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Biology-Botany

Tetrazolium test adaptation for assessing safflower seed vigor and viability

Adaptação do teste de tetrazólio para avaliação de vigor e viabilidade de sementes de cártamo

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ABSTRACT

Safflower appears as an option for crop rotation in Brazil, which makes it an alternative to a well-consolidated system, which is the succession of soybean and corn. However, information is still needed, for instance, in seed analyses, for such inclusion of this species in the country. Thus, the objective was to adapt the tetrazolium test methodology to evaluate the viability and vigor of safflower seeds. The methodology for the tetrazolium test was adapted in three steps (preconditioning, staining, and determination of vigor levels of the seed lots). The experimental design was completely randomized. The data were subjected to analysis of variance at 5% and 1% probability levels by the F test, and the treatments were compared by Tukey test in the first and second steps and grouped by the Scott-Knott method in the third step, when principal component analysis was also used. The tetrazolium test is efficient to evaluate the quality of safflower seed lots. For conducting the tetrazolium test, safflower seeds should be preconditioned for 04h00 on paper at 25 °C, cut longitudinally, and stained for 01h00, with a concentration of 1% in the solution, at 30 °C.

Keywords: *Carthamus tinctorius* L.; Methodology; Seed quality; Viability; Vigor

RESUMO

O cártamo surge como opção de rotação de culturas no Brasil, o que o torna uma alternativa a um sistema bem consolidado, que é a sucessão da soja e do milho. Contudo, ainda são necessárias informações, por exemplo em análises de sementes, para tal inclusão desta espécie no país. Assim, objetivou-se adaptar a metodologia do teste de tetrazólio para avaliar a viabilidade e o vigor de sementes de cártamo. A

metodologia do teste de tetrazólio foi adaptada em três etapas (pré-condicionamento, coloração e determinação do nível de vigor dos lotes de sementes). O delineamento experimental foi inteiramente casualizado. Os dados foram submetidos à análise de variância à 5% e 1% de probabilidade pelo teste F. Os tratamentos foram comparados pelo teste de Tukey na primeira e segunda etapa e agrupados pelo método de Scott-Knott na terceira etapa, quando a análise de componentes principais foi usada. O teste de tetrazólio é eficiente para avaliar a qualidade de lotes de sementes de cártamo. Para a realização do teste de tetrazólio, as sementes de cártamo devem ser pré-condicionadas às 04h00, em papel a 25 °C, cortadas longitudinalmente e coradas por 01h00, com concentração de 1% na solução, a 30 °C.

Palavras-chave: *Carthamus tinctorius* L.; Metodologia; Qualidade das sementes; Viabilidade; Vigor

1 INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an oilseed crop with a high oil content (30-40%), cultivated for centuries in Iran and known for its resistance to drought and low temperatures, making it ideal for semi-arid environments. Its deep root system enables efficient absorption of water and nutrients, making it suitable for rotation with cereals and legumes (Abud et al., 2010; Bonamigo et al., 2013; Coelho et al., 2022; Cheng et al., 2024;). Besides oil production, safflower provides industrial dyes and supports health by controlling glucose and cholesterol levels. Morphological traits like the number and diameter of capitula and biomass are promising for developing high-yield cultivars, especially in regions affected by water scarcity (Cheng et al., 2024; Sabaghnia et al., 2024)a member of the Asteraceae family, is widely used in traditional herbal medicine. This review summarized agronomic conditions, genetic diversity, clinical application, and phytochemicals and pharmacological properties of safflower. The genetic diversity of the plant is rich. Abundant in secondary metabolites like flavonoids, phenols, alkaloids, polysaccharides, fatty acids, polyacetylene, and other bioactive components, the medicinal plant is effective for treating cardiovascular diseases, neurodegenerative diseases, and respiratory diseases. Especially, Hydroxysafflor yellow A (HYSA. Global safflower production faces expansion challenges, such as competition with other oilseeds and limited information on improved management practices. The leading producers globally—Kazakhstan, the USA, Russia, Mexico, and China—collectively

contribute about 79% of world production. In 2019, global safflower seed production was estimated at approximately 573,000 tons, with Kazakhstan leading at 35% of this total (Sajid et al., 2024; Subramani et al., 2021)is cultivated mainly for its seed, which is used for the extraction of high quality edible oil. Traditionally, the crop is grown for its flowers, used for paint industry, textile industry, flavouring foods and making dyes. Safflower is used as cut flowers and also having pharmaceutical potential for the treatment of male and female infertility, cardiovascular diseases, reduction in the blood glucose level, treatment of cancer and reduction in the plasma cholesterol level, etc. Despite having such significant potency, the crop has remained minor, neglected and underutilized. Therefore, there is a need of scientific community to focus the research on this crop and make it popularize as a commercial crop with various values added products. Safflower contains numerous chemical components (secondary metabolites.

Carthamus tinctorius is a species that has potential for inclusion in the well-consolidated system that is the succession of soybean and corn in Brazil, entering as an option for crop rotation, especially in the second season, popularly called 'safrinha'. This fact is corroborated by Ferreira-Santos et al. (2018) planting of safflower has increased because of its potential for cultivation under drought conditions during the off-season in Brazil and its high potential for use in biofuel production (Silva et al., 2021; Gama et al., 2023). This crop has high resistance to lack of water and low relative humidity, adapts to different soil types and tolerates from the highest to the lowest temperatures, except frosts, which enables its cultivation virtually all year round and in different regions of Brazil (Bonamigo et al., 2013). Thus, Gama et al. (2023) state that studies focusing on the evaluation of different aspects of safflower germination and production are promising, as they can shed light on issues related to seed composition, post-embryonic development, seed quality, among others.

In literature, there are several studies on tests for evaluating seed germination and seedling development (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009; Gama et al., 2019; Gama et al., 2023), cold test (Coelho et al., 2021),

accelerated aging test (Coelho et al., 2022), and electrical conductivity test (Silva et al., 2021). However, when evaluating seed lots, it is necessary to perform more than one vigor test (Coelho et al., 2021; Coelho et al., 2022; Silva et al., 2021), in addition to tests that provide quick results for decision-making. Hence the importance of having appropriate methodologies for these evaluations, such as the tetrazolium test, which does not yet have an established methodology for safflower seeds.

Seed quality is evaluated so that the germination process occurs in the best possible way. Seed germination is a vital stage of the plant life cycle, constituting the main form of propagation (Mohamed et al., 2018). Thus, it is necessary to evaluate the vigor of the seeds, and the tetrazolium test stands out, which has been increasingly used in laboratory routines due to its speed in obtaining results and the amount of information expressed on seed quality, as can be observed for sunflower (Nobre et al., 2014), an oilseed like safflower and with an already established methodology.

According to Oliveira et al. (2016), the use of the tetrazolium test to estimate viability becomes essential when the goal is to obtain quick answers about seed quality, because the germination test for example requires days to obtain the results, while the result of the tetrazolium test is obtained in a shorter time. This classification of seeds based on viability is commonly used in tropical forage grasses for commercialization, even replacing the germination test (MAPA, 2010). According to Gaspar-Oliveira et al. (2011), several factors may interfere in obtaining satisfactory results in the tetrazolium test, especially those related to the execution methodology such as seed preparation and preconditioning before staining, concentration of the tetrazolium solution, temperature and period of exposure to the solution, and interpretation criteria. Thus, the objective was to adapt the tetrazolium test methodology to evaluate the viability and vigor of safflower seeds.

2 MATERIAL AND METHODS

The present study was carried out in the Seed Laboratories, was conducted using seeds of the safflower cultivar S-351 from 12 lots, produced in an experimental field in Jataí – Goiás located at latitude 17º 52' 53"S and longitude 51º 42' 52"W. As recommended by the Rules for Seed Analysis - RAS (MAPA, 2009), the lots were initially homogenized, using a soil-type divider, passing three times through the device. Subsequently, all were packed in Kraft-type paper bags and placed in low-density polyethylene plastic bags (45 x 35 cm), which were closed to avoid water losses. Then, they were kept in an air-conditioned environment (3 months), at an average temperature and humidity of 8 ºC and 80%, respectively, until the time of performing the following evaluations and determinations:

Morphological description of seeds - The external and internal morphological characteristics of the seeds from one lot were observed in greater detail, with the aid of a table magnifying glass and a stereoscope microscope 4x. Transverse and longitudinal cuts were made with steel blades after softening and rehydrating the seeds.

Moisture content - Determined by the oven method at 105 ± 3 ºC for 24h00 with two subsamples of 5 g of seeds (MAPA, 2009).

Thousand-seed weight - Four replicates of eight subsamples of 100 seeds were weighed on a precision analytical scale 0,0001 g (MAPA, 2009).

Germination - The test was set up with eight subsamples of 25 seeds, between paper, sown on a sheet of germitest filter paper moistened with a volume of water (mL) equivalent to 3 times the mass of the dry paper (g). It was set up inside transparent plastic boxes (11.0 x 11.0 x 3.5 cm), placed in plastic bags and taken to germination chambers at 25 ºC, where they were exposed to 8 hours of light and 16 hours of darkness to simulate optimal germination conditions, with the first count performed on the 3rd day and the final count performed on the 8th day after sowing (Gama et al., 2019).

The adaptation of methodology of the tetrazolium test was conducted in a completely randomized design and divided into three steps, as described below:

Step 1 - Preconditioning of Seeds

Safflower seeds from three lots with different germination percentages (95, 86 and 77%) representing the maximum (Lot 1), average (Lot 2), and median (Lot 3) germination rates, respectively, determined according to Gama et al. (2019) as described above, were subjected to three methods of preparation: Intact seed (with pericarp and seed coat); Seed with cut in the distal region to the hilum; and Seed soaked with subsequent longitudinal cut, without separating the cotyledons.

After the preparation methods, four replicates of 25 seeds of each method and lot were placed to soak for 04h00 and 06h00 at 25 ºC. This pre-hydration was then tested using two methods: between paper (P) and in water (W), being considered independent experiments in three x three x two factorial scheme, corresponding to three lots, three preparation methods and two soaking periods.

To evaluate the efficiency of the preconditioning methods, the seeds were placed to stain in a tetrazolium solution with concentration of 1% for 04h00 at 30 °C in the darkness – *Helianthus annus* L. adaptation, and this concentration is the most usual in the RAS (MAPA, 2009).

The treatments were subjected to Tukey test was used at 5% probability using Sisvar software (Ferreira, 2019), and the best treatment was the one that promoted the brightest and most uniform color for most of the seeds evaluated. Since color is a parameter evaluated visually, a scale of scores from zero to two was assigned: zero for unstained seeds, one for partially stained seeds and two for completely stained seeds. The best treatment of safflower seeds for the tetrazolium test established in this step was used in the next step.

Step 2 – Seed Staining

This step was conducted in a three x two x two factorial scheme, in which three lots of safflower seeds were stained at two different concentrations (half percent and 1%) and for two periods (01h00 and 02h00) at 30 °C, with four replicates.

In this step, statistical analysis by Tukey was used at 5% probability using Sisvar software (Ferreira, 2019), test was also performed, attributing the classification of viable – seeds without damage; viable vigorous – seeds with damage but not close to the embryonic axis and that do not compromise more than 50% of the cotyledon tissue; viable non-vigorous – damage closer to the axis that compromises the cotyledons; non-viable – seeds with damage to the embryonic axis or dead tissue (white). With this classification, the best concentration and staining period were identified. The best result established in this step was used in the next step of this research.

Step 3 – Levels of Vigor

First, 12 lots of safflower seeds were subjected to determination of moisture content and thousand-seed weight, germination and first count, as described above. After performing the aforementioned steps, the 12 lots were used to obtain four replicates of 25 seeds to evaluate the vigor levels.

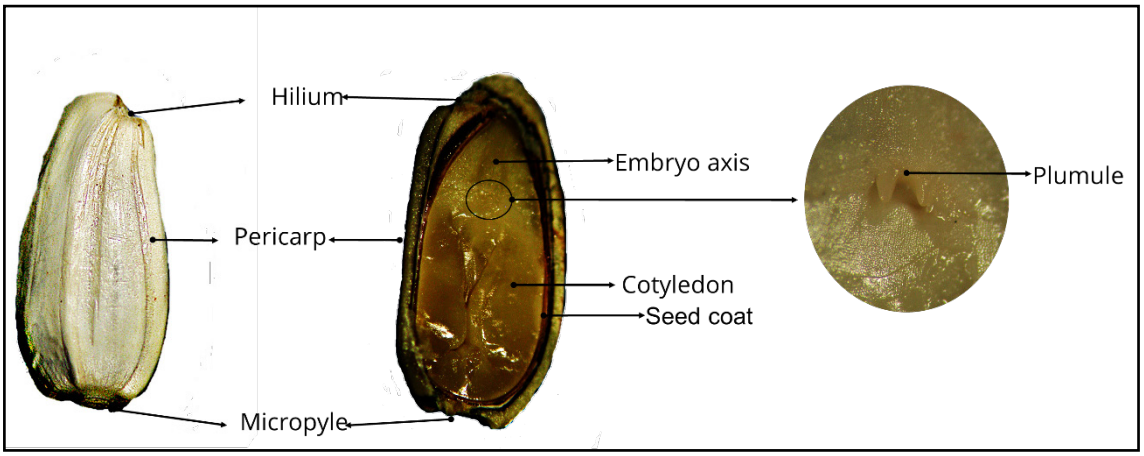
From the visual state of the seeds, five classes were assigned: Class 1 – High-vigor seeds; Class 2 – Medium-vigor seeds; Class 3 – Low-vigor seeds; Class 4 – Non-viable seeds; Class 5 – Dead seeds.

For the adaptation of vigor by the tetrazolium test (Step 3) for safflower seeds, the data were subjected to analysis of variance at 5 and 1% probability levels by the F test, and the treatments were grouped by the Scott-Knott method was used at 5% probability using Sisvar software (Ferreira, 2019).

3 RESULTS AND DISCUSSION

The safflower seed (Figure 1) has embryonic axis, plumule, cotyledons, seed coat and pericarp, the latter two being adhered, micropyle, where water absorption occurs, and hilum, the scar left when the seed detaches from the parent plant.

Figure 1 – External and internal characterization of the safflower seed



Source: Organized by authors (2024)

The dispersal unit of safflower plants, for botany, is the fruit itself, because the seed is located inside it, being classified as an achene (Galastri et al., 2010). Understanding the structures and functions of each component facilitates studies, such as the adaptation of methodology for the tetrazolium test, as a comprehensive understanding of all structures is essential for accurate interpretation.

Step 1 – Preconditioning of Seeds

In the first step of the adaptation of the tetrazolium test methodology, for the preconditioning of safflower seeds on paper (Table 1), differences were observed only in the interactions between lots and preparation methods for unstained seeds and between lots and soaking periods for partially stained and completely stained seeds.

In the evaluation of partial and completely stained seeds (Table 2), the preparation method was significant as a single factor and, among partially stained seeds, seed lot averages, those with cut in the distal region to the hilum (43%) stood out, followed by intact seeds (4%) and seeds with longitudinal cut (0%), the latter two not differing from each other. For completely stained seeds, the best results were obtained by those with longitudinal cut (100%), followed by those with cut in the distal region to the hilum (57%) and intact seeds (2%).

Table 1 – Unstained, partially stained and completely stained seeds in the preconditioning of three lots of safflower seeds on paper in the periods of 04h00 and 06h00 subjected to three preparation methods: Intact seed (with pericarp and seed coat); Seed with cut in the distal region to the hilum, and Seed soaked with subsequent longitudinal cut, without separating the cotyledons, for adapting the tetrazolium test methodology

Source of variation	Unstained seed (%)			Partially stained seed (%)		Completely stained seed (%)	
	Intact	Distal	Longitudinal	04h00	06h00	04h00	06h00
Lot 1	100 aA	0 aB	0 aB	15 abA	13 aA	51 abA	54 aA
Lot 2	89 cA	0 aB	0 aB	21 aA	16 aA	49 bB	55 aA
Lot 3	93 bA	0 aB	0 aB	12 bA	18 aA	56 aA	52 aA
Lot (L)	76.22**			123.56ns		28.67ns	
Preparation methods (M)	70187.55**			13763.56**		57474.67**	
Periods (P)	8.00ns			14.22ns		43.56ns	
L x M	76.22**			91.56ns		113.33ns	
L x P	0.66ns			184.89*		169.56*	
M x P	8.00ns			6.22ns		11.56ns	
L x M x P	0.66ns			80.89ns		85.56ns	
Coefficient of variation (%)	5.55			15.52		4.99	

Means followed by the same letter do not differ according to the Tukey test ($p < 0.05$). Lowercase letters (a) in the column compare lots for the same preparation method or soaking period; uppercase letters (A) in the row compare the preparation method or soaking period for the same lot. ns, * and ** not significant, significant at 5 and 1% probability levels, respectively

A percentage of unstained seeds was verified only for the method of preparation with intact seeds, among which the ones in lot 1 were higher than those in lot 3, and the seeds in these two lots were higher than those of lot 2. For partially stained seeds, the periods of 04:00 and 06h00 showed no difference but, among the lots, it was observed in the period of 04h00 that lot 2 was better than lot 3 and that lot 1 had intermediate values, not differing statistically from the others. For completely stained seeds, difference caused by soaking period was observed only in lot 2, with a lower percentage of stained seeds obtained with 04h00. In this period, it was possible to rank the lots: the percentage of stained seeds from lot 3 was higher than that of lot 2, and lot 1 did not differ from the others.

For the evaluation in water, there was interaction between the three factors for the three variables (Table 2). Higher percentages of unstained seeds were found in the preparation method with the intact seed, and seeds of lot 2 were inferior to the others in the period of 04h00, while those of lot 3 were inferior to the others in the period of 06h00. When comparing the periods, these lots were also the ones that differed statistically, with the lowest percentages of stained seeds in 04h00 for lot 2 and in 06h00 for lot 3. For partially stained seeds, there was a greater occurrence in the method with cut in the distal region, and this percentage was higher in the period of 04h00. The lots could be ranked as follows: lot 2 was superior, but did not differ from lot 3, and lot 3 did not differ from lot 1.

Seeds with cut in the distal region, in the period of 04h00, obtained lower percentages of completely stained seeds, but it was possible to rank the lots: seeds of lots 1 and 3 were superior to those of lot 2. For the seed preparation method with longitudinal cut, there was no difference between the periods, 04:00 and 06h00. However, it should be pointed out that, in the period of 06h00, it was possible to observe with the naked eye excessive staining of some seeds, which could hinder the reading and interpretation of the test.

The treatment with longitudinal cut was superior, as 100% of the seeds were stained regardless of the period, lot used or soaking method. Similar behavior was observed by Gaspar-Oliveira et al. (2009) with castor bean seeds, because the longitudinal cut favored the contact of the inner part of the seed with the tetrazolium solution, resulting in uniform staining.

The treatments with intact seeds and seeds with cut in the distal region to the hilum were inferior, because they showed lower percentages of completely stained seeds, compared to the treatment of seeds soaked with longitudinal cut. This occurred for both methods of pre-hydration (paper and water) and soaking periods (04:00 and 06h00) (Tables 1 and 2), at 25 °C, a temperature used because it is recommended for sunflower, which belongs to the Asteraceae family as safflower, as well as the

temperature for staining, which was 30 °C (MAPA, 2009). It is worth pointing out that the seeds were placed in the 1% tetrazolium solution for 04h00 to optimize the progress of the other steps.

In addition to the treatments with intact seeds and seeds cut in the distal region not being stained efficiently, as the tetrazolium solution does not distribute evenly throughout the seed, the treatment with cut in the distal region to the hilum caused severe damage to the cotyledons, which made the evaluation difficult, rendering it unfeasible.

The treatment with intact seeds in the tetrazolium solution, without any cut, was also not satisfactory, because there was physical impediment for the solution to enter the seeds (MAPA, 2009). Safflower seeds have thick pericarp and seed coat adhered to it (Abud et al., 2010), which possibly gives them this impermeability to the tetrazolium solution. The pericarp makes cutting difficult and the procedure can damage the internal structure of the seeds when they are not placed to soften previously.

Considering the two soaking periods, 04h00 and 06h00, and the two methods of pre-hydration, on paper and in water (Tables 1 and 2), it was verified that all periods and methods were efficient for the treatment with longitudinal cut. Thus, this treatment, the period of 04h00 and the pre-hydration on paper were chosen to be used in the next steps of the study, due to their effectiveness and optimization of laboratory logistics; in addition, seeds of this species have low values of moisture content (Gama et al., 2019), and the soaking on paper may reduce possible damage caused by the rapid entry of water into the seeds.

Table 2 – Unstained, partially stained and completely stained seeds in the preconditioning of three lots of safflower seeds in water in the periods of 04:00 and 06h00 in the preparation for adapting the tetrazolium test methodology

	Unstained (%)					
Variation Sources	**04h00**			**06h00**		
	Intact	**Distal**	**Long.**	**Intact**	**Distal**	**Long.**
Lot 1	97 aAA	0 aBA	0 aBA	94 aAA	0 aBA	0 aBA
Lot 2	86 bAB	0 aBA	0 aBA	91 aAA	0 aBA	0 aBA
Lot 3	94 aAA	0 aBA	0 aBA	82 bAB	0 aBA	0 aBA
Lot x Preparation Method x Soaking Time						48.22**
Coefficient of Variation (%)						8.10
Partially stained (%)						
Lot 1	3 aBA	69 bAA	0 aBA	4 bBA	48 aAB	0 aBA
Lot 2	10aBA	82 aAA	0 aBA	6 bBA	39 aAB	0 aBA
Lot 3	6 aBB	79 abAA	0 aBA	18 aBA	41 aAB	0 aCA
Lot x Preparation Method x Soaking Time						133.00**
Coefficient of Variation (%)						11.76
Completely stained (%)						
Lot 1	0 aCA	31 aBB	100 aAA	2 aCA	52 aBA	100 aAA
Lot 2	0 aCA	18 bBB	100 aAA	3 aCA	61 aBA	100 aAA
Lot 3	0 aCA	21 aBB	100 aAA	0 aCA	59 aBA	100 aAA
Lot x Preparation Method x Soaking Time						89.88*
Coefficient of Variation (%)						4.25

Means followed by the same letter do not differ according to the Tukey test ($p < 0.05$). Lowercase letters (a) in the column compare lots for the same preparation method; uppercase letters (A) in the row compare preparation methods for the same lot; Underlined uppercase letters (A) in the row compare soaking period for the same preparation method. * and ** significant at 5 and 1% probability levels, respectively

Step 2 - Seed Staining

After adopting the best treatment of the previous step, mentioned above, in the second step (Table 3) the seeds were then subjected to staining, testing two concentrations (0.5 and 1%) and two periods (01h00 and 02h00) at 30 °C. The evaluation of non-viable seeds showed effect only of the single factors lots (Pr>Fc 0.0065) and period (Pr>Fc 0.0196). Higher percentages of non-viable seeds were found in lots 2 and 3 (6 and 4%, respectively), and the latter did not differ from lot 1 (2%). In the period of

01h00 in the tetrazolium solution, 5% of the seeds were non-viable, while 3% of non-viable seeds were observed in the period of 02h00.

Table 3 – Staining of three lots of safflower seeds at concentrations of 1 and 0.5%, in the periods of 01h00 and 02h00, in the staining step for adapting the tetrazolium test methodology

Variation Sources	Vigorous viable (%)			
	01h00		02h00	
	0,5%	1,0%	0,5%	1,0%
Lot 1	80 aAA	80 aAA	58 aAB	52 bAB
Lot 2	73 aAA	66 aAB	72 aBA	91 aAA
Lot 3	66 aAA	67 aAB	59 aBA	85 aAA
Batches x Concentration x Soaking time				3.50*
Coefficient of Variation (%)				4.24
Non-vigorous viable seeds (%)				
Lot 1	16 aAB	19 aAB	41 aAA	48 aAA
Lot 2	20 aAA	26 aAA	24 bAA	5 bBB
Lot 3	28 aAB	29 aAA	41 aAA	9 bBB
Lot x Concentration x Soaking time				4.11*
Coefficient of Variation (%)				11.09

Means followed by the same letter do not differ according to the Tukey test ($p < 0.05$). Lowercase letters (a) in the column compare lots for the same preconditioning; uppercase letters (A) in the row compare preconditioning for the same lot; Underlined uppercase letters (A) in the row compare soaking period for the same preconditioning. * and ** significant at 5 and 1% probability levels, respectively

The similar viability between the lots points to the need for differentiation by classes (viable, viable vigorous, viable non-vigorous and non-viable). For vigorous viable seeds, there is no difference in using the concentrations of 1% and half percent for the period of 01h00 of exposure; difference was only observed in lots 2 and 3 compared to lot 1, with the first two being superior, for a concentration of 1% for 02h00, with a decrease in vigorous seeds when comparing the concentrations, which may underestimate the result. Difference was observed for the concentration in the period of 02h00, when lots 2 and 3 had lower percentages of vigorous viable seeds at half percent, when compared to these lots at 1%. In the comparison between the soaking periods, a higher percentage of viable vigorous seeds in lot 1 at both concentrations

was observed in the period of 01h00, when compared to 02h00. For lots 2 and 3, lower percentages of vigorous viable seeds at 1% were obtained in the period of 01h00, when compared to the period of 02h00.

For non-vigorous viable seeds, difference was observed between the lots, with 1 and 3 having higher percentages than lot 2, for a concentration of half percent in the period of 02h00. There was also difference in lot 1, which had higher percentage of non-vigorous viable seeds compared to lots 2 and 3, for a concentration of 1% in the period of 02h00. Difference between concentrations was found in the period of 02h00, when lots 2 and 3 had higher percentages of non-vigorous viable seeds at half percent when compared to 1%.

In the comparison between soaking periods, a higher percentage of non-vigorous viable seeds in lot 1 at both concentrations was observed in the period of 02h00, when compared to 01h00. Lot 2 obtained lower percentages of non-vigorous viable seeds at 1% for the period of 02h00, when compared to the period of 01h00. For lot 3, there were differences in both periods and for both concentrations, with a higher percentage of non-vigorous viable seeds at half percent for 02h00 and at 1% for 01h00.

In a first observation of the results in the table 3, it was possible to indicate the period of 02h00 at both concentrations tested for seeds classified as non-vigorous and at 1% for seeds classified as vigorous, as the lots could be ranked. However, by relating the results to the germination test of these lots, that is, 95, 86 and 77%, respectively, it is possible to infer that the marked staining in the period of 02h00 made the evaluation difficult, causing incorrect differentiation of the lots, since the lot with the highest germination, lot 1 with 95%, was classified as of worse quality in the classification of vigorous seeds. This result points out the peculiarity of the tetrazolium test, which requires visual observation, familiarity of the analyst with the morphology of the studied seed, and ability to interpret the test, as corroborated by Silva et al. (2013), Silva et al. (2016), and França and Krzyzanowski (2018).

Step 3 – Levels of Vigor

All the necessary steps to conduct the tetrazolium test for safflower seeds were performed, with preconditioning of the seeds for 04h00 on paper, at 25 °C, and staining for 01h00, with a concentration of 1% in the solution at 30 °C. Thus, the lots could be ranked according to vigor levels (Table 4).

After this adaptation of the methodology to evaluate safflower seeds in the first and second steps, the third step was performed with the 12 lots of this crop, which showed difference of up to 25 percentage points in germination. In this initial characterization of the 12 lots of safflower seeds, their moisture contents were 5.36 to 6.74%, values close to those found by Gama et al. (2019).

As for thousand-seed weight, the lots were ranked in three classes, with seeds of lots 1, 2, 5, 6 and 9 having the highest values, followed by those of lots 3, 4, 10 and 11, whereas those of lots 7, 8 and 12 were the lightest ones. The variation among the lots was between 29.75 g (Lot 8) and 35.25 g (Lot 2), with an average of 32.75 g, which made it possible to infer that one kilogram contains approximately 30,534 seeds, a value similar to that observed by Abud et al. (2010) and also to what is described in the RAS, according to which one gram contains 30 seeds of *C. tinctorius* L. (MAPA, 2009).

Germination ranged from 70% (Lot 10) to 95% (Lot 1), but there is not yet an established standard in the legislation for the germination of safflower. However, the lots used showed germination higher than 60%, which is the minimum recommended for the production and commercialization of seeds of species of major crops registered in the National Register of Cultivars (RNC) without specific standard (MAPA, 2013). For first germination count, there was a greater differentiation of the lots. Seeds of lot 4 were the most vigorous, followed by those with medium-high vigor of lots 2, 5, 6, 7, 9 and 12; seeds of lots 3, 8 and 11 have medium-low vigor and those of lots 1 and 10 have low vigor. Thus, the lots were differentiated into two classes of viability, with lots from 1 to 7, together with 12, having seeds of higher germination compared to the others. The loss of vigor is noticeable in seed lots well before germination, a fact

observed in studies with safflower (Coelho et al., 2021; 2022), with these losses linked to characteristics specific to the deterioration of each lot.

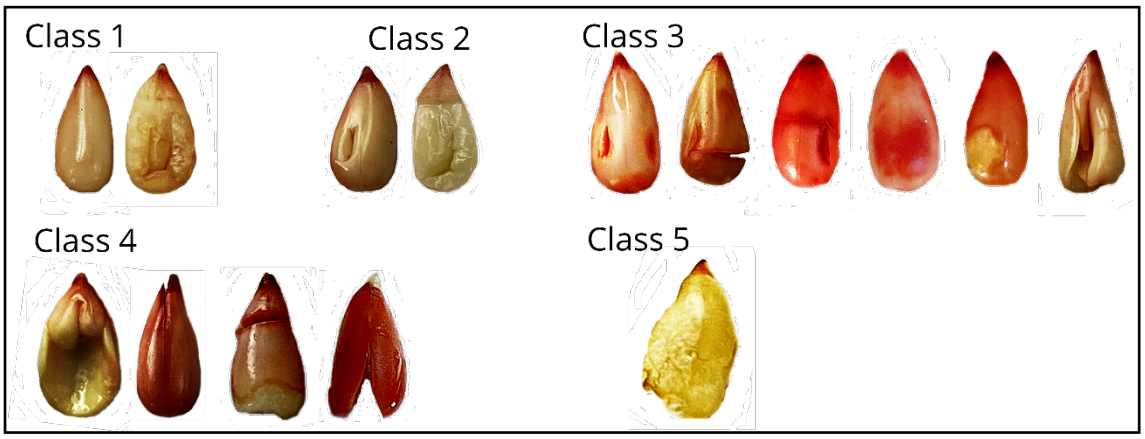
Table 4 – Moisture content (M), thousand-seed weight (TSW), germination (G) and first germination count (FGC) of the 12 lots used in the tetrazolium test for safflower seeds

Lots	M (%)	TSW (g)	G (%)	FGC (%)
L1	5.75	35.13 a	95 a	29 d
L2	5.68	35.25 a	94 a	60 b
L3	5.38	32.98 b	94 a	48 c
L4	6.87	32.58 b	91 a	77 a
L5	5.36	33.80 a	91 a	54 b
L6	5.83	33.71 a	89 a	51 b
L7	5.50	31.19 c	86 a	66 b
L8	6.05	29.75 c	82 b	50 c
L9	5.75	33.93 a	77 b	60 b
L10	5.79	32.79 b	70 b	39 d
L11	6.74	32.13 b	78 b	50 c
L12	6.07	29.77 c	89 a	62 b
F	-	8.05**	6.61**	7.87**
C.V. (%)	-	3.90	7.16	16.44

Means followed by the same letter in the column do not differ according to the Scott-Knott test ($p < 0.05$). Values followed by ** show significant difference by the F test at 1% significance level - not subjected to statistical analysis. F: F value. C.V.: Coefficient of variation

In the ranking of the lots by the tetrazolium test (Figure 2), the classification was made based on the visual appearance of the tissues. For high-vigor seeds (class 1), firm tissues with uniform color were observed, while the classification of medium vigor (class 2) comprised seeds that showed firm tissues with uniform color and minor damage to the basal part of the cotyledon. The low-vigor class (class 3) contained seeds with firm tissues and minor damage to the central part, with no damage to the plumule or embryonic axis, as well as those with major damage to the basal part of the cotyledon (< 50%) and malformed.

Figure 2 – Classification of safflower seeds preconditioned on paper for 04h00 at 25 °C and stained at 1% for 01h00 at 30 °C. Class 1 (high vigor), class 2 (medium vigor), class 3 (low vigor), class 4 (non-viable), and class 5 (dead)



Source: Organized by authors (2024)

Non-viable seeds (class 4) were those with damage affecting more than 50% of the cotyledons, damage to the region of transition between cotyledons and embryonic axis, and damaged or dead plumule, in addition to severe malformation of the reserve tissue. Finally, there was the class of dead seeds (class 5), which had softened tissues and completely whitish color.

The classification in the ranking of the lots by the tetrazolium test is similar to the methodology consolidated by França and Krzyzanowski (2018), routinely performed in seed analysis laboratories for soybean crop, which is also a dicotyledon and oilseed like safflower.

The lots of safflower seeds were also evaluated for viability and classified into vigor classes (Table 5). Lots 1, 3, 4, 7, 8, 9 and 11 had a higher number of viable seeds (91 to 96%). The opposite was observed for lots 2, 5, 6, 10 and 12, which had lower percentages of viable seeds (81 to 88%) and higher percentages of non-viable seeds.

Table 5 – Viability and vigor classes of 12 lots of safflower seeds by the tetrazolium test

Lots	Viability seeds (%)		Vigor classes seeds (%)				
	Viable	Non-viable	High	Medium	Low	Non-viable	Dead
L1	95 a	5 a	43 a	29 b	23 b	5 a	0 a
L2	88 b	12 b	29 b	30 b	29 b	12 b	0 a
L3	92 a	8 a	13 c	50 a	29 b	8 a	0 a
L4	92 a	8 a	21 b	52 a	19 b	8 a	0 a
L5	85 b	15 b	19 c	43 a	24 b	15 b	0 a
L6	87 b	13 b	15 c	44 a	28 b	13 b	0 a
L7	92 a	8 a	19 c	48 a	25 b	7 a	1 a
L8	96 a	4 a	24 b	44 a	28 b	4 a	0 a
L9	91 a	10 a	13 c	45 a	32 b	10 a	0 a
L10	88 b	12 b	16 c	37 b	35 b	12 b	0 a
L11	95 a	5 a	3 d	37 b	56 a	5 a	0 a
L12	81 b	19 b	15 c	33 b	33 b	19 b	0 a
F	3.22**	3.29**	16.30**	2.96**	3.20**	3.53**	2.00ns
C.V.	2.79	24.77	12.75	11.13	16.39	24.24	16.26

Means followed by the same letter in the column do not differ according to the Scott-Knott test ($p < 0.05$). Values followed by ns and** not significant and significant at 1% probability levels, respectively. F: F value. C.V.: Coefficient of variation (%)

For the class of high vigor, lot 1 was superior to the others and it was also the one with highest viability. For low-vigor seeds, lot 11 performed poorly, with a large amount of low-vigor seeds, and the other lots did not differ from each other.

Finally, for dead seeds, no difference was observed between the lots.

For class 1 and 2, high-vigor seeds (Lot 1) and medium vigor (Lot 4 to 9), comprised seeds that superior vigor. From class 3, low-vigor seeds, the seeds showed a more intense and not always uniform color, called strong carmine red. The occurrence of intense red, according to França and Krzyzanowski (2018), is a characteristic of deteriorating tissues, which allow greater diffusion of the tetrazolium solution through the compromised cell membranes, so these seeds are considered less vigorous.

Non-viable seeds were in class 4, with a significant amount of malformation and damage to the regions near the embryonic axis and plumule, which are considered

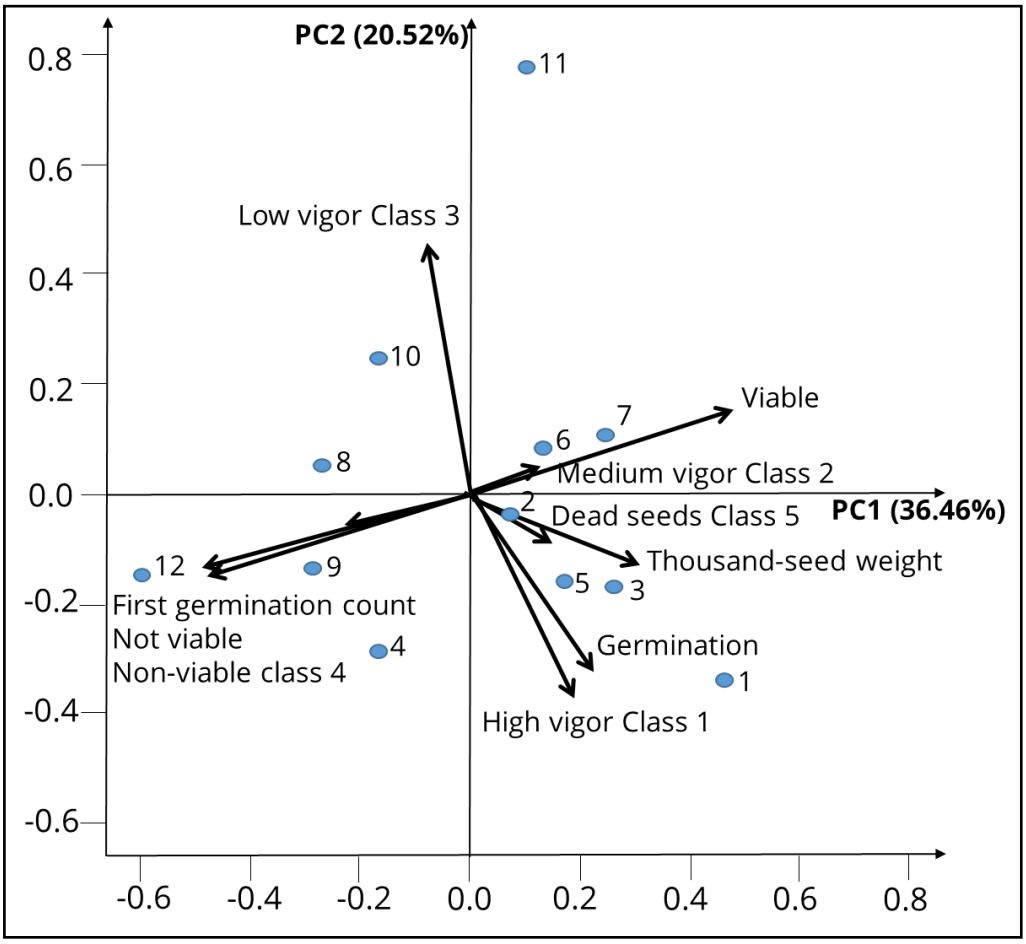
vital, for instance by Silva et al. (2016) in the development of criteria for conducting the tetrazolium test in araucaria seeds. The two regions mentioned are of fundamental importance for seedling development, which caused them to be considered vital since, when damaged, the seeds are no longer viable, because they originate abnormal seedlings or even no seedlings.

Dead seeds (class 5) were softened and whitish, as the whitish spots found in seeds of the other classes, pointing to the death of these tissues. França and Krzyzanowski (2018) made sure that the white color identifies dead tissues, which do not have the enzymatic activity necessary for the production of triphenylformazan.

In the principal component analysis (Figure 3), regarding the classification of the safflower seed lots into vigor classes, lot 1 was superior, with a higher percentage of high-vigor seeds, being the one which also had the highest percentage of germination and standing out among those with the highest thousand-seed weights. On the other hand, seeds from lot 12 had the lowest values of thousand-seed weight and percentage of high-vigor seeds, being mostly classified as with medium and low vigor, in addition to non-viable seeds. On the other hand, seeds from lot 11 were classified as with low vigor, with the lowest values of thousand-seed weight, germination and first count. These data are consistent with the results found in the univariate analyses (Tables 4 and 5).

The adaptation of methodology and classification by the tetrazolium test for seeds in promising crops in the country, such as safflower, is of paramount importance because, according to França and Krzyzanowski (2018), this test is widely adopted for seed quality control in Brazil, assuming proportions never recorded even in the countries where the test was developed.

Figure 3 – Biplot dispersion plane with circle of eigenvectors obtained by the analysis of two principal components (PC1 and PC2) established based on the characteristics of initial physiological quality and vigor classes of 12 lots of safflower seeds



Source: Organized by authors (2024)

4 CONCLUSIONS

The tetrazolium test is efficient in evaluating the quality of safflower seed lots. Tetrazolium test should be conducted with preconditioning of seeds for 04h00, on paper at 25 °C, longitudinal cut and staining for 01h00, with concentration of 1% in the solution, at 30 °C. Based on the results obtained from the tetrazolium test conducted in the present experiment, the Lot 1 reached seeds with greater viability and vigor.

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