# Standardized methodology for germination test on chickpea seeds

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**ABSTRACT.** The present work aimed to establish (update) the methodology for carrying out the germination test on chickpea seeds, with regard to the substrate, ideal temperature and date of the first count. For this, three tests were carried out: I) substrate evaluation; II) evaluation of the seed exposure temperature during germination; and III) verification of the date of the first count. The experiments were organized in a completely randomized design, factorial scheme 6 x 3 (cultivars x substrates), for test I and 6 x 4 (cultivars x temperatures) for test II. Analysis of variances and means were compared by the LSD test. For test III, a graph of normal seedlings was drawn up, evaluating the percentage of germination. The parameters analyzed were: germination, speed germinations index and mean germination time, in trials I and II. In test III, only germination. Higher germination percentages were observed and an increase in germination speed when using the paper roll substrate. Extreme temperatures were not favorable to the seedling formation process. According to the results obtained, it is recommended to use a paper roll, at 20 °C and with an evaluation date of the first count at 6 days after sowing for germination test.

**Keywords**: *Cicer arietinum* L.; substrate; temperature

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## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a legume from the Fabaceae family, considered one of the most important for consumption worldwide. It is an annual plant, well adapted to dry and mild weather and can be grown in winter in tropical regions or in spring and summer in temperate regions (Nascimento et al., 1998; Nascimento et al., 2016).

It is still a poorly cultivated culture in Brazil, and most of what is consumed comes from imports from countries like Argentina and Mexico (Nascimento et al., 2016). To stimulate the production chain of this species it is important that there is basic information on aspects related to the installation of the crop. In this context, adequate knowledge about aspects related to seed quality and its proper measurement, occupy a prominent role (Dias et al., 2019).

To identify the value of a seed lot for sowing, as well as to identify the physiological quality of the seeds, one of the most used methods is the germination test, which is carried out under ideal conditions of temperature and substrates, for each species (Brasil, 2009). In addition, this test is essential for monitoring the viability of seeds in germplasm banks, before and during storage.

According to Bertolin (2011), the germination pattern is conducted under favorable conditions so that the seed lot expresses its maximum potential. Thus, when field conditions are ideal, this test can predict seed performance after sowing.

Germination occurs from a series of metabolic, biochemical and physiological events that culminate in the formation of the seedling. These processes are influenced by external factors such as temperature, water availability, oxygen availability, brightness and internal factors such as the existence of dormancy, synthesis of inhibitors and germination promoters, among others (Marcos-Filho, 2015).

Success in using seeds to multiplicate a plant species depends on the occurrence of rapid and uniform germination, followed by satisfactory seedling development in the field, as the longer the seedling remains in the early stages of development, the more vulnerable it will be to adverse conditions in the environment (Araújo and Souza, 2018).

The germination test is described in the Rules for Seed Analysis - RAS (Brasil, 2009) for a large

number of species, indicating information such as dates for evaluation, substrates to be used and temperature of exposure during the test, and there is a recommendation for carrying out of this test on chickpea seeds. These recommendations must be followed to obtain the maximum germination potential of the seed lot. However, there are many factors that can interfere with the expression of seed potential, such as temperature, substrate (Leão Araújo et al., 2019), and the maximum time for seedling formation (Marcos-Filho, 2015). These factors still deserve to be tested to define the ability to influence the result of the germination test, as well as to seek the standardization of the test.

The substrate is the physical support on which the seed is placed and has the important function of maintaining the right conditions for germination and seedling development. Thus, knowing about the most suitable substrate for the seeds in the substrate is important to adjust the requirements of the seed in relation to its size, a way that affect the absorption of water and consequently the evolution of the metabolic events of the germination process. And these characteristics can vary between species and between cultivars (Brasil, 2009).

Sand is a substrate widely used for germination tests, it is chemically inert, reducing possible problems with the occurrence of phytopathogenic organisms and it has a pH close to neutrality (Brasil, 2009). In addition, the sand has low water retention capacity, good aeration, good drainage and high density (Kämpf, 2000). Despite this, germination paper is still the most used substrate in the laboratory (Barroso, 2010), mainly due to its ease of handling and the smaller space it takes up.

For Wilhelm (2017), the proper positioning of the seeds, in sowing, can decrease the energy costs in germination and in the positioning of the seedlings, making the plants have greater vigor, thus providing greater uniformity in the plant stand, reducing the crossing of plants. Leaves from one plant over the other, optimizing the absorption of light.

According to Bewley and Black (1978), temperature is one of the crucial factors in the germination process. This affects the speed, percentage and uniformity of germination, with the optimum temperature being the one that allows maximum germination in the shortest period of time. As germination occurs with a sequence of chemical reactions, each of these reactions has demands on temperature, as they depend on the activity of specific enzymes (Marcos-Filho, 2015). The temperature interference is due to the fact that it changes the water absorption speed and the metabolic reactions of the reserves necessary for the seedling survival (Alves et al., 2015).

To guarantee the efficiency of a germination test, in addition to the characteristics of the medium such as substrate and temperature that can interfere with the test results, evaluation characteristics, such as counting dates, may be related to the greater precision in defining the physiological quality of a lot of seeds. According to Torres and Minami (2000) the first count test has been used for vegetable seeds, however, checks are necessary to detect their sensitivity in distinguishing the vigor levels of lots of various plant species.

Given the above, the objective of this study was to establish (update) the methodology for carrying out the germination test on chickpea seeds, with regard to the ideal substrate, ideal temperature and date of the first count.

## MATERIAL AND METHODS

The work was carried out at the Seed Analysis Laboratory of the Agronomy Department of the Federal Goiano Institute, Campus Urutaí. Six chickpea cultivars from the kabuli group were used. The cultivars have variations in size, chemical composition and physiological quality, and the names of the cultivars are following: BRS Aleppo; BRS Cícero; BRS Cristalino; BRS Jamu; BRS Kalifa and BRS Toro. The seeds remained stored in kraft paper bags in a cold chamber (16 $^{o}$C and 50-60% RH of air) during the conduction of the experiments.

The study consisted of three subsequent tests as follows: I) substrate evaluation; II) evaluation of the exposure temperature during germination and III) determination of the date for the evaluation of the first count, always based on the results of the previous step. Follows the methodology of each stage.

Test I: In this experiment, three substrates for germinating chickpea seeds were evaluated. For this, the substrates were used: paper roll (PR), between sand (BS) and on sand (OS).

The evaluation was carried out by means of the germination test, speed germinations index and mean germination time. For this, the paper substrate was moistened with the equivalent of 2.5 times the dry mass of the paper and the sand was moistened with 50% of the field capacity, determined according to Brazil (2009).

Eight repetitions of 50 seeds of each cultivar were used for each substrate. The seeds of each cultivar were placed in an equidistant way on the substrates (paper roll, between sand and on sand) kept in a germination chamber regulated at 20 ± 2 $^{o}$C constant (Brasil, 2009). The parameters evaluated were the speed germinations index (SGI) and mean germination time (MGT) - the first was determined according to the formula proposed by Maguire (1962), and the second according to Labouriau and Valadares (1976). These parameters were evaluated simultaneously with the germination test, noting the normal seedlings every 24 hours until the 8$^{th}$ day. At

eight days after sowing, the final count (G) was performed, identifying normal, abnormal seedlings, dead and hard seeds. The results were expressed as a percentage of normal seedlings.

The humidity measurement in the PR's was carried out daily and when necessary, moistening was done with a solution containing fungicide with i.a. Benzimidazole, and p.c. Cercobin 500 Concentrate Solution, diluted in water in the proportion of six grams of the product per liter of water, being applied the same amount of syrup for all treatments and repetitions. The moisture measurement of the substrates BS and OS was carried out daily and the substrates irrigated, when necessary, with pure water.

The experiment was conducted in a completely randomized design with eight replications of 50 seeds, a 6 x 3 factorial scheme (cultivars x substrates). The residues were tested for normality and homoscedasticity. Given the assumptions, the analysis of variance was performed and the means compared by the LSD means test.

Test II: to evaluate the temperature of seed exposure during the germination process, four germination chambers, Mangelsdorf type, were used, regulated at a temperature of 15, 20, 25 and 30 ± 2 ºC constant, each (Brazil, 2009). The seeds were placed in an equidistant manner on the PR substrate, defined as best in Test I, the substrate was moistened as described in Test I. The rolls were kept in the germination chambers with the mentioned temperatures and the seedlings were evaluated daily for SGI and MGT and at eight days the final count (G) was performed. The results were expressed as a percentage of normal seedlings.

The experiment was conducted in a completely randomized design with eight replications of 50 seeds, a 6 x 4 factorial scheme (cultivars x temperatures). The residues were tested for normality and homoscedasticity. Given the assumptions, the analysis of variance was performed and the means compared by the LSD means test.

Test III: in this step, the best date to determine the first count (FC) was evaluated. In this experiment we used the substrate defined in test I and the temperature defined in test II. Daily counts were performed until the 8th day after sowing. Normal seedlings were counted daily. A graph was elaborated detailing the behavior of each cultivar over time. The date analysis was based on the criteria established by Brasil (2009), for the first count.

## RESULTS AND DISCUSSION

### Test I - substrate for seed germination

The data obtained for seed germination of the six cultivars according to the substrate are shown in Table 1.

For this variable, the paper roll (PR) was superior to the other substrates with 78% G in 'BRS Kalifa', while on sand (OS), it proved to be the worst option among the tested substrates. These results corroborate those verified by Gomes et al. (2016), when evaluating the paper roll and between sand for four species of the Myrtaceae family and observed that the paper roll was more conducive to expressing the vigor of the seeds. These authors reported that this occurred as a consequence of the greater capacity of water retention and greater contact area of the substrate with the seeds.

For the cultivars BRS Cristalino and BRS JAMU the substrate between sand (BS) was equal to the PR. Rocha et al. (2014) also revealed similar results for the substrate BS with *Parkia multijuga* seeds (benguê or faveira-benguê), for these authors this is due to the fact that the aeration, the water retention capacity and the increased seed contact with the substrate are high in the substrate BS favoring the germination of the seeds. In the case of our study, this superiority was compared to OS.

The cultivars BRS Kalifa and BRS Toro proved to be superior for germination when exposed to all tested substrates. In general, the cultivar BRS Kalifa was superior for germination, this cultivar showed 78% germination in the PR.

For the speed germination index (SGI), a behavior similar to G was observed when comparing the substrates of the seeds. The RP was higher than the others and OS revealed the worst results for this index (Table 2). The superiority of the PR can be justified by the fact that it promotes a greater contact surface between the seed and the substrate, in order to favor the absorption of water by the seed and thus promoting a higher percentage of germination in less time (Nogueira et al., 2013).

The SGI aims to differentiate the seed lots as to the speed of occurrence of the germination process. According to Peske et al. (2012), this method is based on the principle that lots with the highest seed germination speed are the most vigorous, that is, there is a direct relationship between the speed of seedling formation and seed vigor.

**Table 1**. Germination (%) of six chickpea cultivars (*Cicer arietinum* L.) on three substrates. Urutaí, 2019.

| Cultivars | On sand (OS) | Between sand (BS) | Paper roll (PR) |
|---|---|---|---|
| BRS Aleppo | 1 bB* | 9 bC | 21 aC |
| BRS Cícero | 5 cB | 33 bB | 54 aB |
| BRS Cristalino | 4 bB | 18 aC | 16 aC |
| BRS Jamu | 3 bB | 17 aC | 15 aC |
| BRS Kalifa | 24 cA | 56 bA | 78 aA |
| BRS Toro | 33 cA | 46 bA | 66 aB |
| CV = 30.59% | | | p-value (C x S) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

**Table 2.** Speed germination index of six chickpea cultivars (*Cicer arietinum* L.) on three substrates. Urutaí, 2019.

| Cultivars | On sand (OS) | Between sand (BS) | Paper roll (PR) |
|---|---|---|---|
| BRS Aleppo | 0.134 bB* | 1,312 bC | 3,912 aC |
| BRS Cícero | 0.625 cB | 4,910 bB | 8,571 aB |
| BRS Cristalino | 0.493 bB | 2,632 aC | 2,641 aC |
| BRS Jamu | 0.351 aB | 2,506 aC | 2,221 aC |
| BRS Kalifa | 3.788 cA | 8,841 bA | 13,489 aA |
| BRS Toro | 5.339 cA | 8,113 bA | 12,015 aA |
| CV = 30.07% | | | p-value (C x S) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

**Table 3**. Mean germination time (days) of six chickpea cultivars (*Cicer arietinum* L.) on three substrates. Urutaí, 2019.

| Cultivars | On sand (OS) | Between sand (BS) | Paper roll (PR) |
|---|---|---|---|
| BRS Aleppo | 7.500 cC | 6,764 bB | 5,250aA |
| BRS Cícero | 7.100 aBC | 6,739 aB | 6,535 aCD |
| BRS Cristalino | 7.416 cC | 6,789 bB | 6,125 aBC |
| BRS Jamu | 6.958 aBC | 6,678 aB | 6,972 aD |
| BRS Kalifa | 6.275 aA | 6,546 aB | 5,898 aB |
| BRS Toro | 6.553 aAB | 5,805 aA | 5,599 aAB |
| CV = 23.85% | | | p-value (C x S) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

For the BRS Jamu cultivar, there was no significant difference between the substrates. The cultivar BRS Cristalino showed equality for BS and PR. These two cultivars showed the worst results for G (Table 1) and SGI (Table 2), in the latter case also equaling the BRS Aleppo cultivar. These materials (BRS Aleppo, BRS Cristalino and BRS Jamu) showed very low values for germination (Table 1), this shows that they are lots with a large amount of unviable seeds. Seed lots with these characteristics may reveal greater difficulty in distinguishing between test parameters that assess physiological quality because the viable seeds in the lots are probably at an advanced stage of deterioration and according to Marcos-Filho (2015) evidence of high seed deterioration is the lower capacity to use reserves, altering the synthesis rates, and then generating a smaller number of seedlings.

For the substrate OS the worst values of GSI and G were observed, this can be explained by the fact that the sand presents easy drainage of water (Dousseau et al., 2011), thus generating the dryness of the upper part where the seeds were allocated, impairing the imbibition process and consequently their germination. For Schulz (2013), the low germination in the substrate OS may result from the physical characteristics of the substrate that were not compatible with the size of the seeds, sensitivity to light and the requirements of the same regarding the availability of water, due to the substrate not providing conditions germination of that species.

For the variable mean germination time (MGT) the data are presented in Table 3. In this variable, in four cultivars (BRS Cícero, BRS Jamu, BRS Kalifa and BRS Toro) no significant differences were identified between the substrates, in the other cultivars (BRS Aleppo and BRS Cristalino) it was observed the superiority for PR in the speed of seedling formation (MGT). BS presented intermediate results for this variable. Seed/substrate contact area in the substrate PR and BS are superior to OS, in the latter the seeds keep part of their integument out of contact with the substrate, which provides the necessary water to reactivate the metabolism towards germination. According to Flores et al. (2014) this fact suggests that the contact area of the substrate moistened with the seed is very important and can be critical for the speed of germination.

When evaluating the best substrate for each cultivar, it was found that the cultivars BRS Cícero, BRS Jamu, BRS Kalifa and BRS Toro showed no differences in MGT in relation to the tested substrates. For the cultivars BRS Aleppo and BRS Cristalino, the substrate PR was better in terms of germination time, while BS was considered intermediate and inferior OS.

Although in the Rules for Seed Analysis (Brazil, 2009) there is the possibility of using the substrate BS for germination of chickpea seeds, it was observed that the PR can result in benefits for the final amount of seedlings formed and for speed occurrence of this process.

Thus, the use of paper substrate, in the form of a roll, is the most appropriate option, in agreement with the results of Gomes et al. (2016). For Nogueira (2013), this substrate is also the most suitable since the tests can be conducted in less time, confirming the results obtained for the SGI, in the present work.

In addition, the PR makes it easier for the analyst to distinguish between normal and abnormal seedlings. In addition, it is an easy-to-handle substrate in the germination chamber and occupies less space in the seed analysis laboratory.

**Test II - temperature during germination and seed vigor tests**

Germination at 20 °C stood out from the other temperatures tested (Table 4). As mentioned by Nascimento et al. (2016) this species originates in the southeastern region of Turkey, where temperatures are lower than those observed in a country with a tropical climate, which may justify this better response to germination at lower temperatures. This observation reinforces the hypothesis that the optimum temperature of germination is related to the temperatures of the region of origin of the species at the favorable time for germination, as mentioned by Andrade and Ferreira (2000).

This behavior can extend throughout the life cycle of the plant. Avelar et al. (2018), show high productive potential of this species, especially in the cerrado areas of Cristalina, GO and Brasília, DF, which are regions of the Cerrado that present lower

temperatures throughout the year, when compared with other regions of the state of Goiás and other states such as the North and Northeast regions.

**Table 4**. Germination (%) of six chickpea cultivars (*Cicer arietinum* L.) at four temperatures. Urutaí, 2019.

| Cultivars | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| BRS Aleppo | 13 bcC* | 24 aC | 17 abCD | 5 cCD |
| BRS Cícero | 6 cCD | 37 aB | 24 bC | 9 cC |
| BRS Cristalino | 10 bCD | 20 aC | 13 abD | 8 bCD |
| BRS Jamu | 4 aD | 6 aD | 3 aE | 0 aD |
| BRS Kalifa | 45 bA | 62 aA | 60 abA | 60 aA |
| BRS Toro | 36 bB | 59 aA | 43 bB | 25 cB |
| CV = 25.55% | | | | p-value (C x T) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

**Table 5**. Speed germinations index of six chickpea cultivars (*Cicer arietinum* L.) at four temperatures. Urutaí, 2019.

| Cultivars | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| BRS Aleppo | 1.885 bB | 4.278 aC | 4.416 aB | 1.5 bCD |
| BRS Cícero | 0.817 bB | 6.098 aB | 4.598 aB | 2.009 bC |
| BRS Cristalino | 1.389 aB | 3.165 aC | 2.434 aC | 1.392 aCD |
| BRS Jamu | 0.58 aB | 0.945 aD | 0.469 aD | 0 aD |
| BRS Kalifa | 6.78 bA | 12.072 aA | 11.881 aA | 13.117 aA |
| BRS Toro | 5.195 bA | 12.417 aA | 11.066 aA | 6.983 bB |
| CV = 26.56% | | | | p-value (C x T) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

**Table 6.** Mean germination time (days) of six chickpea cultivars (*Cicer arietinum* L.) at four different temperatures. Urutaí, 2019.

| Cultivars | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| BRS Aleppo | 6.985 aAB | 5.695 abA | 3.959 bcA | 3.275 cB |
| BRS Cícero | 5.212 aBC | 6.222 aA | 5.458 aA | 4.723 aAB |
| BRS Cristalino | 7.125 aA | 6.285 aA | 6.003 aA | 5.625 aA |
| BRS Jamu | 3.5 bC | 6.52 aA | 4.5 bA | 0 cC |
| BRS Kalifa | 6.771 aAB | 5.414 aA | 5.253 aA | 4.856 aAB |
| BRS Toro | 7.037 aAB | 4.945 bA | 4.02 bA | 3.71 bB |
| CV = 26.41% | | | | p-value (C x T) < 0.05 |

*Means followed by the same letter, lower case on the line and upper case on the column, do not differ by the LSD test at 5% significance.

The cultivar BRS Kalifa showed the best results for G, confirming the data obtained in Test I. The cultivar BRS Jamu showed the worst performance among the tested cultivars. In this cultivar there were no differences between the temperatures tested. This can be explained in a similar way to what was mentioned for the substrates in relation to the lots with a high level of deterioration.

For extreme temperatures (15 and 30 °C), lower results were observed for G. According to Oliveira Junior et al. (2015), in case of high temperatures, there may be a reduction of humidity in the germination role, reducing the final percentage of normal seedlings formed. On the other hand, very low temperatures can increase the viscosity and reduce the kinetic energy of the water (Marcos-Filho, 2015), negatively compromising the passage of water from the substrate to the internal tissues of the seed.

The temperatures of 20 and 25 °C were similar to each other (Table 5). The cultivars BRS Cristalino and BRS Jamu showed no significant difference for all temperatures tested, revealing that the temperature factor may have less effect on germination speed when compared to the total number of normal seedlings formed. Shibata et al. (2016) stated that the temperature at which germination occurs is a factor that has an important influence on total germination and also on germination speed.

The mean germination time, in general, at a temperature of 25 and 30 °C were considered promising (Table 6). This is because, at these temperatures, the shortest times necessary to complete the germination process were observed, which is advantageous for the species and the continuity of the plant's development. It is noteworthy the absence of germination for the cultivar BRS Jamu at 30 °C, as previously mentioned, this material had a low physiological quality. In the cultivars BRS Cristalino and BRS Kalifa, the temperature of 20 °C was equal to the temperatures 25 and 30 °C, with the lowest MGT. For most plant species, temperatures between 20 and 30 °C are ideal for all initial seedling development processes (Marcos-Filho, 2015).

The temperatures of 15 and 30 °C were lower compared to the other temperatures tested, as observed for G. The low development of seedlings of these cultivars in extreme temperatures can be explained as a selection of biological nature, which prevents the development of the species in unsuitable environments (Bilio, Guimarães and Caldeira; 2013).

The temperature of 15 °C was lower for MGT. Carvalho and Nakagawa (2000) stated that temperatures below the optimum tend to reduce the percentage of germination and the speed of occurrence of the process. For germination of chickpea seeds, 15 °C was not satisfactory. In agreement with this result, Ferreira and Novembre (2015) concluded that temperatures between 15 ºC and 19 ºC had a negative impact on the germination of annatto seeds, as in this case neither the emission of the primary root occurred.

At a temperature of 15 °C, the cultivars showed no difference in relation to the MGT, for 20 °C the cultivar BRS Toro was superior and for 25 °C and 30 °C the cultivars BRS Aleppo and BRS Toro were superior, the cultivar BRS Jamu showed with lower performance, as well as in other tests.

It is likely that the water retention capacity of each substrate, combined with the intrinsic characteristics that regulate the flow of water to the seeds, may have influenced the results. Thus, it appears that the choice of substrate and temperature is very important to obtain better results in a germination test and in vigor tests, in view, above all, of the great variation that exists between species in relation to the most (Alves, 2015).

**Test III - first count date for germination and seed vigor tests**

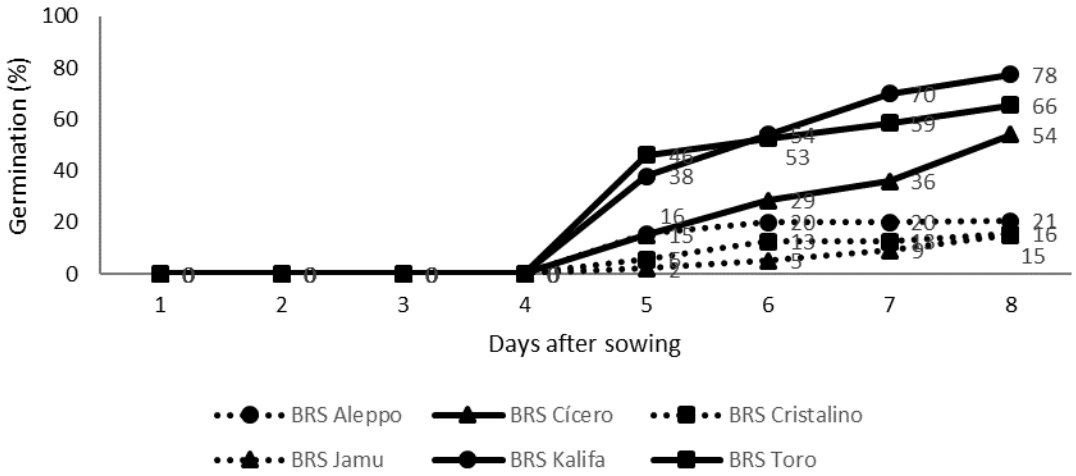


**Figure 1.** Evaluation date of the first and last germination count of six chickpea cultivars (*Cicer arietinum* L.). Urutaí, 2019.

In PR, and with a constant temperature of 20 °C, as suggested in Brazil (2009) and confirmed in Test II, the formation of normal seedlings in the tested cultivars started at five days, even for the cultivar BRS Kalifa, which had physiological potential elevated (Figure 1). Which may be an indication that the date of the first count at five days, as indicated in the Seed Analysis Rules, may not express the purpose of the first count test.

With the data in Figure 1, it can be seen that, on the 6th day after sowing, the lots with high physiological potential (BRS Kalifa and BRS Toro) presented more than 50% of normal seedlings. This criterion was used to determine the best date for the first count, that is, the date when, in the vigorous lots, more than 50% of the sample expressed its potential. Thus, the first count must be carried out 6 days after sowing. As established in the RAS (Brasil, 2009), a variation of 1 to 3 days is allowed for the evaluation of the first count, as long as it is sufficient for the correct evaluation of the seedlings.

Also, according to the Brazilian Rules for Seed Analysis, if the lowest temperature is used, the first count may be postponed. In this work, we realized that 25 °C is also favorable for the species. Possibly at 25 °C, seedling count could be performed at 5 days after sowing (Figure 1). However, at 20 °C it is necessary to postpone this count, taking it 6 days after sowing.

According to Peske (2012), the first count of the germination test can be used as a vigor test, since the germination speed is reduced with the advance of the deterioration of the seed. Thus, samples with higher germination values at the first count can be considered more vigorous.

For Sena et al (2015) the first count test was efficient to detect the physiological quality of corn seeds. For Amaro et al. (2015), the first count test showed sensitivity to identify lots of bean seeds, cultivar Madrepérola, with different levels of vigor. The same results were found by Silva and Cícero (2014) in eggplant seeds, in the first count it was possible to verify differences that were not detected between the data of the final germination percentage.

## CONCLUSIONS

The germination test on chickpea seeds must be carried out on a paper roll substrate, at 20 °C and with the date of evaluation of the first count at 6 days after sowing.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Alves, C. Z., Silva, J. B., Cândido, A. C. S. (2015). Metodologia para a condução do teste de germinação em sementes de goiaba. *Revista Ciência Agronômica*, 46(3), 615-621. DOI: 10.5935/1806-6690.20150045.

Amaro, H. T., David, A. M. S. S., Assis, M. O., Rodrigues, B. R. A., Cangussu, L. V. S., Oliveira, M. B. (2015). Testes de vigor para avaliação da qualidade fisiológica de sementes de feijoeiro. *Revista de Ciências Agrárias*, 38(3), 383-389. DOI: https://doi.org/10.19084/rca.16943.

Andrade, R. N. B., Ferreira, A. G. (2000). Germinação e armazenamento de sementes de uvaia (*Eugenia pyriformis* Camb.) – Myrtaceae. *Revista Brasileira de Sementes*, 22(2), 118-125. DOI:http://dx.doi.org/10.17801/0101-3122/rbs.v22n2p118-125.

Araújo, E. F. L., Souza, E. R. B. (2018). Fenologia e reprodução de *Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae)*. *Scientific Electronic Archives*, 11(3), 166-175. DOI: https://doi.org/10.36560/1122018414.

Araújo, E. F. L., Souza, E. R. B., Peixoto, N., Júnior, F. G. G. (2019). Physiological potential of seeds and morphological

characterization of *Campomanesia adamantium* seedlings. *Revista Brasileira Fruticultura*, Jaboticabal, 41(4), 1-7. DOI: https://doi.org/10.1590/0100-29452019007.

Avelar, R. I. S., Costa, C. A., Rocha, F. S., Oliveira, N. L. C., Nascimento, W. M. (2018). Yield Of Chickpeas Sown At Different Times. *Revista. Caatinga*, 31(4), 900 –906. DOI: https://doi.org/10.1590/1983-21252018v31n412rc.

Barroso, C. M., Franke, L. B., Barros, I. B. (2010). Substrato e luz na germinação das sementes de rainha-do-abismo. *Horticultura Brasileira*, 28(2), 236-240. DOI: https://doi.org/10.1590/S0102-05362010000200018.

Bertolin, D. C., Sá, M. E., Moreira, E. R. (2011). Parâmetros do teste de envelhecimento acelerado para determinação do vigor de sementes de feijão. *Revista Brasileira de Sementes,* 33(1), 104 – 112. DOI: http://dx.doi.org/10.1590/S0101-31222011000100012.

Bewley, J. D., & Black, M. (1978). *Physiology and biochemistry of seeds in relation to germination*. Berlim: Springer-Verlag. DOI: https://doi.org/10.1007/978-3-642-68643-6.

Bilio, R. S., Guimarães, S. C., Caldeira, S. F. (2013). *Qualea grandiflora* Mart.: Temperatura na germinabilidade de sementes. *Ciência Florestal,* 23(1), 245-251. DOI: http://www.redalyc.org/articulo.oa?id=53425660022.

Brasil. (2009). *Regras para análise de sementes.* Brasília: Ministério da Agricultura, Pecuária e Abastecimento.

Carvalho, N. M., & Nakagawa, J. (2000). *Sementes: ciência, tecnologia e produção*. 4.ed. Jaboticabal: FUNEP.

Dias, L. B. X., Queiroz, P. A. M., Ferreira, L. B. S., Santos, W. V., Freitas, M. A. M., Silva, P. P., Nascimento, W. M., Araújo, E. F. L. (2019). Teste de condutividade elétrica e embebição de sementes de grão-de-bico. *Revista Brasileira de Ciências Agrárias*, 14(2), 1-8. DOI: http://www.agraria.pro.br/ojs-2.4.6/index.php?journal=agraria&page=article&op=view&path%5B%5D=agraria_v14i2a5641&path%5B%5D=5172.

Dousseau, S., Alvarenga, A. A., Guimarães, R. M., Lara, T. S., Custódio, T. N., Chaves, I. S. (2011). Ecofisiologia da germinação de sementes de *Campomanesia pubescens*. *Ciência Rural*, 41(8), 1362-1368. DOI: https://doi.org/10.1590/S0103-84782011000800011.

Ferreira, R. L., & Novembre, A. D. L. C. (2015). Teste de germinação de sementes de urucum (*Bixa Orellana L.*). *Multi-Science Journal*, 1(3), 46-52. DOI: 1 https://doi.org/10.33837/msj.v1i3.107.

Flores, A. V., Borges, E. E. L., Gonçalves, J. F. C., Guimarães, V. M., Ataíde, G. M., Barros, D. P., Pereira, M. D. (2014). Efeito do substrato, cor e tamanho de sementes na germinação e vigor de *Melanoxylon brauna*. *Pesquisa Florestal Brasileira*, 34(78), 141-14. DOI: https://doi.org/10.4336/2014.pfb.34.78.558.

Gomes, J. P., Oliveira, L. M., Ferreira, P. I., Batista, F. (2016). Substratos e temperaturas para teste de germinação em sementes de myrtaceae. *Ciência Florestal*, 26(4), 285-293. DOI: http://dx.doi.org/10.5902/1980509821120.

Kämpf An. (2000). Produção comercial de plantas ornamentais. Guaíba: Agropecuária.

Labouriau, L.G.; & Valadares, M.E.B. (1976). On the germination of seeds *Calotropis procera* (Ait.) Ait.f. *Anais da Academia Brasileira de Ciências*, v.48, n.2, (pp.263-284).

Maguire, J. D. (1962). Speed of germination aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, 2(1), 176-177. DOI: http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x.

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

Nascimento, W. M., Pessoa, H. B. S. V., Giordano, L. B. (1998). Cultivo Do Grão-de-bico (Cicer Arietinum L.). Brasília: Embrapa Hortaliças (Instruções Técnicas, 14).

Nascimento, W. M., Silva, P. P., Artiaga, O. P., Suinaga, F. A. (2016). Grão-de-bico. In: Nascimento, W. M. (Ed.). Hortaliças leguminosas. Brasília: Embrapa, (pp.89-118).

Nogueira, N., Ribeiro, M., Freitas, R., Gurgel, G., Nascimento, I. (2013). Diferentes temperaturas e substratos para germinação de sementes de Mimosa *caesalpiniifolia Benth*. *Revista de Ciências Agrarias - Amazon Journal of Agricultural and Environmental Sciences*, 56(2), 95-98. DOI: http://dx.doi.org/10.4322/rca.2013.015.

Oliveira Júnior, J. L., Neta, M. N. A., David, A. M. S. S., Aguiar, A. C. M., Gomes, A. G. O., Amaro, H. T. R., Donato, L. M. S. (2015). Umedecimento do substrato e temperatura na germinação e vigor de sementes de pitaya. *Comunicata Scientiae*, 6(3), 282-290. DOI: http://dx.doi.org/10.14295/CS.v6i3.746.

Peske, S. T., Villela, F. A., Meneghello, G. E. (2012). Sementes: fundamentos científicos e tecnológicos. 3 ed. Pelotas: Ed. Universitária/UFPel.

Rocha, C. R. M., Costa, D. S., Novembre, A. D. L. C., Cruz, E. D. (2014). Morfobiometria e germinação de sementes de *parkia multijuga benth*. *Nativa- Pesquisas Agrárias e Ambientais*, 2(1), 42-47. DOI: https://doi.org/10.31413/nativa.v2i1.1377.

Schulz, D. G., Fey, R., Herzog, N. F. M., Malavasi, M. M., Malavasi, U. C. (2013). Efeito da temperatura e substrato na germinação de sementes de açoita-cavalo (*Luehea divaricata* Mart.). *Revista de Ciências Agroveterinárias*, 12(1), 51-58. DOI: https://www.revistas.udesc.br/index.php/agroveterinaria/article/view/5197/3376.

Sena, D. V. A., Alves, E. U., Medeiros, D. S. (2015). Vigor de sementes de milho cv. 'Sertanejo' por testes baseados no desempenho de plântulas. *Ciência Rural*, 45(11), 1910-1916. DOI: http://dx.doi.org/10.1590/0103-8478cr20120751.

Shibata, M., Pavelski, L. G., Miranda, L., Oliveira, L. M. (2016). Germinação de sementes de *Mimosa flocculosa*, *Magistra*, 28(1), 131-136. DOI: https://magistraonline.ufrb.edu.br/index.php/magistra/article/view/356/240.

Silva, V. N., & Cicero, S. M. (2014). Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de berinjela. *Horticultura Brasileira*, 32(2), 145-151. DOI: http://dx.doi.org/10.1590/S0102-05362014000200004.

Torres, S. B., & Minami, K. (2000). Qualidade fisiológica de sementes de pimentão. Scientia agrícola, 57(1), 109-112. DOI: http://dx.doi.org/10.1590/S0103-90162000000100018.

Wilhelm, C. L. (2017). Densidade de plantas e posicionamento de sementes na cultura do milho. (Trabalho de conclusão de curso) Universidade Federal da Fronteira Sul, Brasil.

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