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Electrical conductivity test for evaluating physiological quality in snap bean (*Phaseolus vulgaris* L.) seeds

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Abstract

Snap bean is a variety of common bean (*Phaseolus vulgaris* L.) that is cultivated and consumed as a vegetable worldwide. In order to optimize the crop's production, germination tests are used to ensure that seeds are high in quality. However, because these tests possess inherent limitations, vigor tests, such as the electrical conductivity test, may be used instead to assess seed quality. The goal of the present study was to develop a standardized methodology for measuring electrical conductivity in snap bean seed that could reliably assess the physiological quality of seed batches. Groups of seeds (n = 50) from each of seven snap bean genotypes (UEL 1, UEL 2, HAV 21, HAV 22, HAV 34, HAV 35, and HAV 42) were immersed in distilled water (75 or 150 mL) for various immersion periods (2, 4, 8, 12, 16, 20, or 24 h), using a completely randomized 7 × 2 × 7 factorial design, with eight replicates per treatment. After immersion, the electrical conductivity of seeds from each treatment group was measured in µS cm^{-1} using a conductivity meter. The group means were subjected to ANOVA and the Scott-Knott test ($p \leq 0.05$). The optimal conditions for the electrical conductivity test in snap bean seeds were a 16-h immersion in 75 mL of water. Under these conditions, genotypes could be clearly differentiated based on their seed vigor, optimizing the measurement of seed quality.

Keywords: *Phaseolus vulgaris*; Genotype; Volume of water; Immersion period; Vigor; Physiological quality.
Abbreviations: cm_centimeter; °C_degrees Celsius; g_grams; h_hours; kJ_kilojoules; µS_microsiemens; min_minutes; mL_millimeter; t_ton.

Introduction

Snap bean, also known as string bean or green bean, is a variety of common bean (*Phaseolus vulgaris* L.) that is cultivated and consumed as a vegetable worldwide. Snap bean differs from common bean in terms of its harvest stage, during which the bean pods are collected. Snap bean is collected at an immature stage and may be consumed either raw or after being processed (Haesbaert et al., 2011). The crop is globally important (Filgueira, 2013), with an annual global production of ~21 million t, and China is the main producer, followed by Indonesia, Turkey, and India (Food and Agriculture Organization of the United Nations, 2017). Snap bean provides fiber and vitamins; help reduce weight in obese individuals; improve colonic transit time, postprandial blood glucose rate and lipid absorption; and reduce the onset of colon cancer. An average fresh pod has a caloric value of 133.9 kJ 100 g^{-1} (Tirilly and Bourgeois, 2002; Salunkhe and Kadam, 2003).

It is well known that seed quality is of utmost importance for stabilizing field cultures, and germination tests have often been used to evaluate seed quality, especially for commercial trading purposes. However, such procedures have limitations, even under ideal conditions, since they require relatively long periods of time to complete and cannot account for potential differences in the performance of seed batches (Carvalho and Nakagawa, 2012). Therefore, during the past few decades, seed companies have used a variety of tests to evaluate seed quality more quickly and efficiently, thereby hastening decision-making processes, especially with regard to harvesting, processing, and trading (Lopes and Franke, 2010). Measuring electrical conductivity is one of the most common of these tests. It satisfies a range of criteria, such as objectivity and quickness, as well as being low-cost, easily repeatable, and easy to both perform and interpret. Vigor is considered a key aspect of seed quality among batches (or genotypes) and is used to make key decisions regarding which batches will be traded, sown, or discarded (Ataide et al., 2012; Marcos Filho, 2015).

The electrical conductivity test is based on the direct relationship between seed vigor and the integrity of a seed's cellular membrane system. Even though the cellular membrane system is the last to be organized during seed maturation, it is the first system to signify deterioration after

seeds reach physiological maturity. When dried seeds are soaked in water, the leaching of electrolytes from cellular constituents indicates the level of disorganization of the seeds' cellular membranes and, consequently, also indicates the physiological quality and vigor of the seeds (Marcos Filho, 2015). However, the results of the electrical conductivity test are affected by a number of variable factors, such as the volume of water in which the seeds are soaked, the immersion period, the number of seeds soaked, the temperature, and the age, integrity, and genotype of the seeds.

The electrical conductivity test is usually conducted by immersing seeds in water for 24 h, but recent studies suggest that shorter immersion periods may also be suitable (e.g., Dias et al., 2006), as optimum immersion periods could vary by species. Furthermore, reducing the time that it takes to complete these tests would hasten decision-making processes, thereby facilitating the optimization of the seed production chain as a whole.

The volume of water in which the seeds are soaked could also affect the results of the electrical conductivity test through dilution. Therefore, when optimizing the test for a specific species, it is crucial to consider the influence of the volume of water used for seed imbibition (Pereira and Martins Filho, 2012).

According to the guidelines of the International Seed Testing Association (2017), the electrical conductivity test has only been standardized for pea seeds (*Pisum sativum* L.). Hence, studies are needed that can provide information to standardize adequate methodologies for other vegetable species, especially since the lack of standardized methods has hindered the application of the test to a great number of valuable species. The goal of the present study was to develop a standardized methodology for measuring electrical conductivity in snap bean seed that could reliably assess the physiological quality of seed batches.

Results and Discussion

Electrical conductivity of snap bean seeds

The three independent variables studied (i.e., genotype, volume of water, and immersion period) exhibited a significant interaction ($p \leq 0.05$; Table 1). Milani et al. (2012) also observed a significant triple interaction in *Brassica napus* L. var. *oleifera* ($p \leq 0.05$), when assessing the effects of seed batch ($n = 4$), volume of water (25 or 50 mL), and immersion period (2, 4, 8, 16, or 24 h) at 25 °C. Our results confirm their findings that a variety of factors influence the optimum methodology for measuring the electrical conductivity of different vegetable species. Tables 2 and 3 summarize the interaction effects of genotype, volume of water, and immersion period on the electrical conductivity of the snap bean seeds. After only 2 h of immersion in 75 mL of water, significant differences could already be observed among the electrical conductivity of the different genotypes. However, for the seeds immersed in 150 mL of water, statistical differences were observed only from 4 h. Again, these results are similar to those of Milani et al. (2012), who reported that differences in the electrical conductivity of *B. napus* batches became apparent more quickly when the seeds were incubated in lower volumes of water (2 h in 25 mL) than when they were incubated in higher volumes of water (8 h in 50 mL).

Other studies have also investigated whether the physiological potential of seeds in different batches can be differentiated using lower immersion periods (i.e., <24 h). Dutra and Vieira (2006) and Fessel et al. (2005) reported that the seed vigor of *Cucurbita pepo* L. and *Brassica oleracea* L. var. *italica* Plenk batches, respectively, could be distinguished after only 1 h, and Dutra et al. (2006) and Batista et al. (2012) both found that the seed vigor of *Vigna unguiculata* (L.) Walp batches could be distinguished after 2 h.

Nevertheless, as shown in Table 2, the immersion of seeds in 75 mL of water for 16 and 24 h was the most effective protocol for differentiating the snap bean genotypes based on physiological quality. The genotypes could be differentiated into six statistically distinct groups under these parameters, whereas after 2 h of immersion, the genotypes could only be separated into three distinct groups. However, when the seeds were immersed in 150 mL of water, the genotypes could only be differentiated into a maximum of four vigor levels, regardless of immersion period. Pereira and Martins Filho (2012) also reported that electrical conductivity decreased with increased volume of water and suggested that dilution of the immersion solution was responsible for reducing the efficiency of the evaluation. Machado et al. (2011) reported that lower volumes of water (e.g., 75 mL) produce less-dilute immersion solutions and thus are more effective for differentiating between batches of *P. sativum* subsp. *arvense*, supporting the findings of the present study. In contrast, when Dias et al. (1998) assessed the seed vigor of four *P. vulgaris* batches after six immersion periods (4, 8, 12, 16, 20, and 24 h), the genotypes could be distinguished only after 20 or 24 h and then could only be distinguished into two distinct levels of vigor.

In the present study, the 16-h, 75-mL immersion treatment was the most effective treatment combination for differentiating the vigor of snap bean genotypes (Table 2). Results that quickly assess physiological seed quality are important because they speed up the decision-making processes involved in the management of seed batches. According to Milani et al. (2012), decreasing the time it takes to evaluate seed vigor is crucial to meeting the needs of the seed industry, which aims to boost seed quality control programs by obtaining more reliable information about batch vigor in a relatively short period (e.g., 8 or 16 h). Dutra and Vieira (2006) also recommended an immersion period of less than 24 h for applying the electrical conductivity test to *C. pepo* seeds, as similar results were obtained after 8 and 24 h. In the present study, the immersion period used for the snap bean seeds had a critical influence on the ability of the electrical conductivity test to differentiate the genotypes based on quality. Previous studies, mainly involving vegetable seeds, have reported that reduced immersion periods can better classify seed batches according to their physiological potential: a 30-min immersion period for *Daucus carota* L. (Ortiz et al., 2014), a 2-h immersion period for *Solanum sessiliflorum* Dunal (Pereira and Martins Filho, 2012), and a 9-h immersion period in *Vigna radiata* (L.) R. Wilczek (Araujo et al., 2011) were all effective.

On the basis of these findings, it is clear that the electrical conductivity methods should be optimized through empirical

Table 1. Effects of genotype (G), volume of water (VW, mL), and immersion period (IP, h) on the electrical conductivity (μS cm^{-1}) of snap bean (*Phaseolus vulgaris*) seeds, according to ANOVA.

Source of variation	DF	SS	MS	F value	*p* (>F)
G	6	70,250,348.50	11,708,391.42	1,390.84	0.0000*
VW	1	34,008,474.96	34,008,474.96	4,039.87	0.0000*
IP	6	87,792,314.03	14,632,052.34	1,738.14	0.0000*
G × VW	6	8,620,828.69	1,436,804.78	170.68	0.0000*
G × IP	36	16,172,123.43	449,225.65	53.36	0.0000*
VW × IP	6	6,010,338.80	1,001,723.13	119.00	0.0000*
G × VW × IP	36	1,319,999.74	36,666.66	4.36	0.0000*
Error	686	5,774,896.63	8,418.22		
Total	783	229,949,324.77			
CV (%)	14.10				

* Significant, $p \leq 0.05$, DF = degrees of freedom, SS = sum of squares, MS = mean square, *p* = *p*-value, CV = coefficient of variation

Table 2. Interactive effects of genotype within each volume of water and immersion period, and interactive effects of immersion period within each genotype and volume of water on the electrical conductivity test (μS cm^{-1}) of snap bean (*Phaseolus vulgaris*) seeds.

Genotype	Volume of water 75 mL						
	Immersion period						
	2 h	4 h	8 h	12 h	16 h	20 h	24 h
UEL 1	385.25 c A	655.88 d B	980.25 d C	1,274.75 e D	1,476.88 e E	1,652.00 d F	1,848.50 e G
UEL 2	278.38 b A	485.25 c B	796.38 c C	1,149.13 d D	1,398.75 d E	1,624.88 d F	1,869.38 e G
HAV 21	253.13 b A	462.88 c B	760.13 c C	1,068.88 c D	1,283.13 c E	1,511.25 c F	1,749.38 d G
HAV 22	80.00 a A	156.38 b A	295.13 b B	424.75 b C	537.75 b D	655.13 b E	772.38 b F
HAV 34	272.75 b A	540.75 c B	955.38 d C	1,330.50 e D	1,603.88 f E	1,870.50 e F	2,097.75 f G
HAV 35	260.50 b A	489.50 c B	784.63 c C	1,047.25 c D	1,260.88 c E	1,463.75 c F	1,641.00 c G
HAV 42	10.63 a A	14.00 a A	28.38 a A	60.75 a A	101.88 a A	152.88 a B	214.63 a B
Genotype	Volume of water 150 mL						
	Immersion period						
	2 h	4 h	8 h	12 h	16 h	20 h	24 h
UEL 1	84.88 a A	244.00 b B	440.00 c C	605.63 c D	748.38 c E	867.75 c F	998.50 c G
UEL 2	87.88 a A	193.13 b B	339.38 c C	567.88 c D	703.25 c E	815.75 c F	972.13 c G
HAV 21	118.50 a A	256.38 b B	413.38 c C	556.00 c D	699.25 c E	810.38 c F	967.63 c G
HAV 22	33.75 a A	70.38 a A	125.88 b A	218.25 b B	285.88 b B	354.38 b C	428.75 b C
HAV 34	81.38 a A	219.13 b B	419.63 c C	691.75 d D	849.63 d E	980.88 d F	1,130.13 d G
HAV 35	118.63 a A	226.63 b B	416.38 c C	594.75 c D	711.88 c E	820.38 c F	941.88 c G
HAV 42	6.13 a A	16.13 a A	30.50 a A	55.88 a A	83.25 a B	116.38 a B	158.63 a B

Averages within each column that are followed by different lowercase letters, for interactive effects of genotype within each volume of water and immersion period, and averages within each row that are followed by different uppercase letters, for interactive effects of immersion period within each genotype and volume of water, differ significantly, according to the Scott-Knott test ($p \leq 0.05$).

tests for each species. Pereira and Martins Filho (2012) observed that a longer immersion period in a greater volume of water did not help to distinguish *S. sessiliflorum* batches because there were no statistical differences in the seeds after an 8-h immersion period in 50 mL water, but the batches could be distinguished into two distinct levels of vigor after immersion for 48 h in 30 mL.

In the present study, HAV 42 was the only genotype that did not show a significant difference in electrical conductivity when different volumes of water were used, regardless of the immersion period (Table 3). For the HAV 22 genotype, there was no significant difference of the volume of water on the electrical conductivity only for the immersion periods of 2 and 4 h. Meanwhile, the remaining genotypes could be distinguished, based on vigor, regardless of the immersion period. Larger volumes of water were associated with reduced conductivity values due to dilution, as expected (Table 3). Other authors have also reported that leachate contents decrease as the volume of water increases, including Milani et al. (2012) with *B. napus*; Machado et al. (2011) with *P. sativum*, and Soares et al. (2010) with *Sorghum bicolor* (L.) Moench.

Final considerations

The results of the present study demonstrate that the optimal conditions for the electrical conductivity test are a 75-mL volume of water and an immersion period of 16 h to evaluate the physiological quality of snap bean seeds. These conditions are most conducive to clearly distinguishing between vigor levels and provide quicker results than the germination test or other physiological tests. Accordingly, the methodology can be used to hasten decision-making in the seed production chain due the effectiveness of the electrical conductivity test for assessing the physiological quality of snap bean seeds.

Table 3. Interactive effects of volume of water within each genotype and immersion period on the electrical conductivity test (μS cm^{-1}) of snap bean (*Phaseolus vulgaris*) seeds.

Genotype	Immersion Period	Volume of water		Genotype	Immersion Period	Volume of water	
		75 mL	150 mL			75 mL	150 mL
UEL 1	2 h	385.25 b	84.88 a	HAV 34	2 h	272.75 b	81.38 a
	4 h	655.88 b	244.00 a		4 h	540.75 b	219.13 a
	8 h	980.25 b	440.00 a		8 h	955.38 b	419.63 a
	12 h	1,274.75 b	605.63 a		12 h	1,330.50 b	691.75 a
	16 h	1,476.88 b	748.38 a		16 h	1,603.88 b	849.63 a
	20 h	1,652.00 b	867.75 a		20 h	1,870.50 b	980.88 a
	24 h	1,848.50 b	998.50 a		24 h	2,097.75 b	1,130.13 a
UEL 2	2 h	278.38 b	87.88 a	HAV 35	2 h	260.50 b	118.63 a
	4 h	485.25 b	193.13 a		4 h	489.50 b	226.63 a
	8 h	796.38 b	339.38 a		8 h	784.63 b	416.38 a
	12 h	1,149.13 b	567.88 a		12 h	1,047.25 b	594.75 a
	16 h	1,398.75 b	703.25 a		16 h	1,260.88 b	711.88 a
	20 h	1,624.88 b	815.75 a		20 h	1,463.75 b	820.38 a
	24 h	1,869.38 b	972.13 a		24 h	1,641.00 b	941.88 a
HAV 21	2 h	253.13 b	118.50 a	HAV 42	2 h	10.63 a	6.13 a
	4 h	462.88 b	256.38 a		4 h	14.00 a	16.13 a
	8 h	760.13 b	413.38 a		8 h	28.38 a	30.50 a
	12 h	1,068.88 b	556.00 a		12 h	60.75 a	55.88 a
	16 h	1,283.13 b	699.25 a		16 h	101.88 a	83.25 a
	20 h	1,511.25 b	810.38 a		20 h	152.88 a	116.38 a
	24 h	1,749.38 b	967.63 a		24 h	214.63 a	158.63 a
HAV 22	2 h	80.00 a	33.75 a				
	4 h	156.38 a	70.38 a				
	8 h	295.13 b	125.88 a				
	12 h	424.75 b	218.25 a				
	16 h	537.75 b	285.88 a				
	20 h	655.13 b	354.38 a				
	24 h	772.38 b	428.75 a				

Averages within each row that are followed by different uppercase letters, for interactive effects of volume of water within each genotype and immersion period, differ significantly, according to the Scott-Knott test ($p \leq 0.05$).

Materials and Methods

Study location and plant materials

The experiment was conducted at the Laboratory of Seed Technology and Production, Department of Agronomy, State University of Londrina (UEL) in Londrina, Paraná, Brazil. Of the seven genotypes of snap bean (*Phaseolus vulgaris*) used for the study, two (UEL 1 and UEL 2) were selected from a UEL breeding program, whereas the remaining five genotypes (HAV 21, HAV 22, HAV 34, HAV 35, and HAV 42) were selected and provided by the International Center for Tropical Agriculture (CIAT).

Experimental design

Groups of seeds (n = 50) from each of seven snap bean genotypes (UEL 1, UEL 2, HAV 21, HAV 22, HAV 34, HAV 35, and HAV 42) were placed in 200-mL plastic containers. The seeds of each genotype were immersed in either 75 or 150 mL of distilled water and incubated at 25 °C for 2, 4, 8, 12, 16, 20, or 24 h. The trial design was completely randomized and involved a 7 × 2 × 7 factorial design: genotypes × volumes of water × immersion periods, with eight replicates per treatment. The electrical conductivity (μS cm^{-1}) was measured using a HI 98331® conductivity meter (Hanna Instruments, Woonsocket, Rhode Island, USA).

Statistical analysis

The separate and combined effects of genotype, volume of water, and immersion period were investigated using analysis of variance (ANOVA; F-test) and the Scott-Knott test ($p \leq 0.05$), both of which were implemented in R v. 3.3.1 (R Development Core Team, 2017).

Conclusion

Electrical conductivity in snap bean (*P. vulgaris*) seeds should be evaluated after a 16-h immersion period in 75 mL of water. This methodology clearly differentiates genotypes based on their seed vigor and, thereby optimizes the evaluation of seed quality.

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