Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.13, n.4, e5585, 2018
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v13i4a5585
Protocolo 5585 - 12/07/2016 • Aprovado em 13/07/2018

Accelerated aging of arugula seeds

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ABSTRACT: This work aimed to study procedures for conducting the accelerated aging test and evaluating the physiological potential of arugula seeds. Five seed lots of cv. Cultivada and four of cv. Folha Larga were firstly subjected to the following tests for characterization of their physiological qualities: moisture content, germination test, first counting, seedling emergence in the field, emergence speed index, seedling shoot length, seedling shoot dry mass, electrical conductivity and potassium leaching. Subsequently, the same lots were subjected to accelerated aging (38 and 41 ºC; 24, 48 and 72 h; with and without the use of saturated NaCl solution). The experiment was conducted in a completely randomized design with four replications of 50 seeds each. The accelerated aging with saturated NaCl solution, using the combination of 41 ºC for 48 h, and traditional accelerated aging, using the combination of 38 ºC for 48 h, were effective for classifying seed lots of cv. Cultivada into different vigor levels. On the other hand, accelerated aging with saturated NaCl solution using the combination of 41 ºC for 24 or 48 h was suitable for classifying the vigor of cv. Folha Larga seeds.

Key words: *Eruca sativa* L.; thermal stress; vigor

Envelhecimento acelerado em sementes de rúcula

RESUMO: O presente trabalho objetivou estudar procedimentos para a condução do teste de envelhecimento acelerado e avaliação do potencial fisiológico de sementes de rúcula. Cinco lotes da cv. Cultivada e quatro da cv. Folha Larga foram inicialmente submetidos às seguintes avaliações para a caracterização das suas qualidades fisiológicas: grau de umidade, teste de germinação, primeira contagem, emergência de plântulas em campo, índice de velocidade de emergência, comprimento da parte aérea da plântula, massa seca da parte aérea da plântula, condutividade elétrica e lixiviação de potássio. Posteriormente, os mesmos lotes foram submetidos ao envelhecimento acelerado (38 e 41 ºC; 24, 48 e 72 h; com e sem o uso de solução saturada de NaCl). O experimento foi conduzido em delineamento inteiramente casualizado com quatro repetições de 50 sementes cada. O envelhecimento acelerado com solução saturada de NaCl, utilizando a combinação 41 ºC por 48 h, e o envelhecimento acelerado tradicional, com a combinação 38 ºC por 48 h, foram eficientes para separação de lotes da cv. Cultivada em diferentes níveis de vigor. Por outro lado, o envelhecimento acelerado com solução saturada de NaCl utilizando a combinação 41 ºC por 24 ou 48 h se mostrou adequado para classificação do vigor de sementes da cv. Folha Larga.

Palavras-chave: *Eruca sativa* L.; estresse térmico; vigor

Introduction

Vegetable crops are grown intensively and should be established with the use of seeds with high germinability. This is one of the main factors to be considered during the agricultural production process, and there is a consensus among researchers, technologists and seed producers on the importance of seed vigor, as well as the need to evaluate it. Thus, seed physiological potential should be demonstrably high, which requires the routine use of vigor tests in quality control programs, with benefits for all segments of arable crops and vegetable production (Marcos Filho, 1999).

Several tests are used to determine the components of seed quality. One of them is the germination test, which usually overestimates the physiological potential of seed lots due to be conducted under favorable humidity and temperature conditions. Therefore, it increases the need for use and improvement of vigor tests (Santos et al., 2011).

In this context, accelerated aging is one of the most studied and recommended vigor tests for several cultivated species. The principle of this test is to increase the rate of seed deterioration by exposing them to high temperatures and relative humidity. These factors have a preponderant influence on the intensity and speed of deterioration (Marcos Filho, 1999). In such conditions, low-quality seeds deteriorate faster than vigorous seeds, presenting a sharp drop in viability after being subjected to artificial aging (Barbosa et al., 2011a).

Studies involving the interaction between temperature and exposure period are found for many vegetable seeds: cucumber, 41 °C/72 h (Abdo et al., 2005); lentil, 41 °C/48 h (Freitas & Nascimento, 2006); radish, 41 °C/48 or 72 h (Ávila et al., 2006); pea, 41 °C/48 h (Nascimento et al., 2007); lettuce and endive, 42 °C/48 h (Santos et al., 2011).

The use of saturated solutions in the accelerated aging test was effective for vigor classification of arugula (Ramos et al., 2004), melon (Torres et al., 2009), lettuce (Barbosa et al., 2011b), endive (Santos et al., 2011), coriander (Tunes et al., 2011) and jilo seed lots (Alves et al., 2012).

For arugula cv. Cultivada, the combination of 48 h at 41 ºC, using the traditional procedures and saturated solution (NaCl), has provided promising results (Ramos et al., 2004). Nevertheless, further research should be carried out, since new cultivars may have different responses to the stress conditions of the test.

Therefore, this work aimed to study procedures for conducting the accelerated aging test to evaluate the physiological potential of arugula (*Eruca sativa* L.) seeds.

Material and Methods

The experiment was carried out at the Seed Analysis Laboratory and at the experimental field of the Department of Plant Sciences of the Universidade Federal Rural do Semi-Árido (UFERSA), located in Mossoró (5° 11’ S, 37° 20’ W, 18 m altitude), Rio Grande do Norte, Brazil. Arugula cv. Cultivada and cv. Folha Larga were represented by five and four seed lots, respectively, and evaluated based on the following tests:

Moisture content: two replicates of 50 seeds per treatment were used, employing the drying method at 105 ± 3 °C for 24 h (Brasil, 2009).

Germination test: four replicates of 50 seeds per treatment were distributed on two sheets of blotting paper moistened with distilled water at a ratio of 2.5 times the paper weight. The sheets were placed inside plastic containers and maintained in a germinator with constant temperature of 20 °C. The final counting was performed seven days after the beginning of the test (Brasil, 2009).

First counting: this was performed along with the germination test, evaluating the percentage of normal seedlings at four days after sowing (Brasil, 2009).

Seedling emergence in the field: four replicates of 50 seeds from each lot were sown in 1.2 m wide beds. Seedlings were then evaluated at 12 days after sowing.

Emergence speed index: this was calculated using the equation proposed by Maguire (1962). Therefore, the number of normal seedlings emerged from the beginning of the emergence process was recorded daily, using four subsamples of 50 seeds from each lot.

Seedling shoot length: this was measured from the shoot apex to the other end of the stem, using a graduated ruler. Only seedlings from the useful plot area were assessed, whereas seedlings on the edges were eliminated. Average results were expressed in centimeters.

Seedling shoot dry mass: after the assessment of seedling shoot length, all seedling shoots were cut and placed in a dry oven at 65 °C for 72 h, and subsequently weighed. The obtained weight was divided by the number of seedlings used, and the results were expressed in mg seedling^{-1}.

Electrical conductivity: this was performed using the mass method, with four subsamples of 50 physically pure seeds, which were weighed and placed to soak in plastic cups (200 mL) containing 50 mL of distilled water for 8 h. After this period, the electrical conductivity of the solution was measured, with results expressed in µS cm^{-1} g^{-1}, as recommended by Torres & Pereira (2010).

Potassium leaching: this was conducted according to the methodology proposed by Alves & Sá (2010). Thus, samples of 200 seeds from each lot were divided into four subsamples of 50 seeds and weighed with precision of 0.001 g. Seeds were placed in plastic cups containing 50 mL of distilled water and maintained at a constant temperature of 30 °C for 2 h. After this period, the amount of potassium leached was determined using a flame photometer. The results were expressed in µg g^{-1} of seed, corresponding to mean values per lot.

Accelerated aging (traditional procedure): this was conducted with 5 g of seed on wire meshes in plastic boxes containing 40 mL of water, maintained at 38 and 41 °C for 24, 48 and 72 h. Then, seeds were placed to germinate according to the methodology described for the germination test. The evaluation was performed four days after sowing and the results expressed as percentages. For monitoring purposes, seed moisture content was determined before and after the test.

Accelerated aging (saturated NaCl solution): this was conducted in a similar manner to that described for the traditional test, except for adding 40 mL of saturated NaCl solution instead of water. This solution was obtained by diluting 40 g of NaCl (P.A.) in 100 mL of water, thus establishing an environment with a relative humidity of 76%.

A completely randomized design with four replicates was used. Data were subjected to analysis of variance and means obtained for each treatment were compared by the Tukey test ($P \leq 0.05$) using the SISVAR software (Ferreira, 2011).

Results and Discussion

Table 1 shows the results for the tests performed in seed lots of each cultivar under study. It was observed for cv. Cultivada that there were germination differences between the assessed lots. Lot 4 was considered to have the lowest physiological potential, with a germination of 89%, whereas percentages between 94 and 99% were observed in the other lots. For cv. Folha Larga, the evaluated lots had similar germinations, ranging from 89 to 93%.

It is important and coherent to compare seed lots with similar germination (Marcos Filho, 1999), especially if they are located in Phase I of the viability loss curve. This is because in Phase II, when a rapid decline in germination occurs, even the germination test (conducted under favorable conditions) is able to detect differences in the physiological potential of samples. However, it is the position of each lot in Phase I that determines its vigor level (Powell, 1986).

The first counting of cv. Folha Larga did not differ between lots, with results similar to those found in the germination test. As for cv. Cultivada, the worst result was observed in lot 4, with a germination of 83% at 4 days. This result was much lower than those obtained for the other lots, which had germination rates equal to or greater than 92%, demonstrating the lower vigor of lot 4.

For seedling emergence of cv. Cultivada in the field, lots 1, 2 and 3 showed the best results, with 73, 76 and 75%, respectively. With 66%, lot 4 did not differ from lot 1, and was similar to lot 5, which had the lowest seedling emergence (59%). In general, these results are lower than those found in the germination test. Nevertheless, they are normally expected due to the optimal laboratory conditions (Ávila et al., 2005). The trend verified for the emergence speed index was similar to that observed for seedling emergence. According to this test, lots 1, 2 and 3 were superior to the others. As for seedling emergence of cv. Folha Larga in the field, no difference was observed between lots. Similar results were obtained for emergence speed index, indicating similar physiological quality between lots.

No differences were found between seed lots of cv. Cultivada when the two other vigor tests, based on seedling performance (shoot length and dry mass), were used. As for cv. Folha Larga, seedling shoot length showed a result similar to that obtained with the other vigor tests discussed so far, which showed lots with the same physiological quality. On the other hand, three vigor levels were verified when seedling shoot dry mass was assessed.

Analyzing the results of the electrical conductivity test, it was observed for cv. Cultivada that, although it did not differ statistically from lots 1, 2 and 3, lot 5 showed the highest value, indicating an advanced seed deterioration (Panobianco et al., 2007). For cv. Folha Larga, using this method, a similar physiological quality was observed for all lots.

The potassium leaching test was not effective to differentiate seed vigor among lots of arugula cv. Cultivada, since all of them were considered to be similar. The same was not observed for cv. Folha Larga, which had the physiological quality of its lots differentiated into two vigor levels. According to this test, lots 3 and 4 had the greatest vigor, but were statistically similar to lot 2, which showed an intermediate quality. Seeds from lot 1 had the highest potassium leaching, thus indicating a lower quality (Alves & Sá, 2010).

Table 2 shows the germination percentage data after traditional accelerated aging and with the use of saturated

Table 1. Germination (G), first counting (FC), seedling emergence in the field (E), emergence speed index (ESI), seedling shoot length (SSL), seedling shoot dry mass (SSDM), electrical conductivity (EC) and potassium leaching (KL) of arugula seeds, cv. Cultivada and cv. Folha Larga.

Cultivar	Lot	G *	FC *	E *	ESI*	SSL	SSDM	EC	KL
			(%)			(cm)*	(mg seedling^{-1})*	(µS cm^{-1} g^{-1})*	(µg g^{-1})*
Cultivada	1	94 ab	92 a	73 ab	14.38 a	0.85 a	13.75 a	11.18 ab	18.16 a
	2	95 ab	92 a	76 a	14.76 a	0.80 a	14.50 a	12.15 ab	18.18 a
	3	99 a	96 a	75 a	15.99 a	0.88 a	11.25 a	10.75 ab	20.85 a
	4	89 b	83 b	66 bc	11.27 b	0.70 a	15.75 a	10.67 b	21.29 a
	5	98 a	97 a	59 c	11.39 b	0.70 a	11.75 a	13.03 a	21.05 a
	CV	4.09	3.95	6.03	6.76	23.20	19.84	9.18	9.59
Folha Larga	1	92 a	91 a	79 a	14.81 a	0.83 a	20.75 a	12.70 a	25.10 a
	2	91 a	89 a	77 a	15.68 a	0.88 a	15.25 b	12.78 a	17.64 ab
	3	93 a	91 a	73 a	15.99 a	0.78 a	11.25 c	11.04 a	15.21 b
	4	89 a	86 a	76 a	14.77 a	0.83 a	9.75 c	12.55 a	12.05 b
	CV	5.25	4.92	7.06	8.67	11.61	12.65	12.40	20.52

*For each cultivar, means followed by the same letter in the column are not significantly different ($P \leq 0.05$) by the Tukey test.

Table 2. Germination percentage after traditional (TS) and saturated NaCl solution (SS) accelerated aging of arugula seeds, cv. Cultivada and cv. Folha Larga.

Cultivar	Lot	Germination (%)*											
		38 °C						41 °C					
		24 h		48 h		72 h		24 h		48 h		72 h	
		TS	SS	TS	SS	TS	SS	TS	SS	TS	SS	TS	SS
Cultivada	1	86 ab	94 a	81 ab	94 a	78 a	95 a	68 bc	93 a	22 b	96 a	8 b	94 a
	2	78 b	93 a	67 cd	92 ab	37 c	93 a	72 b	90 a	13 bc	81 c	7 b	84 b
	3	88 a	94 a	89 a	90 ab	73 ab	94 a	84 a	88 a	60 a	89 ab	29 a	90 ab
	4	78 b	87 a	62 d	87 b	62 b	84 b	53 d	87 a	3 c	81 c	0 c	73 c
	5	91 a	90 a	73 bc	90 ab	84 a	92 a	63 c	87 a	8 bc	88 bc	0 c	89 ab
	CV	5.41	4.32	6.28	3.22	8.00	3.00	6.35	4.32	31.76	3.88	30.91	4.98
Folha Larga	1	44 b	90 a	25 c	88 a	56 b	94 a	53 c	92 a	1 b	93 a	0 b	92 a
	2	85 a	90 a	81 a	88 a	86 a	94 a	82 a	91 a	1 b	91 a	0 b	88 a
	3	84 a	89 a	86 a	93 a	90 a	93 a	86 a	87 ab	3 a	82 b	1 b	84 a
	4	48 b	93 a	52 b	88 a	37 c	86 a	64 b	85 b	2 ab	81 b	8 a	83 a
	CV	14.70	4.76	12.24	3.08	9.30	5.30	6.78	2.96	55.5	4.96	28.87	5.52

*For each cultivar, means followed by the same letter in the column are not significantly different ($P \leq 0.05$) by the Tukey test.

NaCl solution. In general, when seeds were aged with the traditional solution, a great disparity between the results was observed, with different quality levels for the lots. However, when seeds were subjected to the saturated solution, it was possible to differentiate lots without a drastic reduction in germination percentage. This was not observed when seeds were subjected to the traditional solution, which, combined with a high temperature and long exposure period, caused the germination percentage of some lots to be reduced to zero.

For cv. Cultivada, the saturated solution was more effective for vigor classification when seeds were aged at 41 °C for 48 or 72 h, whereas the traditional solution was effective at 38 °C for 48 or 72 h. The combinations of temperature and exposure period that showed results similar to those obtained for emergence and emergence speed index were 41 °C/48 h for the saturated solution and 38 °C/48 h for the traditional solution. According to these combinations, lots 2, 4 and 5 were considered to have the lowest vigor. The ideal combination found for cv. Cultivada using a saturated solution was similar to that obtained by Ramos et al. (2004), but different from those found for other species from the same family, such as broccoli - 45 °C/48 h (Fessel et al., 2005), and radish - 41 °C/72 h (Ávila et al., 2006).

As for cv. Folha Larga, the saturated solution was effective to detect vigor differences using 41 °C for 24 or 48 h. This result was similar to that observed for seedling shoot dry mass, indicating different physiological quality between seed lots. The use of the traditional solution at a temperature of 38 °C was effective to differentiate the vigor of seed lots, regardless of the exposure period (24, 48 or 72 h). At a temperature of 41 °C, the traditional solution in a 24 h exposure period was also effective. However, in general, the results obtained with the traditional solution were not associated with other physiological quality indicators.

The average results for the moisture content reached after performing the accelerated aging test with traditional and saturated solutions, at different temperatures and periods, are shown in Table 3. The initial moisture content was relatively similar for all lots studied, ranging from 5.4 to 6.9%.

Moisture content showed a positive trend in relation to the increase in exposure period, with a considerable increment observed in a 24 h period. After this period, the increases in moisture content became less significant. These

Table 3. Moisture content obtained before and after the combinations of temperature and period for accelerated aging, traditional (TS) and with saturated NaCl solution (SS), of arugula seeds, cv. Cultivada and cv. Folha Larga.

Cultivar	Lot	Initial	Moisture content (%)											
			38 ºC						41 ºC					
			24 h		48 h		72 h		24 h		48 h		72 h	
			TS	SS	TS	SS	TS	SS	TS	SS	TS	SS	TS	SS
Cultivada	1	6.1	25.1	10.3	30.2	10.7	35.9	10.2	28.1	8.6	35.2	10.3	36.4	8.8
	2	6.2	24.8	9.5	32.0	9.2	34.5	9.4	24.8	7.7	35.4	9.9	35.9	7.7
	3	6.3	27.1	9.7	36.5	10.6	36.8	11.3	26.6	7.1	35.1	10.6	34.8	9.0
	4	6.3	25.8	8.6	38.7	9.4	35.0	11.3	25.2	7.2	39.0	10.1	36.5	8.0
	5	6.7	29.3	8.9	35.2	9.2	34.6	13.1	26.2	7.3	35.1	12.2	37.3	8.9
Folha Larga	1	6.5	24.2	9.9	33.3	9.6	36.2	12.8	24.6	9.2	35.2	9.2	31.4	10.2
	2	6.9	25.7	8.5	36.7	10.9	38.2	11.9	25.7	10.2	37.4	10.9	34.2	9.2
	3	5.4	27.0	10.1	36.3	10.0	36.4	10.9	24.8	9.9	38.4	10.6	35.4	9.5
	4	5.5	25.1	8.4	33.0	8.9	31.3	11.5	25.1	7.9	34.5	9.2	34.6	9.5

results were observed mainly for the traditional solution. For the saturated solution, this increment was less substantial.

It was observed that seeds of both cultivars aged by the traditional method reached higher moisture levels and had more pronounced variation, with values between 24.2 and 39%. Similarly, Ramos et al. (2004) found high variation in the seed moisture content of arugula cv. Cultivada at the end of the traditional accelerated aging, with values ranging from 35.6 to 43.3%. In contrast, the use of the saturated solution allowed a reduction in the water uptake rate by the seeds during the exposure period, which led to values between 7.1 and 13.1%. Thus, this condition promoted less severe effects (Table 2). When compared to the conventional method, the use of the saturated solution attenuates seed deterioration by reducing seed moisture content (Kibinza et al., 2006).

A reduction in fungal growth during the test was another advantage verified with the use of the saturated solution. This is because the restriction on relative humidity inhibits the proliferation of microorganisms. In this work, lots aged in solution with the addition of NaCl showed no fungal contamination. Similar observations were made by Ramos et al. (2004), Fessel et al. (2005), Ávila et al. (2006) and Costa et al. (2008).

Conclusions

Accelerated aging with a saturated NaCl solution using the combination of 41 °C for 48 h, and traditional accelerated aging using the combination of 38 °C for 48 h were effective for classifying seed lots of cv. Cultivada into different vigor levels. On the other hand, accelerated aging with saturated NaCl solution using the combination of 41 °C for 24 or 48 h was suitable for classifying the vigor of cv. Folha Larga seeds.

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