A

dados_plant.txt

Arquivo Editar Formatar Exibir Ajuda

Lot	Rep	hypoc	Root
1	1	2.5	8.2
1	1	1.5	5.5
1	2	1.3	8.1
1	2	1.7	7.3
1	3	1.8	7.4
1	3	1.2	6.3
1	4	2.3	7.6
1	4	0.0	0.0
2	1	0.6	1.4
2	1	1.4	4.4
2	2	1.0	2.7
2	2	1.5	5.0
2	3	3.4	6.8
2	3	3.4	6.2
2	4	1.5	0.9
2	4	2.0	2.7

continue...

B

germ.txt

Arquivo Editar Formatar Exibir Ajuda

Lot	Germination
1	100
2	88

continue ...

Figure 2. Presentation of the seedling data organized for processing by SeedCalc. In (A) are the seedling length data and in (B) are the germination values of the treatments/lots, corresponding to the ones in the first column of the data file of seedling length (A). The germination data (B) are optional and necessary for calculation of the corrected vigor index.

Results and Discussion

The collection of functions available in the SeedCalc package implements various methods to describe the duration of the germination process in terms of germination indexes, as well as indexes related to seedling development. Thus, the data obtained in the germination and seedling growth tests were used to exemplify the application of the SeedCalc package. The use of the functions contained in the package and the interpretation of their outputs are better presented in the form of an example of application with real data.

As described, SeedCalc has two main functions: `GermCalc` and `PlantCalc`. The `GermCalc` function allows analysis of the daily counting data and receives the "`Nseeds`" argument, which refers to the number of seeds used in the germination test for each replication. The expression used for calculation of the germination indexes, with the use of the data presented in Figure 1, follows:

```
> dados_ger <- read.table('dados_ger.txt', h=T)

> GermCalc(dados_ger, NSeeds = 50)

> GermCalc
```

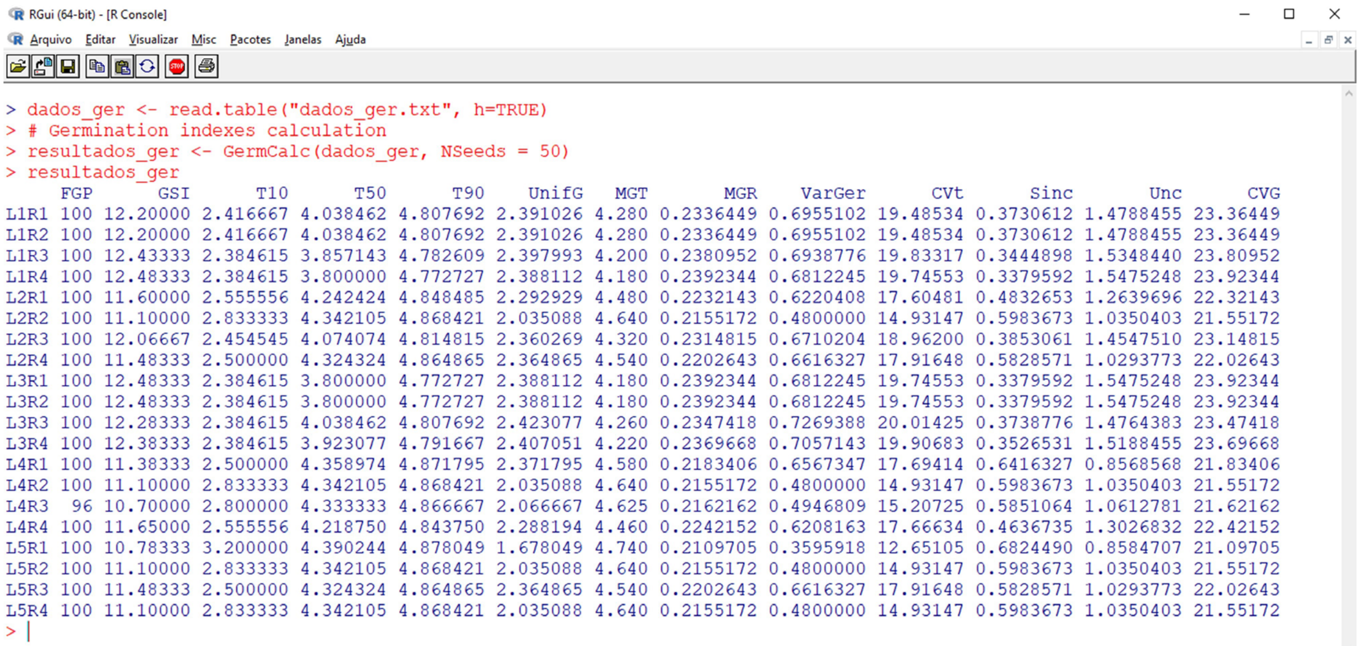
Through this function, the variables presented in Figure 3 were generated.

The `PlantCalc` function allows analysis of the data of seedling length and, optionally, can receive the "`Ger`" argument, which refers to the mean of germination registered for the lots/treatments that is necessary to generate the Corrected vigor index. The expression used to carry out the analysis, using the data presented in Figure 2, follows:

```
> dados_plant <- read.table('dados_plant.txt', h=T)

> germ <- read.table('per_ger.txt', h=T)

> PlantCalc(dados_plant, Ger = germ)

> PlantCalc
```

The variables presented in Figure 4 were generated through these commands. These variables (Figure 4) are normally generated automatically on specific software for image seedling analysis, such as Vigor-S® (Castan et al., 2018), SVIS® (Sako et al., 2001), Groundeye® (previously referred to as SAS) (Pinto et al., 2015) and SAPL® (Medeiros and Pereira, 2018). However, the few systems developed have the disadvantage to be limited to analysis of restricted number of species. Thus, making these quality indexes available would facilitate their use for these and other species.

With SeedCalc, the indexes are implemented automatically, and can be used to generate more detailed information regarding the vigor of seed lots of any species, regardless of the way the data were acquired and without



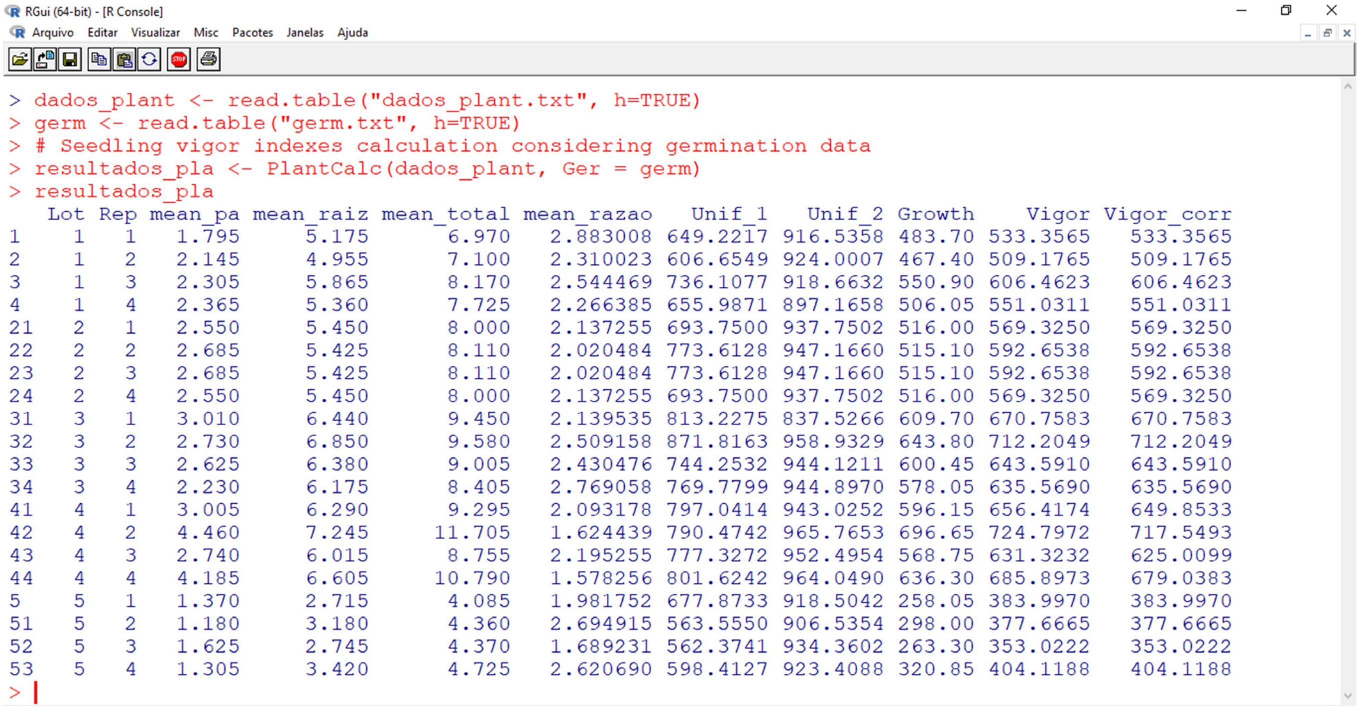

```
> dados_ger <- read.table("dados_ger.txt", h=TRUE)
> # Germination indexes calculation
> resultados_ger <- GermCalc(dados_ger, NSeeds = 50)
> resultados_ger
     FGP      GSI      T10      T50      T90    UnifG   MGT       MGR    VarGer      CVt      Sinc       Unc      CVG
L1R1 100 12.20000 2.416667 4.038462 4.807692 2.391026 4.280 0.2336449 0.6955102 19.48534 0.3730612 1.4788455 23.36449
L1R2 100 12.20000 2.416667 4.038462 4.807692 2.391026 4.280 0.2336449 0.6955102 19.48534 0.3730612 1.4788455 23.36449
L1R3 100 12.43333 2.384615 3.857143 4.782609 2.397993 4.200 0.2380952 0.6938776 19.83317 0.3444898 1.5348440 23.80952
L1R4 100 12.48333 2.384615 3.800000 4.772727 2.388112 4.180 0.2392344 0.6812245 19.74553 0.3379592 1.5475248 23.92344
L2R1 100 11.60000 2.555556 4.242424 4.848485 2.292929 4.480 0.2232143 0.6220408 17.60481 0.4832653 1.2639696 22.32143
L2R2 100 11.10000 2.833333 4.342105 4.868421 2.035088 4.640 0.2155172 0.4800000 14.93147 0.5983673 1.0350403 21.55172
L2R3 100 12.06667 2.454545 4.074074 4.814815 2.360269 4.320 0.2314815 0.6710204 18.96200 0.3853061 1.4547510 23.14815
L2R4 100 11.48333 2.500000 4.324324 4.864865 2.364865 4.540 0.2202643 0.6616327 17.91648 0.5828571 1.0293773 22.02643
L3R1 100 12.48333 2.384615 3.800000 4.772727 2.388112 4.180 0.2392344 0.6812245 19.74553 0.3379592 1.5475248 23.92344
L3R2 100 12.48333 2.384615 3.800000 4.772727 2.388112 4.180 0.2392344 0.6812245 19.74553 0.3379592 1.5475248 23.92344
L3R3 100 12.28333 2.384615 4.038462 4.807692 2.423077 4.260 0.2347418 0.7269388 20.01425 0.3738776 1.4764383 23.47418
L3R4 100 12.38333 2.384615 3.923077 4.791667 2.407051 4.220 0.2369668 0.7057143 19.90683 0.3526531 1.5188455 23.69668
L4R1 100 11.38333 2.500000 4.358974 4.871795 2.371795 4.580 0.2183406 0.6567347 17.69414 0.6416327 0.8568568 21.83406
L4R2 100 11.10000 2.833333 4.342105 4.868421 2.035088 4.640 0.2155172 0.4800000 14.93147 0.5983673 1.0350403 21.55172
L4R3  96 10.70000 2.800000 4.333333 4.866667 2.066667 4.625 0.2162162 0.4946809 15.20725 0.5851064 1.0612781 21.62162
L4R4 100 11.65000 2.555556 4.218750 4.843750 2.288194 4.460 0.2242152 0.6208163 17.66634 0.4636735 1.3026832 22.42152
L5R1 100 10.78333 3.200000 4.390244 4.878049 1.678049 4.740 0.2109705 0.3595918 12.65105 0.6824490 0.8584707 21.09705
L5R2 100 11.10000 2.833333 4.342105 4.868421 2.035088 4.640 0.2155172 0.4800000 14.93147 0.5983673 1.0350403 21.55172
L5R3 100 11.48333 2.500000 4.324324 4.864865 2.364865 4.540 0.2202643 0.6616327 17.91648 0.5828571 1.0293773 22.02643
L5R4 100 11.10000 2.833333 4.342105 4.868421 2.035088 4.640 0.2155172 0.4800000 14.93147 0.5983673 1.0350403 21.55172
> |
```

Figure 3. Example of results output of the functions applied to the daily counting data of the germination test of soybean seeds by SeedCalc.

the need to use specific seedling analysis systems. That way, this information becomes more accessible to the scientific community, and has a direct impact on the seed sector.

To illustrate, in Figure 5, soybean seedlings at three days after the beginning of the germination test can be seen from two samples, with their respective quality indexes. Lot B exhibits seedlings of shorter length and an irregular pattern, which is reflected in lower growth, uniformity, and vigor indexes compared to Lot A.

SeedCalc constitutes an innovative and efficient analysis tool to calculate indexes of seed germination and of seedling performance. The functions developed allow fast and efficient data processing, with a view to offering greater reliability to the variables generated and to facilitating statistical analysis itself, since the processed data have a suitable structure for analysis in R software (R Core Team, 2019). The use of these functions on the R software ensures they can be used freely by the scientific community.




```
> dados_plant <- read.table("dados_plant.txt", h=TRUE)
> germ <- read.table("germ.txt", h=TRUE)
> # Seedling vigor indexes calculation considering germination data
> resultados_pla <- PlantCalc(dados_plant, Ger = germ)
> resultados_pla
   Lot Rep mean_pa mean_raiz mean_total mean_razao   Unif_1   Unif_2 Growth    Vigor Vigor_corr
1    1   1   1.795     5.175      6.970   2.883008 649.2217 916.5358 483.70 533.3565   533.3565
2    1   2   2.145     4.955      7.100   2.310023 606.6549 924.0007 467.40 509.1765   509.1765
3    1   3   2.305     5.865      8.170   2.544469 736.1077 918.6632 550.90 606.4623   606.4623
4    1   4   2.365     5.360      7.725   2.266385 655.9871 897.1658 506.05 551.0311   551.0311
21   2   1   2.550     5.450      8.000   2.137255 693.7500 937.7502 516.00 569.3250   569.3250
22   2   2   2.685     5.425      8.110   2.020484 773.6128 947.1660 515.10 592.6538   592.6538
23   2   3   2.685     5.425      8.110   2.020484 773.6128 947.1660 515.10 592.6538   592.6538
24   2   4   2.550     5.450      8.000   2.137255 693.7500 937.7502 516.00 569.3250   569.3250
31   3   1   3.010     6.440      9.450   2.139535 813.2275 837.5266 609.70 670.7583   670.7583
32   3   2   2.730     6.850      9.580   2.509158 871.8163 958.9329 643.80 712.2049   712.2049
33   3   3   2.625     6.380      9.005   2.430476 744.2532 944.1211 600.45 643.5910   643.5910
34   3   4   2.230     6.175      8.405   2.769058 769.7799 944.8970 578.05 635.5690   635.5690
41   4   1   3.005     6.290      9.295   2.093178 797.0414 943.0252 596.15 656.4174   649.8533
42   4   2   4.460     7.245     11.705   1.624439 790.4742 965.7653 696.65 724.7972   717.5493
43   4   3   2.740     6.015      8.755   2.195255 777.3272 952.4954 568.75 631.3232   625.0099
44   4   4   4.185     6.605     10.790   1.578256 801.6242 964.0490 636.30 685.8973   679.0383
5    5   1   1.370     2.715      4.085   1.981752 677.8733 918.5042 258.05 383.9970   383.9970
51   5   2   1.180     3.180      4.360   2.694915 563.5550 906.5354 298.00 377.6665   377.6665
52   5   3   1.625     2.745      4.370   1.689231 562.3741 934.3602 263.30 353.0222   353.0222
53   5   4   1.305     3.420      4.725   2.620690 598.4127 923.4088 320.85 404.1188   404.1188
>
```

Figure 4. Example of results output of the functions applied to seedling lengths of soybean seeds by SeedCalc.

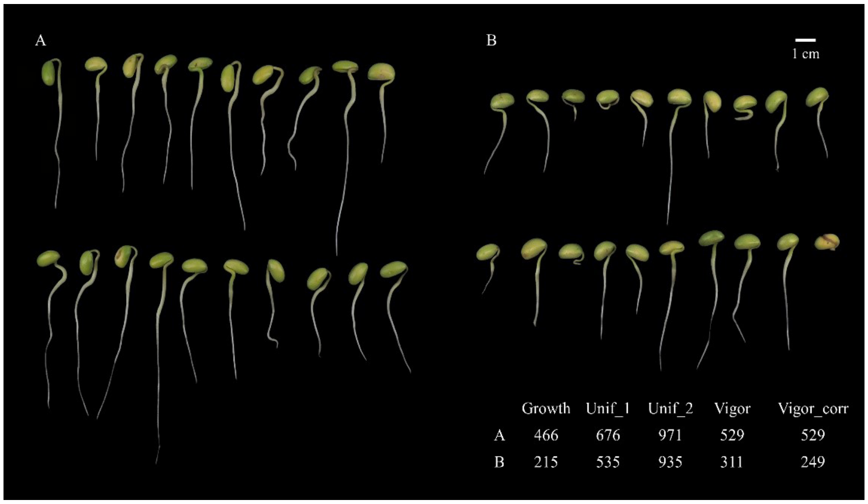


Figure 5. Soybean seedlings originated from seeds with different levels of vigor and their respective quality indexes. (A) higher vigor and (B) lower vigor.

Conclusions

The SeedCalc package is a free access tool and generates indexes based on daily counting data from germination/ emergence and seedling growth tests. It represents a powerful tool for research and will establish new computational approaches within the seed technology sector.

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