TEMPERATURE DURING SOYBEAN SEED STORAGE AND THE AMOUNT OF ELECTROLYTES OF SOAKED SEEDS SOLUTION

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ABSTRACT: The electrical conductivity test measures the electrolytes that leach out of seeds when they are immersed in water and this leakage is an indication of seed vigor. The level of standardization reached by the procedures of this test is such that the test is recommended for pea seeds and suggested for other large seeded legumes, including soybean [*Glycine max* (L.) Merrill]. This study was conducted to contribute to the standardization of this test for soybean seeds by verifying whether the seed storage temperature influences the composition of the leachate from soaked seeds solution. Two soybean seed lots of distinct physiological potential were stored in moisture-proof containers either at constant temperatures of 10°C and 20°C or at the temperature of 20°C during the first seven months of storage followed by a change to 10°C for the rest of the storage time (nine months). The chemical composition of the soaked water was evaluated every three months from January to October 1998. The highest amount of leakage was observed for potassium, followed by calcium and magnesium, iron and sodium regardless of temperature and storage period. The amount of electrolytes in the soaked water increased as the period of time and the temperature of storage increased. On the other hand the amount of leakage decrease along the time for those seeds stored at 10°C or transferred from the temperature of 20 to that of 10°C. The temperature at which soybean seeds remain during storage may affect the amount of electrolytes in the soaked water and consequently the results of the electrical conductivity test.
Key words: *Glycine max*, vigor, deterioration

TEMPERATURA DE ARMAZENAMENTO E QUANTIDADE DE LIXIVIADOS NA SOLUÇÃO DE EMBEBIÇÃO DE SEMENTES DE SOJA

RESUMO: O teste de condutividade elétrica mede a quantidade de eletrólitos liberada das sementes quando imersas em água, sendo um indicador do vigor da semente. O teste é recomendado para sementes de ervilha e sugerido para outras leguminosas, incluindo a soja [*Glycine max* (L.) Merrill]. O presente trabalho visa contribuir para a padronização do referido teste para avaliação do vigor de sementes de soja, procurando verificar se a temperatura de armazenamento da semente pode influenciar a liberação de eletrólitos na solução de embebição das sementes. Dois lotes de sementes de soja de potenciais fisiológicos distintos foram acondicionados em embalagens herméticas e armazenados em três ambientes: 10 e 20°C (constantes) e 20°C por sete meses, com transferência para 10°C até o final do armazenamento (mais nove meses). A composição química da solução de embebição das sementes foi analisada a cada período de três meses, de janeiro a outubro de 1998. Os maiores valores de lixiviação foram observados para potássio, seguido de cálcio e magnésio, ferro e sódio. Verificou-se acréscimo na quantidade de eletrólitos na solução de embebição em função do aumento do período e da temperatura de armazenamento. Por outro lado, observou-se decréscimo na quantidade de lixiviados ao longo do tempo para as sementes armazenadas a 10°C ou transferidas da temperatura de 20°C para 10°C. A temperatura de armazenamento de sementes de soja pode interferir na liberação de eletrólitos na solução de embebição e, conseqüentemente, nos resultados do teste de condutividade elétrica.
Palavras-chave: *Glycine max*, vigor, deterioração

INTRODUCTION

Cell membranes attain their maximum level of organization at seed physiological maturity (Abdul-Baki, 1980). After that point, seed dehydration causes a structural disorganization of cell membranes, and this disorganization is greatest when seeds reach water content levels low enough to allow mechanical harvesting (Bewley & Black, 1994). On the other hand, the integrity of cell membranes, as determined by the degree of biochemical changes and/or physical damage, may be considered the fundamental cause of differences in seed vigor (Powell, 1988).

The electrical conductivity (EC) test was proposed to evaluate the vigor level of pea (*Pisum sativum* L.) seeds due to the fact that the lower the seed vigor, the lesser is its capacity to restore cell membrane integrity which results in increased amounts of electrolytes leached to the soaked water during seed imbibition. This leachate has been reported by several investigators to contain sugars, amino acids, fatty acids, enzymes, and inorganic ions, such as K^+, Ca^{++}, Mg^{++}, and Na^+ (AOSA, 2002; Loomis & Smith, 1980; Givelberg et al., 1984; Woodstock, 1988; Cortes & Spaeth, 1994; Lott et al., 1991; Taylor et al., 1995).

In the EC test, seed quality is indirectly evaluated through the determination of the EC of the solution resulting from the soaking of seeds in water. The amount of electrolytes leached from high vigor seeds is low leading to low conductivities. Low conductivities are considered to be an indication of high vigor because it is thought to represent a low level of cell membrane system disorganization. Some papers have reported significant relationships between the results of the EC test and seedling emergence in the field (Vieira et al., 1999ab, 2004).

Some of this research considered the possibility that the temperatures during seed storage influence the EC test. Ferguson (1988) and Vieira et al. (2001) reported that, after being stored under low temperatures for some period, soybean seeds that showed a significant drop in germination and vigor (accelerated aging) did not show an equivalent drop in physiological quality evaluated by means of the EC.

Thus, having in mind the close relation between the EC test results and the leaching of electrolytes during the imbibition of seeds in water, the objective of this research was to study the chemical composition of the soak water of soybean seeds stored at different temperatures.

MATERIAL AND METHODS

This study was conducted in Lexington, KY, USA between September of 1997 and October of 1998. Two soybean [*Glycine max* (L.) Merrill] seed lots with distinctly different physiological quality were used (Table 1) and also described previously by Vieira et al. (2001).

The seeds were packed in moisture proof containers and stored for 16 months at the following temperatures: (i) constant 10°C, (ii) constant 20°C, and (iii) at the temperature of 20°C during the first seven months which was followed by an additional nine

Table 1 - Seed water content (WC -fresh weight basis) and initial and final seed quality - standard germination (SG), accelerated aging (AA) and electrical conductivity (EC) of soybean seed lots.

Storage temp.	WC	SG	AA	EC
		June/1997		
ºC	----------	%	----------	µS cm^{-1} g^{-1}
		Lot 1 - high vigor		
-	12.3	99	95	58
		Lot 2 - low vigor		
-	11.9	94	65	73
		October/1998		
		Lot 1 - high vigor		
10	12.2	95	82	64
20	12.2	30	0	126
20/10	12.2	94	35	68
		Lot 2 - low vigor		
10	12.1	82	4	80
20	12.0	4	0	172
20/10	12.1	79	0	92

months at 10°C. At three months interval, from January to October 1998, the seeds were sampled and tested for the electrical conductivity (Vieira et al., 2001) until the end of the storage period and at each sampling the chemical composition of the soak water was determined. The procedure for electrical conductivity consisted of imbibing four replicates of 50 seeds in 75 mL of deionised water for 24 hours at a constant temperature of 25°C and the bulk electrical conductivity was measured using a conductivity meter as described by Hampton & TeKrony (1995) and Vieira & Krzyzanowski (1999). After that, the soak water was filtered and the following chemical elements measured: K and Na by means of the flame photometry process, Ca, Mg and Fe by the atomic absorption spectrophotometry method (Bataglia et al., 1983; Tomé Júnior, 1997).

The experimental units were arranged in a completely random design with a split-plot arrangement of treatments in four replicates. The main plots were storage temperatures (10, 20, and 20/10°C) and the split-plots were sampling times (7, 10, 13 and 16 months of storage). The two seed lots were analyzed separately. The comparison between means was accomplished by the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

When stored at 10°C the seeds of both vigor levels showed no difference as to K^+ content in the soak water at any time during the storage period (Table 2). But, when the temperature was of 20°C, the amount of potassium leached after13 and 16 months of storage readings was higher than those after seven and 10 months. This pattern of response was confirmed by the third storage condition (seven months at 20 followed by nine months at 10°C) that is, storing soybean seeds at low temperatures causes a reduction in the leached potassium. These patterns were observed independently of the seed vigor level. Also the electrical conductivity results followed a similar patterns as potassium was leached (Table 3). The reduction of

Table 2 - Potassium content in the soak water of soybean seeds stored for several periods of time.

Evaluation	Storage temperature		
	10ºC	20ºC	20/10ºC
months	Potassium content (mg L^{-1}) (Lot 1 - high vigor)		
7	952 aA	1054 bA	1044 aA
10	832 aA	1049 bA	871 aA
13	737 aC	2001 aA	1445 aB
16	646 aB	2106 aA	866 aB
	Potassium content (mg L^{-1}) (Lot 2 - low vigor)		
7	1239 aA	1530 aA	1305 aA
10	930 aA	1487 bA	987 aA
13	1022 aB	2223 abA	1575 aAB
16	729 aB	2396 aA	976 aB

Means followed by the same small case letter in the column and large case letter in the line do not differ (Tukey's test, $p < 0.05$).

Table 3 - Electrical conductivity of soybean seeds stored for several periods of time.

Evaluation moment	Storage temperature		
	10ºC	20ºC	20/10ºC
months	Electrical conductivity (μS cm^{-1} g^{-1}) (Lot 1 - high vigor)		
7	68 aB	77 bA	71 aAB
10	63 aB	76 bA	63 abB
13	58 aB	84 bA	60 bB
16	64 aB	126 aA	68 abB
	Electrical conductivity (μS cm^{-1} g^{-1}) (Lot 2 - low vigor)		
7	83 aB	101 bA	90 bAB
10	75 aB	112 bA	83 bB
13	72 aB	116 bA	73 bB
16	80 aB	172 aA	92 aB

Means followed by the same small case letter in the column and large case letter in the line do not differ (Tukey's test, $p < 0.05$).

electrical conductivity of soaked seed stored at low temperature (10°C) compared to higher temperatures (20°C) confirm those reported by Ferguson (1988) and Vieira et al. (2001) both working with soybean seeds, however it was not clear for corn seeds (Fessel et al., 2006).

Similar responses were also observed with Ca (Table 4), Mg (Table 5) and Fe (Table 6). The only element without alteration for both seed vigor levels was sodium (Table 7).

Potassium was the most abundant ion in the soak water, this being a confirmation of data published by Loomis & Smith (1980) working with cabbage seeds (*Brasssica Oleracea* L. capitata), Lott et al. (1991) working with peanut (*Arachis hypogaea* L.), pea (*Pisum sativum* L.) and soybean [*Glycine max* (L.) Merrill] and Fessel (2001) working with corn and soybean seeds. Lott et al. (1991) and Fessel (2001) verified that as storage time increased the amount of potassium lost into the imbibing solution also increased. That is one of the reasons why the determination of the amount of K in the soak water has been proposed as a vigor test for soybean (Dias et al., 1997) and peanut (Vanzolini & Nakagawa, 2003) seeds.

It seems that the electrical conductivity variation results is closely related to the amount of the potassium, calcium and magnesium ions into the soaked water of seeds. This was also reported by Fessel (2001) for soybean seeds, who found that when the electrical conductivity values increase the results of potassium, calcium and magnesium also increase in the imbibition solution. The temperature played an important role on soybean seed vigor after stored under low temperatures, such as cold room temperature (10°C).

Why low temperature such as 10°C lead to a reduction on electrical conductivity and also potassium, calcium and magnesium leached from the soaked seeds? Other authors tried to explain this subject. For instance, Vieira et al. (2001) working with soybean seeds mentioned that membranes also stabilized for

Table 4 - Calcium content in the soak water of soybean seeds stored for several periods of time.

Evaluation	Storage temperature		
	10ºC	20ºC	20/10ºC
months	Calcium content (mg L^{-1}) (Lot 1 - hight vigor)		
7	42 aA	56 bA	45 abA
10	27 abB	51 bA	30 bB
13	37 abB	95 aA	56 aB
16	19 bB	83 aA	32 bB
	Calcium content (mg L^{-1}) (Lot 2 - low vigor)		
7	55 aA	66 bA	68 abA
10	51 aA	60 bA	53 abA
13	55 aB	118 aA	80 aB
16	35 aB	104 aA	48 bB

Means followed by the same small case letter in the column and large case letter in the line do not differ (Tukey's test, $p < 0.05$).

Table 5 - Magnesium content in the soak water of soybean seeds stored for several periods of time.

Evaluation	Storage temperature		
	10ºC	20ºC	20/10ºC
months	Magnesium content (mg L^{-1}) (Lot 2 -high vigor)		
7	35 aA	51 bA	45 abA
10	35 aA	62 bA	42 abA
13	44 aB	111 aA	53 aB
16	21 aB	127 aA	24 bB
	Magnesium content (mg L^{-1}) (Lot 2 - low vigor)		
7	38 aA	56 cA	48 aA
10	44 aA	73 bcA	43 aA
13	48 aB	113 abA	69 aAB
16	26 aB	134 aA	34 aB

Means followed by the same small case letter in the column and large case letter in the line do not differ (Tukey's test, $p < 0.05$).

Table 6 - Iron content in the soak water of soybean seeds stored for several periods of time.

Storage temperature	Iron content	
	Lot 1 (high vigor)	Lot 2 (low vigor)
ºC	---------------------------------- mg L^{-1} ----------------------------------	
10	12.35 a	11.13 b
20	12.80 a	13.35 a
20/10	11.93 a	13.33 a

Means followed by the same small case letter in the column do not differ (Tukey's test, $p < 0.05$).

Table 7 - Sodium content in the soak water of soybean seeds stored for several periods of time.

Storage temperature	Iron content	
	Lot 1 (high vigor)	Lot 2 (low vigor)
ºC	---------------------------------- mg L^{-1} ----------------------------------	
10	2.16 a	2.34 a
20	1.83 a	2.23 a
20/10	1.84 a	1.85 a

Means followed by the same small case letter in the column do not differ (Tukey's test, $p < 0.05$).

seeds stored at 10ºC, resulting in no increase in conductivity as a result of no increase in the ions leached. This can raise some questions. Why should seeds stored at 10°C apparently stabilize membranes more than seeds stored at 30°C, for instance, resulting in lower value of EC? Some hypothesis can be raised. First, the transition from a period of relative membrane stability to dynamic seed aging could occur through a loss of the glassy state (Bernal-Lugo & Leopold, 1998). This loss could be influenced by an increase in the water content, in temperature, or by a separation of sugars involved. Besides that, the beginning of deterioration could result from a gradual hydrolysis of the soluble sugars. The hydrolysis of sugars presented in seeds would lead to an accumulation of reducing sugars that would finally threaten the proteins integrity as a result of the formation of Maillard products (Sun & Leopold, 1995). However, it can only be speculated that one or more of the above factors may be involved in the lack of response in EC when seeds were stored at 10°C.

It also can be stated some relationship with seed water content. However it was monitored during the whole period of the experiment and there is no variation on it. The initial and final values of water content were shown on Table 1. It remained close to 12% during the whole storage period (16 months).

Considering these results and others (Ferguson, 1988 and Vieira et al., 2001) the vigor evaluation of soybean seeds using the electrical conductivity test raise some concerns when the seeds are stored at low temperatures such as 10°C. However, in general soybean seeds are stored under this condition very rarely, unless genetic seeds. In this case this situation must be consider. Also some research need to be done studying the period of time after the seeds are removed from 10°C to be evaluated through electrical conductivity test.

CONCLUSION

The temperature at which soybean seeds remain while in storage affects the amount of electrolytes leached during the electrical conductivity test thus having a potential to mask results.

ACKNOWLEDGMENTS

To FAPESP and to CNPq for the granted scholarships.

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Received December 11, 2006
Accepted April 11, 2008