# Assessment of the physiological potential of spinach seeds (*Tetragonia tetragonoides* (Pall.) Kuntze)<sup>1</sup>

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ABSTRACT –The efficiency of vigor tests in assessing the physiological potential of seeds depends on their standardization for different species. In this context, the research aimed to study specific methodologies to evaluate the vigor of spinach seeds (*Tetragonia tetragonoides*). For this purpose, five lots of spinach seed cv. New Zealand were submitted to germination, first count of germination, seedling emergence in the field, accelerated aging (with and without use of saturated solution of NaCl at 41and 45 °C for 24,48 and 72 hours), controlled deterioration(18,21 and 24% seed water content adjustments for 24 hours at 45 °C) and electrical conductivity tests with variations inwater volume (25, 50 and 75 mL), seed quantity (25 and 50) and soaking period (1, 2, 4, 8, 12, 16 and 24 hours) at 25 °C. The first count test allowed obtaining preliminary information about seed vigor, and other tests in general showed similarity with seedling emergence in the field. Thus, it was concluded that traditional accelerated aging test 24 h /41 °C and accelerated aging test with saturated salt solution for 24 h at 41 °C and 45 °C, and controlled deterioration test with 21% seed water content / 24 h / 45 °C were all efficient for evaluating the physiological potential of spinach seeds.

Index terms: vegetable crop, adaptation of procedures, vigor.

## Avaliação do potencial fisiológico de sementes de espinafre (*Tetragonia tetragonoides* (Pall.) Kuntze)

RESUMO – A eficiência dos testes de vigor na avaliação do potencial fisiológico de sementes depende do desenvolvimento e/ou do ajuste de metodologias para as diferentes espécies. Nesse contexto, o objetivo da pesquisa foi adequar procedimentos para avaliação do potencial fisiológico de sementes de espinafre. Para tanto, sementes de cinco lotes, da cultivar Nova Zelândia, foram submetidas aos testes de germinação, primeira contagem da germinação, emergência de plântulas, envelhecimento acelerado (com e sem o uso de solução saturada de NaCl a 41 e 45 °C por 24, 48 e 72 h), deterioração controlada (18, 21 e 24% de teor de água por 24 h a 45 °C) e condutividade elétrica (25 e 50 sementes, 25, 50 e 75 mL de água de embebição por 1, 2, 4, 8, 12, 16 e 24 h, a 25 °C). Pode-se concluir que os testes de envelhecimento acelerado tradicional 24 h / 41° C, envelhecimento acelerado com solução salina por 24 h a 41 e 45 °C e deterioração controlada a 21% / 24 h a 45 °C foram eficientes para avaliar o potencial fisiológico e para classificar os lotes de sementes de espinafre em níveis de vigor.

Termos para indexação:hortaliça, adequação de procedimentos, vigor.

### Introduction

The species *Tetragonia tetragonoides* (Pall.) Kuntze, belonging to the Aizoaceae family, is originally from New Zealand and Australia. It is a plant of creeping growth habit, semi-herbaceous, succulent, branched, woody at the base and bright green. The leaves are fleshy, triangular-shaped, arranged alternately and dark green. The inflorescences are axillary, with one to three flowers, which may be uni or bisexual, of green to yellow color. The fruits are drupe, indehiscent, with small horns.

The propagation is made in trays, and when the seedlings have four to five leaves, they should be transplanted to a permanent site (Filgueira, 2000).

It's most consumed kind of spinach in Brazil, due to its adaptability to the tropical climate. Theacreage in the state of São Paulo is of 652 ha, with production of 10,160 t and productivity of 15,583 kg per hectare (Brazilian Association of Horticulture, 2012). The cultivation occurs mainly in the South, Southeast and Midwest regionsand can be grown year-round, producing best in mild or warm temperatures, not recommended for cold

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winters in altitude regions. Spinach is grown for the use of its leaves, which can be consumed "in natura" or processed into canned or frozen products. It is notable among the vegetables for its nutritional composition, with high levels of iron, excellent source of vitamins A and B2, in addition to providing calcium, phosphorus, potassium and magnesium (Filgueira, 2000).

Although its seeds have high commercial value, it has been noticed, still, the almost inexistence of research directed to developing methods for assessing their physiological potential. High quality seeds provide a suitable stand, and uniform development of plants in the field, allowing the expression of the genetic potential of the cultivar. In view of this, seed companies have been painstaking in search of production, maintenance and quality control techniques. Since decision-making must often be immediate, the demand for tests that provide faster and more reliable results has been increasing. Thus, vigor tests have been a point of support for researchers and the seed industry, as they provide information about the behavior of seeds, reflecting manifestations of decay.

According to Marcos-Filho (2005), due to having lower amounts of stored reserves, the vegetable seeds have a higher propensity to decay after physiological maturity, which is another reason that justifies studies about the vigor of these seeds. Although vigor tests have been widely studied in terms of their standardization for many species, information is still scarce in the literature on the conduction of these tests in vegetable seeds, especially spinach seeds.

Given the above, this study was aimed at determining procedures to assess the vigor of spinach seeds, aiming to classify lots with similar germination pattern, but with different vigor.

#### **Materials and Methods**

The present study was conducted in the Laboratory of Seed Analysis of the Department of Vegetable Production, UNESP, Jaboticabal, using five lots of spinach seed cv. New Zealand. The tests were conducted from March 2010 to January 2011. The seeds were removed from their original packaging, treated with Thiram placed in transparent plastic bags, then kept in a cold chamber at 10 °C. Seeds from all lots were submitted to analyzes of water content, germination and vigor, as per the following descriptions:

*Water content* - was conducted in an oven at  $105 \pm 3$  °C / 24 h (Brasil, 2009), using two replicates of approximately 2 g of each lot. The results were expressed as percentage for each lot (wet basis).

Germination test - was conducted with 200 seeds (four replicates of 50 seeds) for each lot, sown in a plastic box  $(11.0 \times 11.0 \times 3.0 \text{ cm})$  on two sheets of filter paper moistened

with distilled water at a ratio of three times the mass of non hydrated paper. The boxes were placed in a germination chamber at alternating temperatures (20/30 °C). The counts were made at 35 days after sowing, with results expressed as percentage of normal seedlings (Brasil, 2009).

First germination count - was conducted along with the germination test, computing the percentage of normal seedlings, on the seventh day after sowing (Nakagawa, 1999).

Accelerated aging (traditional procedure) - samples were used for each lot of 250 seeds distributed on aluminum screen trays, placed inside plastic boxes (11.0 x 11.0 x 3.0 cm), functioning as individual compartments (mini-chamber). Inside of it, 40 mL of water were added, and then the boxes with the seeds were kept in a water-jacketed chamber. The seeds were aged at 41 and 45 °C for 24, 48 and 72 hours. After these periods, seeds were left to germinate as described for the germination test, with counting on the seventh day after sowing, computing the percentage of normal seedlings. Simultaneously, the water content of the seeds after aging was determined by the oven method (Hampton and TeKrony, 1995; Marcos-Filho, 1999).

Accelerated aging (NaCl saturated saline solution) - the same procedures described for the accelerated aging test were used, except that the 40 mL of distilled water were replaced by 40 mL of NaCl saturated saline solution (Jianhua and McDonald, 1997). This solution was obtained by diluting 40 g of NaCl in 100 mL of water.

Controlled deterioration - initially, the water content of the seeds was adjusted to 18, 21 and 24% using the methodology of the humid atmosphere (Rossetto et al., 1995). Therefore, seed samples from each lot were added on aluminum or stainless steel screens, in a plastic box, containing 40 mL of deionized water. The boxes were capped and kept in a cold chamber at 20 °C, with successive weighings being performed for monitoring the water content of seeds until the desired values were obtained. The final mass of the sample was determined from the equation  $M_f = [(100 - A) \times (100 - B)^{-1}] \times M_i$ , where:  $M_f = \text{final mass}, M_i = \text{final mass}$ initial mass and A and B correspond to the initial and desired water content, respectively. Next, the samples from each lot were packed tightly in aluminized plastic bags and held for five days in a cold chamber (10 °C and 50% RH), to reach moisture content equilibrium. Thereafter, the samples were placed in a water bath at 45 °C for 24 hours (Powell, 1995), and at the end of this period, the containers were removed and placed in a desiccator for 30 minutes to reduce the temperature. The germination test was performed, with counting on the seventh day after sowing normal seedlings. The water content of the seeds after the period in a water bath was also determined for the purpose of monitoring the procedure.

Electrical conductivity - combinations among variables

seed number (25 and 50), soaking water volume (25, 50 and 75 mL) and soaking period (1, 2, 4, 8, 12, 16, 20 and 24 hours) were tested at 25 °C. The evaluations were conducted with four replications, using seeds previously weighed with precision of 0.001 g. Electrical conductivity was determined in conductivity DIGIMED DM-31, and the average values for each lot, expressed in μS.cm<sup>-1</sup>.g<sup>-1</sup>. The reading of each subsample was performed immediately after gradual removal of the material from the germinator, while carefully stirring each container, in order to standardize the lixiviated electrolytes in the solution. The test was conducted according to (Hampton and TeKrony, 1995; Vieira and Krzyzanowski, 1999; AOSA, 2002).

Seedling emergence - expanded polystyrene ("Styrofoam") trays were used with individual cells containing organomineral commercial substrate (type Plantmax), kept in ambient conditions with periodic irrigation. For each lot, there were four replicates of 50 seeds, considering one seed per cell. The evaluation of seedling emergence was done 14 days after sowing, by counting normal seedlings emerged, evaluated according to the criteria used for the evaluation of normal seedling shoot in the germination test, with results expressed as a percentage of normal seedlings.

The experimental design was a completely randomized design with four replications and analyzes conducted separately for each test. Data from germination, seedling emergence, damage control and accelerated aging tests were previously converted to sen root (100 / x), however, the tables present the original values. The comparison of means was done by Tukey test ( $p \le 0.05$ ).

#### **Results and Discussion**

The initial water content of the seeds gave similar results for lots 1, 3 and 5; lot 2 showed higher water content and lot 4 was lower than other lots (Table 1). In the germination test, lots 1, 2 and 4 showed higher germination percentage. Although there is a distinction in the germination percentage, all lots showed average percentage above the minimum established (55%) in the standards for marketing spinach seeds (ABCSEM, 2012).

In the first count test, all lots differed statistically, being lots 1 and 2 of top quality and lots 3, 4 and 5 of low physiological

potential, and however, they all remained within commercially acceptable standards. The first germination count also allowed to differentiate the physiological potential of carrot (Spinola et al., 1998), cucumber (Bhéring et al., 2000), watermelon (Bhéring et al., 2003), lettuce (Franzin et al., 2004) and pepper (Bhéring et al., 2006) seed lots. Data on seedling emergence indicated lot 2 had high vigor and lot 5 had lower vigor. These results agree in general with those obtained from the first germination count. According to Marcos-Filho (2005), seedling emergence is a parameter that indicates the efficiency of tests to assess the physiological potential of seed lots. Therefore, in this study it was found that this efficiency in safely distinguishing lots of high and low vigor.

The results of the traditional accelerated aging test using two different temperatures and three conditioning periods enabled the separation of spinach seed lots in function of the physiological potential. Seed exposure to a temperature of 41 °C for periods of 24, 48 and 72 h was significant for classifying lots (Table 2). In the 24 h period, lots 1 and 2 were considered of superior quality and presented germination above the minimum acceptable commercially, while lot 5 showed less physiological potential. In the 48 h period, although lots 1 and 2 have not presented a significant difference, only lot 2 stood out with considerably acceptable germination. In the period of 72 h, lots 1 and 2 were superior to the other lots.

Table 1. Initial quality of seeds of five lots of spinach, cv. New Zealand, measured by water content (WC), first count (FC), germination (G) and seedling emergence (SE).

T .	WC	FC	G	SE				
Lots								
1	8.4	89 a*	92 a	94 ab				
2	9.9	82 ab	89 a	96 a				
3	8.5	69 cd	73 b	84 abc				
4	7.1	76 bc	82 a	75 bc				
5	8.4	58 d	66 c	65 c				
CV (%)	-	6.99	6.97	11.72				

<sup>\*</sup>Means followed by the same letter in the column do not differ by Tukey test at 5%.

Table 2. Vigor, evaluated by traditional accelerated aging test, of five lots of spinach seeds cv. New Zealand.

		41 °C			45 °C			
Lots	24h	48h	72h	24h	48h	72h		
			0/0					
1	75 a*	47 a	87 a	72 a	24 a	0 a		
2	71 a	62 a	43 a	51 b	18 b	0 a		
3	45 b	46 b	13 b	24 c	3 c	0 a		
4	55 b	26 b	26 b	46 b	3 c	0 a		
5	34 c	18 b	18 b	51 b	0 c	0 a		
CV (%)	8.81	13.71	16.92	16.8	20.2	0		

<sup>\*</sup>Means followed by the same letter in the column do not differ by Tukey's test at 5%.

On aging at 45 °C it was possible to differentiate lots in the 24 h period. Lot 1 stood out for its higher germination, and the others showed no significant germination. The 48 h period was not effective, since the germination percentages were unsatisfactory. In the 72 h, the stress caused by the aging period was drastic to the seeds, leading them to the last consequences of the deterioration process, the total loss of germination capacity.

The water contents before the accelerated aging test were between 7.1 and 9.9%, however, when the seeds were subjected to the test at 41 °C, the average increased, ranging between 21 and 28% according to the period used (Table 3). This intense water absorption confirms the quote by McDonald and Copeland (1997), who claim that the water content of the seeds shows close relationship with high relative humidity in the aging environment, since the seeds present a hygroscopic character. After aging, the maximum variation of water content between lots was one percentage point in the 24 h period, two points in the 48 h period and two points in 72 h. This variation is acceptable, since for Marcos-Filho (2005), the results are considered reliable if the maximum variation is two percentage points. With the temperature at 45 °C, the water contents of the seeds in accordance with the periods presented variation by two percentage points over the 24 h period, in the 48 h period the values were similar for all lots and one percentage point during the 72 h period. Thus, for the 45 °C temperature, the variation in mean water content of the seeds followed the same behavior observed at 41 °C, with similar values in all three periods.

In the accelerated aging with saturated saline solution (Table 4) at temperature of 41 °C the germination percentage was higher when compared to 45 °C; because of this, most of the researchers who are dedicated to studies on this test indicate the use of 41 °C (Marcos-Filho, 1999). At the temperature of 41 °C and in the 24 h period, lot 1 had a superior quality and the other lots were inferior. In the of 72 h period, lots 1, 2 and 4 presented superior germination, while germination in lot 5 was inferior to the others. In the 72 h period, lot 1 showed higher germination percentage than lot 5, even with a low value; the germination percentage was not significant for the remaining lots. The combination of 45 °C temperature and 24 h period was suitable for the separation of lots regarding vigor, lot 1 being superior and the other lots having low physiological potential. In the 48 h period, for all lots the germination percentages were not significant, with values below the established minimum for germination of spinach seeds. The 72 h period was noted for having presented germination, even if low, a fact that has not occurred when the test was conducted with the traditional procedure (Table 2).

Table 3. Water content of spinach seeds, cv. New Zealand, after accelerated aging test (tradicional procedure) at 41 and 45 °C	,
during different periods of time.	

		41 °C		45 °C			
Lots	24h	48h	72h	24h	48h	72h	
				⁄o			
1	22	26	27	22	26	24	
2	22	26	27	21	26	24	
3	22	25	28	22	26	24	
4	22	25	26	22	26	25	
5	23	24	27	23	26	25	
Mean	22.2	25.2	27.0	22.0	26.0	24.4	

Table 4. Vigor of five lots of spinach, cv. New Zealand, assessed by accelerated aging test with saline solution at 41 and 45 °C, during different periods of time.

		41 °C			45 °C		
Lots	24h	48h	72h	24h	48h	72h	
1	87 a*	58 a	54 a	84 a	49 a	28 a	
2	59 b	46 a	37 ab	63 b	23 b	17 b	
3	55 b	26 b	32 ab	45 c	23 b	15 b	
4	56 b	42 ab	31 ab	49 c	19 b	18 b	
5	40 b	9 c	15 b	22 d	1 c	1 c	
CV(%)	11.61	12.62	21.40	10.00	17.35	21.50	

<sup>\*</sup>Means followed by the same letter in the column do not differ by Tukey's test at 5%.

In the accelerated aging test with saturated solution at the temperature of 41 °C, the water content of the seeds remained almost uniform over all periods (Table 5). In the periods of 24 h and 72 h there were no differences among lots. In the 48 h period, variation was of one percentage point. With the temperature at 45 °C, variation in all periods was of one percentage point. The small variation in the results occurred because in the accelerated aging conducted with a saturated NaCl solution, the relative humidity around 76% (Jianhua and McDonald, 1997) is below that verified in aging with water, which is approximately 100%. Thus, as the saline solution provides lower relative humidity, this provided a better uniformity of water content between

lots, providing similar deterioration intensity between lots. In the accelerated aging test using saline solution, a reduction of mold growth was observed during the test, due to the restriction on the relative humidity that disfavors the proliferation of microorganisms. Similar observations were made by Jianhua and McDonald (1997); Rodo et al. (2000) and Panobianco and Marcos-Filho (2001).

In controlled deterioration, the water contents (18, 21 and 24%) remained approximately constant throughout the test period except for lot 5, from whom the methodology became unfeasible in obtaining the water content of 24%, due to the long period that the seeds took to achieve this percentage, as well as the growth of fungi inside the boxes.

Table 5. Water content of spinach seeds, cv. New Zealand, submitted to accelerated aging test in saturated saline solution at 41 and 45 °C, during different periods of time.

	41 °C			45 °C			
Lots	24h	48h	72h	24h	48h	72h	
				%			
1	12	12	12	12	12	12	
2	12	11	12	11	12	12	
3	12	11	12	11	11	11	
4	12	12	12	11	12	12	
5	12	12	12	12	12	12	
Mean	12	11.6	12	11.4	11.8	11.7	

Analyzing the results obtained in the controlled deterioration test (Table 6), it was observed that for the water content of 18% for 24 h at 45 °C, only lot 1 obtained a high physiological potential, lot 2 was of intermediate quality, even if it did not differ from lot 1, and the remaining lots were of lower quality. The combination of 21% / 24 h at 45 °C showed a more satisfactory result, being compatible with the ordering of the lots obtained by the first count test. Thus, in general, lots 1 and 2 were classified as superior and the others were classified as being of lower quality. Not, at 24% / 24 h at 45 °C, while all lots presented low physiological potential, lots 1, 3 and 4 proved to be of superior quality and lots 2 and 5 of inferior quality. According to Hampton and TeKrony (1995), seed lots that have kept their germination after deterioration are considered lots of high vigor and those who decreased their ability to germinate are considered low vigor.

For the electrical conductivity test, the results with 25 seeds and volumes of 25, 50 and 75 mL were considered inconsistent, however, the volume of 75 mL proved to be the most sensitive to in lot stratification from 4 h of soaking (Table 7). To the resulting data of 50 seeds and volume of 25, 50 and 75 mL (Table 8), it was found that a volume of 25 mL enabled the differentiation among lots regarding the vigor. Lots 3 and 4 were superior on

the physiological potential in most periods and the inferiority of lot 5 was confirmed, demonstrating similarity to the results found in other tests. In the combination 50 seeds and 75 mL, lots 1 and 4 stood out with lower leachate values, thus concluding that they are of superior quality in relation to the others. Lot 5 stood out once again as having lower physiological potential, showing similarity to the results found in previous tests.

Table 6. Vigor, by controlled deterioration test, of five lots of spinach, cv. New Zealand, with different water contents.

Lots	18%	21%	24%
1	74 a*	81 a	32 a
2	50 ab	66 a	17 b
3	30 b	13 c	42 a
4	25 b	32 b	43 a
5	31 b	12 c	0 c
CV (%)	28.40	18.48	20.84

\*Means followed by the same letter in the column do not differ by Tukey's test at 5%.

It was further observed that, in the volume of 25 mL, higher readings electrical conductivity were taken compared to volumes of 50 and 75 mL for the amount of both 25 and 50 seeds

(Tables 7 and 8) which was already expected, since soaking in a large volume of water results in greater dilution of leachates. Mello et al. (1999) and Torres and Marcos-Filho (2005) also observed similar results with broccoli seeds, where the ratio of 25 mL of water and 50 seeds at a temperature of 25 °C, provided information comparable to those obtained in germination tests and seedling emergence, in the identification of the physiological potential of the lots assessed. The test when performed with 50 seeds was more efficient for the classification of lots, proving that

recommended by Vieira (1994); Hampton and TeKrony (1995).

Given this, the electrical conductivity test is an important option for evaluating seed vigor of most vegetables, providing consistent results and which allow reliable identification of differences in the physiological potential of seeds of various species. However, for spinach seed there is need for further research in order to identify the most suitable procedures for the species, such as the best combination of temperature, seed number, water volume and soaking period.

Table 7. Electrical conductivity (μS.cm<sup>-1</sup>.g<sup>-1</sup>) of five lots of spinach, cv. New Zealand, depending on the amount of seeds, volume of water and soaking periods at 25 °C.

				eds / 25 mL / 25					
Lots	Imbibition periods								
	1h	2h	4h	8h	12h	16h	24h		
1	118.6 a*	120.1 a	137.0 a	168.7 a	146.9 a	202.6 a	227.9 a		
2	115.3 a	129.4 a	153.6 a	174.4 a	191.1 a	206.8 a	244.6 a		
3	126.4 a	134.9 a	153.5 a	165.1 a	173.4 a	189.6 a	225.8 a		
4	111.4 a	119.3 a	130.9 a	155.8 a	165.0 a	177.2 a	210.5 a		
5	129.8 a	147.8 a	165.7 a	196.1 a	200.8 a	223.5 a	278.8 a		
CV%	14.1	14.8	12.7	14.9	26.4	14.6	21.3		
			25 se	eds / 50 mL / 25	i °C				
Lots	Imbibition periods								
	1h	2h	4h	8h	12h	16h	24h		
1	69.2 a*	73.3 a	80.2 a	92.4 a	92.4 a	106.6 a	117.0 a		
2	61.3 a	72.3 a	78.5 a	87.8 a	87.8 a	100.1 a	121.9 a		
3	62.7 a	72.3 a	76.2 a	78.9 a	78.9 a	95.7 a	98.9		
4	73.5 a	85.6 a	92.5 a	100.9 a	100.9 a	118.3 a	93.9 8		
5	66.1 a	72.9 a	78.5 a	89.2 a	89.2 a	102.5 a	100.0		
CV(%)	16.0	15.0	16.0	15.3	15.3	15.1	14.1		
			25 se	eds / 75 mL / 25	i °C				
Lots	-		Ir	nbibition periods	S				
	1h	2h	4h	8h	12h	16h	24h		
1	49.4 b*	58.9 a	60.5 b	67.7 b	73.5 b	76.7 ab	87.4 a		
2	50.9 b	54.4 a	61.5 b	67.1 b	74.5 b	79.0 b	91.1 a		
3	47.9 ab	53.3 a	57.4 ab	62.7 ab	68.8 ab	73.1 ab	87.3 a		
4	38.9 a	47.1 a	47.9 a	54.9 a	59.3 a	64.0 a	77.5 a		
5	52.6 a	58.3 a	62.5 b	71.1 b	77.8 b	82.2 b	98.1 b		
CV(%)	10.0	11.3	9.8	7.7	7.8	7.9	8.1		

<sup>\*</sup>Means followed by the same lowercase letter in the column do not differ by Tukey's test at 5%.

Table 8. Electrical conductivity (μS.cm<sup>-1</sup>.g<sup>-1</sup>) of five lots of spinach cv. New Zealand, depending on the amount of seeds, water volume and soaking periods.

	50 seeds / 25 mL/ 25 °C Imbibition periods								
Lots									
	1h	2h	4h	8h	12h	16h	24h		
1	83.9 ab*	117.0 a	129.3 ab	143.7 ab	157.0 ab	180.1 ab	200.5 a		
2	96.9 ab	118.9 a	118.1 a	151.1 ab	157.1 ab	188.6 ab	220.0 ab		
3	86.7 a	104.6 a	119.0 abab	124.4 a	144.0 a	159.3 a	197.7 a		
4	95.0 ab	108.9 a	118.8 a	124.0 a	145.9 a	161.9 a	194.1 a		
5	109.9 b	134.5 a	148.1 b	180.7 b	145.9 a	219.3 b	254.3 b		
CV%	10.91	13.94	10.55	13.63	13.86	14.18	11.28		

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			50 s	seeds / 50 mL/ 25	°C				
Lots	Imbibition periods								
	1h	2h	4h	8h	12h	16h	24h		
1	52.2 a*	67.6 a	77.4 a	82.5 a	90.4 a	105.3 a	123.8 a		
2	48.2 a	59.0 a	64.4 a	74.4 a	81.9 a	94.4 a	113.1 a		
3	54.1 a	63.8 a	71.6 a	74.0 a	89.6 a	99.5 a	116.3 a		
4	49.2 a	61.9 a	63.6 a	71.4 a	89.56 a	92.7 a	110.3 a		
5	61.3 a	73.9 a	77.4 a	87.4 a	88.2 a	109.2 a	119.9 a		
CV%	14.0	13.9	9.2	14.4	13.3	10.2	9.9		
	50 seeds / 75 mL/ 25 °C								
Lots	Imbibition periods								
	1h	2h	4h	8h	12h	16h	24h		
1	35.6 a*	43.1 a	46.7 a	55.6 a	58.9 a	64.0 a	73.0 a		
2	38.1 a	47.6 a	50.9 a	56.9 a	62.4 a	67.0 a	80.1 al		
3	41.7 a	49.4 a	53.7 ab	59.5 a	64.1 a	68.8 a	88.5 al		
4	36.8 a	43.8 a	47.1 a	52.7 a	56.9 a	61.3 a	73.6 a		
5	50.5 b	58.5 b	60.2 b	69.8 b	76.3 b	81.5 b	101.0 b		
CV%	9.7	8.3	7.2	6.4	6.3	6.0	11.6		

<sup>\*</sup>Means followed by the same lowercase letter in the column do not differ by Tukey's test at 5%.

#### **Conclusions**

The traditional accelerated aging test in the conditioning period of 24 h at 41 °C and accelerated aging in saline solution for 24 h at 41 and 45° C are best suited for differentiation of lots in vigor levels.

The controlled deterioration test conducted with 21% water content, for 24 hat 45 °C is effective for evaluating the physiological potential of spinach seeds.

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