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Correlation between vigor by accelerated aging at pre-sowing and soybean seedling emergence in the field

Correlação entre o vigor pelo envelhecimento acelerado em pré-semeadura e emergência de plântulas de soja a campo

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Highlights

The 48-hour AA method is indicated for pre-sowing assessment.
There is a correlation between field emergence and 48-hour AA.
AA validates 3 vigor levels: high (>85%), medium (70 and 84%) and low (<70%).

Abstract

Although the accelerated aging test is widely used to determine seed vigor, there are methodological variations in seed exposure time at 41 °C. Therefore, the objective was to indicate the most appropriate seed exposure time to segregate lots that also exhibits a correlation with seedling emergence in the field, and establish vigor levels using the aforementioned test. Soybean seed samples were collected at pre-sowing, representing 100 lots in the 2016/2017 growing season and 125 lots in the 2017/2018 season from different warehouses in 4 regions of Santa Catarina state (SC). Physiological quality was evaluated by germination, accelerated aging (24 and 48 h at 41 °C) and field emergence tests. The results were submitted to descriptive statistical analysis and Pearson's correlation analysis ($p < 0.05$). The rank sum index, associated with the Scott-Knott clustering method, was used to classify physiological quality. The average germination percentage was 90 and 91% in the 2016/2017 and 2017/2018 growing seasons, respectively. Vigor obtained an average of 86 (2016/2017) and 85% (2017/2018), when seeds were submitted to accelerated aging for 48 h, and 89% (both seasons) for 24 h. In regard to field emergence, the average was 85 and 80% in the 2016/2017 and 2017/2018 growing seasons, respectively. Considering the 225 lots, the greatest correlation with field emergence was observed in vigor - 48 h ($r = 0.71$ $p < 0.05$). Rank sum classification made it possible to establish vigor-based categories, whereby batches with over 85% vigor by 48-h accelerated aging were classified as high vigor, 70 to 84% medium vigor, and under 70% low vigor.

Key words: *Glycine max* (L.). Germination. 24 and 48-hour accelerated aging.

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Resumo

Mesmo com a ampla aplicação do teste de envelhecimento acelerado para determinação do vigor das sementes existem variações metodológicas quanto ao tempo de exposição das sementes a temperatura de 41 °C. Diante disso, objetivou-se indicar o tempo de exposição das sementes mais apropriado para segregar lotes e com maior correlação com a emergência de plântulas a campo, e estabelecer níveis de vigor pelo respectivo teste. As amostras de sementes de soja foram coletadas em pré-semeadura, de forma representativa de 100 lotes na safra 2016/2017 e 125 lotes na safra 2017/2018 em diferentes armazéns distribuídos em 4 regiões do estado de Santa Catarina. Avaliou-se a qualidade fisiológica pelos testes de germinação, envelhecimento acelerado (24 e 48 h a 41 °C) e emergência em campo. Os resultados foram submetidos a análise estatística descritiva e a análise de correlação de Pearson ($p < 0.05$). Para classificar a qualidade fisiológica utilizou-se o índice de soma de postos associado ao método de agrupamento de Scott-Knott. O percentual de germinação foi de 90%, na safra 2016/2017 e 91% na safra 2017/2018, e o vigor de 86% e 85% respectivamente, quando as sementes foram submetidas ao envelhecimento acelerado por 48 h, e 89% (ambas as safras) para o tempo de 24 h. Com relação a emergência a campo, observou-se 85% na safra 2016/2017 e 80% na safra 2017/2018. Considerando os 225 lotes, a maior correlação com a emergência a campo foi observada com o vigor 48 h ($r=0.71$; $p < 0,05$). A classificação de soma de postos possibilitou estabelecer categorias com base no vigor, estabelecendo como lotes de alto vigor os que apresentam vigor pelo envelhecimento acelerado 48 h superior a 85%, médio vigor de 70 a 84% e baixo vigor menor que 70%.

Palavras-chave: *Glycine max* (L.). Germinação. EA 24 horas, EA 48 horas.

Introduction

The search for new seed production technologies aims at improving the tests used to assess physiological quality, in order to estimate storage potential and help plan seed commercialization (Marcos, 2015), so that the results obtained indicate the real performance potential of seeds under different field conditions (Dutra & Vieira, 2004).

Physiological quality is usually assessed by the germination test, conducted under optimal environmental conditions, to determine the maximum germination potential of seeds, and can be used to compare different lots and estimate the value of field sowing (Ministério da Agricultura, Pecuária e Abastecimento [MAPA), 2009). However, the widespread use of the germination test to assess physiological quality does not always show a high correlation with field emergence (Schuab, Braccini, França, Scapim, & Meschede, 2002). Seed lots with the same germination percentage, but differences in vigor, exhibit the same behavior under ideal conditions. However, high-vigor lots perform better under unfavorable conditions (Egli & Tekrony, 1996), due to rapid and uniform seedling establishment (Abati, Brzezinski, Zucareli, Werner, & Henning, 2017; Egli & Rucker, 2012).

Vigor tests can accurately identify the differences in physiological potential between seed lots, primarily those with similar germination percentages. The requirements for an efficient vigor test include high sensitivity to differences in physiological potential not detected by germination tests, and the ability to classify lots according to their performance under stress (Marcos, 2015).

The accelerated aging test detects differences in vigor between lots and is an efficient tool in assessing the vigor of seeds from different species. The main applications of the test results are positively correlated with the emergence potential of seedlings in the field, storage time, genotype selection during the breeding process and separation of lots with similar germination (Marcos, 2015). Pereira, Coelho, Sobiecki and Souza (2015) found that the accelerated aging test provided satisfactory results for differentiating vigor between soybean cultivars. Similar results were observed by Gindri, Coelho, Souza, Heberle and Prezzi (2017) for bean seeds and Khan et al. (2017) for rice seeds, as well as classification of maize lots (Castan, Gomes, & Marcos, 2018; Sena & Alves, 2017).

The use of tests that classify lots into different levels of physiological quality help seed producing companies decide which lots to commercialize, directing them to locations with greater performance potential (Amaro et al., 2015), or indicating longer storage time.

Even with the widespread application of the accelerated aging test, care must be taken with testing conditions. The differences in vigor found in seed lots may be a function of meteorology and not physiological potential.

Seed exposure time of 48h may promote severe stress, depending on when the seeds were collected. As such, there are practical indications that 24 h is appropriate for samples assessed after collections in the pre-sowing phase as a function of the length of time the seeds were stored. However, doubts remain regarding this hypothesis. Thus, the aim of this study was to indicate the most appropriate seed exposure time to segregate lots that also exhibits the highest correlation with seedling emergence in the field, and establish vigor levels using the accelerated aging test.

Materials and Methods

This study was conducted by the Seed Analysis Laboratory of the Agricultural and Veterinary Science Center of Santa Catarina State University. Soybean seed samples (100 lots) from the 2016/2017 growing season and 125 lots from the 2017/2018 season produced in Santa Catarina state, Brazil, were assessed.

The lots were randomly sampled during the pre-sowing period in breeding units belonging to soybean seed producers. In order to promote the representativeness of soybean producing regions in Santa Catarina, the number of sampled lots varied according to the production percentage per region of the state, namely 60% in the center-west, 30% in the west, 5% in the highland plateau and 5% in the northern plateau (Mathias et al., 2020).

The number of simple samples was collected according to the size of the seed lots, following the Seed Analysis Rules, where 1 sample per 500 kg was collected from lots weighing between 3001 and 20,000 kg, and 1 sample per 700 kg from those above 20,000 kg. The simple samples were used to obtain the compound sample, which was reduced, resulting in the average sample (1000 g). After homogenization and reduction of the average sample in the laboratory, the working sample was obtained (MAPA, 2009). The degree of seed moisture in the working sample was determined, and germination, accelerated aging and field emergence tests carried out.

Seed moisture content was determined using the oven method at 105 ºC (MAPA, 2009).

The germination method was conducted with four repetitions of 100 seeds. All the samples were submitted to pre-conditioning for 16 h at 25 °C. Next, the seeds were distributed equidistantly on germitest paper moistened with water 2.5 times the weight of the paper, then placed in a Mangelsdorf germinator at a temperature of 25 ± 2 °C. Normal, abnormal and dead seedlings were counted on the fifth (first assessment) and eighth day (second assessment) after sowing. The results were expressed in percentage of normal seedlings (MAPA, 2009).

The accelerated aging test was conducted with four repetitions of 100 seeds. In order to perform the test, the seeds were distributed on a steel screen in a single layer and placed in plastic boxes containing 40 mL of distilled water, with approximately 2 cm between the water level and the seeds. The boxes were closed and placed in an aging chamber with a temperature of 41 ºC for 24 and 48 h (Marcos, Kikuti, & Lima, 2009). Next, the germination test was performed, the first assessment on the fifth day and the second on the eighth day after sowing, with results expressed in percentage of normal seedlings (MAPA, 2009).

Seedling emergence in the field: 400 seeds per lot were sowed, on November 11 and 12, 2017 and October 29 and 30, 2018, in the municipality of Fraiburgo/SC, Brazil. The study design consisted of completely randomized blocks, with four repetitions, and the plots of 4 two-meter-long rows, spaced 0.45 m apart, with 25 seeds per row. Counting occurred at 21 days after sowing, when the seedlings exhibited fully developed and differentiated primary leaves, with results expressed in percentage.

Maximum, average and minimum temperature (°C) and rainfall (mm) in the 21 days between sowing and emergence assessment were obtained from the National Meteorological Institute (Inmet, 2018).

The results were submitted to descriptive statistical analysis. Data normality was tested by the Kolmogorov-Smirnov test, homogeneity of variance applying the Levene test, and variable distribution frequency using deviations from the group average (treatments). With a view to identifying the highest correlation between the vigor test and emergence in the field, Pearson´s linear correlation was applied ($p<0.05$). In order to classify the physiological quality of the 225 lots studied in the 2016/2017 and 2017/2018 growing seasons, the rank sum index (Mulamba & Mock, 1978) was associated with the Scott-Knot clustering method. All the analyses were carried out in R software (R Core Team [R], 2016). The magnitude of the correlations was classified as follows: $| r | \leq 0.20$, low; $0.20 < | r | \leq 0.50$, weak; $0.40 < | r | \leq 0.60$, moderate; $0.60 < | r | \leq 0.80$, strong; and $| r | > 0.80$, very strong. In addition to the significance of r, z-scores were transformed, as follows: $z(r1)=1.1513 \log((1+r1)/(1-r1))$, and $z(r2)=1.1513 \log((1+r2)/(1-r2))$, where r1 and r2 represent the correlation coefficients (value in percentage/100) for each variable. The variance of the difference between the following values was calculated: $V=1/(N1-3)+1/(N2-3)$, where N1 and N2 represent the degrees of freedom associated with the observations of each variable, which made it possible to calculate the value of "t", $(t= (z(r1)+z(r2))/\sqrt{V})$, where t values > 1.96 represent significant differences at 5% between two correlations (Pimentel-Gomes & Garcia, 2002)

Results and Discussion

The average germination percentage remained at around 90% in both growing seasons (Table 1). These data indicate that the physiological quality of soybean seeds produced in Santa Catarina was similar to the results obtained by other authors, such as Carvalho, Uarrota, Souza and Coelho (2017), who reported mean germination of 92 and 94% in the 2011/2012 and 2012/2013 growing seasons, respectively.

Germination varied from 82 to 98% in the two crops (Table 1), and approximately 55% of the lots showed above average germination (90%) in the 2016/2017 growing season (Figures 1a and 2a). All the lots assessed met the germination standards required for commercialization, which is 75% for the basic category, and 80% for the other categories (C1, C2, S1 and S2) (Instrução Normativa nº 45, 2013).

The average percentage in the 48-h accelerated aging (AA) test remained at around 85%, with high dispersion in the 2017/2018 growing season. For 24h AA, the average percentage was 89%, with low dispersion in both crops. Field emergence exhibited values between 80 and 85% in both growing seasons, with dispersion values similar to those observed for 48h AA vigor (Table 1). The higher dispersion and similarity in the percentage of field emergence for 48h AA indicates better segregation of lots with establishment potential under field conditions, given that the test condition requires high vigor seeds to exhibit greater tolerance to possible adverse field conditions.

Values of 58 to 95% were observed for 48h vigor in the 2016/2017 growing season (Table 1), with 48% of the lots obtaining results of more than 86% (Figure 1b). With respect to 2017/2018, AA values between 39 and 97% were reported (Table 1), with 60% of the lots above the overall average (Figure 2b). Although the 48h AA condition promoted significant lot segregation in both seasons, lots with high physiological potential were observed, with vigor percentage of more than 80%, which, according to Mbofung, Goggi, Leandro and Mullen (2013), indicates that seeds must exhibit average vigor greater than or equal to 80% in order to establish an ideal seedling stand.

Accelerated aging for 48h was efficient in segregating lots in terms of physiological quality. Similar results were obtained by authors conducting the accelerated aging test at temperatures of 41 and 42 °C, who observed that the combination of 42 °C for 48 h was indicated to determine vigor differences between lots (Dutra & Vieira, 2004; Santorum et al., 2013). The authors underscore that longer test duration (72h) causes severe stress, precluding differentiating lots in terms of quality (Dutra & Vieira, 2004).

Table 1
Descriptive statistics (DS), number of samples (N), average, minimum and maximum coefficient of variation (CV), variance (VAR), standard deviation (SD), emergence (%), germination (%) and vigor (%) of soybean seed lots produced in Santa Catarina state, Brazil.

Variable	DS	Growing season	
		2016/17	2017/18
Germination	N	100	125
	Average	90	91
	Minimum	82	82
	Maximum	98	98
	CV (%)	5.24	4.33
	VAR	22.29	15.41
	SD	4.72	3.92
Vigor - 48 h	Average	86	85
	Minimum	58	39
	Maximum	95	97
	CV (%)	7.64	10.58
	VAR	42.97	80.39
	SD	6.55	8.96
Vigor - 24 h	Average	89	89
	Minimum	72	65
	Maximum	98	98
	CV (%)	5.74	6.75
	VAR	26.14	36.05
	SD	5.11	6.00
Emergence	Average	85	80
	Minimum	57	51
	Maximum	92	91
	CV (%)	7.03	9.94
	VAR	35.44	62.65
	SD	5.93	7.91

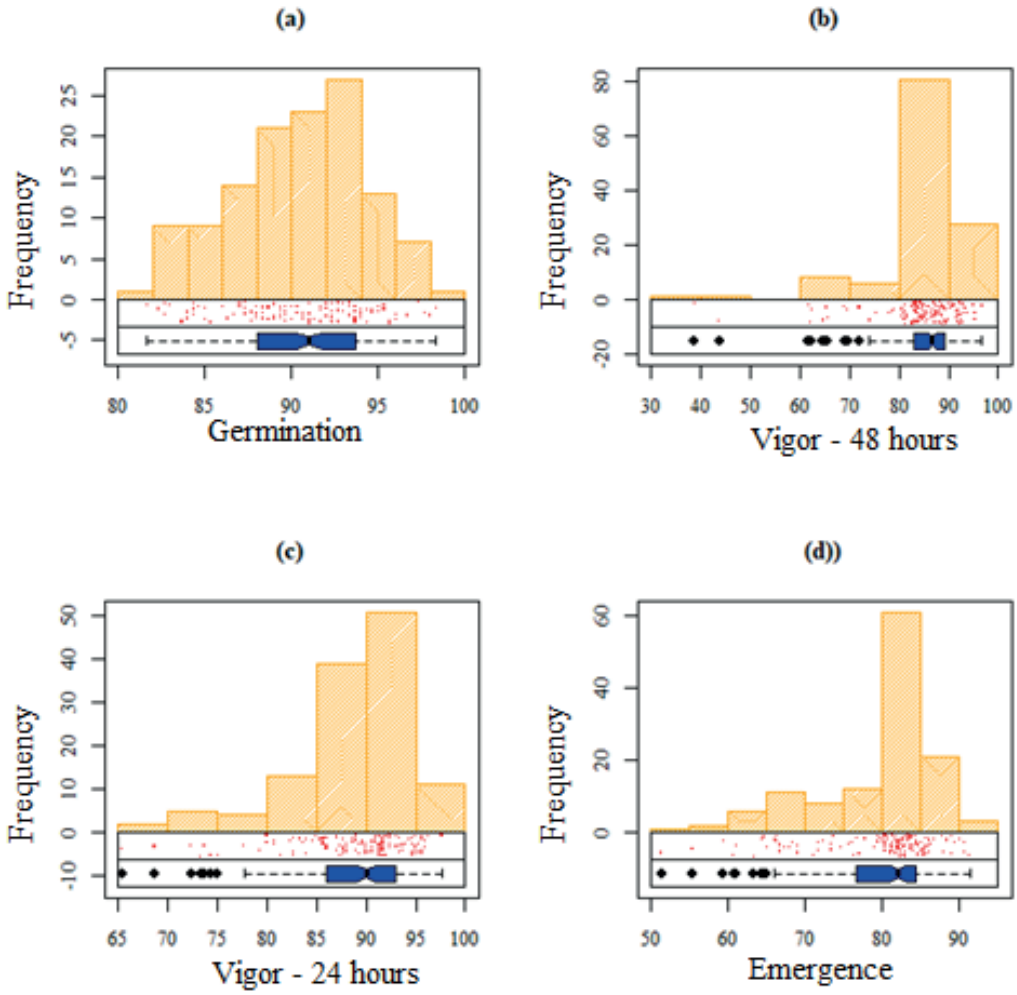

Figure 1. Germination frequency (a), vigor - 48 h (b), vigor - 24 h (c) and field emergence (d) of 100 soybean seed lots produced in Santa Catarina state in the 2016/17 growing season.

The results of 24h AA showed increased vigor between 72 and 98% in the 2016/2017 growing season, and from 65 to 98% for 2017/2018 (Table 1). It was found that 54 and 61% of the lots displayed higher-than- average vigor in 2016/2017 and 2017/2018, respectively (Figures 1c and 2c). It was concluded that 24h was not effective for separating lots in relation to vigor, since the results were similar to those obtained by the germination test with low lot segregation, confirming that the test condition did not cause the stress needed for seed vigor to manifest itself (Table 1).

Diversity between lots was also observed via the frequency distributions of emergence in the field, where average values varied from 57 to 92% in 2016/2017 and 51 to 91% in 2017/2018 (Table 1). Additionally, 59% of the lots showed more than 85% emergence in 2016/2017 (Figure 1d). In 2017/2018, 70%

of the lots obtained a higher-than-average field emergence percentage (Figure 2d).

The correlation test was applied to identify the seed exposure time in the accelerated aging test that exhibited the highest correlation with field emergence (Table 2). All the correlations showed significant effects. The correlation between germination and field emergence obtained the lowest value, irrespective of growing season. In 2016/2017, the highest correlation with field emergence was recorded for 48h AA (r=0.67*), similar to that observed in 2017/2018 (r=0.76). Considering the 225 lots, there was greater correlation between emergence and 48h AA (r=0.71*).

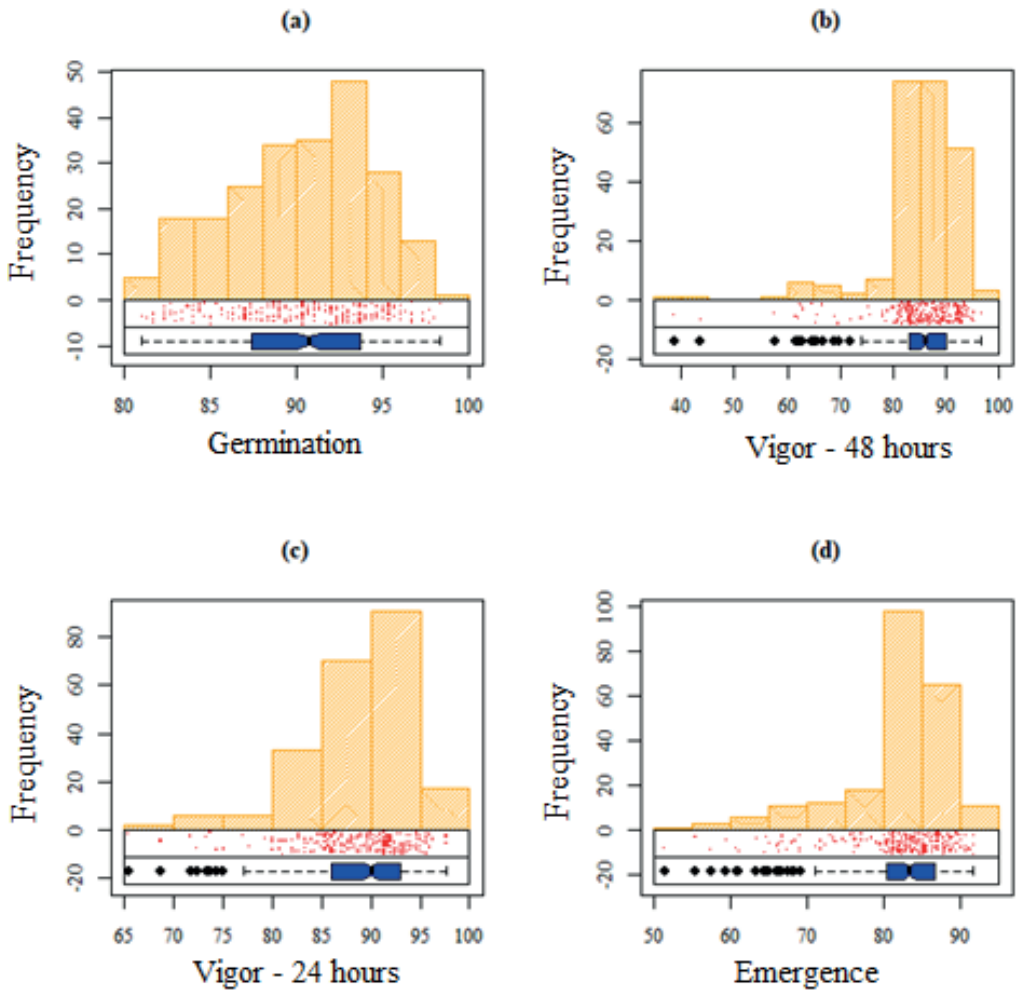

Figure 2. Germination frequency (a), vigor - 48 h (b), vigor - 24 h (c) and field emergence (d)of 125 soybean seed lots produced in Santa Catarina state in the 2017/18 growing season.

Table 2
Coefficients of correlation between emergence, germination and vigor of soybean seed lots produced in Santa Catarina state.

	Emergence		
	100[1]	125[2]	225[3]
Germination	0.24*	0.50*	0.35*
Vigor - 48 h	0.67*	0.76*	0.71*
Vigor - 24 h	0.39*	0.82*	0.65*

*significant at 5% probability; [1] lots produced in the 2016/17 growing season; [2] lots produced in the 2017/18 growing season; [3] number of lots considering the results of the two growing seasons. All the (r) values were significantly different after z transformation and the t-test.

The correlation stability observed between 48h AA and field emergence, in both crops, indicates that 48h was the ideal time to estimate the field establishment potential of seedlings. In addition, the results show greater reliability in the test conducted for this duration, since the correlation with field emergence remained high irrespective of growing season. Other studies found a relationship between vigor in the accelerated aging test (41°C/48 h) and seedling performance parameters in the field (Egli, Hamman, & Rucker, 2010), observing that seed vigor has a negative effect on the uniformity index, with low vigor lots emerging more slowly under stress and therefore resulting in uneven stands.

The low correlation between germination and field emergence was directly influenced by stress time in the accelerated aging test, since, despite the significant correlation between 24h AA and field emergence, it did not remain high in the two seasons, which demonstrates the weakness of 24h AA in discriminating lots, aimed at tolerating adverse field conditions. Similar behavior was observed by Corbineau (2012). When soybean seedlings emerge quickly, the effects of vigorous seeds on emergence cannot be identified, showing only germination potential, but low vigor lots demonstrate a high negative correlation with field emergence (Egli et al., 2010).

Climate conditions during emergence accounted for the performance differences of lots in the field for the growing seasons assessed. Conditions in the initial 21-day period in 2017 were more stressful than those observed for the same period in 2018. The main differences were minimum temperature and rainfall (Figure 3).

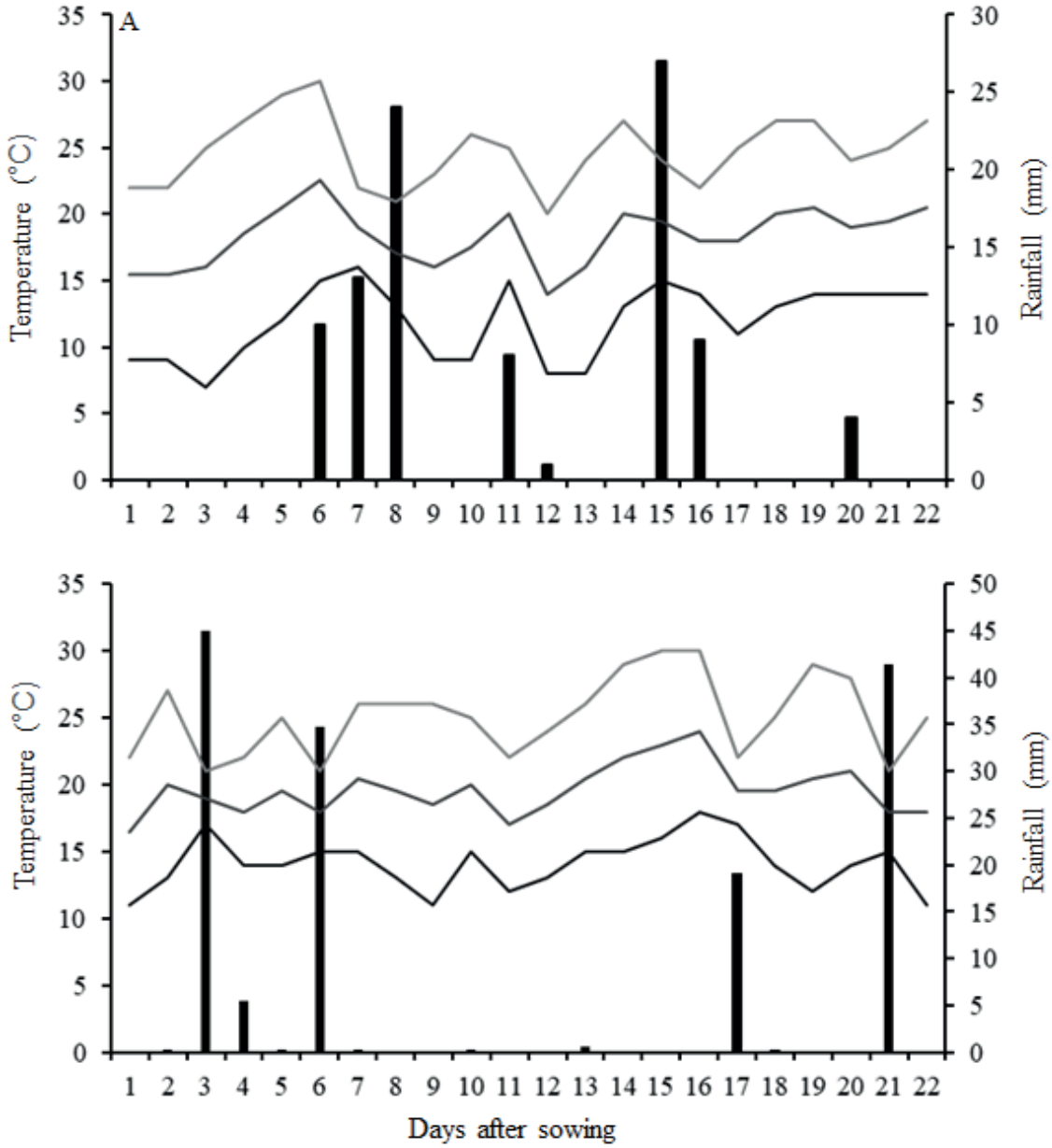

Figure 3. Maximum, average and minimum temperature and rainfall recorded 21 days after sowing in 2017, (sowing on 11/12) (A) and 2018 (sowing on 10/29) (B), Fraiburgo, Santa Catarina state.

In 2017, the minimum temperature reached values below 10 °C, while in the same period in 2018, they were near 12 °C. With respect to rainfall, 2017 saw homogeneous rainfall distribution; however, it coincided with low temperature days (< 10°C), which supposedly were responsible for the higher stress on seeds during that year. Although rain was less intense in 2018, it did not characterize drought stress.

The stress conditions observed were similar to those reported by Albuquerque and Carvalho (2003), exhibiting greater impact on soybean seedling emergence in the field. The authors underscore that the effect of seed vigor on field emergence depends on the type of climate stress, with the greatest impacts caused by low (15 °C) and high temperatures (35 °C) and pathogen infestation.

Ideal sowing conditions were observed in 2018, justifying the greater correlation between the 24h vigor test and field emergence, similar to that observed by other authors. França, Krzyzanowski, Pádua, Henning and Costa (2003) report that the test can be conducted for 24h when carried out near sowing time. This recommendation is based on the study performed in the 2002/2003 growing season in Londrina, Párana state, in which the results of the 24h accelerated aging test were correlated with the field emergence of 100 samples, obtaining a coefficient of determination of 0.80. The authors emphasize that for near-ideal temperature and soil moisture conditions, the coefficient of determination reveals the reliability of the 24h accelerated aging test (França et al., 2003).

The classification of lots into different levels of physiological quality is an important tool in helping seed producing companies decide the destination of lots. In order to classify the physiological seed quality of 225 lots, the rank sum index (Mulamba & Mock, 1978) was associated with the Scott-Knott clustering algorithm, where the lots analyzed were grouped into four classes, defined as low, medium, high and very high physiological quality (Table 3). This classification revealed that 46% of the lots are in the high physiological quality class, with 86 and 90% emergence and germination for 48h AA, and 85 and 88% for 24 h AA, respectively. The highest variations were observed in the low-quality category. The classification determined by the Scott-Knott clustering method made it possible to establish categories based on vigor, defining high vigor as more than 85% after 48 h of accelerated aging, medium vigor between 70 and 84% and low vigor less than 70%.

After the lots were classified, the correlation coefficient was determined for each class, in order to identify which class exhibits the highest correlation between the vigor tests and field emergence. There was a significant correlation between 48 h AA and field emergence in the low physiological quality class (average values of 64%) (Table 4), indicating that the use of the vigor test with longer exposure (48 hours) to stress made it possible to identify lots that exhibit low potential for field establishment. This is more evident when there was no significant correlation with field emergence when 24 h AA vigor results were used, which proves that this parameter is very weak in discriminating the physiological performance of the lots under adverse field conditions. In order to guarantee the commercialization of seeds with high field establishment potential, it is recommended that the 48-h accelerated aging test be used in association with the other physiological quality parameters, which should remain near those observed for the high-quality class, ensuring high vigor (above 85%) and germination standards above those legally established (above 80%).

Table 3
Descriptive statistics and classification of the 225 lots in terms of physiological seed quality considering the four variables studied in the 2016/17 and 2017/18 growing seasons.

Classes	Emergence						
	n	Average	Minimum	Maximum	CV	VAR	SD
Low	13	63	45	73	11.77	53.69	7.32
Medium	25	74	65	84	8.95	43.36	6.58
High	103	83	73	91	4.65	14.80	3.84
Very High	84	86	81	92	3.49	9.10	3.02
	Germination						
	n	Average	Minimum	Maximum	CV	VAR	SD
Low	13	85	82	90	3.18	7.33	2.70
Medium	25	86	81	94	4.10	12.54	3.54
High	103	90	81	96	4.05	13.20	3.63
Very High	84	93	86	98	3.13	8.52	2.92
	Vigor – 48 h						
	n	Average	Minimum	Maximum	CV	VAR	SD
Low	13	64	43	79	12.76	67.15	8.19
Medium	25	79	62	84	7.10	31.37	5.60
High	103	85	80	94	3.27	7.79	2.79
Very High	84	91	83	97	3.15	8.15	2.86
	Vigor – 24 h						
	n	Average	Minimum	Maximum	CV	VAR	SD
Low	13	76	65	91	8.52	41.73	6.46
Medium	25	83	74	87	3.40	7.99	2.82
High	103	88	82	95	3.31	8.56	2.92
Very High	84	93	88	98	2.33	4.72	2.17

Table 4
Coefficients of correlation between emergence, germination and vigor of soybean seed lots classified in the low physiological quality category.

	Emergence
Germination	0.24
Vigor - 48 h	0.68*
Vigor - 24 h	0.21

*significant at 5% probability.

Conclusion

The accelerated aging test conducted for 48 hours showed a similar correlation (r=0.70*) with field emergence in both crops.

Classifying the lots resulted in four physiological seed quality classes, with the highest number of lots in the high-quality category.

Based on the vigor test using accelerated aging (41°C/48 hours), lots with percentages above 85% were classified as high vigor, 70 to 84% medium vigor, and below 70% low vigor.

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