

RESEARCH NOTE

Accelerated aging parameters in the prediction of physiological and sanitary quality of birdsfoot trefoil (*Lotus corniculatus* L.) seeds¹

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ABSTRACT - The objective of this study was to evaluate the accelerated aging test, with and without NaCl solution, predicting the physiological health and quality of birdsfoot trefoil (*Lotus corniculatus* L.) seed. The experimental design was completely randomized. Ten seed lots were submitted to three conditioning periods (24, 48 and 72 h at 41 °C). The physiological characterization of the lots was the determination of water content (before and after accelerated aging), germination, first germination count, germination speed index, seedling emergence and seed health test. The data were submitted to variance analyze and the averages were compared by the Scott-Knott test, at 5% probability. The accelerated aging methodologies with saline solution and saturated NaCl solution, conditioned for 48 h, were correlated with seedling emergence test (0.52 and 0.69, respectively), allowing the prediction of physiological quality of birdsfoot trefoil seeds. The use of saturated solution for 48 h decreases incidence of fungi. The traditional method is not suitable because it provides water content variation between samples above the tolerable.

Index terms: fungi, germination, saline solution, vigor.

Parâmetros do envelhecimento acelerado na predição da qualidade fisiológica e sanitária de sementes de cornichão (*Lotus corniculatus* L.)

RESUMO – O objetivo deste trabalho foi avaliar o teste de envelhecimento acelerado, com e sem solução de NaCl, na predição da qualidade fisiológica e sanitária de sementes de cornichão (*Lotus corniculatus* L.). O delineamento experimental foi o inteiramente casualizado, foram utilizados dez lotes de sementes submetidos a três períodos de condicionamento (24, 48 e 72 h, a 41 °C). A caracterização fisiológica dos lotes consistiu na determinação do teor de água (antes e depois do envelhecimento acelerado), germinação, primeira contagem de germinação, índice de velocidade de germinação, emergência de plântulas e teste de sanidade de sementes. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Scott-Knott, a 5% de probabilidade. As metodologias de envelhecimento acelerado com solução salina e solução saturada de NaCl, condicionadas por 48 h, correlacionaram-se com o teste de emergência de plântulas (0,52 e 0,69, respectivamente), permitindo a predição da qualidade fisiológica de sementes de cornichão. A utilização de solução saturada durante 48 h diminui a incidência de fungos. O método tradicional não é recomendado por proporcionar variação do teor de água entre as amostras, superior ao tolerável.

Termos para indexação: fungo, germinação, solução salina, vigor.

Introduction

The birdsfoot trefoil (*Lotus corniculatus* L.) has positive environmental effects and is beneficial for ruminant nutrition making it suitable for pasture based cattle production; its high content of tannins make it usable in pure pastures (as single

species pasture) without risk of bloat (Hunt et al., 2015). It is adapted to low fertility soils and has the ability of symbiotic nitrogen fixation. However, the species has slow establishment: the seeds have a long germination period and seedlings have reduced early growth, reaching its highest peak only after one year (Artola et al., 2003).

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The use of high quality seeds is a determining factor during sowing and establishment period, influencing growth, development and productivity of the plants (Souza et al., 2009). Therefore, it is necessary to improve the tests that estimate seed vigor, enabling the selection of the best seed lots for marketing and thus providing more accurate information for sowing.

One of the most used vigor tests is accelerated aging (Almeida et al., 2014), which is to increase the rate of deterioration of the seeds through their exposure to high levels of temperature and relative humidity (Kapoor et al., 2011). Therefore, seeds with higher vigor are deteriorated slower than those with low vigor (Tunes et al., 2011).

However, for most of the species with small seeds, the accelerated aging test has limitations, differences in water absorption by the seeds causes sudden changes in the moisture content of samples and may accelerate the deterioration process, revealing inconsistent results (Bhering et al., 2006). In order to minimize this problem, Jianhua and McDonald (1996) proposed the use of saline solutions in the test conditions and thereby reduce the absorption of water by the seeds, improving the uniformity of the results and reducing the incidence of fungi. The efficacy of this methodology wasn't verified only for small seeds but also for bigger seeds (Lehner et al., 2008; Tunes et al., 2012; Yagushi et al., 2014).

The objective of this work was to optimize the accelerated aging test to predict the physiological quality of birdsfoot trefoil seeds and verify the possibility of the use of NaCl solution to control the water uptake and fungi incidence in seeds.

Material and Methods

The experiment was conducted at the Seed Analysis Laboratory, Department of Forage Plants and Agrometeorology of the Universidade Federal do Rio Grande do Sul, Brazil. Ten lots of birdsfoot trefoil cv. "São Gabriel" (*Lotus corniculatus* L.) were used. The seeds were obtained from a company specialized in processing and selling forage seeds in Uruguaiana, RS, Brazil (29°46'55 "S, 57°02'18" W). The seeds were scarified using an electric scarifier (1.750 rpm and ½ HP), coated with sandpaper n.º180. In this device, fifteen grams of seeds were processed for 30 seconds.

The water content before and after the accelerated aging treatment, was determined using two subsamples approximately $4.5 \pm 0.5 \text{ g}^{-1}$ for each seed lot in an oven at $105 \pm 3 \text{ }^\circ\text{C}$ for 24 h (Brasil, 2009). The germination test was carried out with four replications of 100 seeds for each lot, on sheets of "germitest" paper moistened with water to 2.5 times the weight of dry paper. The seeds were kept in a germination chamber at a constant temperature of $20 \text{ }^\circ\text{C}$.

The percentage of normal seedlings was evaluated after 12 days (Brasil, 2009). The first germination count was carried out considering the number of normal seedlings on the fourth day of the germination test. The results were expressed as a percentage. The germination speed index was calculated according to the formula proposed by Maguire (1962) from this germination test, where the number of seeds with root protrusion was computed daily until the twelfth day. The seedlings emergence test was performed in polystyrene trays (EPS) with 128 individual cells containing Carolina Soil II™ commercial substrate, and were kept in a greenhouse (average temperature $18 \text{ }^\circ\text{C}$). Four replicates of 50 seeds per lot were used. The assessment of seedling emergence was performed 12 days after sowing, by counting the emerged seedlings.

The traditional accelerated aging test (SAAT) was conducted using plastic boxes ($11 \times 11 \times 3.5 \text{ cm}$) containing 40 mL of distilled water, covered with an aluminum screen tray, where the seeds were distributed into a single uniform layer. The boxes were maintained in BOD chamber at $41 \text{ }^\circ\text{C}$ for 24, 48 and 72 h. After each aging period, four replicates of 100 seeds were submitted to a germination test, following the methodology described above, with evaluation performed on the fourth day. The salt solution accelerated aging test (SSAA) was performed similarly to SAAT, however, 40 mL unsaturated salt solution (11 g of NaCl diluted in 100 mL of distilled water) was added to the bottom of the plastic containers, establishing an environment with approximately 94% relative humidity, adapting the methodology described by Jianhua and McDonald (1996). The saturated salt accelerated aging test (SatSAA) was performed similarly to SAAT, however, 40 mL of saturated NaCl solution (40 g of NaCl diluted in 100 mL of distilled water) was added to the bottom of plastic containers, establishing an environment with approximately 76% relative humidity, following the methodology described by Jianhua and McDonald (1996).

The seed health test was carried out with a bulk sample composed of a subsample of seeds from each lot, corresponding to the conditioning periods of each studied methodology. The method used was blotter and freezing (Brasil, 2009), 25 seeds were distributed per plate, totaling 100 seeds per treatment, placed on three blotter paper, previously moistened with distilled water. The incubation was performed at $20 \pm 2 \text{ }^\circ\text{C}$ in a light regime of 12 h of white light and 12 h of dark following a period of 24 h at $-20 \text{ }^\circ\text{C}$ and subsequent return to initial conditions for four days. After the incubation period, the seeds were examined individually in a stereoscopic microscope, and the identification of fungi was made based on the morphological characteristics of their growth on the seeds, and also by the optical microscope using a slide made from the

material contained in seeds. The results were expressed as the percentage of seeds with different fungal growth.

The experiment was conducted in a completely randomized design with four replications per treatment for all characteristics evaluated, except for the water content, which used two replications. The data was subjected to analysis of variance and F test, in a factorial 10×3 (ten lots and three periods of conditioning), setting the methodology (SAAT, SSAA and SatSAA) to identify the most vigorous lots. To determine the effectiveness of each methodology, a Pearson correlation was used between the seedling emergence at the end of each time of accelerated aging and the vigor observed by the first germination counting, germination speed index and seedlings emergence test. The data was submitted to tests of normality and homogeneity of variance, which did not indicate the need for changes. These analysis procedures were performed using the GENES software (Cruz, 2013). The data relating to the health test was not statistically analyzed.

Results and Discussion

The water content in the non-aged seeds varied between lots, with values of 8.2 to 8.9%, amplitude of 0.7% (Table 1), however this difference did not compromise the test (Marcos-Filho, 2005). In SAAT, with 24 h of conditioning, the seeds showed a significant increase in water content, reaching values between 16.1 and 21.1%, with a variation of 5%, exceeding the recommended limit of 2% (Marcos-Filho, 2005). The seed moisture content remained high with increased conditioning period, so in 48 h of conditioning, the displayed values were between 33.5 and 36.7% (3.2% variation), exceeding the maximum accepted amount (2%) by 1.2%. After 72 h of conditioning, the minimum and maximum water content values were 48.0 and 49.3% respectively (1.3% variation), remaining within acceptable limits for consistency of results. The high water content obtained after periods of conditioning is due to high humidity environment ($\approx 100\%$ relative humidity). According to Krishnan et al. (2004), the smaller the seed potential (Ψ_M), the smaller the water potential (hydropotential) of the seed and more water will be absorbed from the environment, to the extent that there is a pressure counterforce generated by the pressure potential (Ψ_p) of the cell wall.

After submitting seeds to SSAA lower water levels were observed than those found in SAAT, ranging between 13.9 and 14.5% (0.6% of variation) in 24 h, 17.7 and 18.5% (0.8% of variation) in 48 h and 18.3 and 19.2% (0.9% of variation) in 72 h of conditioning (Table 1). By using SSAA, the effect of moisture is attenuated by retarding the absorption of water by the seed without exceeding the tolerable limit of 2% of range between lots. According to Hussain et al. (2006), the use of

solutions with different osmotic potentials (Ψ_o) regulates the rate of hydration of the seeds by decreasing the absorption of humidity once they come into balance with the osmotic potential of the environment.

The seeds submitted to the SatSAA methodology showed a markedly lower availability of water, reducing the rates of absorption between periods of conditioning (Table 1). In 24 h of conditioning the values ranged from 8.9 to 9.6% (0.7% variation), 48 h between 11.1 and 12.0% (0.9% variation) and 72 h between 13.1 and 13.7% (0.6% variation). Consequently, the seeds presented greater uniformity of moisture content and minor variations between lots, the same as SSAA, except for the magnitude of values. Thus, the most evident stress condition, using the methodology SatSAA, is mainly caused by the influence of high temperature, while still not affecting the consistency of the results (Almeida et al., 2014). According to Essemine et al. (2010), the high temperature favors the oxidation of cells producing free radicals that act on cell membranes and proteins causing stress on seeds.

The data relating to the germination test (G) indicated two categories for physiological quality in the birdsfoot trefoil lots (Table 2). Lots 5, 9 and 10 were superior, the second category of lots (1, 2, 3, 4, 6, 7 and 8) were within the standard for commercialization of *L. corniculatus* seeds, that means 70% (Brasil, 2010). It is important that the evaluated lots had similar germination or compatible with the standards established for marketing, as one of the accelerated aging test objectives is to identify significant differences in physiological quality of marketable lots, especially among those with similar germination (Marcos-Filho, 2005).

In the first germination count (FGC) significant differences between lots were observed, classifying them in different categories of physiological quality (Table 2). Despite being conducted along with the germination test, which basically provides that the seeds express their maximum germination (Brasil, 2009), the lots were divided into seven physiological quality categories, being the lot 10 classified as superior than the others. Similar results were observed in lots of *Coriandrum sativum* and *Raphanus sativus* seeds, demonstrating the sensitivity of the FGC to differentiate the physiological quality between lots (Vieira et al., 2013).

The results of the germination speed index (GSI) showed similarities to the FGC, identifying the lot 10 as the most vigorous and the others, again separated into six existing categories (Table 2). Although FGC and GSI are considered indicative of vigor, the process of reducing the speed of germination is not one of the first events occurring internally in seeds (Delouche and Baskin, 1973); therefore, they cannot detect the early events of reduced seed quality.

Table 1. Average water content of ten lots of birdsfoot trefoil seeds, before and after accelerated aging test, depending on the conditioning periods.

Lots	Initial water content (%)	Standard Accelerated Aging Test (%)			
		24 hours	48 hours	72 hours	Means
1	8.2 b	21.0 Ca	36.7 Ba	48.8 Aa	35.5 a
2	8.2 b	16.9 Cb	33.7 Bb	48.5 Aa	33.0 b
3	8.3 b	21.1 Ca	36.7 Ba	48.9 Aa	35.5 a
4	8.5 b	17.1 Cb	33.7 Bb	48.2 Aa	33.0 b
5	8.9 a	16.5 Cb	33.6 Bb	48.8 Aa	33.0 b
6	8.3 b	16.9 Cb	33.7 Bb	48.0 Aa	32.9 b
7	8.2 b	21.1 Ca	36.7 Ba	49.0 Aa	35.6 a
8	8.4 b	21.0 Ca	36.6 Ba	48.9 Aa	35.5 a
9	8.7 a	16.9 Cb	33.6 Bb	49.3 Aa	33.3 b
10	8.8 a	16.1 Cb	33.5 Bb	48.3 Aa	32.6 b
Means	8.5	18.5 C	34.9 B	48.7 A	--
F (initial water content)	4.30*	--	--	--	
F (lots)	--		32.70**		
F (periods)	--		2022.32**		
F (lots × periods)	--		6.86**		
C.V. (%)	2.15		1.69		
Lots	Initial water content (%)	Salt Solution Accelerated Aging (%)			
		24 hours	48 hours	72 hours	Means
1	8.2 b	13.9	17.7	18.3	16.7 a
2	8.2 b	13.9	17.9	18.3	16.7 a
3	8.3 b	13.9	18.1	18.3	16.7 a
4	8.5 b	13.9	18.3	19.2	17.1 a
5	8.9 a	14.1	18.5	19.1	17.2 a
6	8.3 b	14.3	17.7	18.5	16.8 a
7	8.2 b	14.1	17.7	18.6	16.8 a
8	8.4 b	14.5	18.1	18.3	16.9 a
9	8.7 a	14.4	18.3	18.6	17.1 a
10	8.8 a	14.5	18.4	18.4	17.1 a
Means	8.5	14.2 B	18.1 A	18.6 A	--
F (initial water content)	4.30*	--	--	--	
F (lots)	--		2.10 ^{ns}		
F (periods)	--		835.06**		
F (lots × periods)	--		1.48 ^{ns}		
C.V. (%)	2.15		1.80		
Lots	Initial water content (%)	Saturated Salt Accelerated Aging (%)			
		24 hours	48 hours	72 hours	Means
1	8.2 b	9.2	11.8	13.3	11.4 a
2	8.2 b	9.0	11.1	13.1	11.1 a
3	8.3 b	9.2	11.1	13.7	11.3 a
4	8.5 b	9.0	11.8	13.5	11.4 a
5	8.9 a	9.6	11.7	13.3	11.5 a
6	8.3 b	9.3	11.8	13.6	11.6 a
7	8.2 b	9.1	11.6	13.5	11.4 a
8	8.4 b	8.9	12.0	13.3	11.4 a
9	8.7 a	9.1	11.7	13.5	11.5 a
10	8.8 a	9.2	11.9	13.3	11.5 a
Means	8.5	9.2 C	11.7 B	13.4 A	--
F (initial water content)	4.30*	--	--	--	
F (lots)	--		2.07 ^{ns}		
F (periods)	--		836.08**		
F (lots × periods)	--		1.75 ^{ns}		
C.V. (%)	2.15		2.19		

Values followed by the same letter in columns (lower case) and rows (uppercase) do not differ ($p > 0.05$) by the Scott-Knott test. ** and * Significant 1 and 5%, by F-Test, ^{ns} not significant.

Table 2. Germination (G), first germination count (FGC); germination speed index (GSI) and seedling emergence (SE) of ten lots of birdsfoot trefoil seeds.

Lots	G (%)	FGC (%)	GSI	SE (%)
	Means			
1	73 b	35 f	8.39 g	53 c
2	71 b	50 d	10.21 d	57 c
3	70 b	31 g	8.25 g	41 d
4	73 b	47 e	9.62 e	54 c
5	79 a	60 b	11.47 b	69 b
6	74 b	44 e	9.14 f	51 c
7	70 b	31 g	8.17 g	43 d
8	71 b	35 f	8.69 g	43 d
9	78 a	55 c	10.92 c	68 b
10	83 a	71 a	12.83 a	80 a
C.V.	4.45	4.23	2.14	6.70

Values followed by the same letter in columns do not differ ($p>0.05$) by the Scott-Knott test.

The percentage of seedling emergence (SE) allowed separation of the lots into four levels of vigor, lot 10 with the highest physiological quality, followed by lots 5 and 9 (close to the standard of 70%, with 69 and 68%, respectively) and in descending vigor order, lots 1, 2, 4, 6, 3, 7 and 8 (Table 2). According to Marcos-Filho (2005), the SE is an indicator of the vigor test efficiency in the evaluation of the physiological quality of seed lots. Thus, there were different responses of the lots when subjected to different specific situations in each test, which demonstrates the importance of using several tests to evaluate seed vigor.

The results from SAAT showed significance for the purposes of lots, conditioning periods and interaction lots x conditioning periods (Table 3). However, only the most vigorous lots (10, 9, 5 and 2) achieved satisfactory results and the other means did not allow a consistent categorization of the lots by vigor levels, depending on conditioning periods. This is due to the relatively high water content obtained by the seeds (Table 1), resulting in a greater degree of deterioration, physiological changes, and consequently not uniform results. These results support the hypothesis of inactivation of enzymes (CAT, catalase) and release of free radicals (SOD, superoxide dismutase) during the conditions imposed by SAAT, reducing breathing capacity, reducing the supply of energy (ATP, adenosine triphosphate) and assimilates for seed germination (Lehner et al., 2008; Demirkaya et al., 2010). According to Silva et al. (2010), the SAAT showed little reproducible results, with less homogeneity and increased coefficient of variation, preventing the separation of lots in levels of vigor for *Cynodon dactylon* seeds, reducing on average, 58% of the germination after the conditioning of the seeds.

Analysis of variance for SSAA results presented significance only for the isolated effects of lots and conditioning periods (Table 3). The results showed that the use of solution with the addition of NaCl provided greater uniformity and delay in the absorption of water by the seeds (Table 1). Thereby, SSAA conditions promoted less drastic effects, because once lower water content was achieved the degree of deterioration was attenuated compared to that observed with the use of SAAT. The values found in this study ranged from 56% (lot 10), 48% (lots 5 and 9), 41, 43, 43 and 44% (lots 1, 4, 6 and 2) and 31, 33 and 34% (lots 7, 8 and 3) germination after the SSAA (Table 3). The main effect of lots, regardless of period effect, allowed the categorization of the lots into four levels of vigor, agreeing with the values observed in the SE (Table 2). Tunes et al. (2011), analyzing the results of SSAA, observed that this method allowed the stratification of lots of *Lolium multiflorum* seeds for vigor, providing the same separation of lots verified by SE.

The SatSAA indicated significant interaction between lots x conditioning periods, as well as for the separate effects of lots and periods (Table 3). In general, the effect of lots showed similar vigor values between the levels of period effects, with the exception of 24 h, where lot 6 was categorized differently from the others tests performed. The SatSAA provided more consistent results due to lower water content absorbed by the seed and the lack of apparent growth of microorganisms, which concurs with the findings in the literature (Vieira et al., 2013; Almeida et al., 2014).

The incidence of fungus was observed in all the combinations of lots and conditioning periods, to a greater or lesser extent, depending on the treatments applied to the seeds (Table 4). In SSAT there was 100% of *Aspergillus* spp., 21% of *Rhizopus* spp. and 18% of *Penicillium* spp. during the larger conditioning period (72 h). During the conditioning period of the seeds the high relative humidity, seed moisture and temperature, promote favorable conditions for the acceleration of biochemical processes, favoring fungal action (Kapoor et al., 2011) especially in *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp., that contribute to the deterioration of the seed (Demirkaya et al., 2010).

The use of NaCl in SSAA and SatSAA methodologies decreased fungal growth, when compared to SAAT (72 h). There was a decrease of 98% in *Aspergillus* spp. (SatSAA 24, 48 and 72 h), 89% in *Penicillium* spp. (SSAA 48 h) and 95% in *Rhizopus* spp. (SatSAA 48 h). When the seeds are submitted to SSAA and SatSAA, it is likely that the saline solution releases chlorine and sodium ions to the media, since these ions have antifungal action, they help to reduce the fungi proliferation (Ávila et al., 2006).

Table 3. Average germination (%) of ten lots of birdsfoot trefoil seeds after accelerated aging (methodology with and without NaCl), depending on the conditioning periods.

Lots	Standard Accelerated Aging Test (%)			Means
	24 hours	48 hours	72 hours	
1	36 Ad	33 Ac	16 Bb	28 b
2	47 Ab	45 Ab	22 Ba	38 a
3	35 Ad	31 Ac	16 Bb	27 b
4	42 Ac	40 Ab	14 Bb	32 b
5	51 Ab	46 Ab	20 Bb	39 a
6	41 Ac	35 Bc	18 Cb	31 b
7	31 Ad	27 Ac	17 Bb	25 b
8	34 Ad	34 Ac	19 Bb	28 b
9	50 Ab	46 Ab	27 Ba	41 a
10	61 Aa	54 Aa	23 Ca	46 a
Means	42 A	39 A	19 B	--
F (lots)		31.07**		
F (periods)		92.84**		
F (lots × periods)		3.79**		
C.V. (%)		8.95		
Lots	Salt Solution Accelerated Aging (%)			Means
	24 hours	48 hours	72 hours	
1	44	43	37	41 c
2	48	37	38	44 c
3	37	34	30	34 d
4	45	46	38	43 c
5	53	47	44	48 b
6	48	43	38	43 c
7	34	30	29	31 d
8	37	31	30	33 d
9	50	50	45	48 b
10	64	57	52	56 a
Means	46 A	43 A	38 B	--
F (lots)		111.63**		
F (periods)		51.72**		
F (lots × periods)		1.65 ^{ns}		
C.V. (%)		4.54		
Lots	Saturated Salt Accelerated Aging (%)			Means
	24 hours	48 hours	72 hours	
1	43 Ac	41 Ac	34 Bc	39 c
2	45 Ac	42 Ac	36 Bc	41 c
3	39 Ad	33 Bd	29 Bd	33 d
4	47 Ac	41 Bc	36 Cc	41 c
5	53 Ab	49 Bb	46 Bb	49 b
6	55 Ab	42 Bc	35 Cc	44 c
7	39 Ad	33 Bd	30 Bd	34 d
8	37 Ad	32 Bd	30 Bd	33 d
9	51 Ab	49 Ab	47 Ab	49 b
10	63 Aa	60 Aa	55 Ba	59 a
Means	47 A	42 B	38 B	--
F (lots)		79.58**		
F (periods)		38.12**		
F (lots × periods)		2.10*		
C.V. (%)		5.42		

Values followed by the same letter in columns (lower case) and rows (uppercase) do not differ ($p > 0.05$) by the Scott-Knott test. ** and * Significant 1 and 5%, by F-Test, ^{ns} not significant.

According to the Pearson correlation analysis, some methods had positive and negative correlations with seedling

emergence (SE) and other variables (Table 5). It was possible to establish positive associations at 5% probability

Table 4. Average percentage (%) of fungi in ten lots of birdsfoot-trefoil seed after accelerated aging test, depending on the methodology and conditioning periods.

Fungi	SAAT (%)			SSAA (%)			SatSAA (%)		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
<i>Alternaria</i> sp.	2	-	-	4	-	2	4	-	-
<i>Aspergillus</i> sp.	11	14	100	3	6	9	2	2	2
<i>Fusarium</i> sp.	4	2	2	2	1	-	-	-	-
<i>Nigrospora</i> sp.	-	-	-	1	-	7	1	-	-
<i>Penicillium</i> sp.	-	-	18	5	2	-	-	-	-
<i>Rhizoctonia</i> sp.	2	-	-	-	-	-	-	-	-
<i>Rhizopus</i> sp.	2	14	21	4	3	-	2	1	-

SAAT, standard accelerated aging test; SSAA, salt solution accelerated aging; SatSAA, saturated salt accelerated aging.

Table 5. Correlation coefficient values (r) between first germination counting (FGC), germination speed index (GSI) and seedling emergence (SE) depending on the accelerated aging methodology.

Method	Period	FGC	GSI	SE
SAAT – Standard Accelerated Aging Test	24 hours	0.43 ^{ns}	0.53 ^{**}	0.25 ^{ns}
	48 hours	0.52 ^{**}	0.51 ^{**}	0.45 [*]
	72 hours	-0.12 ^{ns}	0.30 ^{ns}	0.22 ^{ns}
SSAA - Salt Solution Accelerated Aging	24 hours	0.51 ^{**}	-0.05 ^{ns}	-0.02 ^{ns}
	48 hours	0.28 ^{ns}	0.59 ^{**}	0.52 ^{**}
	72 hours	0.12 ^{ns}	0.53 ^{**}	0.43 ^{ns}
SatSAA - Saturated Salt Accelerated Aging	24 hours	0.59 ^{**}	0.02 ^{ns}	-0.08 ^{ns}
	48 hours	0.43 ^{ns}	0.39 ^{ns}	0.69 ^{**}
	72 hours	0.35 ^{ns}	-0.03 ^{ns}	0.24 ^{ns}

** and * Significant 1 and 5%, by F-Test; ^{ns} not significant.

between the SE and the SAAT methodology ($r = 0.45$) and at 1% significance level to the methodologies of SSAA and SatSAA ($r = 0.52$ and $r = 0.69$, respectively), both for 48 h conditioning period. The correlation coefficients between the FGC and SE have not shown consistency in certain observed associations, particularly for those previously mentioned between SSAA and SatSAA methodologies (48 h) and SE. The GSI was positively correlated with the SE in SAAT methodologies (24 and 48 h) and SSAA (48 and 72 h). The highest rate found in this study, SatSAA/48 h ($r = 0.69$) was not significant for the FGC and GSI tests. This partnership between laboratory tests and SE does not necessarily reflect the corresponding precision in the estimation of lot quality and according to Marcos-Filho (2005) the results of this analysis should not be interpreted alone.

Studies in vigor tests to evaluate the physiological quality of forage species seeds are scarce and the methodology used is, most of the time, adapted from field crop seeds. Therefore, this adjustment will allow quality control for forage seeds being produced, and may result in faster and more uniform establishment of pastures.

Conclusions

The accelerated aging methods with saline solution (SSAA) and saturated solution (SatSAA) with 48 h of conditioning, allow the prediction of physiological quality of birdsfoot trefoil seeds.

The variation in water content during periods of accelerated aging utilizing traditional method interfere in the birdsfoot trefoil seeds' quality.

Making use of saturated solution (SatSAA) for 48 h can be applied to assess the birdsfoot trefoil seeds.

The incidence of fungus decreases when using saturated solution for 48 h.

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