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Physiological quality and enzymatic activity of crambe seeds after the accelerated aging test

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ABSTRACT. Crambe is a promising crop for biodiesel production, mainly due to the high oil content of its seeds. However, there have been no effective methodologies for evaluating the physiological quality of crambe seeds. Seed lots have not been compared, especially by vigor tests, such as the accelerated aging. The objective of this study was to evaluate the effects of high temperature and exposure periods on the physiological quality and enzymatic activity of crambe seeds during the accelerated aging test. Two lots of crambe seeds of the Brilhante cultivar were analyzed by tests of moisture content, weight of 1,000 seeds, germination, first count, electrical conductivity, enzymatic activity (peroxidase and superoxide dismutase) and seedling length. All tests were carried out before and after the accelerated aging, which evaluated different temperatures (38, 40 and 42ºC) and exposure periods (24, 48 and 72 hours). The experimental design was completely randomized with four replications. Variance analysis and mean comparison through the Tukey and Dunnet tests ($p \leq 0.05$) were applied for statistical analysis and means were also submitted to the linear correlation test. It was concluded that the interaction between temperature and exposure period affects the physiological quality and enzymatic activity of crambe seeds. We also conclude that the best conditions of temperature and exposure period for evaluating the physiological quality of crambe seeds through the accelerated aging test depend on genotype.

Keywords: *Crambe abyssinica*, germination, vigor, deterioration.

RESUMO. Qualidade fisiológica e atividade enzimática de sementes de crambe, após o envelhecimento acelerado. O crambe é uma cultura promissora para produção de biodiesel, principalmente pelo alto conteúdo de óleo de suas sementes. No entanto, não há metodologias estabelecidas para avaliar a qualidade fisiológica das sementes desta espécie e lotes não podem ser comparados, especialmente por testes de vigor, como o de envelhecimento acelerado. O objetivo da presente pesquisa foi avaliar o efeito da alta temperatura e do período de exposição durante o teste de envelhecimento acelerado na qualidade fisiológica e atividade enzimática de sementes de crambe. Dois lotes de sementes de crambe, cultivar Brilhante, foram analisados por meio dos testes de teor de água, massa de 1.000 sementes, germinação, primeira contagem, condutividade elétrica, atividade enzimática (peroxidase e superóxido dismutase) e comprimento de plântulas. As avaliações foram conduzidas antes e após o envelhecimento acelerado, que foram testadas diferentes temperaturas (38, 40 e 42ºC) e períodos de exposição (24, 48 e 72h). O delineamento experimental foi o inteiramente casualizado com quatro repetições. Os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Tukey ($p \leq 0.05$). O teste de Dunnet ($p \leq 0.05$) foi utilizado para comparar os valores da testemunha (antes do envelhecimento acelerado) com cada valor médio individualmente. O teste de correlação linear simples também foi aplicado. Conclui-se que a interação temperatura x período de exposição afeta a qualidade fisiológica das sementes e a atividade enzimática e que as melhores condições durante o teste de envelhecimento acelerado são dependentes do genótipo.

Palavras-chave: *Crambe abyssinica*, germinação, vigor, deterioração.

Introduction

Concerns about the environment and the imminent risk of an energy crisis have encouraged the search for an energy model that is based on biomass use, which is a clean and renewable source of energy. In Brazil, it is estimated that there are more than 200 species that may be potentially cropped for biodiesel production. Crambe (*Crambe abyssinica* L.) is an oil crop of the *Brassicae* family, and it is usually used as forage (OPLINGER et al., 1991).

Crambe is a winter crop originally from the Mediterranean. It is tolerant to dry periods and frost after its establishment. This species shows short annual cycle and uniform maturation. Preliminary studies show that crambe seeds contain about 35% high-quality oil (LAGHETTI et al., 1995).

However, few studies have been developed about ideal conditions for seed germination. The physiological quality and storage of crambe seeds have also not been studied.

Evaluating seed physiological potential is essential in a program for quality control. It supplies information during the production process, increasing the seed's final quality. The germination test is the official procedure for evaluating the potential of seeds to have normal seedlings in ideal conditions. Nevertheless, this test does not always reveal performance differences among lots during storage or in the field (CARVALHO; NAKAGAWA, 2000). Therefore, it is important to evaluate seed vigor as a complement to the information supplied by the germination test. Accelerated aging is one of the many procedures that have been used (TORRES; NEGREIROS, 2008). This test is based on the artificial deterioration of seeds through exposure to high temperatures and relative humidity, which plays an important role in the intensity and speed of declining quality (MARCOS FILHO, 1999).

The mechanisms that lead to seed deterioration are still not well known, but studies show that the decrease in physiological quality is related to biochemical modifications that compromise metabolic activities. Among them, are respiratory and enzymatic changes, and synthesis processes, reserve compounds, cell membranes and chromosomes are all affected (ABDUL-BAKI; ANDERSON, 1972). According to Abdul-Baki and Anderson (1972), enzymatic activity decreases in seeds submitted to the accelerated aging test because protein synthesis is affected.

As for germination evaluated after the accelerated aging test, many factors affect seed performance. The stress caused by the interaction temperature/exposure period are the most studied. Dutra and Vieira (2004) reported that the best combination is 45ºC $72h^{-1}$ for maize and 42ºC $48h^{-1}$ for soybean. According to Anfinrud (1997), seeds submitted to artificial stress before the germination is evaluated may effectively simulate soil temperatures up to 40ºC when the crop is sown in the field.

The objective of the present study was to evaluate the effects of high temperature and exposure period on the physiological quality and enzymatic activity of crambe seeds during the accelerated aging test.

Material and methods

Two lots of crambe seeds of the cultivar Brilhante were used. These had been cropped in different locations and harvested under distinct environmental conditions, and were provided by the "Mato Grosso do Sul Foundation", Maracajú, Mato Grosso do Sul State, Brazil. The seeds were stored in paper bags and evaluated at the "Laboratório de Análise de Sementes, Departamento de Produção Vegetal – Agricultura, Faculdade de Ciências Agronômicas, UNESP", in Botucatu, São Paulo State, Brazil. The seeds were under an environmental condition (without any control of temperature and relative humidity).

Table 1 shows the physiological quality of the crambe seeds before the accelerated aging test. The seeds were previously analyzed though the following tests:

Table 1. Characterization of two lots of crambe seeds.

Evaluation	Lot A	Lot B	Variation coefficient (%)
Moisture content (%)	6.83	6.02	-
Weight of 1,000 seeds (g)	6.97 b	8.16 a	2.69
Germination (%)	84 a	42 b	11.88
First count (%)	68 a	40 b	6.79
Abnormal seedlings (%)	7 a	2 a	10.77
Electrical conductivity (μS cm^{-1} g^{-1})	125.09 b	44.61 a	3.90

Means followed by the same letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$).

Moisture content: Two replications with similar weight were evaluated using an oven at 105 ± 3°C for 24h (BRASIL, 1992). The results were expressed as a mean percentage for each lot.

Weight of 1,000 seeds: To obtain the mean weight of 1,000 seeds in grams, eight replications of 100 seeds from each lot were weighed, and the average for each seed lot was calculated by multiplying by 10.

Germination: four replications of 50 seeds per lot were distributed in plastic boxes (11.0 x 11.0 x 3.5 cm) on two sheets of blotter paper moistened with 12 mL of water. The boxes were placed inside plastic bags and left for germination at 25ºC. Normal and abnormal seedlings were evaluated seven days after sowing, according to Brasil (1992), and the results were expressed as a mean percentage of normal seedlings.

First count of germination: This was performed along with the germination test. The percentage of normal seedlings was recorded on the fourth day after sowing.

Electrical conductivity: Fifty seeds from each replication were weighed and soaked in 200-mL

plastic cups containing 100 mL of deionized water for 24h at 25ºC (VIEIRA; KRZYZANOWSKI, 1999). Afterwards, the electrical conductivity of the solution was determined through a reading in a conductivimeter, and the average values obtained for each lot were expressed as $\mu S\ cm^{-1}\ g^{-1}$.

Enzymatic activity: As described by Ekler et al. (1993), the seeds were homogenized under ice-cold conditions in 5 mL of extraction buffer TRIS-HCl (0.2 mol L^{-1}, pH 7.8) containing 1 mmol L^{-1} of EDTA, 7.5% (w v^{-1}) of polyvinylpolypyrrolidone (PVPP) and sterilized, washed sand. The homogenates were centrifuged at 14,000 *g* for 30 min. at 4ºC. The supernatant was collected and centrifuged once more. Samples were stored at -20ºC. The extracts were used to evaluate superoxide dismutase and peroxidase activity. The soluble protein of the enzymatic extracts was analyzed according to Lowry et al. (1951) and determined using a spectrophotometer (660 nm absorbance). The protein used for reference was the Bovine Serum Albumin (BSA). Then, the activity of the following enzymes was determined: a) superoxide dismutase (SOD, EC 1.15.1.1): According to Beauchamp and Fridovich (1971), the following reagents were used: sodium phosphate buffer (50 mmol L^{-1}, pH 7.8); nitro bule tetrazolium (NBT, 33 µmol L^{-1}) + EDTA (0.66 mmol L^{-1}, 5:4); L-metionina (10 mmol L^{-1}) + riboflavina (0.0033 mmol L^{-1}, 1:1); and the enzymatic extract. The mixture remained under illumination at 25ºC for 10 minutes before measurement at 560 nm using a spectrophotometer. b) peroxidase (POD, EC 1.11.1.7): according to Teisseire and Guy (2000), the mixture consisted of the enzymatic extract; a sodium phosphate buffer (50 mmol L^{-1}, pH 6.5); pyrogallol (20 mmol L^{-1}); and hydrogen peroxide (H_2O_2, 5 mmol L^{-1}). The mixture remained in environmental conditions for 5 min. before measurement at 430 nm using a spectrophotometer. The activity was calculated using the extinction coefficient of 2.5 mmol $L^{-1}\ cm^{-1}$ due to purpurogalline formation.

After initial characterization, the seeds were submitted to the accelerated aging test, as follows:

Accelerated aging: According to Marcos Filho (1999), four replications of 50 seeds were arranged on accelerated aging trays. These were placed in plastic boxes (11.0 x 11.0 x 3.5 cm), without any contact, with 40 mL of water at the bottom. The boxes were closed and placed inside plastic bags for the artificial aging. Specific equipment (Hitachi MT10) provided controlled temperature and relative humidity. For this test, three temperatures (38, 40 and 42ºC) and three exposure periods (24, 48 and 72h) were evaluated.

After this procedure, the seeds from each treatment were analyzed through the germination test. Normal and abnormal seedlings and non-germinated seeds were evaluated. In this experiment, dormant seeds were counted as non-germinated because there are no specific methodologies for the tetrazolium test to be performed on crambe seeds. The first counts of germination, seedling length and the enzymatic activity of peroxidase and superoxide dismutase were also evaluated after aging. The test to determine seedling length is described as follows:

Seedling length: Four replications of 10 seeds per lot were sown on a line drawn on two sheets of blotter paper moistened with 12 mL of water. The boxes were placed inside plastic bags and left for germination at 25ºC for 7 days (NAKAGAWA, 1999).

The experimental design was completely randomized with four replications. Variance analysis and mean comparison through the Tukey test ($p \leq 0.05$) were applied for statistical analysis considering a factorial 3 x 3 (temperature x exposure period) for each seed lot. For data obtained after the accelerated aging test, the Dunnet test ($p \leq 0.05$) was also applied. This compared each mean value to a control (seeds without any artificial deterioration) considering LSD values (least significant difference). Mean values were submitted to the linear correlation test (r).

Results and discussion

The initial evaluations of physiological quality (Table 1), showed that the two lots of crambe seeds were different in germination and vigor, considering that they were cropped in different locations and harvested under distinct environmental conditions.

The seeds of lot A showed higher germination compared to the other lot. Similarly, the first count of germination and the percentage of abnormal seedlings showed a difference in quality among the lots. Lot A had a better initial performance. Carlson et al. (1996) reported that crambe is barely domesticated, and it exhibits a post-harvest dormancy. Such mechanisms are typical in undomesticated species to improve longevity and success. In an annual crop, however, such a trait makes it difficult to accurately estimate germination percentages. This may have influenced initial values obtained through germination and vigor tests similarly to Gutormson et al. (1992), who reported 18 and 12% for dormant seeds cultivars Indy and Meyer, respectively.

Differences in physiological quality among seed lots are relevant when evaluating the accelerated aging test. This is because some

characteristics may influence the tolerance level to the stress imposed by the test. Although the seeds of lot B had a lower percentage of normal seedlings in the first and last counts of germination, the test of electrical conductivity showed a higher quality in this lot. This is because lower values found in this evaluation mean a better integrity of cell membranes and, consequently, higher vigor (MARCOS FILHO, 2005). The physical conditions of the seeds influence the results of the electrical conductivity and speed of deterioration (VIEIRA; KRZYZANOWSKI, 1999). Conversely, the seeds of lot B also showed a higher weight, which may affect the results of electrical conductivity. Another possible explanation would be that the seeds of lot B showed post-harvest dormancy, which influenced both the percentage of germination and the results of electrical conductivity. In this case, the seeds would have high quality, but would not yet be established due to dormancy effects.

According to Table 2, the seed moisture content before the accelerated aging test was low due to the relation with the relative humidity of the air (MARCOS FILHO, 2005) quickly reaching the hygroscopic equilibrium. As for moisture after the accelerated aging test, the values were uniform in each exposure period at any temperature, but they increased as time passed. Marcos Filho (2005) found that seeds with higher moisture are more sensitive to stressful conditions and, consequently, are subject to intense deterioration. Therefore, some of the results of this study may have been affected by the moisture content reached by seeds in higher exposure periods.

Table 2. Means of moisture content (%) of crambe seeds before and after the accelerated aging test.

Lot	Moisture before the aging	Temperature (ºC)	Exposure period (h)		
			24	48	72
A	5.95	38	21.30	24.73	30.71
		40	23.32	26.77	31.17
		42	22.38	26.05	31.41
B	5.79	38	21.13	24.84	30.34
		40	22.98	26.30	27.69
		42	22.15	24.76	31.96

Variance analysis carried out after the accelerated aging test (Table 3) showed the effects of the interaction between temperature and exposure period and the isolated influence of both.

Table 4 shows the physiological quality of crambe seeds before and after the accelerated aging test.

The results of germination and first count showed different responses to stress depending on the combination of temperature and exposure period.

The physiological quality of the seeds in lot A was significantly affected by the exposure to high temperatures, standing out at 38ºC, which resulted in lower values. The exposure periods also affected the quality of this seed lot; in this case, the higher period resulted in a higher percentage of germinated seeds. According to the results, the higher longevity and germination of the seeds may be related to the conditioning effects provided by the test conditions. Tilden and West (1995) also observed that conditioning reverts the effects of accelerated aging in soybean seeds.

Table 3. Variance analysis and variation coefficient for means of germination (G), first count (FC), abnormal seedlings (AS), non germinated seeds (NGS), seedling length (SL) and activity of the enzymes superoxide dismutase (SOD) and peroxidase (POD) of crambe seeds after the accelerated aging test.

Evaluation	Lot	F values			Variation coefficient (%)
		Temperature	Exposure period	Interaction	
G	A	12.518**	31.198**	0.985ns	17.14
	B	34.707**	13.637**	4.843**	29.40
FC	A	16.505**	36.256**	1.378ns	17.95
	B	27.873**	17.083**	5.269**	32.49
AS	A	7.073**	91.187**	4.004*	4.52
	B	3.692*	9.523**	2.306ns	9.93
NGS	A	19.417**	0.660ns	0.626ns	29.47
	B	42.544**	17.933**	6.019**	11.00
SL	A	24.624**	19.370**	6.815**	65.01
	B	5.848**	6.897**	0.655ns	78.69
SOD	A	0.457ns	0.631ns	2.920ns	9.71
	B	0.543ns	0.372ns	0.331ns	6.35
POD	A	1.896ns	1.507ns	4.293*	10.81
	B	7.211**	9.613**	10.937**	7.99

* and ** significant at a probability level of 5 and 1%, respectively; ns: non significant.

The seeds of lot B were influenced by temperature at all exposure periods. The combination of 42ºC $48h^{-1}$ increased the percentage of normal seedlings evaluated in the tests of germination and first count. Lower values were found whenever seeds remained under any temperatures for a longer period (72h).

Independently of the seed lot, all results of the germination and first count differed significantly from the control by the Dunnet test. Next, the negative conditions of the accelerated aging test were observed. Maeda et al. (1986) similarly reported that different lots of sunflower seeds showed distinct resistance to stress conditions (mainly high temperature) during the accelerated aging test.

In the non-germinated seeds of lot A, there was an isolate effect of temperature. The exposure at 38ºC, then, differed from the others, resulting in higher mean percentages. The values of this seed lot were lower than the control. As for the seeds of lot B, the significant effect of the interaction showed higher values for 38ºC combined with the periods of 48 and 72 hours. These values did not differ from the control.

Table 4. Means of germination (G), first count (FC), abnormal seedlings (AS), non-germinated seeds (NGS) and seedling length (SL) of crambe seeds before and after the accelerated aging test.

Test	Lot	Temperature (°C)	l.s.d.	Control	Exposure period (h)			Mean
					24	48	72	
G (%)	A	38	9.77	58	14★	19★	23★	19 b★
		40			18★	25★	33★	25 a★
		42			18★	28★	32★	26 a★
		Mean	-	-	17 C★	24 B★	29 A★	-
	B	38	9.74	48	12 b★	4 cB★	3 bB★	6★
		40			13 abAB★	18 bA★	10 aB★	14★
		42			19 aB★	26 aA★	13 aB★	19★
		Mean	-	-	15★	16★	8★	-
FC (%)	A	38	9.18	54	12★	15★	21★	16 b★
		40			16★	19★	32★	22 a★
		42			18★	24★	31★	24 a★
		Mean	-	-	15 C★	20 B★	28 A★	-
	B	38	9.90	46	11 bA★	3 cB★	2 bB★	6★
		40			14 abAB★	18 bA★	9 aB★	14★
		42			19 aA★	25 aA★	9 aB★	17★
		Mean	-	-	15★	15★	7★	-
AS[1] (%)	A	38	6.37	11	18 aB★	9 bA	8 bA	12
		40			23 bC★	14 aB	8 bA	15
		42			21 abC★	11 abB	4 aA★	12
		Mean	-	-	20★	11	7	-
	B	38	4.89	3	3	1	1	2 a
		40			5	1	4	4 b
		42			5	3	1	3 ab
		Mean	-	-	4 B	1 A	2 A	-
NGS (%)	A	38	10.57	32	19★	22	19★	20 b★
		40			9★	12★	9★	10 a★
		42			12★	12★	14★	12 a★
		Mean	-	-	13★	15★	14★	-
	B	38	10.69	52	35 bA★	46 cB	46 bB	42
		40			32 abA★	31 bA★	37 aA★	33★
		42			27 aA★	22 aA★	37 aB★	28★
		Mean	-	-	31★	33★	40★	-
SL (cm)	A	38	1.55	1.14	0.16 aA	0.23 bA	0.74 bA	0.38
		40			0.26 aA	0.41 bA	1.03 bA	0.56
		42			0.18 aC	2.40 aB	3.71 aA★	2.09
		Mean	-	-	0.20	1.01	1.83	-
	B	38	2.28	4.18	1.55★	0.28★	0.24★	0.69 b★
		40			1.31★	0.29★	1.12★	0.90 b★
		42			2.94	1.25★	1.45★	1.88 a★
		Mean	-	-	1.93 a	0.61 b★	0.93 b★	-

Means followed by the same small letter in the column and capital letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$). [1]Data transformed in arc sin $\sqrt{(x/100)+0.5}$. ★Means followed by this symbol do not differ from the control by the Dunnet test ($p \leq 0.05$) considering LSD values (least significant difference).

The accelerated aging test provides stress conditions that accelerate the deterioration process. It is to be expected, then, that the percentage of non-germinated seeds will be higher after the test. Nevertheless, it was observed that the crambe seeds after the test showed values which were lower than the control, probably due to an indirect conditioning effect (KHAN, 1992). Nascimento (1998) considers that the effects of conditioning depend on stress conditions.

As for the abnormal seedlings, the seeds of lot A were affected by the interaction between temperature and exposure period. The combination of 42°C 72h^{-1} resulted in values that were lower than the other combinations and the control. When each mean value was compared to the control, there was no difference for the period of 48h at any temperatures or 72h at 38 and 40°C. The period of 24h at any temperature resulted in higher values for seeds of lot A compared to the control, which was expected to happen after the aging. Although variance analysis showed positive isolate effects of the treatments, the seeds of lot B resulted in low percentages of abnormal seedlings.

According to Marcos Filho (2005), the interpretation of the accelerated aging test does not include any information for detecting the decrease in speed and intensity of growth, usually observed throughout seed deterioration. Hence, the evaluation of seedling growth was carried out to reveal other effects of the accelerated aging test.

As for length of crambe seedlings, lots A and B were affected by the combination and isolate factors, respectively. The data of lot A showed that as time passed, the temperature of 42°C resulted in an increase of seedling length. Also, the values were statically different from each other. For the seeds of this lot, the temperature of 42°C combined with 48 and 72h stood out. However, only the combination of 42°C 72h^{-1} was significantly different and resulted in values higher than the control. When lot B was

evaluated, the combination of 42ºC 24h^{-1} was the best for the development of crambe seedlings. Binotti et al. (2008) reported that bean seeds maintained at 41ºC for 72h produced seedlings with a shorter primary root and hypocotil.

Table 5 shows the activity of the enzymes superoxide dismutase (SOD) and peroxidase (POD). According to Matés (2000), the cell possesses antioxidant enzymes such as SOD and POD to defend the organism against redox-active oxygen species (ROS).

Although the SOD enzyme catalyses superoxide radicals (O_2^-), produced in different parts of the cells, to oxygen and H_2O_2 (RABINOWITCH; FRIDOVICH, 1983), there was no modification in the activity of this enzyme provided by any of the stress conditions. Similarly, Menezes et al. (2008) did not observe any effects of the controlled deterioration on the SOD activity of maize seeds of different genotypes.

As for the activity of the POD enzyme, it was observed that the interaction significantly affected the results. Therefore, higher values were observed for the combination of 40ºC 24h^{-1}. Although there was no influence from temperature, it was found that the increase in time for 48h resulted in higher enzymatic activity. Typical behavior of the antioxidant enzymes was observed for the seeds of lot B. The increase in the exposure period at 42ºC resulted in higher enzymatic activity, increasing the stress level. The POD enzyme is considered the most stable enzyme to heat (FREITAS et al., 2008), but according to Lu and Whitaker (1974), the enzymes can be reactivated after thermal inactivation. This phenomenon is known as renaturation.

The correlation test (Table 6) showed different significances depending on the evaluation. For the seeds of lot A, it was observed that the activity of the POD enzyme correlated to the percentage of germinated and non-germinated seeds at the first and last count of germination. Peroxidase has been implicated in a variety of physiological processes, and response to injury is among them (GILLIKIN; GRAHAM, 1991). Considering the seeds of lot B, a significant correlation was found only for the first count of germination. Nevertheless, a correlation between the enzymes POD and SOD was reported. The activity of SOD explains the results of the non-germinated seeds.

Table 5. Activity of the enzymes superoxide dismutase (SOD) and peroxidase (POD) of crambe seeds before and after the accelerated aging test.

Test	Lot	Temperature (ºC)	l.s.d.	Control	Exposure period (h)			Mean
					24	48	72	
SOD	A	38	8.67	33.17	38.02	38.33	33.57	36.64
		40			34.42	32.47	38.49	35.13
		42			36.62	37.92	32.05	35.53
		Mean	-	-	36.35	36.24	34.70	-
	B	38	4.34	28.54	27.11	27.30	28.73	27.71
		40			27.37	27.01	27.38	27.25
		42			26.42	27.31	26.86	26.86
		Mean	-	-	26.96	27.21	27.66	-
POD	A	38	11.98	23.73	46.14 aB★	43.11 abAB★	35.20 aA★	41.48★
		40			50.06 aB★	38.61 aA★	45.52 bAB★	44.73★
		42			42.09 aA★	48.54 bA★	46.25 bA★	45.63★
		Mean	-	-	46.10★	43.42★	42.32★	-
	B	38	11.05	26.25	59.60 bA★	51.06 aA★	51.70 aA★	54.12★
		40			41.16 aA★	56.12 aB★	54.89 aB★	50.72★
		42			47.86 aA★	59.09 aB★	68.52 bC★	58.49★
		Mean	-	-	49.54★	55.42★	58.37★	-

[1]Means followed by the same small letter in the column and the capital letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$). [2]Data transformed in arc sen $\sqrt{(x/100)+0.5}$. ★Means followed by this symbol do not differ from the control by the Dunnet test ($p \leq 0.05$) considering LSD values (least significant difference).

Table 6. Correlation test among germination (G), first count (FC), abnormal seedlings (AS), non-germinated seeds (NGS), seedling length (SL) and activity of the enzymes superoxide dismutase (SOD) and peroxidase (POD) of crambe seeds after the accelerated aging test.

		FC	AS	NGS	SL	SOD	POD
Lot A	G	0.99★★★	-0.47ns	0.54ns	0.40ns	-0.37ns	-0.71★
	FC	-	-0.44ns	0.51ns	0.43ns	-0.34ns	-0.68★
	AS	-	-	-0.28ns	-0.65★	0.17ns	0.24ns
	NGS	-	-	-	-0.08ns	-0.17ns	-0.79★★
	SL	-	-	-	-	-0.30ns	0.13ns
	SOD	-	-	-	-	-	0.44ns
Lot B	G	0.99★★★	0.33ns	0.05ns	0.76★★	0.41ns	-0.60ns
	FC	-	0.38ns	0.02ns	0.75★	0.41ns	-0.64★
	AS	-	-	-0.39ns	0.61ns	-0.08ns	-0.41ns
	NGS	-	-	-	0.06ns	0.75★	-0.48ns
	SL	-	-	-	-	0.20ns	-0.56ns
	SOD	-	-	-	-	-	-0.67★

★, ★★ and ★★★ significant at a probability level of 5, 1 and 0.1%, respectively; ns: non significant.

Conclusion

The interaction between temperature and exposure period affects the physiological quality and enzymatic activity of crambe seeds.

The best conditions of temperature and exposure period for evaluating the physiological quality of crambe seeds through the accelerated aging test depend on genotype.

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