

Physiological quality and seed storage potential of four soybean cultivars

Qualidade fisiológica e potencial de armazenamento de sementes de quatro cultivares de soja

Calidad fisiológica y potencial de almacenamiento de cuatro cultivares de soja

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Larissa Aparecida Giasson

ORCID: <https://orcid.org/0000-0003-1447-3506>
Federal Technological University of Paraná, Brazil
E-mail: lari.giasson@hotmail.com

Adriana Paula D'Agostini Contreiras Rodrigues

ORCID: <https://orcid.org/0000-0001-7076-2092>
Federal Technological University of Paraná, Brazil
E-mail: adrianap@utfpr.edu.br

Daniela Aparecida Dalla Costa

ORCID: <https://orcid.org/0000-0002-7479-5702>
Federal Technological University of Paraná, Brazil
E-mail: danidallacosta27@gmail.com

Betania Brum de Bortolli

ORCID: <https://orcid.org/0000-0003-0088-5681>
Federal Technological University of Paraná, Brazil
E-mail: bbrum@utfpr.edu.br

Aline Ferreira Coelho

ORCID: <https://orcid.org/0000-0003-0802-8115>
Anhanguera University - Uniderp, Brazil
E-mail: eng.agro.aline.coelho@gmail.com

Silvia Rahe Pereira

ORCID: <https://orcid.org/0000-0002-9604-5232>
Anhanguera University - Uniderp, Brazil
E-mail: silviaraha@gmail.com

Abstract

The objective of this study was to evaluate physiological seeds quality of four soybean cultivars during storage, under controlled and uncontrolled conditions. Seeds of Brasmax Lança IPRO, Brasmax Raio IPRO (está diferente do Material e Métodos), Pioneer 95y52 and TMG 7062 IPRO cultivars were stored in controlled (20 ± 1.2 °C) and uncontrolled (producer's warehouse) environments and the following traits were assessed: percentage of germination (GERM), germination speed index (GSI), germination speed (GS) and accelerated aging (AA) at 0, 60, 120 and 180 days of storage. Covariance matrices structure of storage time was tested through repeated measures analysis, adopting a bifactorial model. Data were submitted to ANOVA ($\alpha=5\%$) and when the means presented significant differences, regression analysis and Scott-Knott test ($\alpha=5\%$) were performed. Differences in soybean seeds physiological quality are not related to storage environment. Lança cultivar was the most tolerant to storage, while Ray and TMG 7062 cultivars were more sensitive.

Keywords: *Glycine max*; Vigor; Germination.

Resumo

Objetivou-se avaliar a qualidade fisiológica de sementes de quatro cultivares de soja durante armazenamento em condições de ambiente controlado e não controlado. Sementes das cultivares Brasmax Lança IPRO, Brasmax Raio IPRO, Pioneer 95y52 e TMG 7062 IPRO foram armazenadas em ambiente controlado ($20 \pm 1,2$ °C) e não controlado (armazém de produtor) e realizaram-se análises de percentual de germinação (GERM), índice de velocidade de emergência (IVE), velocidade de germinação (VG) e envelhecimento acelerado (EA) aos 0, 60, 120 e 180 dias de armazenamento. Testou-se a estrutura das matrizes de covariância para tempo de armazenamento através de análise de medidas repetidas, adotando-se modelo bifatorial. Os dados foram submetidos a ANOVA ($\alpha=5\%$) e quando as médias apresentaram diferenças significativas, procedeu-se análise de regressão e teste de Scott-Knott ($\alpha=5\%$). As diferenças na qualidade fisiológica das sementes de soja estão mais relacionadas aos fatores genéticos do que ao ambiente de armazenamento. A cultivar Lança mostrou-se a mais tolerante ao armazenamento, enquanto as cultivares Raio e TMG 7062, demonstraram-se mais sensíveis.

Palavras-chave: *Glycine max*; Vigor; Germinação.

Resumen

El objetivo de este estudio fue evaluar la calidad fisiológica de semillas de cuatro cultivares de soja durante el almacenamiento en condiciones ambientales controladas y no controladas. Semillas de cultivares Brasmax Lança IPRO, Brasmax Raio IPRO, Pioneer 95y52 y TMG 7062 IPRO fueron almacenados en un ambiente controlado ($20 \pm 1,2$ °C) y no controlados (almacén del productor) y se realizaron análisis de porcentaje de germinación (GERM), índice de velocidad de emergencia (IVE), velocidad de germinación (VG) y envejecimiento acelerado (EA) a los 0, 60, 120 y 180 días de almacenamiento. La estructura de las matrices de covarianza para el tiempo de almacenamiento se probó mediante el análisis de medidas repetidas, adoptando un modelo de dos factores. Los datos fueron sometidos a ANOVA ($\alpha = 5\%$) y cuando las medias presentaban diferencias significativas se realizó análisis de regresión y prueba de Scott-Knott ($\alpha = 5\%$). Las diferencias en la calidad fisiológica de las semillas de soja no están relacionadas con el entorno de almacenamiento. El cultivar Lança demostró ser el más tolerante al almacenamiento, mientras que los cultivares Raio y TMG 7062, fueron más sensibles.

Palabras clave: *Glycine max*; Fuerza; Germinación.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is the most important agricultural species in the world, representing the oleaginous species with the greatest cultivation and consumption on the whole planet. This is due to its great economic value, because of China's high demand for grain, as well as its wide use by the chemical, pharmaceutical and agro-industrial industry (Oliveira et al., 2017). In this context, Brazil is considered one of the world's largest soybeans producers, due to a series of advantages arising from edaphoclimatic, cultural and environmental factors, as well as productivity gains arising from the high-standard technologies introduction, both related to culture and management (Pellenz, Almeida & Freitas, 2019).

Among the pertinent aspects of crop technologies, using seeds with high quality stands out (Gazzola Neto et al., 2017). According to Popinigs (1985) and França Neto (2009), a seed to be considered of quality, must present a set of genetic, physical, sanitary and physiological factors that ensure its viability. With regard to physiological quality, this characteristic is related to the ability of a seed to perform its vital functions (Cardoso et al., 2012; Kappes et al., 2012), so it is one of the main characteristics to be considered in the crop implantation (Dias et al., 2010).

Obtaining quality seeds is one of the main challenges faced by the seed sector, especially regarding the production in tropical and subtropical regions, as there is wide range of climatic variations in these sites (Mathias et al., 2017). These variations in temperature and humidity are responsible for causing seeds deterioration that occur in molecular, genetic, cellular, tissue and seed population levels (Pontes et al., 2006), which culminates with quality drop and consequently their death (Marcos Filho, 2005).

Storage represents one of the main tools that seed producers, especially of soy, can use to control climatic variations and to reduce seed deterioration in order to ensure the seed quality maintenance (Azevedo et al., 2003). To Garcia et al. (2014) and Guedes et al. (2012) this process is an efficient alternative to prolong the seeds physiological quality, since it reduces deterioration processes speed, maintaining their vigor and viability during the period between harvest and sowing. However, as certified by Fanan et al. (2009), storage is an efficient process, it is essential to know seeds physiological potential during the whole period in which they remain stored, as each species presents particular requirements regarding the environmental conditions for their conservation.

In this context, it is known that in Brazil, most of the soybean seed producers, especially small and medium-sized farmers who use the legal right to save seeds, carry out the storage within their properties, which often does not ensure ideal storage conditions, exposing seeds to factors responsible for causing deterioration and reduction of their physiological quality. Thus, the objective of this study was to evaluate physiological quality of seeds of four soybean cultivars during storage under controlled and uncontrolled conditions.

2. Methodology

The experiment was carried out in the Laboratório Didático de Análise de Sementes of Universidade Tecnológica Federal do Paraná - UTFPR, *Campus* Pato Branco, in a completely randomized design with four replications. It was carried out in a 4 x 8 bifactorial scheme [four storage times versus eight environmental cultivars (four soybean cultivars in two storage environments – controlled – C and uncontrolled – UC)].

Soybean seeds used were from Brasmax Lança IPRO, Brasmax Raio IPRO, Pioneer 95y52 and TMG 7062 IPRO cultivars, from a producer in the municipality of Mariópolis – PR and from the agricultural harvest of 2017/18.

After collection, seeds were sampled and homogenized, where the weight of one thousand seeds (WTS) was determined, according to the methodology described by the Rules for Seed Analysis (Brazil, 2009). They were placed in polyethylene bags and stored for 180 days in two different conditions: environment with controlled temperature (C) (20 ± 1.2 °C) and uncontrolled environment (UC)- in big bags kept shaded in a warehouse of a producer in the municipality of Mariópolis – PR.

Seeds physiological quality was evaluated in the newly harvested seeds and during storage by percentage of germination (GERM), germination speed index (GSI), germination speed (GS) and accelerated aging (AA).

For determination of GERM (%), four subsamples of 50 seeds were used in germitest paper rolls, moistened with distilled water 2.5 times the weight of dry paper, which were kept in Mangelsdorf germinator, for eight days at 25°C. Counts were performed at five and eight days, following the normal seedling criteria described by RAS (BRAZIL, 2009).

For GSI and GS determination (in days), subsamples of 50 seeds were used, arranged in the same molds as GERM test. Daily counts were made up to the eighth day, of the number of seeds that presented root protrusion greater than 2 mm (Juntilla, 1976), with a result calculated according to the methodology proposed by Nakagawa (1999).

AA (%) was conducted with 200 seeds placed on stainless steel screen, kept inside gerbox boxes containing 40 ml of water, which remained in BOD for 48 hours at 41°C (Marcos Filho, 1999). After this period, seeds were arranged in germitest paper rolls with four replicates of 50 seeds, as described for the germination test, with evaluation performed at five days, considering the normal seedlings (Brazil, 2009).

Results obtained for the variables GERM and AA were transformed using transformation $Y_{ij}^* = (\arcsine(\sqrt{(Y_{ij}/100)}))$, while the results of GSI and GS were transformed by Box & Cox (Osborne, 2010). Afterward, the structure of the covariance matrices for storage time was tested through repeated measures analysis, adopting a bifactorial model. Finally, data were submitted to Analysis of Variance (ANOVA) and when the means presented significant differences in the level of 5% probability of error, they were submitted to regression analysis and grouping of means by the Scott-Knott test ($\alpha=5\%$), using the Genes software (Cruz, 2013).

3. Results and Discussion

A significant interaction was found between the cultivars-environment and storage times ($\alpha=5\%$) for the variables GSI and GS, which indicates that for these, soybean cultivars stored in the two environments behave differently during storage times (Table 1).

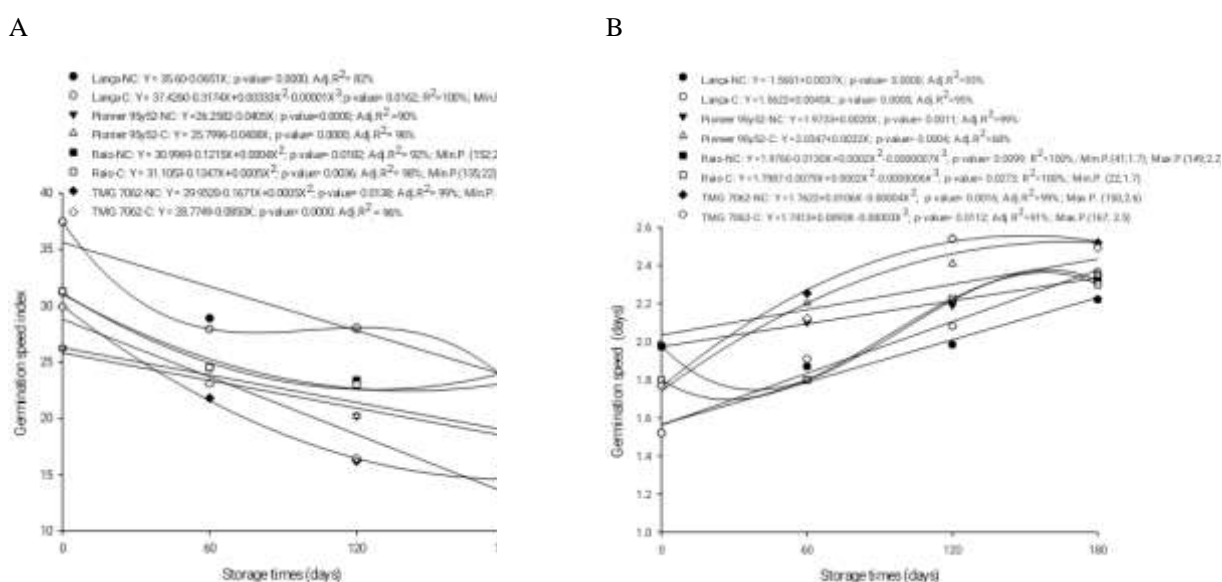
Table 1. Summary of Analysis of Variance (GL and statistics F) of a bifactorial experiment conducted in a completely randomized design, where four storage times versus eight environmental cultivars were evaluated (four soybean cultivars in two storage environments – controlled and uncontrolled) for the variables germination - GERM (%), accelerated aging test- AA (%), germination speed index - GSI and germination speed - GS (days). Mariópolis, 2018/2019.

Causes of variation	GL	Variables			
		GERM ¹	AA ¹	GSI ²	GS ²
		Statistics F			
Storage time ³	3	6.2722*	6.3396*	123.8479*	88.0999*
Cultivars-Environment	7	6.1398*	3.2977*	35.2578*	12.1381*
Cultivars-environment X storage time	21	0.3120 ^{ns}	0.3668 ^{ns}	2.8641*	2.5624*
Error	96	-	-	-	-
Overall average		88.59	80.98	24.58	2.11
Coefficient of variation (%)		9.24	15.34	6.40	12.77

* significant at a level of 5% probability of error ; ^{ns}non-significant at a level of 5% probability of error; ¹ variable transformed using transformation $Y_{ij}^* = (\arcsin(\sqrt{Y_{ij}/100}))$; ² Variable transformed with Box=Cox transformation. ³ Before carrying out the analysis of variance, the structure of the covariance matrices for storage time was tested by means of repeated measures analysis, in which, using the AIC and BIC criteria, it was verified that the structure was of the diagonal type, that is, with the independant errors, thus being the best ANOVA strategy, the bifactorial model. Source: Authors.

For storage time, all equations adjusted to express the relation of GSI and GS showed high adjusted coefficients of determination (R² adjusted); Most of them, above 80%, indicating that storage times satisfactorily explain the variability in these variables (Figures 1A and 1B).

Figure 1. Germination speed index (A) and germination speed - days (B) due to the interaction between storage times (days) and storage cultivars (four soybean cultivars in two storage locations - controlled and uncontrolled) in a factorial experiment, conducted in a completely randomized design. Mariópolis, 2018/2019.



Source: Authors.

The speed germination is one of the main indications of its vigor (Marcos Filho, 2009). Thus, based on the assumption that seed choice is directly associated to the fast and uniform development of the culture, the faster the establishment of a seedling in the field, the greater the utilization of the available resources (Oliveira et al., 2009).

In this context, the behavior expressed by the variable GSI (Figure 1A) of the cultivars Lança-NC, Pioneer 95y52-NC, Pioneer 96y52-C and TMG 7062-C as a function of storage times was explained by decreasing linear models, with reduction of GSI corresponding to 0.0651 (Lança-NC), 0.0405 (Pioneer 95y52-NC), 0.0408 (Pioneer 95y52-C) and T7062-MG (T7062-C) (T7062-C). For each day that they were stored GSI is linearly reduced, and consequently, the time required for the seed to emerge in the field is increased.

For the cultivar-environment Lança C, a cubic adjusted model was obtained, with minimum GSI point (27.5) at 80 days of storage and maximum point (27.9) at 121 days. Whereas for the cultivars-environment Raio-NC, Raio-C and TMG 7062-NC, the behavior as a function of storage times was quadratic, with a minimum point obtained at 152, 135 and 157 days of storage, respectively, which correspond to GSIs of 22, 22 and 16.

Regarding GS (Figure 1B), cultivars-environment Lança-NC, Lança-C, Pioneer 95y52-NC and Pioneer 95y52-C, presented increase of 0.004, 0.004, 0.002 and 0.002 days to germinate each extra day of storage, respectively. Cultivar-environment Raio-NC, showed cubic behavior as a function of storage times with a minimum point obtained at 41 days when the seed needed 1.7 days for germination. Maximum point was expressed at 149 days, when the seeds took 2.2 days to germinate. For Raio-NC, only a minimum point was obtained, corresponding to 22 days of storage where GS resulted in 1.7 days. For this situation, the maximum point is outside the interval of days of storage studied and therefore does not have adequate practical interpretation.

The cultivar TMG 70G2, both in the UC environment and in the C environment, showed results that it is the one that undergoes the most increase in days for germination with the increase in storage time. In an UC environment, the maximum number of days for the seeds of this cultivar to germinate was 2.6 days when they were stored for 150 days. In C environment, the maximum number of days for germination was given at 167 days of storage, when the seeds needed 2.5 days for germination.

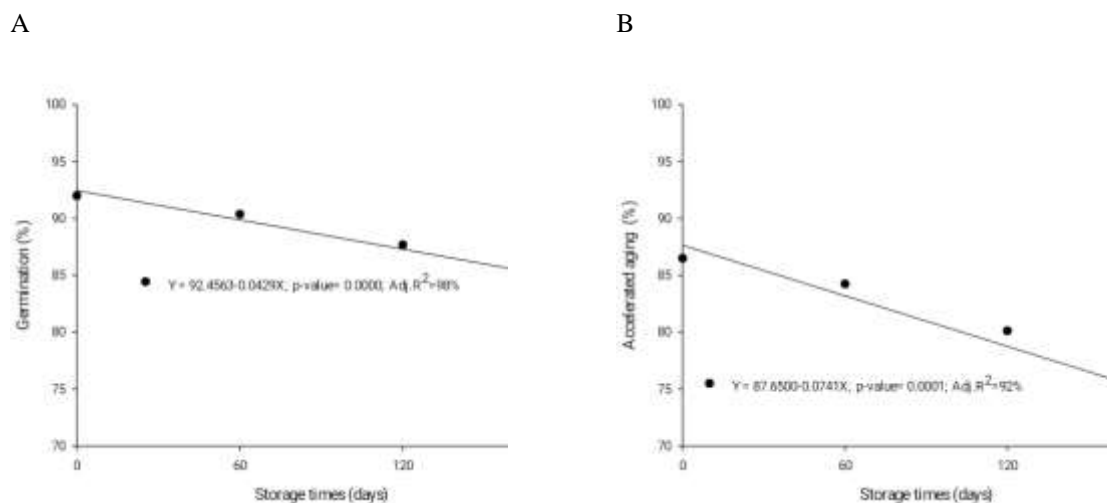
Concerning the storage environments, it is noted that, although the treatments where cultivars were stored in UC environment, they presented lower results than those stored in C environment, both for GSI (Figure 1A) and GS (Figure 1B), the variation was shown to be not significant, which indicates that the difference between temperature and humidity of the UC environment and of the producer's warehouse (environment C) was not enough to cause changes in the speed of the seeds biochemical and metabolic reactions (Silva et al., 2011). One of the possible reasons for this result is that in the uncontrolled environment (producer's warehouse), *the big bags* where the seeds were kept were in a place with little sun exposure, ensuring a milder and more constant temperature, avoiding marked changes in the seeds physiological quality.

The results demonstrated for all cultivars-environment, both for GSI and GS, are in accordance with the results described by Pontes et al. (2006), who claim that the longer a soybean seed is stored, the longer the time it will need to emerge in the field. According to Ludwig et al. (2011), this is because the changes caused by the storage period result in less vigorous seeds and consequently have lower germination performance, with reflexes seen in the germination speed. Thus, it is understood that the increase in the time required for a seed to germinate is capable of resulting in cultures productive losses. This premise was confirmed by Pinthus and Kimel (1979) who, upon assessing the final productivity of soybean culture, observed a direct relationship between seedling emergence speed and final productivity, so that the seedlings that emerged in four and five days after sowing, presented higher productivity than those that needed more time for emergency.

For GERM and AA variables, the interaction effect is not significant, indicating cultivars had shown similar behavior stored in both environments when changing the evaluated storage times. However, there is a significant effect of the isolated effects of the cultivars-environment factor and the storage times factor (Table 1). The equations adjusted for the GERM and AA variables (Figures 2A and 2B) as a function of storage time presented high adjusted coefficients of determination (R^2 adjusted), both higher than 80%, indicating that storage times satisfactorily explain their variability. A linear decrease in the GERM was observed of 0.0429% for each extra day of storage, regardless of the cultivar and storage environment (Figure 2A), while for AA

(Figure 2B), a linear decrease of 0.0741% was obtained for each extra day of storage. Similar behavior to this was observed by Santos et al. (2005), who evaluated the effect of soybean seed size of the cultivars Splendor and UFV-19 on physiological quality during storage for 240 days, obtained linear and decreasing germination for all the evaluated seed sizes, with daily reduction between 0.0628401 and 0.101105%. In addition, Barbosa et al. (2010), in a study evaluating the quality of soybean seeds of BRS Tracaja cultivar after storage, observed that the average germination values up to the four months of storage were between 80 and 81.8%, but reduced to 74.3% when stored for six months.

Figure 2. Germination, in % (A) and accelerated aging test of soybean, in % (B) as a function of storage times (days) in a factorial experiment (storage times X storage situations - four soybean cultivars in two storage locations - controlled and uncontrolled) conducted in a completely randomized design. Mariópolis, 2018/2019.



Source: Authors.

The results obtained for GERM and AA indicate that the storage of soybean seeds of different cultivars in different environments is responsible for reducing their physiological quality, which can be explained by possible biochemical and structural changes that this process causes. According to Fanan et al. (2009), one of the main problems generated by the storage of seed of oil species such as soyabean, is the lipids oxidative rancidity, because of the low chemical stability of these substances which makes the increase in temperature provoked by the respiratory process be sufficient to decompose lipids and consequently cause seed deterioration.

Another aspect in which the storage of soybean seeds is capable of producing deterioration of these seeds is the cell membranes disstructuring. This is related to factors such as damage to chromosomes and nucleic acids and changes in membrane and enzyme systems that degrade the respiratory system, reducing ATP production and disorganizing the cell membranes. As a result, in addition to the loss of cellular compartmentalization, the uncontrolled metabolism and water and solute exchange between the cells and the outer environment, causing a drop in the seeds vigor and viability (Marcos Filho, 1999).

The cultivar Lança, both in UC and in C, presented the highest mean values for GERM and AA (Table 2), which differed significantly from the others ($\alpha = 5\%$). The lowest GERM was obtained for TMG 7062-NC, which did not differ from the cultivars-environment, Pioneer 95y52-NC, Pioneer 95y52-C, Raio-NC, Raio-C and TMG 7062-C.

Table 2. Grouping of averages by Skott-Knott test ($\alpha= 5\%$) of the cultivars-environment factor for the variables germination - GERM (%), accelerated aging test - AA (%), germination speed index - GSI and germination speed - GS (days) of a bifactorial experiment, storage time *versus* local cultivars, conducted in a completely randomized design. Mariópolis, 2018/2019.

Cultivars-Environment	Variables			
	GERM ¹ (%)	AA ¹ (%)	GSI ²	GS ²
Lança-NC	94.50 a	90.38 a	29.74 a	1.90 c
Lança-C	93.38 a	88.88 a	29.24 a	1.97 c
Pioneer 95Y52-NC	89.25 b	79.88 b	22.61 c	2.15 a
Pioneer 95Y52-C	87.25 b	77.38 b	22.13 c	2.23 a
Raio-NC	88.88 b	81.88 b	25.49 b	2.08 b
Raio-C	87.88 b	79.63 b	25.66 b	2.03 b
TMG 7062-NC	82.63 b	75.38 b	20,64 d	2.27 a
TMG 7062-C	85.00 b	74.50 b	21,13 d	2.23 a

¹ Transformed variable using transformation $Y_{ij}^* = (\arcsin(\sqrt{(Y_{ij}/100)}))$; ² Transformed variable with Box=Cox transformation. Source: Authors.

Lança cultivar (UC and C) also resulted in higher values of GSI, differing from the others, where the lowest results of GSI were observed with the cultivar TMG 7062, for both environments, which differed significantly from the other cultivars-environment. These results reflected in GS, which is faster for seed of the cultivar Lança in both storage environments and is statistically different from the other cultivars. The greatest need for days to germinate occurred for the TMG 7062-NC, TMG 7062-C, Pioneer 95y52-NC and Pioneer 95y52-C cultivars, which differed significantly from the others.

The superiority of the results obtained by the cultivar Lança in relation to the others can be explained by its WTS (Table 3) obtained immediately after harvest, and consequently by its oil content. The cultivar Lança presents the lowest WTS among the four cultivars (170.99 grams), while Pioneer 95y52, Raio and TMG 7062, presented WTS of 202.42, 203.58, and 2225.25 grams, respectively. Thus, it is understood that lighter seeds present lower oil content in their constitution compared to heavier seeds, which makes them less prone to deterioration caused by oxidative rancidity, one of the main factors of reduction in the physiological quality of soybean seeds (Fanan et al., 2009).

Table 3. Weight of one thousand seeds - WTS (g), of cultivars Bramax Lança IPRO, Pioneer 95y52, Brassax Ray IPRO and TMG 7062 of a bifactorial experiment, storage time versus cultivars-environment, conducted in a completely randomized design. Mariópolis, 2018/2019.

PMS (g)	Cultivars			
	Brasmax Lança IPRO	Brasmax Raio IPRO	Pioneer 95y52	TMG 7062
	170.99	202.42	203.58	225.25

Source: Authors.

The results observed for the variables GERM, AA, GSI and GS, allow to ensure that the cultivar Lança is tolerant to storage for long periods, without losses in the seeds physiological quality, while the cultivars Raio and TMG 7062, when stored, present a significant reduction in physiological quality indicators. This result allows to infer that Lança cultivar can remain stored longer, regardless of the environment. This fact increases its possibilities of use, that is, from harvest to harvest or from out-of-

season to harvest. The cultivars Raio and TMG 7062, in turn, can be stored only from harvest to out-of-season, since they should be used as soon as possible after harvest, since they present great susceptibility to storage with marked reduction of physiological quality.

4. Conclusion

1. Differences in soybean seeds physiological quality are not related to storage environment;
2. Physiological quality of soybean seeds of Lança, Pioneer 95y52, Raio and TMG 7262 cultivars decreased during storage for 180 days;
3. Soybean Lança cultivar showed to be the most tolerant to storage;
4. In both storage environments, controlled and uncontrolled, Raio and TMG 7262 cultivars were shown to be more sensitive to storage, resulting in a marked reduction in physiological quality.

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