The methodology of the electrical conductivity test for *Carthamus tinctorius* L. seeds

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ABSTRACT: The electrical conductivity test stands out for generating quick responses for lot differentiation; however, studies are needed to improve this practice in safflower seeds. This study aimed to verify the efficiency and establish a methodology of the electrical conductivity test for safflower seeds. Initially, 12 lots were characterized regarding physical properties and physiological potential. For electrical conductivity, the experiment was carried out in three stages with the 12 lots: firstly, the soaking period was determined (2, 4, 6, 8, and 16 hours), then the volume was evaluated (50, 75, 100, 150, and 200 mL), and finally the number of seeds (25 and 50 seeds) was determined. The electrical conductivity test using 25 seeds in 50 mL of distilled water for six hours of soaking enables the differentiation of safflower seed lots. Multivariate analysis of principal components is efficient in discriminating the vigor of safflower seed lots.

Key words: deterioration, physiological potential, safflower, vigor

RESUMO: O teste de condutividade elétrica destaca-se por gerar respostas rápidas para diferenciação de lotes, no entanto, necessita-se de estudos visando o aprimoramento desta prática em sementes de cártamo. Objetivou-se verificar a eficiência e estabelecer metodologia do teste de condutividade elétrica para sementes de cártamo. Inicialmente, 12 lotes foram caracterizados quanto às propriedades físicas e potencial fisiológico. Para condutividade elétrica, o experimento foi efetuado em três etapas com os 12 lotes: primeiro determinou-se o período de embeciação (2, 4, 6, 8, e 16 horas), em seguida avaliou-se o volume (50, 75, 100, 150 e 200 mL) e por fim determinou-se a quantidade de sementes (25 e 50). O teste de condutividade elétrica utilizando 25 sementes embebidas em 50 mL de água destilada no período de seis horas possibilita a diferenciação de lotes de sementes de cártamo. A análise multivariada dos componentes principais é eficiente na discriminação do vigor de lotes de sementes de cártamo.

Palavras-chave: deterioração, potencial fisiológico, cártamo, vigor

HIGHLIGHTS:
The electrical conductivity test was adapted for safflower seeds; it is easily reproducible and allows reliable results.
The six-hour soaking period for safflower seeds represents a significant reduction in the seed conditioning period.
The use of 50 mL of water for the safflower electrical conductivity test is economical and practical in the routine analysis of seed laboratories.

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**Introduction**

The safflower (*Carthamus tinctorius* L.), originally from Asia and Africa, is a branched annual herbaceous plant belonging to the Asteraceae family, with a seed oil concentration between 35 and 50% (Flemmer et al., 2015). This oilseed is grown on all continents and has high economic value, as it has great versatility of properties (Sarto et al., 2018).

The dissemination is carried out by seeds. For several years, their physiological quality has been one of the most researched aspects because it is fundamental for making decisions regarding the storage and/or marketing of this product (Frandoloso et al., 2017). After maturation, the seeds are subject to a series of degenerative changes, thus reducing germination capacity as one of its final consequences (Sena et al., 2015).

Among the vigor tests considered most important by the ISTA (2014), the tests based on membrane permeability may be highlighted, with the electrical conductivity test, due to its objectivity and speed, as well as the ease of execution in most seed analysis laboratories, without major expenses in equipment and personnel training (Krzyzanowski et al., 1999). However, there is an established methodology of this test only for pea seeds.

The electrical conductivity test is based on the indirect evaluation of the organization of cell membrane state by determining the quantity of leachate released by the seeds in the soaking solution (Krzyzanowski et al., 1999). For safflower seeds, there is no adequate method to evaluate vigor. Its standardization and adaptation to each species are necessary, so it is easily reproducible and allows reliable results. Given the above, the purpose of this study was to verify the efficiency of the electrical conductivity test and establish a methodology to evaluate the physiological potential of safflower seed lots.

**Material and Methods**

Twelve lots of safflower seeds from the S-351 cultivar were obtained through experiments carried out in the 2017/2018 harvest at the School Farm of the Federal University of Jataí (Universidade Federal de Jataí), Jataí, GO, Brazil - 29°43’ S, 53°43’ W, and altitude of 670 m. The seed analyses were carried out in the Seed and Chemistry Laboratories of the same University.

According to the Rules for Seed Analysis (Brasil, 2009), the lots were initially homogenized using the soil divisor. Afterward, they were all packed in Kraft paper bags and placed in closed plastic bags (45 x 35 cm) to avoid dehydration. Then, they were placed in an acclimatized environment, at an average temperature and relative air humidity of 8 °C and 80%, respectively, until the moment of execution of the tests.

Soon after, the seeds were characterized for the following tests and determinations conducted in an entirely randomized design, except for the test of seedling emergence under field conditions that was conducted in randomized block design:

- **Moisture content** - determined by the oven method at 105 ± 3 °C for 24 hours with two sub-samples of 300 g of seeds for each lot (Brasil, 2009).

- **1000-seed weight** - seeds were weighed on a precision analytical scale (0.001 g) in four replicates of eight sub-samples of 100 seeds for each lot (Brasil, 2009), corrected for 8% humidity.

- **Germination** - eight replicates of 25 seeds were sown, on a substrate of blotting paper, on one sheet of paper moistened with a volume of water (mL) equivalent to 2.5 times the dry mass of the paper (g) (Brasil, 2009), covered with a sheet of the same paper, in a transparent plastic box (11.0 x 11.0 x 3.5 cm), with final counting at the eighth day, according to Gama et al. (2019). In the BOD-type germination chambers, after the installation of the tests, the transparent plastic boxes were kept in closed plastic to avoid dehydration. First Count - performed along with the germination, previously described, with counting at three days (Gama et al., 2019) including the number of normal seedlings according to the Brasil (2009).

- **Seedling emergence under field conditions** – it was carried out from November to December 2018 by sowing four sub-samples of 50 seeds per lot, in furrows of 1 m long, 2 cm deep, spaced with 45 cm between rows, and irrigated only on the day of sowing. The meteorological information of temperature and precipitation was made available by INMET (2019) during the conduction period (temperature: 23.2 ± 3 °C and relative air humidity of 84 ± 10%).

The evaluations were performed with the counting of emerging seedlings, and the results expressed in emergence percentage (Krzyzanowski et al., 1999). Also, the first count of the emergence was taken when 50% + 1 of the total seedlings of the lots emerged.

- **Emergence speed index** - through daily counts from the emergence of the first seedling to the stabilization of the emergence, calculated according to the methodology described by Maguire (1962).

- **Initial, final, mean time, and synchrony of seedling emergence** – from the daily count data of seedling emergence under field conditions, the initial (IT), final (FT), and mean time (MT), and synchrony (Z) of emergence were determined. For this, the formulas described in Santana & Ranal (2004) were used.

The methodology for the electrical conductivity test was performed in three stages, conducted in randomized block design:

- **First stage - period determination**: The aim was to determine the soaking time (2, 4, 6, 8, or 16 hours). Four samples of 50 seeds soaked in 75 mL were used for each lot, conducted in a factorial scheme, 12 lots x 5 times. It is important to emphasize that the quantity of 50 seeds was used in the volume of distilled water of 75 mL, due to being the most usual, as for pea seeds (ISTA, 2014). The soaking times were pre-established according to the soaking curve of safflower seed according to Gama et al. (2020) and appropriate for the laboratory working hours.

- **Second stage - determination of volume**: it consisted of determining the volume of soaking for the safflower seeds (50, 75, 100, 150, and 200 mL). For each lot, four samples of 50 seeds soaked in the period determined in the first stage were used, conducted in a factorial scheme of 12 lots x 5 volumes.

- **Third stage - determination of seed quantity**: the purpose was to indicate the number of seeds to perform the test. For
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The methodology of the electrical conductivity test for Carthamus tinctorius L. seeds was conducted in a factorial scheme of 12 lots x 2 number of seeds. The four sub-samples of each lot were randomly taken from the samples and subsequently weighed using an analytical scale with an accuracy of 0.1 mg. They were then placed in plastic cups to soak in distilled water in the volumes and periods mentioned above.

At the end of the programmed periods for the soaking, the electrical conductivity of the solution was determined with a benchtop conductivity meter, model CG 1800. After the readings, the electrical conductivity values per gram of seeds were calculated and the results expressed in μS cm⁻¹ g⁻¹ (Krzyzanowski et al., 1999).

The data from the initial quality characterization tests were submitted to normality and homogeneity tests, followed by analyses of variance for all characteristics evaluated. The means of treatment were compared using the Scott-Knott test at p ≤ 0.05. For electrical conductivity, the data were submitted to analysis of variance at p ≤ 0.05, and the means of the lots were compared by the Scott-Knott test. The data regarding the volumes and periods of soaking were submitted to regression analysis for linear and quadratic equations at p ≤ 0.05. The principal component analysis was also processed using the R software (R Core Team, 2017).

**Results and Discussion**

Among all the variables analyzed (Table 1), only germination, abnormal seedlings, dead seeds, and first germination count showed no statistical difference between lots. It is worth noting that there was no difference between the lots analyzed for the germination test results, whose germination was maintained between 87 and 95%. It is interesting not to have a difference between the germination because the reduction of germination power is one of the last steps of the deterioration sequence. In general, the average germination percentage obtained was higher than that found in the literature, which is 60% for safflower seeds (Freitas et al., 2018). The lots also did not differ in the higher percentage of 32% was recorded for seeds from lots 3 and 9, differing from those that presented 42% for 6 and 11, and 43% for the 12 (Table 1). The other lots did not differ between them, suggesting that there are differences in germination speed, being more vigorous, therefore, those with higher emergence speed (Santos et al., 2017).

For the data referring to the first emergence count, a percentage of 32% was recorded for seeds from lots 3 and 9, differing from those that presented 42% for 6 and 11, and 43% for the 12 (Table 1). The other lots did not differ between them, being higher than those mentioned, presenting a percentage above 46%. The highest emergence synchrony obtained was 0.59 for lot 7, which also presented one of the lowest 1,000-seed weight, reaffirming the verification of Marcos Filho (2016) that there was no difference between the lots analyzed for the 12 (Table 1). The other lots did not differ between them, showing no statistical difference between lots.

The 12 lots of safflower seeds showed moisture between 5.41 and 6.73% (Table 1). This similarity is essential so that the quality analysis tests are not affected by differences in metabolic activity and humidification speed (Silva et al., 2017). Girardi et al. (2013) found moisture content between 6.90 and 8.80% for safflower seed lots. Such values, recommended for oilseeds, once higher values can increase the respiratory process of the seeds, the mobilization of reserves, and the release of energy, thus accelerating the deterioration (Marcos Filho, 2016).

The seeds of lots 2, 3, 4, and 5 presented higher masses, while lots 8, 9, and 10 presented lower masses (Table 1). Omidi et al. (2012) evaluated the 1000-seed weight of safflower lots and found the highest value of 31.15 g. According to Marcos Filho (2016), the largest and most dense seeds usually have well-formed embryos and larger amounts of reserves, potentially the most vigorous. From what was reported by Brasil (2009), 1 g of safflower seeds corresponds to 30 seeds, so that 1,000 seeds correspond to 33 g, a value lower than the one that was observed in lots 1 to 5.

In the seedling emergence under field conditions, seeds from lots 1, 2, and 4 to 11 were most vigorous, with an average of 85% of emergence (Table 1). Menegaes et al. (2017) observed in safflower seeds an average of 87% of seedling emergence under field conditions, under conditions similar to those of this study. The emergence speed index classified the lots into three levels of vigor, with the seeds of the lot 12 being of poorer quality, those of the lot 3 intermediate, and the others with higher quality. Although the seed lots had similar germination percentages, there are often differences in emergence speed, suggesting that there are differences in vigor among them, being more vigorous, therefore, those with higher emergence speed (Santos et al., 2017).

For the data referring to the first emergence count, a percentage of 32% was recorded for seeds from lots 3 and 9, differing from those that presented 42% for 6 and 11, and 43% for the 12 (Table 1). The other lots did not differ between them, showing no statistical difference between lots.

**Table 1. Means of moisture content (MC), 1000-seed weight (1000SW), seedling emergence under field conditions (SEF), first count of the emergence (FCE), emergence speed index (ESI), mean time (MT) and synchrony (Z) of twelve safflower seed lots**

| Lots | MC (%) | 1000SW (g) | SEF (%) | FCE (%) | ESI | MT (d) | Z |
|------|--------|------------|---------|--------|-----|--------|---|
| 1    | 6.73   | 33.482 b   | 83 a    | 54 a   | 9.54 a | 4.18 c | 0.52 b |
| 2    | 6.00   | 37.222 a   | 84 a    | 49 a   | 9.54 a | 4.13 c | 0.48 b |
| 3    | 6.34   | 36.447 a   | 76 b    | 32 c   | 8.32 b | 4.53 b | 0.47 b |
| 4    | 6.27   | 38.230 a   | 84 a    | 46 a   | 9.20 a | 4.10 c | 0.44 c |
| 5    | 6.38   | 38.453 a   | 86 a    | 51 a   | 9.85 a | 4.01 c | 0.50 b |
| 6    | 6.14   | 30.004 c   | 87 a    | 42 b   | 9.64 a | 4.03 c | 0.45 c |
| 7    | 6.21   | 30.456 c   | 80 a    | 58 a   | 9.32 a | 4.35 b | 0.59 a |
| 8    | 5.41   | 27.966 d   | 89 a    | 46 a   | 9.89 a | 3.88 c | 0.42 c |
| 9    | 6.07   | 26.624 d   | 87 a    | 32 c   | 9.31 a | 3.97 c | 0.40 c |
| 10   | 5.46   | 28.096 d   | 86 a    | 56 a   | 9.38 a | 4.01 c | 0.51 b |
| 11   | 6.02   | 29.084 c   | 83 a    | 42 b   | 9.20 a | 4.17 c | 0.42 c |
| 12   | 5.62   | 28.745 c   | 86 c    | 43 b   | 7.53 c | 5.27 a | 0.50 b |
| CV (%) |        | 3.67       | 16.00   | 38.68  | 0.21  | 0.03  | 0.01 |

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at p ≤ 0.05; CV - Coefficient of variation.
median since this value can range from 0 to 1. Based on this observation, it was found that lots 4, 6, 8, 9, and 11 obtained the smallest emergence synchrony among all the lots.

The initial emergence time of safflower seeds was on the third day after sowing and lasted up to six days; the final time, however, showed no significant effect between the lots. The mean emergence time ranged from 3.88 to 5.27 days, classifying the lots into three levels of vigor, with the most vigorous lots being 1, 2, 4, 5, 6, 8, 9, 10, and 11 because they obtained the shortest mean time of emergence (Table 1). In lot 12, there was the longest mean time of emergence, resulting in less vigor. The other lots had intermediate values, classifying the seeds of them as average vigor.

During the seedling emergence test under field conditions, the temperature was favorable to meet the crop’s needs. According to Ramos et al. (2018), the favorable temperature range for safflower seeds at initial development is 15 to 30 ºC. The temperature ranged daily, between 20 and 35 ºC, with higher temperatures during the emergency stabilization period. However, the amount of precipitation was relatively low during the execution of the emergence test under field conditions. From October 23, 2018, to the end of the test, precipitations of only 2.4 to 12.2 mm were recorded. Bidgoly et al. (2018), when evaluating lots of safflower, found that the precipitations of only 2.4 to 12.2 mm were recorded. As a result, there was a better separation of the lots in quality levels, which differs from the data observed in the present study.

In two hours, there was a ranking in three levels of vigor. The seeds of lots 1 and 12 were detected as the least vigorous, with higher values of leached electrolytes. Lots 4, 10, and 11 stand out as the best quality lots with the most intact cell membrane system or with the greatest capacity to restore their integrity during soaking since lower conductivity values were recorded, indicating greater vigor. Lots 2, 3, 5, 6, 7, 8, and 9 exhibited intermediate behavior related to the physiological quality compared to the others (Figure 1).

In four hours of soaking, the ranking is similar to the previous period, in which with lower physiological quality are lots 1 and 12 with higher EC values, lots 4, 10, and 11 showed higher vigor and the other lots 2, 3, 5, 6, 7, 8, and 9 showed intermediate vigor.

Evaluating the 12 lots during the six-hour soaking period, it is possible to verify four levels of vigor, in which the lots 8, 10, and 11 stand out with greater physiological potential, therefore, with lower values of electrical conductivity, followed by lots 2 and 3, and lots 4, 5, 6, 7, and 9 that show intermediate vigor. Lot 1 shows a reduction in the cell membranes' reorganization capacity, characterizing it with the lowest vigor among the 12 lots.

Similarly, the eight-hour period stratifies the lots into four levels of vigor and again indicates lots 10 and 11 with greater vigor. In contrast, lot 1 still stands out as the one with the worst physiological quality. Lots 2, 3, 4, 5, 6, 7, 8, 9, and 12 were exposed with average vigor. Lot 1 continues to behave inferiorly to the others in 16 hours; lots 8 and 11 stand out again in terms of physiological quality. Lots 2, 3, 4, 5, 6, 7, 9, 10, and 12 demonstrated an intermediate physiological potential, thereby allowing the separation into three levels of vigor in 16 hours. As a result, there was a better separation of the lots in six and eight hours, where the lots are differentiated into four levels of vigor. It is observed that the electrical conductivity maintained the same tendency during these periods. It allows the test to be performed in the shortest possible time and, consequently, to obtain faster results.
Different periods have been observed for different seed species. The soaking period of six hours for safflower seeds represents a significant reduction in the period of seed conditioning concerning the period of 24 hours adopted by the research as a standard for pea tests (ISTA, 2014). This reduction in the period proposed by Soleymani (2019) for electrical conductivity in safflower seeds in the 24-hour period is fundamental. The possibility of reducing the time of the electrical conductivity test without affecting the results was also verified by Oliveira et al. (2012) for sunflower, belonging to the same family as safflower. After determining the six-hour period, the volume of soaking was evaluated for the 12 lots, and for this stage, there was also interaction among the factors evaluated. An increase in the distilled water volume results in the dilution of the solution in a linear manner (Figure 2).

In this way, with the use of the volume of 50 mL, higher electrical conductivity readings were obtained concerning the volume of 200 mL. This fact is justified since soaking in a larger volume of water implies greater dilution of the leachates. Consequently, there is a greater concentration of the solution by reducing the water content. Results in this direction were found by Oliveira et al. (2012) in sunflower seeds. Another influence of the volume of water used on the electrical conductivity test is that as the volume of water increases, the test loses sensitivity in the differentiation of the evaluated lots.

When the seeds were submitted to soaking in a volume of 50 mL, the lots were separated into six classes of vigor, classifying those of lot 1 with the lowest vigor. In this volume, seeds from lot 8 were graded with higher physiological quality among the 12 lots because less leaching of solutes was detected in the solution. Thus, lots 2 to 12 expressed an intermediate physiological quality.

The soaking of the seeds in 75 mL resulted in the separation of the lots in four levels of vigor, indicating seeds of lots 8, 10, and 11 with the highest vigor, and again those of lot 1 with the lowest vigor. The seeds of lots 2, 3, 4, 5, 6, 7, 9, and 12 exhibited a median behavior concerning vigor.

The soaking volumes of 100, 150, and 200 mL characterize the lots in two levels of vigor. Even not being the most appropriate volumes for the evaluation, Kaya et al. (2019) used 200 mL of distilled water in safflower seeds. In these three volumes, lots 1 and 2 exhibited similar behaviors, differing from the others with higher release of ions, therefore, with less vigor.

The volume of 50 mL of distilled water in the six-hour soaking period was efficient in ranking safflower seeds at different vigor levels compared to the higher volumes. Also, lower water use consists of the demand for savings and practicality in routine analyses of seed laboratories.

In the last stage, the number of seeds for the 12 lots of safflower was evaluated, setting the period of six hours and the volume of 50 mL. However, there was no interaction of factors at this stage, only an isolated effect of them.

Stratification of the lots was verified, being the seeds of lot 1 (206.24 μS cm⁻¹ g⁻¹) of low vigor with a higher quantity of leachates in the solution and the seeds of lots 2, 10, and 12 of intermediate vigor (182.06, 126.27 and 193.45 μS cm⁻¹ g⁻¹, respectively). The seeds of the other lots were classified as higher vigor (between 142.98 and 159.85 μS cm⁻¹ g⁻¹, in lots 10 and 4, respectively), with lower electrical conductivity in the evaluated solutions.

The number of seeds provided a significant difference between the electrical conductivity values, in which a higher value was obtained using 50 seeds (212.22 μS cm⁻¹ g⁻¹) and a lower value in the test with 25 seeds (115.24 μS cm⁻¹ g⁻¹), explained by the fact that the higher number of seeds will result in the greater release and, consequently, in a greater concentration of ions. The stratification of the lots with the lowest number of seeds corroborates the results obtained by Carvalho et al. (2009) in soybean seeds.

As one of the objectives of the laboratory tests is, besides the reduction of the period necessary to conduct the test, to reduce the volume of water and the number of seeds used, considering the results observed in the tests of means, it would be better to use 25 seeds per replicate, due to the cost of seeds.

The first two principal components had a variance of 44.83% and 29.14%, respectively, totaling 73.97% of the accumulated variance (Figure 3), demonstrating that the first two principal components explained a value greater than 70% of the total.

**Figure 3.** Scatter of eigenvectors along a circular plane and group formation obtained by principal component analysis according to the variables: first count of the germination (FCG), germination (G), seedling emergence under field conditions (SEF), first count of the emergence (FCE), emergence speed index (ESI), mean time (MT), synchrony (Z), electrical conductivity with 25 seeds (EC25) and with 50 seeds (EC50)

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safflower seeds lots components and evaluation of physiological quality of 12

Table 2. Matrix of eigenvectors and eigenvalues of principal components were higher than one.

According to Hongyu et al. (2015), the eigenvalues of these total sample variance and can be used to study the data set. Similarly, studies on vigor tests of Urochloa brizantha seeds (Silva et al., 2017) also found that two principal components were sufficient to explain 74.23 and 73.47% of the variance in the discrimination of variables.

The larger the indicative vectors of the variables, the greater the discriminatory power of these in the evaluation of the lots, the fact also verified in Table 2, in which the representative variables of the component are based on the modulus of the ratio 0.5/(λ₀.₅), being λ the eigenvalue of the component (Ovalles & Collins, 1988), to understand the importance of each variable in the construction of the two components adopted. The autovectors higher than 0.25 and 0.31 for components 1 and 2, respectively, were considered discriminating variables of the components.

Based on the results obtained by the principal component analysis (Table 2), it can be inferred that the variables germination, seedling emergence under field conditions, emergence speed index, mean time, synchrony, and electrical conductivity with 25 and 50 seeds had higher discriminatory power for the first principal component 1. The variables first germination count, first emergence count, emergence speed index, synchrony, and electrical conductivity with 25 seeds had higher discriminatory power for the second principal component.

Thus, it is observed the efficiency of the tests of electrical conductivity using 25 seeds in differentiating the physiological quality of the 12 safflower lots, since they are in the quadrant opposite of germination and vigor tests, so that the lower the germination, the higher the seedling emergence, the lower the emergence speed index, the lower the synchrony, and the lower the electrical conductivity.

Therefore, group 1 consists of lot 8 with seeds of higher viability and vigor by the previous tests. Group 2 was characterized by intermediate lots (4, 5, 6, 10, and 11) regarding the physiological quality. Group 3 comprises the seed lots with lower vigor (1, 2, 3, 7, 9, and 12) because it is in the quadrant opposite these variables. Also, it is possible to verify that the behavior of these lots was similar to that observed in the univariate analyses (Tables 1 and 2).

Conclusions

1. The electrical conductivity test using 25 seeds soaked in 50 mL of distilled water for six hours enables identifying lots with different vigor levels of safflower seeds.

2. Multivariate analysis of principal components is efficient in discriminating the vigor of safflower seed lots.

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