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Physiological quality of soybean seeds grown under different low altitude field environments and storage time

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Abstract: The use of high-quality seeds is essential to maintain high rates of production and productivity. The physiological quality of seeds obtained in the field is directly correlated to storage conditions and storage time. This study aimed to evaluate the physiological quality of soybean seeds in relation to different field environments (seed lots) and storage time. Commercial lots of seeds of the soybean cultivar M8349 IPRO were stored for three and six months. Seed moisture content was determined before and after accelerated aging, along with seed germination percentage and vigour evaluations performed before and after each storage period. The experiment was carried out as a completely randomised factorial design (10 × 3): with ten seed lots and three storage periods. The data were analysed by ANOVA, and the means of four independent replicates for each parameter evaluated were compared using the Scott-Knott test at 5% probability ($P \leq 0.05$). Our results revealed that the low altitude regions where the seed samples were collected are suitable for soybean seed production with high physiological quality. Seed storage for six months does not cause a significant reduction in subsequent soybean seed field performance.

Keywords: *Glycine max* (L.) Merrill; deterioration; edapho-climatic conditions; germination; vigour

Soybean (*Glycine max* (L.) Merr.) stands out in the world as one of the top cultivated and commercialised crops (FAO 2020). However, suitable areas for expanding soybean cultivation are limited (Battisti et al. 2017). To achieve and maintain high grain production and productivity rates in tropical regions, the use of certified and high-quality seeds is essential (Cattelan and Dall'Agnol 2018). Since Brazil is the largest soybean producer in the world (FAO 2020), overtaking the United States during the 2019/20 season (USDA 2020) with an estimated production of 123.2 million tons, the crop has great importance for the country's economy (MAPA 2020).

The physiological quality of any seed is based on its germination and vigour, and it is used to describe the value of a seed lot for its intended purpose (Ghassemi-Golezani et al. 2015). The initial establishment of any crop is directly correlated to the physiological potential of the seeds. The percentage, speed and uniformity of seedling emergence in the field depend on the potential of this physiological characteristic (Ebone et al. 2020). Germination failures are normally associated with the use of low-quality seeds and are commonly caused by low initial vigour. The use of high-quality seeds, on the other hand, results in strong, more vigorous and well-developed seed-

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lings, improving the establishment of the crop under different edapho-climatic conditions. Faster seed emergence and seedling development also result in more efficient weed control (Ebone et al. 2020).

Although all stages of the production chain are important to ensure high-quality soybean seeds, storage in a controlled environment is considered one of the most important, specially for orthodox seeds. Soybean seeds are among the group of least storable seeds in the "relative storability index" classification (Ebone et al. 2019). It is hypothesised that if the environmental conditions in the field are not favourable during seed production and storage conditions are sub-optimal, the physiological quality of the seeds will be reduced as irreversible deterioration commences progressively. The aim of this study was to evaluate the physiological quality of soybean seeds in relation to different field environments during seed production and to storage time following harvesting.

MATERIAL AND METHODS

Experimental materials and locations. The experiment was carried out at the State University of Santa Cruz (UESC), Ilhéus, Bahia, Brazil. Ten commercial samples of soybean seeds (cultivar M8349 IPRO) were provided by Oilema Sementes for the experiment. This cultivar is recommended for this region (Alliprandini et al. 2009). The samples were produced in different locations in the western region of the Bahia state during the 2014/2015 season; three (1, 2 and 3) from Barreiras, four (4, 5, 6 and 7) from Formosa do Rio Preto, and three (8, 9 and 10) from São Desidério (Figure 1, Table 1).

Climate data. During the soybean growing season, monthly average air temperature and rainfall were obtained from automatic weather stations of the Brazilian National Institute of Meteorology in Santa Rita de Cássia and Barreiras (Figure 1), and are presented (Figures 2 and 3). The meteorological station located in Barreiras (Figure 2) was used for two different municipalities (Barreiras and São Desidério) as they are in a similar climatic range (Figure 1), and the meteorological station located in Santa Rita de Cássia was used for the production sites in Formosa do Rio Preto (Figure 3).

Seed harvesting and processing. At the end of the growing season, seeds were mechanically har-

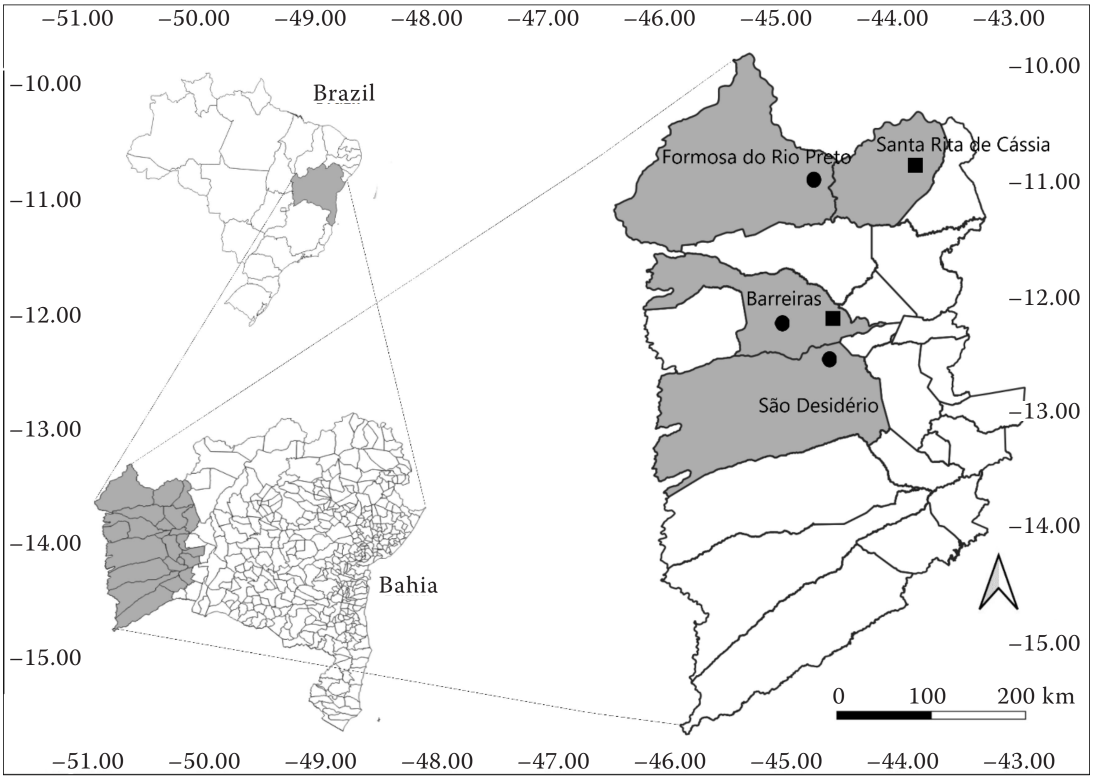

Figure 1. Map indicating the location of sites and meteorological stations used to obtain climatic data during the soybean growing season for the different production sites in the western region of Bahia. Black circles (●) – sample sites; black squares (■) – climate sites (meteorological stations)

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Table 1. Production site locations of soybean seed samples in the western region of Bahia state with climate classification according to Köppen-Geiger

Location	Barreiras	Formosa do Rio Preto	São Desidério
Latitude	12°09'10''S	11°02'54''S	12°21'48''S
Longitude	44°59'24''W	45°11'35''W	44°58'24''W
Altitude (m a.s.l.)	452	490	497
Climate[a]	Aw	Aw	Aw

[a]Tropical climate (Aw) with dry winter

vested and taken to a processing unit where they underwent the processes of pre-cleaning, cleaning, size classification, density classification (through the use of a densimetric table), and shape classification (through the use of a spiral separator). Finally, after being packed, stored and sampled, the seeds were taken to the phytotechnology laboratory of UESC.

Physiological quality evaluations: before and after storage. In the laboratory, seeds were stored in a refrigerator (7 °C). Evaluations of the physiological quality were carried out prior to and after three and six months of storage. The following tests were conducted on the seeds: seed moisture content before and after accelerated aging (Brasil 2009), standard germination (Brasil 2009), first germination count (Nakagawa 1999), germination speed index (Maguire 1962), accelerated aging (Marcos-Filho 1999), electrical conductivity (Marcos-Filho and Vieira 2009), field emergence of seedlings (Nakagawa 1999), and emergence speed index (Maguire 1962).

Statistical procedure. The experiment was carried out in a completely randomised factorial design (10 × 3): ten seed lots and three storage treatments. Statistical analysis was performed using the software AgroStat (v. 1.1.0.694) (Barbosa and Maldonado 2015). Analysis of variance (ANOVA) was calculated for each parameter measured to identify differences between samples. When significant, the means of four independent replicates for each parameter evaluated were compared using the Scott Knott post hoc test at 5% probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

Field environmental conditions during seed development and in post-harvest storage are considered two of the most important factors that can affect the physiological quality of seeds. During the soybean crop cycle, plant water requirement varies between 450 mm and 800 mm, depending on climatic conditions, crop management and cycle duration. The most critical periods in relation to water availability are those corresponding to germination/emergence and flowering/grain filling (Fuganti-Pagliarini et al. 2017). When producing grain,

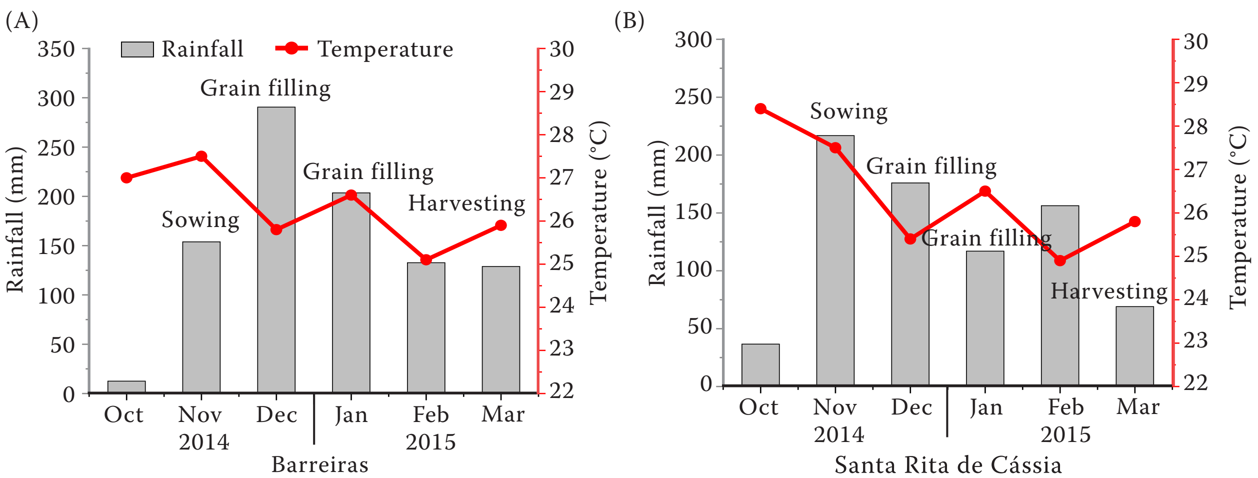

Figure 2. Average monthly rainfall and temperature data were obtained from the meteorological station located in (A) Barreiras for the seeds produced in Barreiras and São Desidério and (B) Santa Rita de Cássia for the seeds produced in Formosa do Rio Preto during the 2014/15 soybean growing season. Source: Brazilian Meteorological Service (INMET)

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growers adjust the sowing date to obtain maximum yields. For seed production, quality is more important than productivity (James and Lawn 2011).

The values recorded for average monthly rainfall by the meteorological stations at Barreiras and Santa Rita de Cássia (Figure 2) are above the recommended plant water requirements during sowing (Fuganti-Pagliarini et al. 2017). In addition to this, deficit and excess water can negatively affect soybean grain yield during pod filling (Ghassemi-Golezani et al. 2015, Lima et al. 2018). In Barreiras and São Desidério (Figure 2A), rainfall was high throughout the growing season, and in Formosa do Rio Preto (Figure 2B), although rainfall decreased for the months following germination, it was not enough to negatively affect the crop during grain filling (Lima et al. 2018).

The production of high-quality seeds requires low temperatures associated with dry climatic conditions during ripening and harvesting. These conditions are not easily found in tropical regions but can occur either at altitudes above 700 m a.s.l. or by matching the sowing time for suitable conditions during seed production (James and Lawn 2011). All sites evaluated in this study have an altitude below 500 m a.s.l. (Table 1), and the regional climate is classified as tropical with a dry winter, according to the Köppen-Geiger classification (Köppen 1936). Therefore, in low-altitude regions, growers must pay particular attention to the time of sowing to plan harvest for the cooler and dryer months to ensure high-quality seeds (Hartmann-Filho et al. 2016), as shown in Figure 2 for the sites in this study.

Low average rainfall values, associated with dry weather conditions, favoured the ripening and natural drying process of the seeds (Figure 2). In addition, low temperatures are also desirable during harvesting, improving seed quality (Hartmann-Filho et al. 2016), and it was also observed in this study (Figure 2). To preserve the physiological quality obtained in the field, seeds must be artificially dried to achieve the desired moisture content before storage and to avoid subsequent chemical and physical damage (Ebone et al. 2019). The ten different lots of soybean cultivar M8349 IPRO seeds presented initial seed moisture contents below 12% with little variation before and after each storage period (Table 2). This similarity among the lots is very important because it ensures standardisation of seed evaluations, providing consistent results for the vigour tests.

Table 3 shows that the soybean seeds had initial germination and germination after storage rates within the recommended levels for commercialisation in Brazil (Brasil 2009). However, high germination percentages (above 80%), as obtained in this study, do not necessarily mean that the lots have high seed vigour since the germination test is performed under controlled conditions. Therefore, although very useful, the germination test has limitations, providing information that overestimates the physiological potential of the seeds under adverse field conditions.

A reliable evaluation of physiological potential identifies seed lots that are most likely to present the desired performance in the field even after storage (Zuffo et al. 2017). The first count test demonstrated that storage after three months reduced the germination speed of lot 7, but did not drop further after six months, and progressively reduced the germination speed of lot 10 after three and after six months, which may be considered a sign of vigour reduction (Table 3). The performance of lots 5 and 9, on the other hand, was only affected after storage for six months. The other lots maintained germination speed over the storage period, indicating that seed vigour was maintained (Table 3). Significant differences were recorded between seed lots for germination speed index only after storage (increasing after six months) (Table 3). Overall, high values recorded for this test are an indication that none of the lots had low initial physiological quality (Zuffo et al. 2017).

The field emergence of seedlings and the field emergence speed index presented very different

Table 2. Seed moisture content before (ISMC) and after accelerated aging (SMCAA) of ten soybean seed samples before and after different storage durations: zero (I), three (II) and six months (III)

Site/Lot		I		II		III	
		ISMC	SMCAA	ISMC	SMCAA	ISMC	SMCAA
		(%)					
B	1	10.5	23.4	7.8	14.5	5.9	20.9
	2	9.5	22.5	7.8	24.1	6.1	24.9
	3	9.4	21.8	7.8	21.2	6.0	18.4
FR	4	9.2	25.2	7.9	19.2	6.2	22.4
	5	9.3	25.5	7.9	16.5	6.7	23.8
	6	9.0	24.6	7.6	18.5	6.1	14.6
	7	9.3	19.4	8.0	21.6	6.0	20.1
SD	8	8.4	22.0	7.7	24.9	6.3	23.5
	9	8.8	16.8	7.5	22.4	6.0	18.0
	10	9.4	23.3	7.1	16.9	5.8	21.9

B – Barreiras; FR – Formosa do Rio Preto; SD – São Desidério

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Table 3. Germination, first count and germination speed index tests of ten soybean seed samples before and after different storage durations: zero (I), three (II) and six months (III)

Site/Lot		Germination			First count			Germination speed index		
		I	II	III	I	II	III	I	II	III
						(%)				
B	1	99^{aA}	98^{aA}	99^{aA}	99^{aA}	94^{bA}	98^{aA}	9.9^{aA}	9.7^{aA}	9.9^{aA}
	2	99^{aA}	98^{aA}	97^{aA}	99^{aA}	97^{aA}	96^{aA}	9.9^{aA}	9.8^{aA}	9.7^{bA}
	3	98^{aA}	100^{aA}	99^{aA}	98^{aA}	100^{aA}	99^{aA}	9.8^{aA}	10.0^{aA}	9.9^{aA}
FR	4	98^{aA}	96^{aA}	96^{aA}	97^{aA}	93^{bA}	94^{aA}	9.8^{aA}	9.5^{bA}	9.6^{bA}
	5	100^{aA}	99^{aA}	97^{aA}	99^{aA}	98^{aA}	94^{aB}	10.0^{aA}	9.9^{aA}	9.6^{bA}
	6	97^{aA}	97^{aA}	96^{aA}	94^{bA}	94^{bA}	92^{bA}	9.6^{aA}	9.6^{bA}	9.5^{bA}
	7	98^{aA}	91^{cB}	87^{cC}	93^{bA}	88^{cB}	85^{cB}	9.7^{aA}	9.0^{cB}	8.6^{dB}
SD	8	99^{aA}	99^{aA}	99^{aA}	97^{aA}	98^{aA}	99^{aA}	9.8^{aA}	9.9^{aA}	9.9^{aA}
	9	96^{aB}	99^{aA}	93^{bB}	94^{bA}	98^{aA}	91^{bB}	9.5^{aB}	9.9^{aA}	9.2^{cB}
	10	99^{aA}	95^{bA}	91^{bB}	98^{aA}	93^{bB}	88^{cC}	9.9^{aA}	9.4^{bB}	9.0^{cC}
CV (%)			2.81			3.23			2.77	

B – Barreiras; FR – Formosa do Rio Preto; SD – São Desidério; *CV* – coefficient of variation. Means in the same column followed by the same lower-case letter and means in the same line followed by the same capital letter are not significantly different according to the Scott-Knott test at $P \leq 0.05$

results compared to the germination test (Tables 3 and 4), being below 80% for some seed lots (6, 3, 8 and 9) after storage (Table 4). This highlights that seeds with the same germination percentage may not have the same performance in the field due to differences in seed vigour (Ebone et al. 2020). The emergence speed index results varied between seed lots over the storage time, with no clear pattern being present (Table 4). Therefore, similar and high-performance seeds, demonstrated by the germination speed index (Table 3), may present a slow, reduced, or uneven emergence under field conditions, leading to standing failure, delays in plant development and weed control problems (Ebone et al. 2020).

Following the accelerated aging test, all lots showed a decrease in performance compared to the germination test (Table 5). Lot 7, and only before storage, recorded a lower germination percentage after accelerated seed aging (Table 5). This may have occurred due to a high incidence of fungi in this lot, which, together with the test conditions (high humidity and temperature), were favourable to the proliferation of pathogens during the test (Zuffo et al. 2017). According to Ramos et al. (2014), pathogen infections can influence the results of vigour tests, resulting in misleading results. Since fungal contamination was high in this sample, this can explain the false higher germination for lot 7 after being stored, which should have been maintained or reduced over time (Diniz et al. 2013, Zuffo et al. 2017).

An increase in the electrical conductivity with storage time is thought to be due to the deterioration process, causing irreversible damage to seed quality (Zuffo et al. 2017, Ebone et al. 2019) and therefore, it was expected that the electrical conductivity would increase with storage time. However, this did not occur for all seed lots (Table 5). Lots 6 and 10 showed a decline in the electrical conductivity after three months of storage and maintained this level after six months of storage. Lot 7 was the only lot that presented a progressive decline after three and then six months of storage. Lots 4 and 8 showed an increase in the electrical conductivity after three months of storage but a decline after six months of storage. These differences may be explained by the low temperature at which the seeds were stored in this study (7 °C). Storage of soybean seeds at low temperatures (< 10 °C) has been shown to influence the electrical conductivity as the leaching of K^+, Ca^{2+} and Mg^{2+} ions are influenced by temperature and storage time (Fessel et al. 2010). In addition, Wain-Tassi et al. (2012) found that seed pathogens can influence electrical conductivity results, producing misleading positive results with lower values with increased storage time.

The high physiological quality of soybean seeds verified by the vigour tests performed in this study was attri-

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Table 4. Field emergence of seedlings and emergence speed index tests of ten soybean seed samples before and after different storage durations: zero (I), three (II) and six months (III)

Site/Lot		Field emergence of seedlings			Emergence speed index		
		I	II	III	I	II	III
		(%)					
B	1	96^{aA}	96^{aA}	98^{aA}	6.8^{aA}	6.8^{aA}	7.0^{aA}
	2	98^{aA}	93^{aA}	88^{bA}	6.9^{aA}	5.8^{bB}	5.6^{bB}
	3	81^{bA}	76^{bA}	85^{bA}	4.9^{dA}	4.5^{dB}	5.2^{bA}
FR	4	96^{bA}	81^{bB}	96^{aA}	6.7^{aA}	5.7^{bB}	6.6^{aA}
	5	93^{aA}	92^{aA}	87^{bA}	5.8^{cA}	5.6^{bA}	5.4^{bA}
	6	74^{cB}	83^{bA}	86^{bA}	5.3^{dB}	6.0^{bA}	6.0^{bA}
	7	86^{bA}	91^{aA}	93^{aA}	6.1^{bA}	6.5^{aA}	6.6^{aA}
SD	8	93^{aA}	78^{bB}	95^{aA}	6.4^{bA}	5.2^{cB}	6.6^{aA}
	9	86^{bA}	79^{bA}	78^{bA}	5.0^{dA}	4.8^{dA}	5.4^{bA}
	10	90^{aA}	88^{aA}	89^{bA}	6.4^{bA}	6.4^{aA}	6.4^{aA}
CV (%)			7.00			6.14	

B – Barreiras; FR – Formosa do Rio Preto; SD – São Desidério; *CV* – coefficient of variation. Means in the same column followed by the same lower-case letter and means in the same line followed by the same capital letter are not significantly different according to the Scott-Knott test at $P \leq 0.05$

buted to the good environmental conditions during plant development and harvesting (Zuffo et al. 2017). Although the results showed that, in general, the seeds maintained high germination levels after storage (Table 3), after six months of storage, seed vigour declined, as indicated by the tests of the first count, field emergence and accelerated aging (Tables 3–5). Therefore, it is possible to conclude that large-scale seed producers in low altitude regions must pay particular attention to the time of sowing and initial seed quality at harvest, as these factors can compromise seed viability during the storage. Overall, regions with such environmental

Table 5. Accelerated aging and electrical conductivity tests of ten soybean seed samples before and after different storage durations: zero (I), three (II) and six months (III)

Site/Lot		Accelerated aging			Electrical conductivity		
		I	II	III	I	II	III
			(%)			(μS/cm/g)	
B	1	96^{aA}	90^{bA}	94^{aA}	45.1^{aA}	51.6^{aA}	49.4^{aA}
	2	94^{aA}	94^{aA}	92^{aA}	46.9^{aA}	75.2^{bB}	95.1^{cC}
	3	88^{bA}	97^{aA}	92^{aA}	85.7^{bA}	74.9^{bA}	82.1^{bA}
FR	4	88^{bA}	86^{bA}	85^{bA}	46.2^{aA}	75.2^{bB}	49.2^{aA}
	5	92^{aA}	93^{aA}	90^{aA}	80.5^{bA}	76.1^{bA}	117.7^{dB}
	6	89^{bA}	94^{aA}	92^{aA}	100.8^{cB}	70.2^{bA}	75.9^{bA}
	7	68^{cB}	86^{bA}	84^{bA}	118.9^{dC}	90.5^{cB}	69.2^{bA}
SD	8	94^{aA}	95^{aA}	95^{aA}	45.9^{aA}	58.0^{aB}	46.2^{aA}
	9	88^{bA}	90^{bA}	85^{bA}	76.8^{bA}	75.9^{bA}	83.0^{bA}
	10	85^{bA}	85^{bA}	90^{aA}	90.4^{bB}	71.2^{bA}	75.7^{bA}
CV (%)			5.9			10.39	

B – Barreiras; FR – Formosa do Rio Preto; SD – São Desidério; *CV* – coefficient of variation. Means in the same column followed by the same lower-case letter and means in the same line followed by the same capital letter are not significantly different according to the Scott-Knott test at $P \leq 0.05$

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conditions are excellent for large-scale, high-quality soybean seed production. All seed lots evaluated in this study presented high physiological quality, as demonstrated by the vigour tests. The storage for six months is not capable of causing any drastic reductions in soybean seed field performance.

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