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Sample size for determination of the physiological potential of coriander (*Coriandrum sativum* L.) seeds

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Abstract

The objective of this work was to determine the minimum number of replications and seeds per replication to perform the germination and seed vigor tests with coriander seeds. Two seed lots were compared in terms of water content, vigor and viability. Eight hundred seeds per lot were used. Values of germination first count, germination, germination speed index and mean germination time were analyzed. Sample size scenarios were developed using different combinations of number of replications (from 2 to 10) and the number of seeds per replication (from 20 to 80) by means of a resampling with replacement technique. The reference sample consisted of four replications of 50 seeds, as it is commonly used in researches with this species. Determination of the minimum number of replications and seeds was based on comparing the 95% bootstrapped confidence intervals for the index of variation ($CV/n^{0.5}$) of each scenario with the confidence interval of the reference sample. It is reasonable to reduce the number of seeds per replication from 50 to 30 in order determine germination first count, germination and germination speed index. Forty seeds per replication are recommended to quantify the mean germination time. However, reductions in the number of replications can affect negatively the accuracy of germination and vigor tests.

Keywords: resampling; seed viability; seed vigor.
Abbreviations: GSI_germination speed index; IV_index of variation; MGT_mean germination time.

Introduction

The Brazilian Manual/Rules for Seed Analysis recommends the use of 400 seeds to perform the germination test. These 400 seeds can be arranged in four replications of 100, eight of 50 or sixteen replications of 25 seeds (Brasil, 2009). The manual of the International Seed Analysis Association (International Seed Testing Association-ISTA) also recommends the use of 400 seeds for the germination test. However, this manual allows the use of fewer seeds in certain circumstances, in which case at least 100 seeds should be tested with replications of 25 or 50 seeds (ISTA, 2008). The manual also advocates the use 200 seeds for the germination test, when so requested by the test applicant.

Researchers conducted with vegetable seeds have typically used four replications of 50 seeds to determine the percentage of germination of seed lots (Silva and Cícero, 2014; Justino et al., 2015; Armondes et al., 2016; Nascimento et al., 2016). In studies with coriander seeds, four replications of 50 have also been commonly used for the germination test (Nascimento et al., 2006, Pereira et al., 2011, Torres et al., 2012, Silva et al., 2012). According to Ribeiro-Oliveira and Ranal (2016), the number of seeds used to carry out the germination test can affect the information extracted from the germination process.

The standardization of laboratory analyses is important for the correct determination of seed lot quality. However, there are particularities in some groups of plant species, such as some native forest plants that present seasonality in seed production (Sousa-Silva et al., 2001; Ribeiro-Oliveira et al., 2013) and some vegetables whose production of hybrid seeds involves high costs (Nascimento and Melo, 2015), that merit studies aimed at reducing the sample size required for the test, whilst still guaranteeing accuracy of results.

In view of the variation in sample size and the need to standardize the germination test, studies shall be carried out to determine the minimum number of seeds to estimate viability and vigor of a seed lot. In a study by Ribeiro-Oliveira et al. (2016), it was feasible to produce germination test protocols for several species with a reduced number of seeds.

Studying the germination of sucupira-preta seeds with samples of 100, 200 or 400 seeds arranged in four replications, Ribeiro-Oliveira et al. (2013) observed that the measurements of time, uniformity, synchrony, mean velocity and germinability were unaffected by different sample sizes.

In an investigation of the effect of sample size on germination tests conducted with six Brazilian Savanna species, it was reported that germination and mean

germination time presented robustness in relation to changes in sample size (Ribeiro-Oliveira and Ranal, 2016).

The production of vegetable seeds in Brazil has shown progress in the last few years, because of the investment in technological innovation of cropping systems and the increasing use of modern, efficient machinery and other inputs, such as high value hybrid seeds, in particular. The price of these seeds is high and for this reason there is a trend of trading seeds per unit instead of per weight. Currently, packages are filled with 500, 1000 or 2000 seeds (Nascimento and Melo, 2015). In view of the high price of vegetable seeds, reduction in the number of seeds required to carry out the germination test would be an advantage for seed producers, researchers and producers who acquire them.

The objective of this study was to determine the minimum number of replications and seeds per replication to perform the germination and seed vigor tests with coriander seeds (*Coriandrum sativum* L.) through resampling techniques.

Results and discussion

Water content and physiological potential of lots

The initial water content of seeds was 7.16% for lot A and 6.92% for lot B. The similarity in water content between lots makes it reasonable to compare them in terms of tests for initial quality. The initial characterization of the lots showed that Lot A presented high physiological potential while Lot B had low physiological potential (Figure 1). These differences are important because, according to Marcos-Filho (2015), deficiencies in germination synchrony, lack of uniformity in seedling emergence and reduction in the percentage of emerged seedlings are clear manifestations of low physiological potential of seed lots. In addition, according to Ribeiro-Oliveira et al. (2016), the sample size required for tests depends on the initial physiological quality of the samples. Thus, the characteristics of the seed lots used in this study makes it reasonable to compare them in terms of the effects of the number of seeds and the number of replications for performing tests that account for physiological potential.

Sample size for the germination test

Concerning the germinability of coriander seeds, that is, the ability to form normal seedlings at 21 days after sowing, it was observed that four replications of 30 seeds were sufficient to estimate this parameter in lots with high or low physiological potential (Figure 2). Freitas et al. (2011) investigated the effect the number of seeds (20, 25, 30, 50, 70 and 100) of *Mimosa caesalpiniifolia* per replication and reported that when four replications were used, variations in the number of seeds had no effect on the germination tests. Contrasting data were obtained for *Brachiaria brizantha* Stapf cultivar Marandú in an investigation of the influence of the number of seeds (50, 100, 150 and 200) per replication (four) on germinative performance (Carneiro, 1994). It was concluded that the ideal number of seeds per replication was 200. However, it is worth noting that the morphological, physiological and production characteristics of coriander and brachiaria seeds are very different.

As the number of seeds per replication increased, the index of variation was appreciably low (Figure 2), for both seed lots. Freitas et al. (2011) also observed a reduction in the IV (%) with increasing number of seeds per replication in a study of germination of *Mimosa caesalpiniifolia* seeds.

The effect of the number of seeds on the germination of each sample of *Bowdichia virgilioides* Kunth was studied using samples comprising 100, 200 or 400 seeds arranged in four replications. It was concluded that the number of seeds does not interfere with indicators of germinability and using four replications of 25 seeds or four replications of 100 seeds resulted in similar germination values (Ribeiro-Oliveira et al., 2013). Similarly, in a later study which used 100, 200 and 400 seeds in each of four replications, Ribeiro-Oliveira and Ranal (2016) demonstrated that germination was not influenced by sample size.

In this study, less than four replications of 50 seeds reduced the accuracy of estimates of germination of both coriander seed lots. Similar results were obtained by Freitas et al. (2011) who investigated number of replications ranging from three to ten for estimating germination of *Mimosa caesalpiniifolia* seeds. The authors concluded that with three replications it was not possible to define the ideal number of seeds. With five or more replications, excepting nine, it was possible to identify reduced numbers of seeds suitable for the test.

Sample size for the germination first count

When studying the germination first count, there was a general trend with both seed lots for decreases on variability (IV) of the percentage of normal seedlings at seven days after sowing to be associated with increasing numbers of seeds and replications (Fig 3). These reductions were more evident and consistent with increasing number of replications (using 50 seeds per replication) than with increasing number of seeds (using four replications). With both seed lots, it was reasonable to admit a reduction in the number of seeds from 50 to 20. High IV values were obtained for lot B with four replications of 30 seeds; this finding is likely related to a bias in the resampling process, since the same number of replications with 20 seeds presented IV values similar to the reference sample.

In general, the IV of lot A was lower than that of lot B. Ribeiro-Oliveira et al. (2016) reported that seed lots with a high germination percentage require fewer seeds to compose a sample of sufficient size than those with low germination percentage.

Carneiro (1996) found a significant effect of differences in physiological potential of the lots on the sample size. This author, working with seed lots of *Stevia rebaudiana* with low physiological potential, concluded that the number of seeds should be higher when compared to lots of seeds with high physiological potential. This work investigated different number of seeds (50, 100, 150 and 200) per replication (eight). The conclusion was that for the seed lot produced in greenhouse (upper initial quality), the ideal number of seeds per replication was 100, while for the lot produced in field (lower initial quality), the ideal number of seeds per replication was 200.

In this study, reduction in the number of replications caused considerable differences in seed vigor estimates through the germination first count with both seed lots. In fact, the IV appeared to stabilize from four replications upwards (Figure 3).

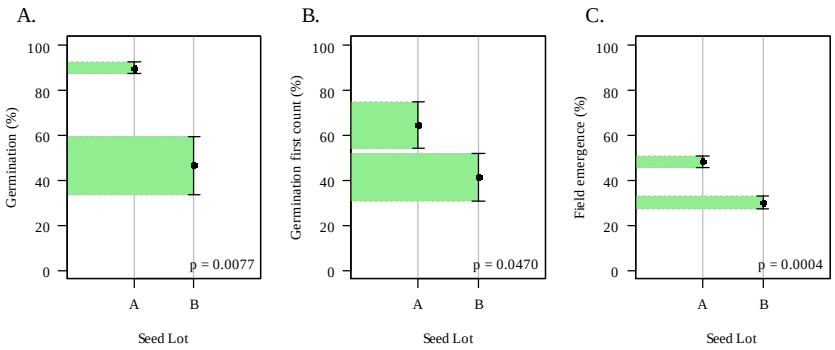

Fig 1. 95% confidence intervals for the means of A.: germination, B.: germination first count and C.: field emergence of two lots of coriander seeds.

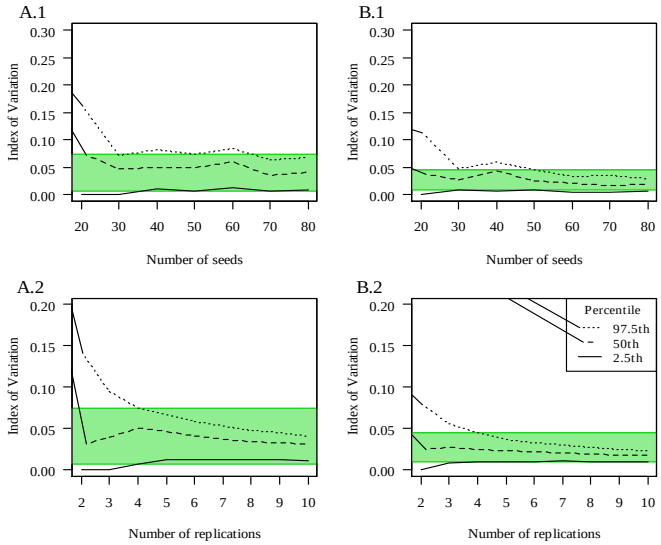

Fig 2. Bootstrap intervals of 95% confidence for analysis of the minimum number of seeds (with 4 replications: A.1 and B.1) and replications (with 50 seeds per replication: A.2 and B.2) of two lots, A.: high initial germination and B.: low initial germination, for the germination test with coriander seeds. The green strip delineates the confidence interval for the reference sample (4 replications of 50 seeds).

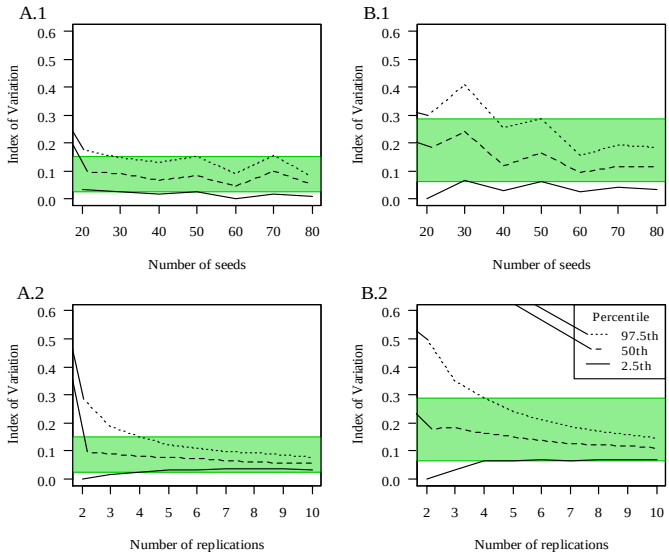

Fig 3. Bootstrap intervals of 95% confidence for analysis of the minimum number of seeds (with 4 replications: A.1 and B.1) and replications (with 50 seeds per replication: A.2 and B.2) of two lots, A.: high initial germination and B.: low initial germination, for the germination first count test with coriander seeds. The green strip delineates the confidence interval for the reference sample (4 replications of 50 seeds).

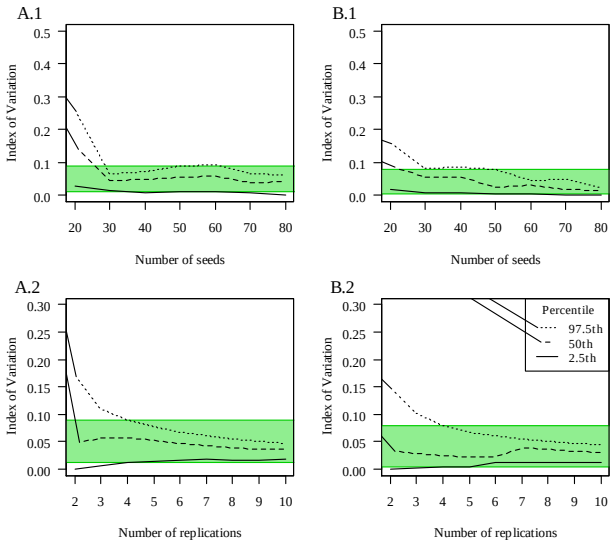

Fig 4. Bootstrap intervals of 95% confidence for analysis of the minimum number of seeds (with 4 replications: A.1 and B.1) and replications (with 50 seeds per replication: A.2 and B.2) of two lots, A.: high initial germination and B.: low initial germination, for determining the germination speed index (GSI) of coriander seeds. The green strip delineates the confidence interval for the reference sample (4 replications of 50 seeds).

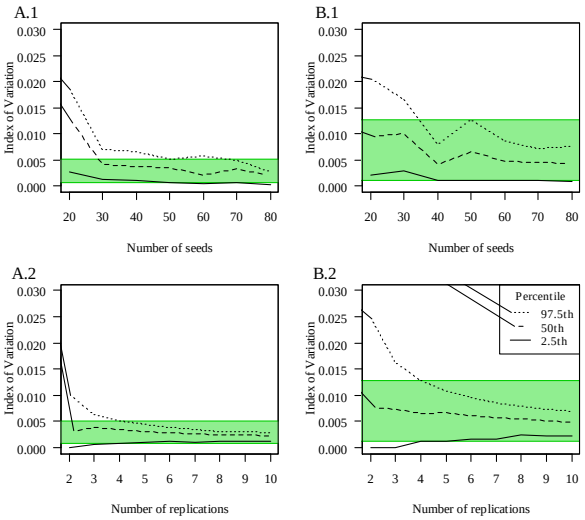

Fig 5. Bootstrap intervals of 95% confidence for analysis of the minimum number of seeds (with 4 replications: A.1 and B.1) and replications (with 50 seeds per replication: A.2 and B.2) of two lots, A.: high initial germination and B.: low initial germination, for determining the mean germination time (MGT) of coriander seeds. The green strip delineates the confidence interval for the reference sample (4 replications of 50 seeds).

Sample size for the germination speed index

Similar results were found for both vigor variables: germination first count and germination speed index. It is feasible to reduce the number of seeds per replication from 50 to 30, but the minimum number of replications should remain at four (Figure 4). And the subsample is expected to have the sample accuracy. Contrasting data were obtained by Freitas et al. (2011) in an investigation with *Mimosa caesalpiniifolia* seeds: three replications with 75 seeds each were considered sufficient for the estimation of seed vigor through GSI.

The evaluation of seed vigor by the GSI should be used with caution, since the number of seeds per replication interferes directly with the result once it is used in the calculation. In the studies of Ribeiro-Oliveira et al. (2013), the germination speed estimated by GSI was affected by the number of seeds in the sample for *Bowdichia virgilioides* Kunth. These authors concluded that, as sample size increases from 100 to 400 seeds, the GSI also increases, leading to overestimates of germination speed values in this species. The possibility of errors using this index was also cited by Ranal and Santana (2006). An alternative is to follow the recommendation of Maguire (1962), that is 100 seeds per replication or, as it was done in this research, computing the percentage of normal seedlings per day.

Sample size for the mean germination time

Increasing the number replications and of seeds per replication has promoted reductions on the index of variation of MGT (Figure 5). For both seed lots, four replications of 40 seeds shall produce accurate estimates of mean germination time. It was observed that MGT

presented, in general, lower values of IV (<0.002) than the other variables (Figures 2-5). This finding suggests that, based on MGT, there is less chance of the variability presented by the reference sample (4 replications of 50 seeds) to be reduced. Results presented in Figure 5 indicate that increasing the number of seeds (>50) does not make the confidence intervals to shrink significantly. The stability of this measure of vigor is noted, particularly, for seed lots with high physiological potential.

According to Ribeiro-Oliveira and Ranal (2016), MGT and germinability can be used in any germination study, for they are considered to be precise and efficient measures for quantifying the physiological potential of seeds. The authors concluded that MGT has an excellent ability to detect differences between lots and then to provide inferences about the vigor.

It is suggested to take at least four replications of 50 seeds to determine MGT, as well as for the other variables evaluated in this study. In general, the lot with high physiological potential (A) presented lower values of IV than lot B, as observed for germination first count.

General considerations on number of seeds and number of replications

Increases in both the number of seeds per replication and the number of replications for each vigor test evaluated in this study are effective in reducing the IV. Nonetheless, reducing the number of seeds per replication from 50 to 40 or 30 allows one to estimate seed vigor with equal or similar accuracy, confirming the premise of Ribeiro-Oliveira et al. (2016), that it is feasible to have protocols for germination tests for seeds of several species which involve using reduced numbers of seeds.

Material and methods

Vegetal material and characterization of lots

The experiment was conducted using two commercial seed lots of coriander (*Coriandrum sativum* L.) cultivar Super Verdão. Initial characterization of the lots was carried out according to the methodologies described as follows, involving determination of water content, germination, and field emergence.

Water content: determined using the oven-drying method at 105 ± 3 °C for 24 h. Two replications of approximately 2 g were used for each lot, weighed on a scale with 0.001 g precision. Data are expressed on a wet weight basis, as percentage of the fresh weight of the seed (Brasil, 2009).

Germination: four replications of 50 seeds per lot were distributed in gerbox plastic boxes (11 × 11 × 3.5 cm) between sheets of germination paper, two sheets beneath the seeds and one covering the seeds. The substrate was previously moistened with deionized water, using the equivalent of 2.5 times the mass of the dry paper. The seed boxes were maintained at 20 °C in a Mangelsdorf-type germinator. Seedling evaluations were performed according to the criteria established in the Rules for Seed Analysis (Brasil, 2009). Evaluations were performed seven days after sowing (first count data) and after 21 days (germination data). The results were expressed as percentage of normal seedlings.

Field emergence: four replications of 50 seeds per lot were sown in an unmanaged soil bed in rows of 2.5 m length, spaced 0.5 m apart. Sowing was conducted so that seeds remained at 2.0 cm depth. A sprinkler irrigation system was installed. Evaluations were carried out 21 days after sowing, counting only those seedlings that presented hypocotyl greater than 1 cm to determine the percentage of normal seedlings.

Sample size scenarios and resampling process

The two seed lots were used to analyze the number of replications and seeds per replication. Eight hundred (800) seeds per lot were used. These were arranged in gerbox-type boxes using the substrate and wetting procedure previously reported. Five rows of 10 seeds were arranged in each box. The, sixteen boxes were used in the experiment, with rows numbered from 1 to 80, totaling 800 seeds from each lot. Sample size scenarios were developed using different combinations of the number of replications (from 2 to 10) and the number of seeds per replication (20, 30, 40, 50, 60, 70 and 80) by means of resampling with replacement the numbered lines. Thus, the simplest scenario consisted of 2 replications of 20 seeds and the most complex scenario, 10 replications of 80 seeds.

Daily counts were conducted for up to 21 days after sowing. Germination was evaluated according to the criteria of normal seedlings (Brasil, 2009). The following variables were analyzed: percentage of normal seedlings at seven days (germination first count) and at 21 days (germination), GSI (Maguire, 1962) and MGT (Labouriau and Valadares, 1976).

Computing and statistical procedures

The characterization data were submitted to analysis of variance and the assumptions of normality and residual homoscedasticity were verified. Confidence intervals of 95% were built for comparison of means for each variable.

Determination of the minimum number of replications and seeds per replication was based on the 95% bootstrapped confidence interval analysis for the index of variation (IV) of each scenario. And,

$$IV = \frac{s}{\bar{x}\sqrt{n}}$$

Where *s* represents the standard deviation, $\bar{x}$ is the mean and *n* is the number of data. It should be noted that IV is used as a measure of variability by taking into account the sample size (*n*) of each scenario.

The reference sample consisted of four replications of 50 seeds, since the majority of researches involving seeds from large crops and vegetables has been carried out using 200 seeds arranged in four replications. Determination of the minimum number of seeds required was based on comparing the confidence intervals of each scenario with the confidence interval of the reference sample.

All analyses were performed using R software (www.R-project.org). The codes used to perform the resampling can be obtained with the corresponding author.

Conclusion

Considering four replications of fifty seeds as reference sample for researches with coriander, subsamples can be taken to estimate the physiological potential of seed lots. It is reasonable to reduce the number of seeds per replication from 50 to 30 in order determine germination first count, germination and germination speed index. Moreover, 40 seeds per replication are recommended to quantify the mean germination time. On the other hand, reductions in the number of replications are not recommended, for they can affect negatively the accuracy of germination and vigor tests.

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References

Armondes KAP, Dias DCFS, Martinez PAH, Silva LJ, Hilst PC (2016) Condicionamento osmótico e desempenho de sementes de repolho com diferentes níveis de vigor. Hortic Bras. 34:428-434.

Brasil - Ministério da Agricultura e da Reforma Agrária. Regras para análise de sementes (2009) Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS, 399p.

Carneiro JWP (1994) Determinação do número de sementes para avaliar o desempenho germinativo de sementes de capim braquiária (*Brachiaria brizantha* cv marandú). Rev Bras Sementes. 16:156-158.

Carneiro JWP (1996) Determinação do número de sementes para avaliar o desempenho germinativo de Stevia (*Stevia rebaudiana* (bert.) Bertoni). Rev Bras Sementes. 18:1-5.

Freitas TAS, Mendonça AVR, Freitas TP, Santana SPB (2011) Tamanho de amostra para teste de germinação de *Mimosa caesalpiniifolia* Benth, espécie nativa da Caatinga. Cien Agron. 42:714-724.

ISTA - International Seed Testing Association (2008) The germination test. In: ISTA - International Seed Testing Association, International rules for seed testing. Bassersdorf, Zurich. 5.

Justino EV, Boiteux LS, Fonseca MEN, Silva Filho JG, Nascimento WM (2015) Determinação da maturidade fisiológica de sementes de pimenta dedo de moça *Capsicum baccatum* var. *pendulum*. Hortic Bras. 33:324-331.

Labouriau LG, Valadares MEB (1976) On the germination of seeds *Calotropis procera* (Ait.) Ait.f. An Acad Bras Cienc. 48:263-284.

Maguire JD (1962) Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

Marcos-Filho J (2015) Fisiologia de sementes de plantas cultivadas. 2nd edn. Abrates, Londrina.

Nascimento WM, Andrade KP, Freitas RA, Silva GO, Boiteux LS (2016) Germinação de sementes de tomateiro em diferentes temperaturas: Variabilidade fenotípica e heterose. Hortic Bras. 34:216-222.

Nascimento WM, Melo PCT (2015) Desafios e oportunidades na produção de sementes de hortaliças no Brasil. Revista Seed News. XIX: 26-32.

Nascimento WM, Pereira RS, Freitas RA, Blumer L, Muniz MFB (2006) Colheita e armazenamento de sementes de coentro. Pesqui Agropecu Bras. 41:1793-1801.

Pereira MFS, Torres SB, Linhares PCF, Paiva ACC, Paz AES, Dantas AH (2011) Qualidade fisiológica de sementes de coentro [*Coriandrum sativum* (L.)]. Rev Bras Plantas Med. 13:518-522.

Ranal MA, Santana DG (2006) How and why to measure the germination process? Rev Bras Bot. 29:1-11.

Ribeiro-Oliveira JP, Ranal MA (2016) Sample size in studies on the germination process. Botany. 94:103-115.

Ribeiro-Oliveira JP, Ranal MA, Santana DG (2013) Amplitude amostral interfere nas medidas de germinação de *Bowdichia virgilioides* Kunth? Cienc Florest. 23:623-634.

Ribeiro-Oliveira JP, Ranal MA, Santana DG, Pereira LA (2016) Sufficient sample size to study seed germination. Aust J Bot. 64:295-301.

Silva MAD, Coelho Júnior LF, Santos AP (2012) Vigor de sementes de coentro (*Coriandrum sativum* L.) provenientes de sistemas orgânico e convencional. Rev Bras Plantas Med. 14:192-196.

Silva VN, Cicero SM (2014) Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de berinjela. Hortic Bras. 32:145-151.

Sousa-Silva JC, Ribeiro JF, Fonseca CEL, Antunes NB (2001) Germinação de sementes e emergência de plântulas de espécies arbóreas e arbustivas que ocorrem em Mata de Galeria. In: Ribeiro JF, Fonseca CEL and Sousa-Silva, JC (eds) Cerrado: caracterização e recuperação de mata de galeria, 1st edn. Embrapa Cerrados, Planaltina. 10.

Torres SB, Dantas AH, Pereira MFS, Benedito CP, Silva FHA (2012) Deterioração controlada em sementes de coentro. Rev Bras Sementes. 34:319-326.