



RESEARCH ARTICLE

ADEQUATION OF THE ELECTRICAL CONDUCTIVITY TEST FOR ARUGULA SEEDS AND CORRELATION WITH SEEDLING EMERGENCE

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ARTICLE INFO

Article History:

Received 10th August, 2016
Received in revised form
08th September, 2016
Accepted 23rd October, 2016
Published online 30th November, 2016

Key words:

Eruca sativa,
Electrical conductivity,
Seeds deterioration,
Stratification of lots,
Vigor.

ABSTRACT

Currently, due to the growth of vegetable crops seeds market there is a demand for tests to evaluate the physiological potential quickly and efficiently for selecting vigorous commercial lots. The study aimed to adapt the electrical conductivity test methodology to stratify lots of arugula seeds into vigor levels according to seedling emergence. Five lots of arugula seeds were used. The initial quality of seeds were evaluated by the water content of the seeds, germination, first count, seedling emergence. After that, were analyzed the following methodologies for the electrical conductivity test: six periods of soaking (1, 2, 4, 6, 8 and 24 hours), combined with three volumes of deionized water (25, 50 and 75 mL), and repeat number of seeds (25 and 50) at soaking temperature of 20 °C. When using fewer seeds and an amount of water that promotes the reading of the leachate without over- or underestimation values in shorter periods of time, the use of this test becomes compatible routinely seed analysis laboratories. The conductivity test using 25 seeds and 50 mL of water at 20 °C, whether for 6 or 8 hours were effective to stratify lots of arugula seeds into vigor levels according to the seedling emergence test.

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Citation: Aline Klug Radke, Vanessa Nogueira Soares, Andrea Bicca Noguez Martins, Leticia Winke Dias, Paulo Eduardo Eberhardt Rocha and Francisco Amaral Villela, 2016. "Adequation of the electrical conductivity test for arugula seeds and correlation with seedling emergence", *International Journal of Current Research*, 8, (11), 40912-40916.

INTRODUCTION

Arugula (*Eruca sativa*) is a vegetable whose leaves are used mainly in salads, being rich in vitamins and minerals. This vegetable belongs to Brassicaceae family. Characteristics such as rapid vegetative growth and short life cycle making this vegetable economically viable alternative for farmers (Linhares et al., 2011). In the last decade arugula has been showing significant growth both in cultivation and consumption when compared to other similar vegetables (Freitas et al., 2009). Research institutions and seed companies face a challenge in evaluating physiological potential of seeds and selection of commercial lots. The market for vegetable seeds has been growing fast and the need for seeds with high quality is important. The use of seeds with high vigor provides greater percentage of emergence in the field, homogeneous plant stands, and early seedling growth (Pego et al., 2011). The germination test is the standardized procedure to assess the ability of seeds to produce normal seedlings under favorable conditions. However, that does not always shows differences

in performance among lots of seeds during storage or in field conditions (Carvalho & Nakagawa, 2000). Therefore, responsive and efficient methodologies to estimate more accurately the quality of seeds will speed up the marketing decisions in critical stages of seed production (Martin et al., 2011). Thus, the International Seed Testing Association (ISTA) highlights the electrical conductivity test as one of the most important tests due to fast and objective execution (Gonzales et al., 2009). Results can be obtained within 24 hours (Pereira & Martins Filho, 2012). The electrical conductivity test is considered a fast test that indirectly evaluates the quality of seeds. Seeds when hydrated exude ions, sugars, and other metabolites in the beginning of the soaking period. This happens because of changes in the integrity of the cell membrane system, which is depending of the level of seed deterioration (Carvalho & November, 2011). Seeds with low vigor tend to present disorganization of the cell membranes, which allow an increase in leaching of solutes (McDonald, 1999). Studies with vegetable crops seeds have shown that decrease in germination and vigor is directly proportional to an increase in concentration of electrolytes released by the seeds during the imbibition (Martins et al., 2002).

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Nonetheless, studies with many species of vegetables like arugula do not provide appropriate methodologies to evaluate physiological quality of seeds. Researchers have sought to adapt methodologies for the electrical conductivity test for different species (Cruz *et al.*, 2013). Different species may have variation in the amount of seed used, volume of deionized water and seed soaking times (Kikuti and Marcos-Filho, 2008; Souza *et al.*, 2009; Milani *et al.*, 2012). Although the test provides reliable results in shorter times, the 24 hours period remains the most used to evaluate seed vigor for certain crops (Milani *et al.*, 2012; Cruz *et al.*, 2013). The objective of the study was to identify a method of electrical conductivity test to stratify lots of arugula seeds according to seedling emergence.

MATERIALS AND METHODS

The experiment was conducted at Didactic Laboratory of Seed Analysis 'Flávio Farias Rocha', of College of Agronomy 'Eliseu Maciel', Federal University of Pelotas (UFPEL), Pelotas – RS, Brazil. Five lots of arugula (cultivate broadleaf) seeds were used in this study. Seeds were untreated, and the following analysis were realized:

Moisture content: the oven method was used in accordance with the Seed Analysis Rules (Brazil, 2009). Two sub-samples from each lot were used with approximately 4 g in each sample. The sub-samples were submitted to temperature of $105 \pm 3 \text{ }^{\circ}\text{C}$ for 24 hours. The results were expressed as mean percentage of lots. **Germination:** four sub-samples of 50 seeds per lot were distributed on two sheets of blotting paper. The paper was previously moistened with water equivalent to 2.5 times the weight of dry paper and placed inside transparent plastic boxes (11.5x11.5x3.5 cm). The transparent boxes were then placed in germination chamber with temperature set at $20 \pm 0.5 \text{ }^{\circ}\text{C}$. The evaluations were performed at four and seven days after sowing and the results expressed as mean percentage of normal seedlings for each lot (Brazil, 2009). **First count:** held together with the germination test. Four days after sowing the percentage of normal seedlings was counted. **Seedling emergence:** four replicates of 50 seeds were distributed in polystyrene trays with individual cells filled with sand. Daily measures during 21 days were realized to determine the percentage of seedlings (Nakagawa, 1999). **Electrical conductivity:** to evaluate electrical conductivity combination of soaking period, seed number and deionized water volume were used in this study. The treatments were: six soaking periods (1, 2, 4, 6, 8, and 24), three deionized water volume (25, 50, and 75 mL), and seed number (25 and 50 seeds) at the soaking temperature of $20 \pm 0.5 \text{ }^{\circ}\text{C}$. Four sub-samples seeds of the pure fraction of seeds for each lot were used in this study. The seeds were weighed at 0.0001g precision balance and placed to soak in plastic cups containing deionized water. The plastic cups were then placed in a growth room with controlled temperature of $20 \pm 0.5 \text{ }^{\circ}\text{C}$. The readings of conductivity were carried out in conductivity (Digimed DM-31 model) and the mean values obtained for each lot were expressed in $\mu\text{S cm}^{-1} \text{ g}^{-1}$ seed. The experimental design was completely randomized design with five replications. Analysis of variance (ANOVA) was performed using Winstat software, version 2.0 (Machado & Conception, 2005). Mean comparison using Tukey test at 5% of probability was realized. The Pearson's correlation between seedling emergence and the different electrical conductivity methodologies was performed using the Winstat software, version 2.0 (Machado & Conception, 2005).

RESULTS AND DISCUSSION

Analysis of variance was realized in the data and due to significant results a mean comparison was realized (Table is not presented). The mean comparison for the parameters measure in this study is presented in Table 1. Data regarding the water content of the arugula seeds were similar for all five lots with variations between 7.6 and 7.9 % (Table 1). The germination test was not possible to verify differences among the evaluated lots (Table 1). In this case the identification of differences in the physiological quality can be made by testing vigor (Ramos *et al.*, 2004). The first count test was more sensitive in differentiate lots than the germination test. Lots 1 and 3 were superior for first count test than lot 4. Similar results were obtained by Dantas & Torres (2010) where the first count of germination test was sensitive to classify lots of arugula seeds (cultivars Folha Larga and a cultivar Courtesy of ISLA Sementes) by vigor level of seeds. However, the first count test was not efficient in stratify other lots evaluated in this study (Table 1). Although, the first count may be considered an indicative of vigor, it is known that the reduction in the speed of germination is not one of the first events in the seed deterioration process (Delouche & Baskin, 1973). Thus, this could be the reason for the lower efficiency of this test in detecting smaller differences in vigor and subsequent grouping lots.

The seedling emergence results indicated significant differences among the seed lots, allowing the classification of lots 1 and 3 as the largest vigor, with seedling emergence of 76 and 73 %, respectively. Lots 2 and 5 with seedling emergence from 64 and 67 %, respectively, with median vigor plot and 4 lower vigor to emergence of seedlings 49 % (Table 1). The seedling emergence is the main reference for the stratification of seed lots in vigor levels, which can be understood by the amount of energy that a seed is available for fast, uniform germination and proper establishment of seedlings under different environmental conditions (SOUZA *et al.*, 2013).

Table 1. Initial quality of arugula seeds for five lots evaluated for germination, first count germination, seedling emergency means analysis, and quantified the water content

Lots	Moisture content ² (%)	Germination (%)	First count germination (%)	Seedling Emergence (%)
1	7.8	94a	92a	76a
2	7.6	95a	88ab	64b
3	7.6	96a	93a	73a
4	7.7	93a	83b	49c
5	7.9	94a	89ab	67b
CV ¹ (%)		2.68	4.73	4.15

* Means followed by the same letter in column do not differ by Tukey test at 5% probability. ¹CV - Coefficient of variation. ²Moisture content- Tukey test was not used.

The combination of 25 seeds and 50 mL of deionized water in periods of 6 and 8 hours highlighted lots 1 and 3 as greater vigor. Lots 2 and 5 with intermediate vigor and lot 4 as inferior vigor, according to the physiological characterization of seed lots (Table 2). During the process of imbibition, the lower seed vigor requires greater amount of nutrients such as sugars, amino acids and essential fatty acids for the restoration of cell and reactivating the metabolism membranes. Thus, seeds with lower physiological potential tend to have higher lixiviation of inorganic ions and enzymes (Vanzolini & Nakagawa, 2005).

Table 2. Mean comparison for electrical conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$) using combinations 25 seeds and 25; 50 to 75 mL per 1; 2; 3; 4; 6; 8 and 24 hours of soaking at 20°C of five lots of arugula seeds

Lots	Soaking period (hours)						
	25 mL and 25 seeds						
	1	2	3	4	6	8	24
1	274.33b	304.59b	322.74b	339.73b	352.44ab	375.65ab	424.27a
2	245.49ab	277.36ab	318.01b	350.55b	370.74b	400.01b	505.54b
3	232.22a	259.74a	282.28a	290.07a	333.83a	349.46a	409.07a
4	309.29c	351.17c	372.53c	390.30c	429.04c	451.65c	556.33c
5	250.45ab	263.74a	296.28ab	330.81b	344.89ab	369.57ab	441.17a
CV ¹ (%)	5.07	5.30	4.36	3.38	4.16	4.25	3.59
Lots	50 mL and 25 seeds						
	1	2	3	4	6	8	24
	1	136.99a	142.60a	157.08a	172.17a	187.34a	200.35a
2	169.54b	177.31b	216.62b	233.18b	238.75b	254.39b	336.36c
3	141.33a	152.08a	170.50a	180.45a	196.58a	215.09a	304.79b
4	195.49c	222.06c	239.74b	256.28b	283.05c	296.56c	368.13d
5	177.36bc	209.66c	222.25b	234.77b	244.81b	263.33b	331.85c
CV ¹ (%)	7.02	5.64	7.11	7.39	5.71	4.24	2.89
Lots	75 mL and 25 seeds						
	1	2	3	4	6	8	24
	1	56.71b	70.47a	109.98b	106.19a	142.00a	148.51a
2	44.21a	72.02a	93.12a	110.34a	139.10a	153.28a	248.77b
3	40.74a	69.54a	87.85a	100.43a	135.43a	143.60a	228.00a
4	70.79c	92.87b	119.89b	127.66b	157.67b	173.56b	252.66b
5	38.27a	66.18a	91.85a	108.19a	133.68a	149.42a	220.38a
CV ¹ (%)	6.31	5.38	5.78	4.78	4.52	3.87	3.83

*Means followed by the same letter in column do not differ by Tukey test at 5% probability. ¹CV - Coefficient of variation.

Table 3. Mean comparison for electrical conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$) using combinations 50 seeds and 25; 50 to 75 mL per 1; 2; 3; 4; 6; 8 and 24 hours of soaking at 20°C, five lots of arugula seeds

Lots	Soaking period (hours)						
	25 mL and 50 seeds						
	1	2	3	4	6	8	24
1	598.69	594.24a	704.70a	700.15a	738.91a	811.15ab	930.24a
2	641.04ab	640.59ab	698.34a	727.11a	784.58a	801.15a	1020.50ab
3	642.60ab	682.91b	716.97ab	719.56a	788.75a	819.31ab	1043.22bc
4	747.58c	813.62c	822.89c	859.71b	916.48b	938.07b	1151.40c
5	710.91bc	763.26c	778.06bc	787.17ab	838.92a	867.71ab	1031.39ab
CV ¹ (%)	5.31	4.96	4.50	6.60	5.17	7.25	4.80
Lots	50 mL and 50 seeds						
	1	2	3	4	6	8	24
	1	131.86b	196.04ab	267.86a	2080.08a	353.84a	388.51a
2	123.49b	231.19bc	303.19b	335.72bc	433.27b	460.01bc	647.00bc
3	89.75	182.00a	246.71a	276.76a	355.57a	423.98ab	620.07c
4	126.48b	236.52c	305.62b	345.37c	426.63b	482.41c	657.88d
5	84.50a	230.53bc	268.61a	312.74b	376.62a	430.98ab	579.58b
CV ¹ (%)	10.16	7.69	4.49	4.52	5.64	4.63	2.70
Lots	75 mL and 50 seeds						
	1	2	3	4	6	8	24
	1	39.47a	117.43a	167.37a	183.35a	257.05a	345.44a
2	46.24a	142.67b	225.54b	243.90b	318.47bc	351.62ab	553.51c
3	43.71a	131.55ab	211.39b	240.44b	291.51ab	374.43ab	470.64b
4	84.31c	183.43c	242.17b	281.04b	358.41c	404.52b	544.69c
5	54.19b	169.55c	223.47b	262.29b	343.86c	376.06ab	532.45c
CV ¹ (%)	6.47	6.15	8.53	8.29	6.90	6.64	3.70

* Means followed by the same letter in column do not differ by Tukey test at 5% probability. ¹CV - Coefficient of variation.

Table 4. Pearson's correlation coefficient (r) between the test results in electrical conductivity combinations 25 seeds and 25; 50 and 75mL, 50 seeds and 25; 50 and 75mL of 1; 2; 3; 4; 6; 8 and 24 hours of soaking with the seedling emergence arugula test plants

Hours	Seedling Emergence					
	25 seeds			50 seeds		
	25 ml	50 ml	75 ml	25 ml	50 ml	75 ml
1h	-0.62**	-0.80**	-0.80**	0.15 ^{ns}	-0.25 ^{ns}	-0.84**
2h	-0.62**	-0.80**	-0.80**	-0.03 ^{ns}	-0.65**	-0.75**
3h	-0.73**	-0.77**	-0.77**	0.56 ^{ns}	-0.70**	-0.59**
4h	-0.76**	-0.77**	-0.77**	0.27 ^{ns}	-0.81**	-0.65**
6h	-0.80**	-0.86**	-0.86**	0.32 ^{ns}	-0.70**	-0.71**
8h	-0.78**	-0.87**	-0.87**	0.31 ^{ns}	-0.85**	-0.47*
24h	-0.87**	-0.86**	-0.86**	0.21 ^{ns}	-0.69**	-0.72**

^{ns} not significant; ** significant at 1% and; * significant at 5% probability.

Tables 2 and 3 show the results for the electrical conductivity methodologies testes in this study. Lot 3 showed the best seed vigor for all the soaking period for the combination of 25 seeds and 25 mL of deionized water (Table 2). Lot 4, for the same treatment, in all soaking periods has the lowest seed vigor. However, it was not possible to stratify lots for vigor. In the treatment with 75 mL of water there was no stratification of lots for seed vigor (Table 2 and 3). Using the electrical conductivity test as defined shape as observed with 50 mL of distilled water, 6 and 8 hours of soaking seeds 25 and 6 hours with 50 seeds, seen in tables 2 and 3 respectively. As the reduction in the volume of water, keeping constant the number of factors and seed imbibition, the increase in amount of leakage was observed. These results are consistent with those observed by Torres & Pereira (2010) in arugula seeds and Vieira & Dutra (2006) pumpkin seeds, which was attributed to the dilution caused by the increased amount of water used to soaking the seeds. At the volume of water of 75 mL in combination with 25 or 50 seed there were no stratification of the lots for vigor (Table 2 and 3). Similar result to emergence test (Table 1). For the other combinations were possible classify better the lots for electrical conductivity. Increase in leached products were observed when the water volume was reduced keeping all the other factors constant. This results agree with Torres & Pereira (2010) studies with arugula seeds and Vieira & Dutra (2006) pumpkin seeds. A possible reason for the low sensibility of the test could be due to increasing water would increase the dilution and as consequence decreasing the sensibility of the test that classify seed vigor.

For the seed soaking was found that there was a progressive increase in value as it increased the soaking period. Pereira Martins & Son (2012) found similar results in cubiu seeds. After an hour of soaking, as reported by Torres & Pereira (2010), it was possible to stratify lots of arugula seeds by vigor. However, the classification of the lots became more evident after eight hours of soaking. Correlation results between the electrical conductivity in the imbibition of 25 seeds at 25 mL, 50 seed at 75 mL, and 50 seeds at 50 mL and 75 mL in all soaking periods for seedling emergence are presented on Table 4. The results indicated a significant correlation among the tests. On the other hand, there was no significant correlation ($P > 0.05$) between the electrical conductivity (50 seeds at 25 mL) and seedling emergence. This combination is probably not suitable to evaluate the physiological potential of arugula seeds. Overall analysis of the results, the combinations of 25 seeds with 50 ml of water at 20 °C and different periods of 6 and 8 hours are suitable for driving the electric conductivity test arugula seeds. When using smaller number of seeds and the quantity of water which promotes the reading of the leachate, without over- or underestimation values in shorter periods becomes compatible to this test for routine seed analysis laboratories.

Conclusion

The electrical conductivity test using 25 seed at 50 mL of water at 20 °C, independently if was 6 or 8 hours, were efficient in stratify lot of arugula seed into level of vigor based on seedling emergence.

Thanks

The author would like to acknowledge the National Council for Scientific and Technological Development for their support for this work.

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