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Study on some aspects of seed vigor for sesame

[1] Lorena Leão de Jesus, [1] Marcela Carlota Nery, [1] Adriana de Souza Rocha, [1] Sara Michelly Cruz, [2] Cíntia Maria Teixeira Fialho, [3] Fernanda Carlota Nery, [4] Denise Cunha Fernandes dos Santos Dias

[1] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Rodovia MGT 367, Km 583, n 5.000 Alto da Jacuba, CEP 39100-000, Diamantina, MG, Brasil. E-mail: lorenalj3@yahoo.com.br, nery.marcela@ufvjm.edu.br, saramichellycruz@gmail.com
[2] Universidade Tecnológica Federal do Paraná, Prolongamento da Rua Cerejeira, s/n, CEP 85892-000, Bairro São Luiz, Santa Helena, PR, Brasil. E-mail: cintiamtfialho@yahoo.com.br
[3] Universidade Federal de São João del-Rei, Praça Dom Helvécio, 74, Fábricas, CEP 36301-160, São João del-Rei, MG, Brasil. E-mail: fernandacarlota@yahoo.com.br
[4] Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n, Campus Universitário, CEP 36570-900, Viçosa, MG, Brasil. E-mail: dcdias@ufv.br

Abstract: The selection of high quality sesame seed lots is essential to obtain high productivity in the field. This assessment of the physiological potential of the seeds can be obtained by the vigor tests, as they are able to detect more subtle differences in the quality of the seed lots and are fast, when compared to the germination test. However, there are no standardized vigor tests for sesame cultivation. Thus, the objective of this study was to adapt the methodologies for the testing of electrical conductivity, accelerated aging and potassium leaching, in order to evaluate the quality of sesame seeds. For the electrical conductivity test, the seeds were submitted to imbibition periods of 4; 8; 12 and 16 hours, and a number of combinations of seeds/volume of water. For the potassium leaching test, the effects of immersion periods and a number of combinations of seeds/volume of water were evaluated. In the electrical conductivity test, it was demonstrated that the imbibition period of 4 hours and the combination of 25/75 mL of water and 50/75 mL of water is efficient to distinguish the physiological quality of sesame seeds. For the accelerated aging test, the aging periods of 48 hours by the traditional method or 72 and 96 hours with the saturated solution allow the separation of sesame cultivars in different levels of vigor. For the potassium leaching test, a combination of 25/25 mL and 60 or 90 minutes of imbibition is adequate to evaluate seed physiological potential.

Keywords: electrical conductivity, accelerated aging, potassium leaching.

Estudo sobre alguns aspectos do vigor da semente de gergelim

Resumo: A seleção de lotes de sementes de gergelim de alta qualidade é essencial para o obtenção de alta produtividade em campo. Essa avaliação do potencial fisiológico das sementes pode ser obtido pelos testes de vigor, pois estes são capazes de detectar diferenças mais sutis na qualidade dos lotes de sementes e são rápidos, quando comparados ao teste de germinação. No entanto, não existem testes de vigor padronizados para a cultura do gergelim. Assim, objetivo do trabalho foi adequar às metodologias do teste de condutividade elétrica, envelhecimento acelerado e lixiviação de potássio para avaliar a qualidade de sementes de gergelim. Na avaliação da condutividade elétrica, as sementes foram submetidas a períodos de embebição 4; 8; 12 e 16 horas e combinações número de sementes/volume de água. Para o teste de lixiviação de potássio foram avaliados os efeitos dos períodos de embebição e das combinações número de sementes/volume de água. As condições mais eficientes para a distinção da qualidade fisiológica das sementes de gergelim foram: teste de condutividade elétrica com período de embebição de 4 horas e a combinação de 25/75 mL e 25/75 mL; teste de envelhecimento acelerado com período de envelhecimento de 48 horas em água e 72 e 96 horas com solução saturada; teste de lixiviação de potássio com a combinação 25 sementes em 25 mL durante 60 ou 90 minutos.

Palavras chave: condutividade elétrica, envelhecimento acelerado, lixiviação de potássio.

Introduction

Sesame, *Sesamum indicum* L., belongs to the family Pedaliaceae and is one of the oldest known oleaginous seeds (Beltrão et al., 2010). The commercial product is its seeds, containing about 44% to 58% oil content, used for the production of biodiesel, cooking oil, paints, margarines, varnishes, soaps, cosmetics and medicines (Borchani et al., 2010).

For the success in agriculture and the establishment of suitable populations in the field, the use of high quality seeds is essential. However, there are no standardized vigor tests for sesame seeds that allow a rapid assessment of the physiological potential of its seeds.

The importance of studying and standardizing vigor tests is related to the fact that they are able to detect more subtle differences in the quality of seed batches, when compared to the germination test. Among the available vigor tests, those of electrical conductivity and accelerated aging are the most studied for several cultivated species, since they are efficient in the comparison of vigor and in the estimation of the storage potential of seed batches, showing a good relation with the emergence test for seedlings in field, in several cultivated species (Krzyzanowski et al, 1999). Another test less frequently used is potassium leaching; however, it has shown to be promising in the obtention of rapid information on seed vigor (Favarato et al., 2011).

Some studies aimed at adapting the electrical conductivity test to different species evaluated different seed numbers, water volumes and imbibition times. A number of 25 or 50 seeds is frequently used, as well as a water volume of 25 mL, 50 mL or 75 mL (Alves et al., 2012 & Oliveira et al., 2012). The recommended for large crops is the 24-hour imbibition period. However, it is possible to obtain results with only 6 hours for seeds of physic nut (Araújo et al., 2011) and 8 hours for canola (Milani et al., 2012).

Regarding the accelerated aging test, the interaction between exposure period and temperature is an important factor to verify the efficiency of the test. Small seeds absorb water more quickly, which causes further deterioration and may compromise test results. One of the alternatives is the replacement of water by saturated salt solutions, such as sodium chloride (NaCl), which retains part of the moisture (Tunes et al., 2012). The traditional method with 100% relative humidity is recommended for canola at 42 ºC for 24 hours (Ávila et al., 2006) and, for physic nut, a temperature of 41 ºC is used for 24 hours (Pereira et al., 2012). By the alternative method with saturated NaCl solution, 45 ºC for 36 hours is recommended for forage turnip seeds (Morais & Rossetto, 2013).

The potassium leaching test has been used to evaluate the physiological potential of seeds of different species such as wheat, peanuts, soybeans and, as well as the electrical conductivity test, the relationship between seed number and the ideal water volume is variable (Favarato et al., 2011 & Kikuti et al., 2008b).

The objective of this research was to adapt testing methodologies of electrical conductivity, accelerated aging and potassium leaching to evaluate the quality of sesame seeds.

Material and methods

The study was conducted in the Seed Laboratory of the Department of Agronomy at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina-MG, from April 2012 to October 2013. Four sesame cultivars were used, provided by Embrapa Algodão: BRS G2 (2011 harvest) (Cultivar 1), BRS Seda (2011 harvest) (Cultivar 2), Seridó (2006 harvest) (Cultivar 3) and BRS G4 (2011 harvest) (Cultivar 4). These cultivars were submitted to the following determinations and tests:

The **moisture content** was determined by the greenhouse method at 105 for 24 hours (Brasil, 2009), with two replicates of 0.5 g seeds for each cultivar.

For the **germination test**, sowing was carried out on blotting paper (substrate), moistened with an amount of water equivalent to 2.5 times the dry weight of the paper, in boxes (Gerbox®), placed in a Biochemical Oxygen Demand (BOD) germination chamber, regulated at 25 °C, with a 12-hour photoperiod. Four replicates of 50 seeds were used and the results were expressed as percentage of normal seedlings on day 3 (**First count**); the test was terminated on day 6 (Brasil, 2009). Counts were made daily to determine the **germination speed index (IVG)**, calculated according to the formula proposed by Maguire (1962), computing the number of seeds emerged from the emission of 1 mm radicle.

The **emergence test** was conducted with four replicates of 50 seeds per cultivar in trays with substrate sand and earth at a ratio 2:1. Seedling emergence was computed on day 9 (**Initial stand**) and on day 15 (**Final stand**) after sowing, and the number of emerged seedlings was evaluated. The results were expressed in percentage. The number of seedlings emerging from the onset of emergence was calculated daily for the **emergence speed index (IVE)**, and the calculation was performed according to Maguire (1962).

The effects of imbibition periods (4; 8; 12 and 16 hours) and combinations seed number/volume of deionized water (25/25 mL; 25/50 mL; 25/75 mL; 50/25 mL; 50/50 mL; 50/75 mL) were evaluated for the **electrical conductivity** test. Four replicates were used for each combination which, after counting, were weighed in a 0.001-g precision scale and put to soak in 200-mL plastic cups added with deionized water. The seeds were kept in BOD, at a constant temperature of 25 °C (Torres et al., 2009), where they remained during each imbibition period, defined according to the seed imbibition curve. After the conditioning time, the electrical conductivity of the solution was measured by reading in a conductivity meter, and the results were expressed as $\mu S\ cm^{-1}.g^{-1}$. The electrical conductivity of the solution was measured immediately after removal of the material from the BOD, and each vial was carefully agitated in order to standardize the electrolytes leached in the solution.

For the **accelerated aging test**, four replicates of 50 seeds, arranged in a single layer on a metal screen coupled to a box (Gerbox®) containing 40 mL of distilled water or saturated sodium chloride (NaCl) solution, were used at a ratio 40 g NaCl to 100 mL water. The boxes were kept in BOD germination chambers, at 41 °C, for 0; 24; 48; 72 and 96 hours. After the exposure period to accelerated aging, the seed moisture content and the percentage of normal seedlings were determined on the 6th day of evaluation. However, under these conditions, seed aging did not occur, and the use of a new temperature was required. Subsequently, the aging temperature was set at 45 ºC.

The effects of imbibition periods (30; 60; 90; 120; 150 and 180 minutes) and combinations between seed number/volume of distilled water (25/25 mL; 25/50 mL; 50/25 mL; 50/50 mL) were evaluated for the **potassium leaching** test. The seeds were counted, weighed in a 0.01-g precision scale and put to soak in 200-mL plastic cups added with distilled water. The seeds were kept in BOD, at a constant temperature of 25 °C, where they remained during each imbibition period. After the conditioning time, the amount of potassium leached from the solution was measured by reading in a flame photometer (Analyser 910). Potassium leaching was calculated by multiplying the reading obtained in the flame photometer (mgK/L) by the water volume (mL) and dividing the result by the weight of the sample (g). The results were expressed as mg potassium/g seed (Alves & Sá, 2010).

A completely randomized design was used to analyze the results. A 4x4x6 factorial scheme (4 cultivars, 4 imbibition periods and 6 treatments combining seed number and water volume) was used for the electrical conductivity test. For the accelerated aging test, data were analyzed in a 4x5x2 factorial scheme (4 cultivars, 5 aging periods and 2 aging methods). For the potassium leaching test, a 4x6x4 factorial scheme (4 cultivars, 6 imbibition periods and 6 treatments combining seed number and water volume) was used.

For the purpose of comparison, the Tukey test was used at 5% probability for electrical conductivity and accelerated aging. For the potassium leaching test, the means were compared by the Scott-Knott test at 5% probability. Data for electrical conductivity and potassium leaching were transformed into log(x), thus allowing normalization, while the other tests did not undergo transformations. Statistical analyses were performed using the statistical program SISVAR® (Ferreira, 2010).

Results and discussion

Seed moisture content ranged from 4.6% to 5.4% (Table 1), an ideal value for storage of sesame seeds, which must be lower than 6%. In addition, a similar water content between batches is paramount so that the tests are not affected by differences in metabolic activity, wetting rate and seed deterioration rate (Marcos-Filho, 2015).

Table 1 - Results in percentage (%) of moisture content – U; normal seedlings in the first count – PC; germination – G; germination speed index – IVG; initial stand – EI; emergence – E and emergence speed index – IVE, obtained in 4 sesame seed cultivars. Diamantina,MG, 2016.

Cultivars	**U**	**PC**	**G**	**IVG**	**EI**	**E**	**IVE**
1	5.4 a	65 b	94 a	32.67 b	24 a	61 a	5.49 a
2	5.4 a	24 c	74 b	23.16 c	14 b	29 b	2.63 b
3	4.7 ab	92 a	97 a	44.06 a	27 a	64 a	5.85 a
4	4.6 b	55 b	89 a	37.17 b	20 ab	50 ab	4.71 a
CV(%)	3.45	13.85	7.09	8.26	21.90	20.69	17.29
F	12.61	46.89	10.62	38.36	7.05	8.90	12.72
Pr>Fc	0.02	0.00	0.00	0.00	0.01	0.00	0.00

Means followed by the same lowercase letter in the column do not differ by the Tukey test at 5% probability.

By the percentage of normal seedlings obtained by tests for first germination count and IVG, it was possible to distinguish the cultivars in three quality levels: cultivar 3 had a superior quality; cultivars 1 and 4 were of intermediate quality and cultivar 2, of inferior quality (Table 1). Germination tests, initial stand and IVE allowed to differentiate only cultivars 1, 3 and 4 as superior in quality in relation to cultivar 2 (Table 1). Vigor tests for first germination count and IVG were more sensitive when evaluating seed quality, showing greater differences among cultivars that were not identified by the germination test.

The results of the electrical conductivity test involving the treatments seed number combined with water volumes and imbibition times in the different cultivars can be observed in Tables 2 and 3.

Cultivar differentiation in three quality levelscan be observed from 4 hours of imbibition, demonstrating the superiority of cultivar 3 and the inferiority of cultivar 2 (Table 2). These results are in agreement with those obtained in the first germination count and IVG (Table 1). Torres et al. (2009) worked with sesame seeds from four batches of cultivar Preta and concluded that an efficient imbibition period for the evaluation of the physiological potential was 8 hours. However, it was also possible to observe a smaller imbibition period for other species, such as arugula and eggplant seeds with 4 hours (Alves et al., 2012) and 1 hour for ryegrass seeds (Lopes & Franke, 2010). In other oleaginous species such as castor bean and physic nut, a 6-hour period was adequate to detect differences between seed batches (Araújo et al., 2011).

With the increase in imbibition volume, there was a decrease in the values of electrical conductivity, independent of imbibition period (Table 3). Higher water amounts provide higher ion dilutions due to the low concentration of solutes, especially when using smaller amounts of seeds (Santos & Paula, 2005). Therefore, the treatments with 25 seeds and 75 mL water, and 50 seeds with 75 mL water at 4 hours of imbibition were those that had the lowest conductivity (Table 3).

Table 2 - Results of electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) for different imbibition periods of sesame seeds. Diamantina, MG, 2016.

Cultivars	**Imbibition period (hours)**			
	4	8	12	16
1	79 aB	105 bAB	114 cAB	138 dA
2	96 aC	130 bC	141 cC	185 dB
3	67 aA	97 bA	109 cA	132 dA
4	81 aB	106 bB	123 cB	186 dB

Means followed by the same lowercase letter in the row and uppercase in the column do not differ by the Tukey test at 5% probability.

Table 3 - Results of electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) for the different treatments; 25 seeds (stes) in 25 mL of deionized water; 25 seeds in 50 mL of deionized water; 25 seeds in 75 mL of deionized water; 50 seeds in 25 mL of deionized water and 50 seeds in 50 mL of deionized water in different imbibition periods of sesame seeds. Diamantina, MG, 2016.

Treatments	**Imbibition period (hours)**			
	4	8	12	16
25stes/25 mL	129 aC	186 bD	185 bF	279 cF
25stes/50 mL	70 aB	96 bB	125 cD	151 dD
25stes/75 mL	44 aA	69 bA	88 cB	109 dB
50stes/25 mL	134 aC	157 bC	160 bE	190 cE
50stes/50 mL	65 aB	88 bB	103 cC	141 dC
50stes/75 mL	43 aA	62 bA	73 cA	93 dA

Means followed by the same lowercase letter in the row and uppercase in the column do not differ by the Tukey test at 5% probability.

Silva and Martins (2002) worked with castor bean seeds, and also observed that the most appropriate condition for the electrical conductivity test was the use of 25 seeds in 75 mL water. In sunflower seeds, the use of 50 seeds in 75 mL water was also the combination that allowed better results (Oliveira et al., 2012).

Table 4 shows that the seed moisture content had a significant interaction only between the methods and periods after the accelerated aging test, and there were no significant differences among cultivars. It is verified that, by the traditional method, the seeds had an increase in the water content with the increase in aging period, reaching up to 12% humidity in a period of 72 hours.

Table 4 - Results of moisture content (%) in different periods of traditional accelerated aging and with saturated NaCl solution of sesame seeds. Diamantina, MG, 2016.

Aging periods	**Treatments**	
(hours)	Water	NaCl
Control	5,0 aC	5 bA
24	9,0 aB	6 bA
48	9,0 aAB	6 bA
72	12,0 aA	5 bA
96	5,0 aC	3 bB

Means followed by the same lowercase letter in the row and uppercase in the column do not differ by the Tukey test at 5% probability.

Regarding the moisture content of seeds exposed to the saturated NaCl solution, smaller and more uniform values were observed (Table 4). The use of a saturated solution led to less drastic effects than the traditional method, as there was a decrease in the degree of seed deterioration when reaching lower water contents, as observed in fennel seeds (Torres, 2004). However, some authors reported that, even with a lower water content, there is sufficient stress to reduce germination (Ávila et al., 2006).

The results of normal seedlings obtained after traditional accelerated aging (Table 5) show that, after 24 hours, there was a reduction in the percentage of normal seedlings of sesame seeds for all cultivars and, at 48 hours, it was possible to separate the cultivars in three vigor levels: cultivar 3 had the highest quality, cultivars 1 and 4 were of intermediate quality and cultivar 2, of inferior quality. There is a similarity between these results and those obtained in the first germination count, IVG and electrical conductivity (Table 1 and 2). Santos et al. (2011) also observed similar results for lettuce and chicory seeds, in which the 48-hour period was adequate to evaluate seed physiological potential.

Table 5 - Results of normal seedlings (%) obtained in the germination test for sesame seeds submitted to different periods of traditional accelerated aging and saturated NaCl solution. Diamantina, MG, 2016.

	Treatments/Imbibition period (hours)									
Cultivars	**Traditional**					NaCl				
	0	24	48	72	96	0	24	48	72	96
1	96aA	75bA	65bAB	52cA	54cA	96aA	87abA	83bA	67bcAB	75bA
2	79aB	69bA	51cC	43cdA	37dB	79aB	69aB	68aB	38bC	50bC
3	98aA	76bA	70bA	54cA	54cA	98aA	88aA	83aA	74bA	54cBC
4	96aA	75bA	55cBC	52cA	43cAB	96aA	84abA	80bAB	60cB	62cB

Means followed by the same lowercase letter in the row and uppercase in the column do not differ by the Tukey test at 5% probability.

By the method using saturated NaCl solution, the periods that separated the cultivars in greater number of vigor levels were 72 and 96 hours (Table 5). At the 72-hour period, it was possible to detect the superiority of cultivar 3, followed by cultivars 1 and 4, and cultivar 2, of inferior quality. In the 96-hour period, cultivar 1 was considered to be of superior quality, followed by cultivars 4 and 3, of intermediate quality, and cultivar 2, of inferior quality.

Martins et al. (2002) worked with broccoli seeds and reported that aging with water (100% RH) further impaired seed quality, since the water absorption rate by the seed and, consequently, seed deterioration rate, was lower when working with the saturated NaCl solution. Alves and Sá (2010) also reported that the use of saline solution in arugula seeds contributed to delay water absorption by the seeds. These authors observed that the accelerated aging test with saturated NaCl saline solution with 72 and 96 hours has sensitivity for the evaluation of the physiological potential of arugula. In peanut seeds, the 72-hour period in saline solution was also efficient to detect differences between batch vigor (Rossetto et al., 2004).

In order to adapt the methodology of the potassium leaching test, a new characterization of cultivar profile was carried out, since oleaginous plants have a lower storage potential (Fanan et al., 2009) and were stored for 1 year and 5 months. It can be observed that seed moisture content ranged from 5.0% to 5.9% (Table 6). This variation in the degree of humidity is acceptable, once differences below 2% do not affect the results of vigor tests, allowing consistent and important results for test standardization (Marcos-Filho, 2015).

Table 6 - Results in percentage (%) of moisture content – U; normal seedlings in the first count – PC; germination – G; germination speed index – IVG; initial stand – EI; emergence – E and emergence speed index – IVE, obtained in 4 sesame seed cultivars. Diamantina, MG, 2016.

Cultivars	U	PC	G	IVG	EI	E	IVE
1	5.9 a	49 a	83 a	23.86 a	20 a	35 a	4.76 a
2	5.9 a	29 b	52 b	19.57 a	21 a	27 a	2.97 a
3	5.0 b	57 a	84 a	22.27 a	30 a	42 a	5.67 a
4	5.8 a	36 b	71 a	23.66 a	19 a	32 a	5.39 a

Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at 5% probability.

By the percentage of normal seedlings obtained in the test for first germination count, it was possible to distinguish the cultivars in two quality levels: cultivars 1 and 3 of superior quality and cultivars 2 and 4 of inferior quality (Table 6). The germination test was less sensitive, since it allowed to differentiate only cultivar 2 as of inferior quality in relation to the other cultivars. Tests for IVG, initial stand, emergence and IVE did not show significant differences among cultivars.

There was an interaction between seed number/water volume, imbibition period and cultivars (Table 7), that is, the cultivars have a differentiated performance at each combination of water volume and imbibition period.

The combination seed number/water volume and imbibition period that allowed the best results was 25 seeds with 25 mL water in 60 and 90 min imbibition (Table 7). Under these conditions, it was possible to verify cultivar differentiation in three quality levels. At the 60-min imbibition period, cultivars 1 and 3 had superior quality, cultivar 4 intermediate quality and cultivar 2, inferior quality. In the 90-min period, cultivar 3 had the best quality, 1 and 4, intermediate quality and 2, inferior quality. Similar results were obtained in the first germination count and germination (Table 6), in which the superiority of cultivar 3 and the inferiority of cultivar 2 were verified.

Table 7 - Results of potassium leaching (mgK/g) for sesame seeds according to treatment – 25 seeds in 25 mL of deionized water; 25 seeds in 50 mL of deionized water; 50 seeds in 25 mL of deionized water and 50 seeds in 50 mL of deionized water – and imbibition period. Diamantina, MG, 2016.

Treatments	Cultivars	Imbibition period (minutes)					
		30	60	90	120	150	180
25/25	1	8.75bA	20.50cA	5.25aB	24.25cA	33.25cB	6.00aA
	2	19.25bA	112.25dC	16.25bC	28.75cA	50.25dB	9.25aB
	3	13.00bA	19.50cA	2.50aA	17.50bA	21.75cA	4.25aA
	4	13.75bA	54.25cB	6.25aB	27.50bA	36.75cB	8.50aB
25/50	1	10.00bA	27.25dA	5.50aB	9.75bA	18.25cA	3.00aA
	2	34.25cB	29.25cA	5.50aB	16.00bA	52.50cB	16.75bB
	3	9.25bA	18.25cA	3.00aA	8.00bA	20.75cA	3.25aA
	4	12.00bA	29.50cA	6.00aB	14.00bA	32.25cB	3.75aA
50/25	1	10.50bB	44.75cA	4.25aA	12.50bA	45.00cA	5.00aA
	2	12.50bB	45.50cA	7.50aB	17.75bA	51.25cA	5.50aA
	3	6.00aA	36.00cA	3.50aA	9.00bA	29.00cA	5.00aA
	4	12.25bB	40.25cA	6.00aB	13.25bA	46.00cA	5.25aA
50/50	1	10.75bA	24.25cA	4.25aA	9.00bA	27.50cA	5.00aA
	2	27.00bB	29.25bA	5.50aA	30.00bB	51.75cB	9.00aB
	3	11.75bA	21.50cA	4.00aA	10.25bA	20.75cA	4.00aA
	4	11.75bA	27.00cA	5.25aA	12.50bA	28.50cA	7.50aB

Means followed by the same lowercase letter in the row and uppercase in the column do not differ by the Skott-Knott test at 5% probability.

The results obtained with sesame seeds corroborate those observed for other species. Kikuti et al. (2008) observed that the 60-minute imbibition period is efficient in distinguishing the vigor of peanut seed batches. In soybeans, the evaluations carried out for 60 and 90 minutes are also adequate to identify batches with different vigor levels.

This rapidity in obtaining the results compared to the tests for electrical conductivity and accelerated aging occurs due to the fact that the potassium leaching test allows the evaluation of seed vigor in a shorter time, and the beginning of immersion is accompanied by the rapid leaching of exudates, mainly the potassium ion, which is the cation that is in higher concentration in the membranes and more available in plant cells (Taiz & Zeiger, 2009).

The use of 25 seeds to detect differences in seed physiological potential was also observed in other species such as cauliflower (Kikuti & Marcos-Filho, 2008a) and peanut (Kikuti et al., 2008b).

In the potassium leaching test, it was possible to separate sesame seeds in different vigor levels with the use of a smaller seed number in a short period of time. This fact is of great importance for the seed quality control program, since it allows for greater agility in decision making.

Conclusions

For the electrical conductivity test, imbibition for 4 hours and the combination of 25 seeds in 75 mL of water and 50 seeds in 75 mL of water is efficient to distinguish the physiological quality of sesame cultivars.

For the accelerated aging test, the aging periods of 48 hours by the traditional method or 72 hours and 96 hours with saturated NaCl solution allow to separate sesame cultivars at different levels of vigor.

For the potassium leaching test, the combination of 25 seeds, 25 mL of water and the periods of 60 or 90 minutes of imbibition is adequate to evaluate seed physiological potential.

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