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Accelerated aging test and antioxidant enzyme activity to assess chickpea seed vigor

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ABSTRACT: The chickpea crop has been expanding in Brazil and, thus, the demand for high quality seeds along with it. The aim of this study was to adapt the methodology for evaluation of chickpea seed vigor by the accelerated aging test and to analyze whether antioxidant enzyme activity is related to the physiological potential of these seeds. Seeds from five seed lots of the cv. BRS Aleppo and from four seed lots of the cv. Cícero were initially tested to characterize their physiological potential. The accelerated aging test was conducted by the traditional method (100% RH) and with a saturated NaCl solution (76% RH), both at 41 °C, for 24, 36, 48, and 72 h. The activity of the SOD, CAT, and APX enzymes were also determined. The accelerated aging test using 41 °C/48 h with a saturated NaCl solution is recommended for assessment of chickpea seed vigor. The activity of CAT and APX enzymes may be related to the physiological potential of these seeds, but this is not the case with SOD.

Index terms: artificial aging, *Cicer arietinum* L., enzymatic analysis, methodology, physiological potential.

RESUMO: O cultivo de grão-de-bico vem se expandindo no Brasil e, com isto, a demanda por sementes de alta qualidade também aumenta. Neste trabalho, objetivou-se adequar metodologia para avaliação do vigor de sementes de grão-de-bico pelo teste de envelhecimento acelerado, bem como analisar se a atividade de enzimas antioxidantes apresenta relação com o potencial fisiológico das sementes. Sementes de cinco lotes, cv. BRS Aleppo, e de quatro lotes da cv. Cícero, foram inicialmente submetidas a testes para a caracterização do seu potencial fisiológico. O teste de envelhecimento acelerado foi conduzido pelo método tradicional (100% UR) e com solução saturada de NaCl (76% UR), ambos a 41 °C, por 24, 36, 48 e 72 h. Foram determinadas também a atividade das enzimas SOD, CAT e APX. Para o teste de envelhecimento acelerado, recomenda-se a utilização de 41 °C/48 h com solução saturada NaCl para a avalição do vigor das sementes de grão-de-bico. A atividade das enzimas CAT e APX pode ser relacionada ao potencial fisiológico das sementes, o que não ocorre para a SOD.

Termos para indexação: envelhecimento artificial, *Cicer arietinum* L., análise enzimática, metodologia, potencial fisiológico.

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INTRODUCTION

Pulses, or dry edible seeds of leguminous plants, are important sources of plant protein. Chickpea (*Cicer arietinum* L.) is one of the most prominent pulses as it is the third most consumed leguminous seed in the world (Ullah et al., 2020). In Brazil, the Central-West, Southeast, and South regions have climate and altitude characteristics that are favorable to growing it, and this has been encouraged both for increasing production for internal consumption, reducing imports, and for increasing exports to consumer countries (Nascimento and Silva, 2019), especially India, where a considerable part of the population has a vegetarian diet.

Brazilian domestic production of chickpea has gradually increased in recent years. In 2017, growing area in Brazil was less than 1,000 hectares (Nascimento and Silva, 2019), increasing to more than 10,000 hectares in 2019 (Cavalcanti-Mata et al., 2020). In this context, the availability of high-quality seeds on the market is important for obtaining uniform and high-yielding crop fields. Thus, reliable information on the vigor of seed lots for estimating seed performance in the field and in storage is essential.

Among the vigor tests available, the accelerated aging test has proven to be one of the most sensitive and effective for assessment of seed vigor (Marcos-Filho, 2016). It is a stress test based on the principle that the seed deterioration rate increases when seeds are exposed to high temperature and relative humidity conditions (Baalbaki et al., 2009). Temperature, relative humidity, and period of exposure of seeds to the test conditions and their interactions are important factors to establish for effectiveness of the test in seed vigor evaluation (Marcos-Filho, 2020). Very high temperatures may lead to denaturation of proteins and excessive decline in seed viability. In this regard, conducting the test at 41 °C for soybean seeds (Marcos-Filho et al., 2001) and common bean seeds (Bertolin et al., 2011) has been recommended.

The traditional accelerated aging test (TAA) uses water within the gerbox (germination box) to obtain relative humidity of around 100%. Differences in water absorption by seeds when exposed to this humid atmosphere may result in sharp variations in seed moisture content and, consequently, in the deterioration rate (Jianhua and McDonald, 1996). To get around this problem, these authors proposed an alternative methodology, replacing the water with a saturated salt solution, so as to obtain relative humidity lower than that used in the TAA. That way, water absorption by seeds is slower, with the additional possibility of reducing the occurrence of microorganisms that may interfere in the results of the test. Saturated salt accelerated aging (SSAA) was proposed for small seeds of vegetable crops and proved to be effective for other species as well, such as field pea (Machado et al., 2019), sweet corn (Alvarenga et al., 2013), and cowpea (Dourado et al., 2013).

There is still little information regarding the most appropriate methodology for conducting the accelerated aging test in chickpea seeds. Kapoor et al. (2010) used specific accelerated aging conditions, such as 45 °C and 100% RH for 72 h, to bring about seed deterioration and monitor the physiological and biochemical changes resulting from this process. Samarah and Abu-Yahya (2008) used 40 °C and 100% RH for 96 h to evaluate the vigor of chickpea seeds harvested at different stages of maturity. Neither study had the objective of fitting the methodology of the accelerated aging test to chickpea seeds.

Considering that seed vigor declines as the deterioration process advances and that the formation of reactive oxygen species (ROS) generated by lipid peroxidation (Kumar et al., 2015) has been attributed as the main cause, an interesting alternative for classifying seed lots for vigor would be evaluation of enzymes of the antioxidant system. Among these enzymes, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) play a fundamental role in neutralization of the ROS, and they are the most studied and relevant in aiming to perform evaluations related to oxidative stress (Anjum et al., 2017; Ebone et al., 2019).

Reduction in antioxidant enzyme activity has been associated with reduction in the quality of seeds during deterioration (Ebone et al., 2019; Oliveira et al., 2020). However, studies related to antioxidant enzyme activity for selection of lots in regard to vigor are not widely reported in the literature, but they have had promising results. Bandeira et al. (2014) found that evaluation of the SOD, CAT, and APX enzymes was an effective alternative for differentiating soybean seed lots with small vigor differences.

In light of the above, the aim of this study was to adapt the methodology for evaluation of chickpea seed vigor by the accelerated aging test and to analyze whether antioxidant enzyme activity is related to the physiological potential of these seeds.

MATERIALS AND METHODS

The study was carried out in the seed research laboratory of the *Universidade Federal de Viçosa*, Minas Gerais, Brazil. Five seed lots of the chickpea cultivar BRS Aleppo and four lots of the cultivar Cícero were used, which were supplied by *Embrapa Hortaliças*. The seeds from each lot were first characterized for their physiological potential through the following tests:

Moisture content: determined by the laboratory oven method at 105 °C for 24 h (Brasil, 2009).

Germination test: four replications of 50 seeds were distributed on paper toweling moistened with water in the amount of 2.0 times the weight of the dry paper. Rolls were formed and kept in a seed germinator at 20 °C. The percentage of normal seedlings at 8 days after sowing was determined (Brasil, 2009).

Emergence percentage and emergence speed index (ESI) of seedlings: conducted in a growth chamber using four replications of 50 seeds sown at 2.0 cm depth in plastic trays containing a mixture of soil and sand at the proportion of 1:2 moistened until reaching 60% retaining capacity (Brasil, 2009). Daily counts were made of the number of emerged seedlings up to complete stabilization of stand, calculating the percentage of emergence and the ESI, according to Maguire (1962).

Cold test: conducted in a way similar to the germination test, adding 40 mL of soil over the seeds after sowing. The rolls were placed in plastic bags and kept in BOD at 10 °C for seven days. After that period, they were removed from the plastic bags and kept in a seed germinator at 20 °C for five days to then evaluate the percentage of normal seedlings.

Germination at low temperature: conducted according to the methodology described for the germination test; however, the rolls were placed in plastic bags and kept in BOD at 15 °C for 5 days. The result was expressed in percentage of normal seedlings with size ≥ 4.0 cm (Dias and Marcos-Filho, 2020).

Trial I – Adaptation of the methodology for the accelerated aging test

The traditional method and alternative method (with saturated NaCl solution) were used, following the procedures recommended by the AOSA (Baalbaki et al., 2009).

Traditional accelerated aging test (TAA): conducted with 250 seeds distributed in a uniform layer on metallic screens in gerboxes (plastic germination boxes) containing 40 mL of water at the bottom. Lids were placed on the boxes to obtain 100% RH within them and they were kept in a BOD incubator at 41 °C for 24, 36, 48, and 72 h. After each period, the germination test was conducted as described above, obtaining the percentage of normal seedlings on the fifth day after sowing. The moisture content of the seeds was also determined after the aging periods.

Saturated salt accelerated aging (SSAA): conducted in a way similar to the traditional method, using the same temperature conditions and aging periods, however, replacing water with 40 mL of saturated NaCl solution (40 g of NaCl / 100 mL of water) to obtain approximately 76% RH within each gerbox (Jianhua and McDonald, 1996).

Trial II – Quantification of antioxidant enzymes

Six replications of 10 seeds from each seed lot were used, which were pre-soaked in moistened paper toweling and kept in a seed germinator at 20 °C for 24 h. After that, the seed coat was removed and the embryos were frozen in liquid nitrogen and lyophilized to later be macerated using a TissueLyser. The activity of the enzymes superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC. 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11) was quantified by absorbance readings on a microplate spectrophotometer, Multiskan GO.

Crude enzyme extracts were obtained from 80 mg of embryos followed by the addition of 1.000 µL of the extraction medium, and then they were centrifuged at 15,000 g for 15 min. at 4 °C. The extraction medium consisted of a potassium phosphate buffer (50 mM, pH 7.0), ethylenediamine tetraacetic acid (EDTA) (2 mM), ascorbate (20 mM), triton (0.1%), dithiothreitol (DTT) (2 mM), and polyvinylpolypyrrolidone (PVPP) 200% (w/w). The protein contents of the enzymatic extracts were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. SOD activity was determined, monitoring the inhibition of photochemical reduction of the NBT according to Giannopolitis and Ries (1977). CAT activity was estimated measuring the rate of H_2O_2 decomposition in 240 nm (Havir and McHale, 1987). Total activity of the APX was estimated monitoring the decline in absorbance at 290 nm (Nakano and Asada, 1981).

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design. Analysis of variance was performed on the data, and the means were compared by Tukey's test at 5% probability. In Trial I, for each cultivar, the data were analyzed in a triple factorial arrangement: seed lots (five for the cv. BRS Aleppo and four for the cv. Cícero) × aging methods (TAA and SSAA) × aging periods (24, 36, 48, and 72 h). Regression analysis was performed for the quantitative factor. Furthermore, the simple Pearson correlation coefficients (r) were calculated for all the combinations among the variables obtained in the tests of characterization of the seed lots and in Trial I. In addition, multivariate principal component analysis (PCA) was performed among the data on characterization of physiological potential of the seeds and of Trial II. The R statistical software, version 3.5.2, was used for all the analyses.

RESULTS AND DISCUSSION

Characterization of seed physiological potential

The seed moisture content of both cultivars was similar for all the lots, ranging from 12.2% to 12.8% (Table 1). According to Marcos-Filho (2016), uniformity in seed moisture content is important to allow standardization of the evaluations and reliable comparison of the physiological potential of the seed lots analyzed.

From the tests performed in characterization of the physiological potential of the seeds of the cv. BRS Aleppo (Table 1), different levels of seed quality are seen among the lots when evaluated by the germination test, with the highest value for Lot A2, which did not differ from Lot A1, and the lowest value was for Lot A4. Lot A4 was also lower than the others by the seedling emergence results. The results obtained in the emergence speed index, cold test, and germination at low temperature were similar in terms of classification of the seed lots in vigor levels, with Lot A4 as the least vigorous and Lots A1 and A2, in general, as the most vigorous. Lots A3 and A5 were in an intermediate position.

The cv. Cícero (Table 1) showed lower germination for the seeds of Lot C1 in relation to the others, which did not differ from each other. Similar results were also obtained in the cold test and in germination at low temperature. In general, except for the emergence speed index, lower physiological potential was found for the seeds of Lot C1.

Trial I – Accelerated aging

Within each period of aging, regardless of the methodology, there was little variation in the moisture content of the seeds after accelerated aging with 100% RH and 76% RH (Table 2). The maximum was 2.4 percentage points (p.p.) and minimum was 1.1 p.p. for the cv. BRS Aleppo, and 2.8 p.p. maximum and 0.7 p.p. minimum for the cv. Cícero. These variations are within the tolerable limit of up to 3 p.p. (Marcos-Filho, 2020), indicating that the results obtained were consistent.

Comparison of the two methods of accelerated aging (Table 2) shows that with use of the saturated NaCl solution (76% RH), there was lower moisture absorption by the seeds, resulting in less drastic deterioration, which can be confirmed by the higher values of germination after accelerated aging in relation to the values obtained with 100% RH (Table 3).

Table 1. Moisture content (MC), germination (G), emergence (E), emergence speed index (ESI), cold test (CT), and germination at low temperature (GLT) of seed lots of two chickpea cultivars.

Lot	MC (%)	G (%)	E (%)	ESI (index)	CT (%)	GLT (%)
			BRS Aleppo			
A1	12.7	95 ab	99 a	5.7 a	97 a	87 a
A2	12.8	97 a	98 a	5.5 a	96 a	90 a
A3	12.8	90 b	94 a	5.2 ab	89 ab	73 b
A4	12.7	80 c	84 b	4.1 c	73 c	44 c
A5	12.2	91 b	92 a	4.8 b	88 b	80 ab
F	-	23.51*	12.49*	19.00*	24.82*	64.47*
CV (%)	-	3.01	3.63	5.51	4.31	6.17
			Cícero			
C1	12.5	68 b	64 b	3.1 a	57 b	34 b
C2	12.3	78 a	79 ab	3.7 a	82 a	55 a
C3	12.5	80 a	81 a	3.7 a	80 a	57 a
C4	12.4	81 a	78 ab	3.6 a	76 a	55 a
F	-	9.49*	4.47*	1.26 ^{ns}	47.16*	67.11*
CV (%)	-	5.05	9.73	14.91	4.62	5.47

*, ^{ns} = significant and non-significant by the F test at 5% probability; F = F value calculated; CV = coefficient of variation. Mean values followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability.

Table 2. Moisture content (%) obtained after the periods of traditional accelerated aging (TAA) and saturated salt accelerated aging (SSAA) in seed lots of two chickpea cultivars.

Lot	TAA (100% RH)				SSAA (76% RH)			
	24 h	36 h	48 h	72 h	24 h	36 h	48 h	72 h
				BRS Aleppo				
A1	19.6	23.6	24.6	26.5	13.6	13.6	13.5	13.5
A2	20.6	21.4	25.2	27.6	13.7	13.7	13.6	14.1
A3	20.8	23.8	25.1	26.6	13.6	13.8	14.0	13.9
A4	20.0	21.9	24.3	26.0	13.5	13.8	13.4	13.0
A5	19.2	23.0	24.1	26.5	13.4	13.6	13.8	14.0
				Cícero				
C1	19.6	22.2	23.9	26.8	13.0	13.5	13.8	13.7
C2	19.4	22.4	23.9	25.1	13.1	13.4	13.6	13.5
C3	19.7	22.2	24.5	27.9	13.3	13.1	13.5	13.3
C4	19.2	21.4	23.9	26.7	13.3	13.6	13.4	13.3

The TAA and the SSAA allowed separation of the seed lots regarding vigor in the four aging periods analyzed, for both cultivars (Table 3). However, for the cultivar BRS Aleppo, the lots could be classified in three vigor levels in all the aging periods with salt solution. This was not observed in the test conducted by the traditional method, in which there was stratification of the lots in only two vigor levels in the periods of 36 and 72 h. Thus, when using the saturated NaCl solution, in general, greater vigor was observed for Lots A1 and A2. Lower vigor was clearer in Lot A4; and Lots A3 and A5 were classified as having intermediate vigor.

For the cv. Cícero (Table 3) in the TAA for 24 h, there was separation of the seed lots in two levels of physiological potential, with greater vigor for Lots C2, C3, and C4 compared to Lot C1. A similar result was found in the period of 48 h; however, there was no statistical difference between Lots C4 and C1. With 36 and 72 h, in general, there was greater stratification of the lots, with Lot C1 remaining as that of lowest vigor, but variations occurred regarding the lot of greatest vigor. Nevertheless, it is noteworthy that in the period of 72 h, the values obtained were very low, indicating excessive deterioration of the seed lots, leading to loss of viability of the seeds of Lot C1. In the period of 48 h, although the TAA did not have as drastic effect as that observed in the period of 72 h, the values obtained were also low, ranging from 18% to 31%, which may be an indication that for lots that do not have high physiological potential, such as lots of the cv. Cícero, 48 h of aging may be excessive. There are reports of conducting the accelerated aging test in chickpea seeds using 100% RH for 96 h (Ram et al., 1989); however, this also caused severe reduction in seed germination, especially in the lots that did not have high physiological potential. It should be emphasized that these authors did not test other accelerated aging times.

The results of the SSAA for the cv. Cícero (Table 3) in the period of 24 h were similar to those obtained in the same period of the TAA, with lower vigor for Lot C1 compared to the others, which also occurred in the periods of 36 and 48 h. In these periods, it was possible to separate the seed lots into two levels of physiological quality, in a way similar to that observed in most of the vigor tests used for characterization of seed physiological potential (Table 1). At 72 h, there was better performance for Lots C2 and C4, which did not differ from each other, followed by Lot C3; and Lot C1 was inferior to the others. Considering that the vigor results obtained for the seed lots of this cultivar were relatively low, it should be emphasized that in a quality control program, such lots could be discarded as grain or recommended for immediate sowing, and should not be stored.

There was a linear reduction in vigor with the increase in aging time for all the seed lots in both methods studied (Figure 1). Nevertheless, there was a sharper decline in percentage of normal seedlings after accelerated aging when TAA

Table 3. Germination (%) of seed lots of two chickpea cultivars after different times of traditional accelerated aging (TAA) and saturated salt accelerated aging (SSAA).

Lot	TAA (100% RH)				SSAA (76% RH)			
	24 h	36 h	48 h	72 h	24 h	36 h	48 h	72 h
				BRS Aleppo				
A1	95 aA	93 aA	87 aA	60 a B	93 aA	93 aA	83 aA	86 aA
A2	93 aA	90 aA	86 aA	68 aB	91 abA	92 aA	84 aA	85 aA
A3	66 cB	62 bB	50 bcB	19 bB	81 cA	76 bA	73 bA	55 bcA
A4	57 cB	58 bA	42 cB	11 bB	64 dA	54 cA	59 cA	48 cA
A5	76 bA	64 bB	52 bB	16 bB	82 bcA	76 bA	72 bA	61 bA
CV (%)				2.91				
				Cícero				
C1	37 bB	35 cA	18 bB	1 cB	48 bA	32 bA	30 bA	25 cA
C2	56 aA	48 bB	31 aB	16 bB	60 aA	57 aA	50 aA	51 aA
C3	60 aA	59 aA	29 aB	21 abB	66 aA	55 aA	47 aA	39 bA
C4	55 aB	48 bA	25 abB	25 aB	64 aA	50 aA	50 aA	48 aA
CV (%)				3.41				

Mean values followed by the same lowercase letters in the column and uppercase letters in the row comparing the methods in each time do not differ from each other by Tukey's test at 5% probability. CV = coefficient of variation.

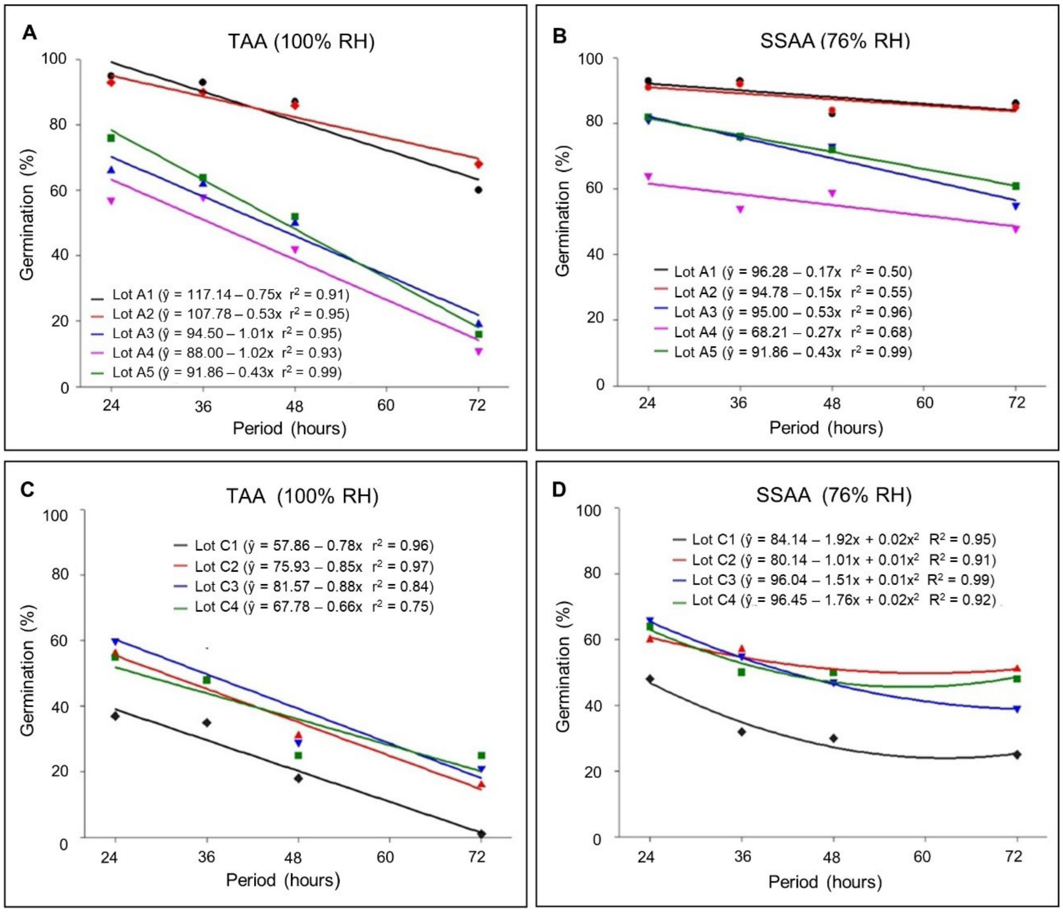

Figure 1. Germination of the chickpea seed lots, cv. BRS Aleppo (A and B) and cv. Cícero (C and D) after traditional accelerated aging (TAA) and saturated salt accelerated aging (SSAA) for different periods.

(100% RH) was used (Figures 1A and C), due to the greater stress and deterioration from TAA compared to conducting the test with salt solution (76% RH) (Figures 4B and D). It is noteworthy that in traditional accelerated aging for 72 h, there was a sharp decrease in germination, especially in Lots A3, A4, and A5 of the cv. BRS Aleppo and Lot C1 of the cv. Cícero, which practically lost all vigor, indicating that this period had a drastic effect on chickpea seeds. Freitas and Nascimento (2006) also reported that traditional accelerated aging for 72 h had a drastic effect on lentil seeds.

For the accelerated aging test in soybean and common bean seeds, the temperature of 41 °C for 48 h is recommended for the traditional method (Baalbaki et al., 2009). However, for chickpea seeds in this same method and period, there was a high incidence of microorganisms and many dead seeds, with excess of exudate around them, indicating excessive deterioration mainly caused by high relative humidity.

In general, a clear increase in the deterioration rate is seen with an increase in the period of exposure to the test, above all when the TAA is used, a result also observed in common bean seeds (Binotti et al., 2008; Bertolin et al., 2011). Kapoor et al. (2010) in a study on deterioration of chickpea seeds after aging with 100% RH at 45 °C for 24, 48, and 72 h, observed a reduction in seed physiological potential, as well as in protein and sugar contents as the time of accelerated aging increased. Based on the results observed with 100% RH at 41 °C, the temperature of 45 °C would be relatively high for the species as it would cause excess deterioration and an increase in the incidence of microorganisms in the seeds.

The correlation between the results of the accelerated aging test and those of the other tests for evaluation of seed quality (Figure 2) reinforces its effectiveness for evaluation of chickpea seed vigor. It should be emphasized that high and significant correlations were obtained between the SSAA for 36 and 48 h and seedling emergence, ESI, the cold test, and germination at low temperature. In other leguminous plants, such as pea (Nascimento et al., 2007), lentils

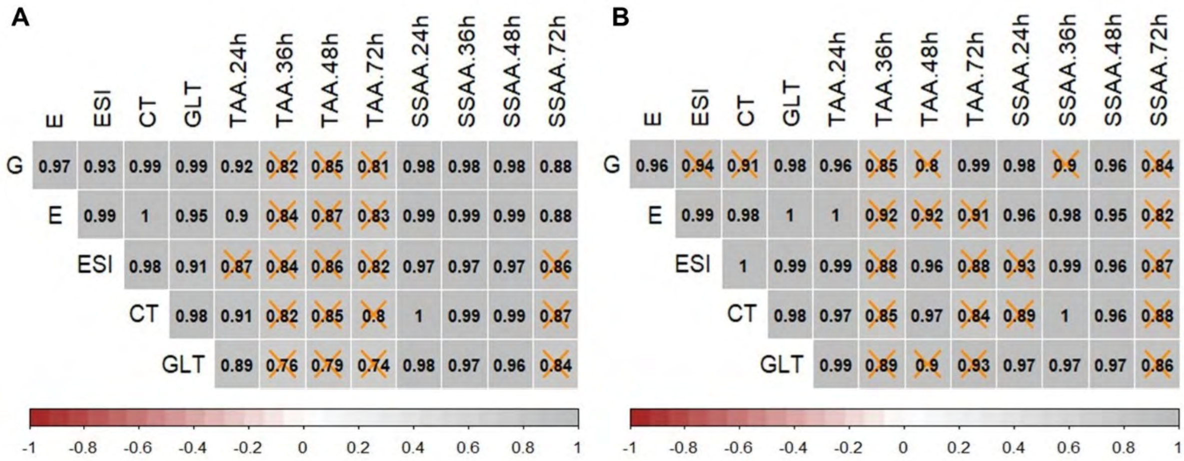

Figure 2. Pearson simple correlation coefficients (r) estimated among the variables of germination and vigor with the different procedures of the accelerated aging test performed on seeds from five chickpea seed lots, cv. BRS Aleppo (A), and on seeds from four lots, cv. Cícero (B). Quadrants marked with "X" represent non-significant correlation at 5% probability by the *t*-test.

(Freitas and Nascimento, 2006), and cowpea (Dourado et al., 2013), there was also greater effectiveness of the SSAA in relation to the TAA, and the time of 48 h at 41 °C is most recommended.

Considering the lower occurrence of microorganisms in the SSAA with 76% RH, this procedure can be considered effective for evaluation of the vigor or chickpea seed lots. Although both 36 h and 48 h were effective for both cultivars, and both periods cited provided similar results, the period of 48 h can be considered more appropriate due to practicality, allowing the test to be conducted within the commercial schedule of operation of seed analysis laboratories.

Trial II – Enzyme activity

The activity of the antioxidant system enzymes does not show significant difference between the seed lots regarding SOD activity for either cultivar (Figures 3A and D). However, significant variations were observed in the activity of the CAT (Figures 3B and E) and APX (Figures 3C and F) enzymes between the seed lots of different vigor levels. For the cv. BRS Aleppo, the activity of the CAT and APX enzymes was greater for Lots A1 and A2 compared to Lot A4, although the APX activity in the seeds of Lot A3 was also superior to that obtained for Lot A4. These results are consistent with those obtained in most of the tests for evaluation of physiological quality (Table 1), in which Lots A1 and A2 were generally superior to Lot A4. The cv. Cícero, Lot C1, was classified as having lowest vigor in most of the tests performed in initial characterization of the quality of the lots, and it was also that which obtained the lowest activity of the CAT and APX enzymes.

In general, considering these results, it can be inferred that the seed lots of greater physiological potential had greater activity of the CAT and APX enzymes. According to Deuner et al. (2011), the balance between the production and intracellular removal of ROS, especially of hydrogen peroxide, is directly related to cell capacity in maintaining high SOD, CAT, and APX activity. To maintain cellular homeostasis, preventing oxidative damage, the seed antioxidant defense system is activated, which includes the antioxidant enzymes (Kumar et al., 2015). Effective operation of the antioxidant defense system is directly related to success in seed germination (Gomes and Garcia, 2013).

Thus, antioxidant enzyme activity can be an interesting tool for separating seed lots into different levels of physiological quality. Bandeira et al. (2014) found that activity of enzymes such as SOD, CAT, and APX was more sensitive than germination, first count, and seedling growth tests for differentiating soybean seed lots regarding physiological potential. These authors found high activity of these enzymes in the shoots of seedlings at 8 days of germination in the highest vigor lots. But upon evaluating the activity of these enzymes in the primary root, they did not obtain results related to the vigor of the lots, with greater activity of SOD and APX for the lot of lower physiological potential, and no

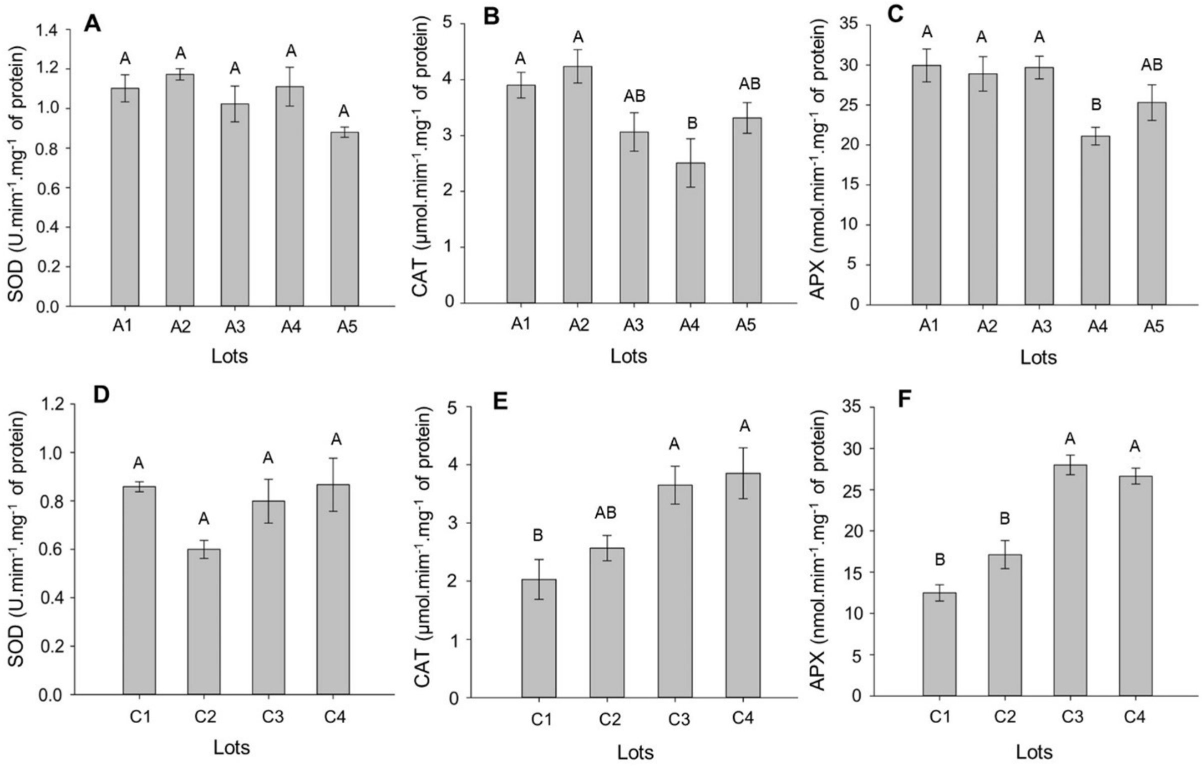

Figure 3. Activity of the enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) in seeds from five chickpea seed lots cv. BRS Aleppo (A, B, and C) and four lots of cv. Cícero (D, E, and F). The same letters do not differ by Tukey's test ($p \leq 0.05$). Bars: standard deviation.

significant difference for CAT. For Borba et al. (2014), evaluation of the enzymes of the antioxidant system (SOD, CAT, and APX) was also an effective alternative for differentiating maize seed lots with small differences in vigor. Reductions in SOD activity due to deterioration were observed in sunflower (Bailly et al., 1996) and soybean (Xin et al., 2014) seeds, and the results were associated with loss of vigor. In a similar manner, Sharma et al. (2018) observed that SOD and CAT activity significantly declined in mung bean seeds subjected to deterioration. In contrast, Yin et al. (2015) did not observe changes in SOD and CAT activities in rapeseed under artificial aging compared to non-aged seeds.

Principal component analysis (PCA) was performed in the present study with the aim of associating the results obtained in the enzyme analyses and those of the traditional tests performed for evaluation of seed physiological quality. The first two components of the PCA explained more than 90% of the total variability of the data (Figure 4). When the sum of the principal components (PC1 and PC2) is greater than 80%, the results observed in the PCA can be considered effective for explaining the total variability of the data (Jolliffe and Cadima, 2016). The more the vector of the variable is distant from the lot, the lower the performance of that lot in relation to the corresponding variable. Lot A4 of the cv. BRS Aleppo and Lot C1 of the cv. Cícero were located in the quadrant opposed to (PC1-/PC2+) the vectors of CAT and APX, confirming the results presented in Figure 3, which show lower enzyme activity in less vigorous seed lots. According to Ebone et al. (2019), depression of protective ability against ROS can result in physiological imbalances, such as lipid peroxidation and increase in respiration, leading to loss of seed vigor and viability.

The proximity between the vectors of the physiological quality variables with the vectors corresponding to CAT and APX activity indicates high correlation between these two enzymes especially with the results of the cold test and of germination at low temperature. Thus, the results obtained in the present study suggest that antioxidant enzyme activity has sensitivity in classifying chickpea seed lots in relation to physiological potential since they were consistent with the results obtained in traditional vigor tests.

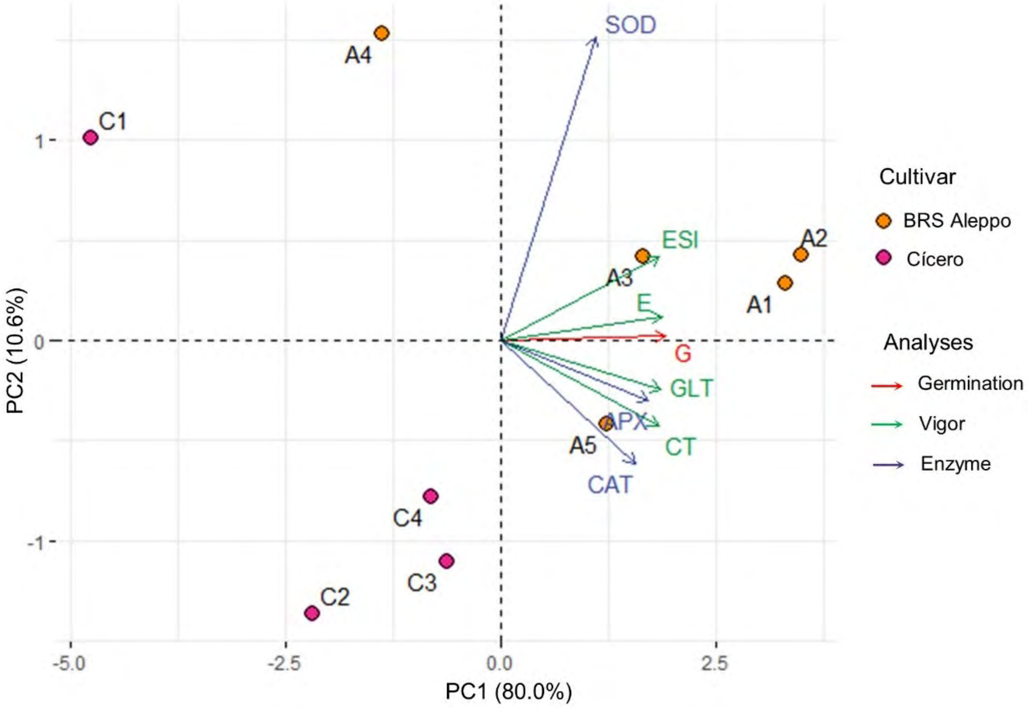

Figure 4. Biplot of principal component analysis obtained from the linear combination of physiological variables (germination and vigor) and antioxidant enzymes in seeds of two chickpea cultivars.

CONCLUSIONS

The accelerated aging test with saturated NaCl solution (76% RH) conducted at 41 °C for 48 h is effective for evaluation of chickpea seed vigor.

The activity of the CAT and APX enzymes is related to seed physiological potential, unlike that which occurs for SOD.

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