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Vigor of soybean seeds stored under long-term refrigeration

Vigor de semillas de soja almacenadas en refrigeración a largo plazo

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Abstract

Seed deterioration is a natural process over time; however, it can be mitigated by appropriate storage conditions that help preserve seed physiological characteristics. This study aimed to evaluate the vigor of soybean seeds from different cultivars stored under refrigeration for a period of five years. Seeds were produced by the company Uniggel® on properties located in the Lagoa da Confusão region, in the state of Tocantins, Brazil. Samples from seven cultivars were provided: 84I86RSF IPRO (Brasmax®), 8579RSF IPRO (Brasmax®), 81I81RSF IPRO (Brasmax®), GH 8383 RR (Golden Harvest®), 80I82RSF IPRO (Brasmax®), DM80I79RSF IPRO (Donmario®), and M 8644 IPRO (Monsoy®), and sent to the Plant Morphology Laboratory at Fundação Educacional de Andradina (FEA), in the Municipality of Andradina, state of São Paulo. The samples were placed in labeled paper boxes and stored in an Eletrolux® refrigerator for a period of five years at a constant temperature of 5 °C. Among the cultivars evaluated, 84I86RSF IPRO showed the lowest seed quality, whereas M 8644 IPRO exhibited the best performance. Long-term storage resulted in greater leaching of calcium, magnesium, and potassium. Electrical conductivity was correlated with the vigor of soybean seeds after long-term refrigerated storage.

Keywords: Cultivars, electrical conductivity, germination, *Glycine max* (L.) Merrill.

Resumen

El deterioro de las semillas es un proceso natural que ocurre con el tiempo. Sin embargo, puede mitigarse ajustando las condiciones de almacenamiento de forma que permitan conservar las características de las semillas. Este estudio tuvo como objetivo evaluar el vigor de semillas de soja almacenadas en refrigeración durante un período de tiempo prolongado. Las semillas fueron producidas por la empresa Uniggel® en propiedades ubicadas en la región de Lagoa da Confusão en el Estado de Tocantins, Brasil. Se proporcionaron muestras de semillas de soja de siete cultivares: 84I86RSF IPRO (Brasmax®), 8579RSF IPRO (Brasmax®), 81I81RSF IPRO (Brasmax®), GH 8383 RR (Cosecha dorada®), 80I82RSF IPRO (Brasmax®), DM80I79RSF IPRO (Donmario®) y M 8644 IPRO (Monsoy®). Las muestras fueron enviadas al Laboratorio de Morfología Vegetal de la Fundación Educacional Andradina (FEA) ubicada en el municipio de Andradina, Estado de São Paulo. Allí, fueron embaladas en cajas de papel etiquetadas y almacenadas en un refrigerador Eletrolux® a una temperatura constante de 5 °C durante un período de cinco años. Entre las variedades evaluadas, las semillas del cultivar 84I86RSF IPRO tuvieron la menor calidad, mientras que el cultivar M 8644 IPRO presentó los mejores resultados. El almacenamiento a largo plazo permitió una mayor extrusión de calcio, magnesio y potasio. La conductividad eléctrica se correlacionó con el vigor de las semillas de soja después de ser almacenadas en refrigeración por un periodo de tiempo prolongado.

Palabras clave: cultivares, germinación, *Glycine max* (L.) Merrill, conductividad eléctrica.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a crop of global importance in the grain sector. It is used in animal feed formulations, oil production, and, increasingly, for fresh consumption, which has expanded in recent years (Kumari *et al.*, 2025). In Brazil, the areas cultivated with soybean require seeds that meet high physiological quality standards. High-quality seeds must exhibit high vigor, strong germination potential, and good health, as well as assurance of purity and the absence of weed seeds. These factors contribute to strong field performance, allowing seeds to establish the plant population required by the cultivar and, consequently, support higher productivity (Embrapa, 2010; Mattioni *et al.*, 2015).

Seed deterioration is inevitable; however, storage conditions and intrinsic seed characteristics can delay this process (Cardoso *et al.*, 2012). Storage is an essential practice for maintaining and preserving seed physiological quality, ensuring viability and sustaining high vigor until the next sowing cycle (Azevedo *et al.*, 2003).

Some factors, such as inadequate temperature and seed moisture content at harvest, may affect seed quality during storage (Juarez de Sousa e Silva *et al.*, 2008). Low moisture and temperature levels reduce cellular respiration, thereby limiting microorganism activity. Therefore, maintaining appropriate temperature and relative humidity is essential during long-term storage (Malviya and Gayen, 2025).

Soybean cultivars may exhibit variations in quality and in their physiological responses during cultivation (Lisboa *et al.*, 2022; Yu *et al.*, 2023). These differences can also be observed in seed quality, as some cultivars tend to produce heavier seeds (Luo *et al.*, 2023; Nair *et al.*, 2023), and larger cotyledons, which nourish the embryo during the initial stages of development (Lapaz *et al.*, 2017). In addition, certain cultivars possess thicker cell walls, which help reduce water loss during storage (Vianna *et al.*, 2023).

The physiological alterations that occur during long-term seed storage can reduce the concentrations of malonyl glycosides, which are converted into α-glucones, thereby affecting germination rates. These responses highlight the importance of implementing methods that enhance seed tolerance to storage-related stresses (Televičiųtė *et al.*, 2020). Any change in seed physiology can affect the initial development of soybean seedlings, influencing emergence speed, germination rate, and especially shoot and root architecture (Bláha and Pazderu, 2013). Such alterations may also impact seedling dry mass accumulation and final crop productivity (Ebone *et al.*, 2020). When changes in seed vigor are observed, seed health must also be evaluated.

The electrical conductivity (EC) test is correlated with vigor tests used to detect reductions in seed physiological quality after storage (Couto *et al.*, 2021). There is a negative correlation between EC and seed viability parameters: higher conductivity values indicate lower seed vigor. Therefore, the EC test can be used to evaluate the viability of soybean seeds (Santin and Aguiar, 2023).

EC values measured in seed-soaking solutions vary according to the leaching of nutrients from the cotyledons and are directly related to cell membrane integrity. Disorganized membranes—whether affected by insects, mechanical damage, or prolonged storage—are often associated with seed deterioration processes (Vieira *et al.*, 2004). It is important to note that storing seeds from different soybean cultivars for extended periods can alter seed quality and vigor. Some cultivars exhibit morphological adaptations in seed formation, such as thicker seed coats and cell walls in cotyledons tissues, which can provide greater resistance during long-term storage.

In light of these considerations, the present study aimed to evaluate the vigor of soybean seeds stored under refrigeration for an extended period.

Material and methods

Seed acquisition and study location

Soybean seeds were produced by the company Uniggel® on properties located in the Lagoa da Confusão region, in the State of Tocantins, Brazil. Samples from seven cultivars were provided: 84I86RSF IPRO (Brasmax®), 8579RSF IPRO (Brasmax®), 81I81RSF IPRO (Brasmax®), GH 8383 RR (Golden Harvest®), 80I82RSF IPRO (Brasmax®), DM80I79RSF IPRO (Donmario®), and M 8644 IPRO (Monsoy®), which were sent to the Plant Morphology Laboratory at Faculdades Integradas Stella Maris de Andradina (FISMA), located in the Municipality of Andradina, state of São Paulo. The seeds were placed in labeled paper boxes and stored in an Eletrolux® refrigerator for a period of five years at a constant temperature of 5 °C.

Experimental setup and procedures

The experiment was conducted using a completely randomized design (CRD), with seven treatments (soybean cultivars) and four replicates. Each replicate consisted of 50 pure seeds, totaling 200 seeds per treatment. All seeds were re-homogenized, and working samples were collected to perform seed vigor tests according to the methodology described by Sá *et al.* (2011), adapted from the Rules for Seed Testing (Brazil, 2009). The experiment was set up and conducted under laboratory conditions, where the following parameters were evaluated:

Electrical conductivity (EC)

Electrical conductivity (EC) was determined using four subsamples of 50 seeds per cultivar, totaling 200 seeds per treatment. The seeds were weighed with precision to the nearest 0.01 g using an analytical balance and then placed to soak in plastic cups containing 75 mL of deionized water at 25 °C. EC readings were taken after 3 and 24 hours of soaking (EC3h and EC24h) using an Analyser 600® benchtop conductivity meter with a constant-cell electrode (Fessel *et al.*, 2010).

Nutrient concentrations in the solution

After the 24-hour seed immersion period, the soaking solutions were filtered and prepared according to the methodology described by Silva (2009). An aliquot of each solution was collected and diluted at a 1:10 ratio. The concentrations of calcium (Ca), magnesium (Mg), and potassium (K)—expressed in mg kg^{-1} of seeds—were determined by atomic absorption spectrophotometry using a Thermo Scientific® ICE 3300 instrument (Fessel *et al.*, 2010). Calcium chloride, magnesium chloride, and potassium chloride were used as analytical standards.

Germination test

For each treatment, 200 pure soybean seeds were taken from the working sample and divided into four subsamples of 50 seeds. The seeds were then placed in Germitest ® paper towels, moistened with distilled water at an amount equivalent of 2.5 times the dry weight of the paper. The paper rolls were then placed in a germination chamber at a constant temperature of 25 °C. Evaluations were performed on the fifth and eighth day, recording the number of normal (%NS) and abnormal (%AS) seedlings, and non-germinated seeds (%NGS) (Sá *et al.*, 2011; Rodrigues *et al.*, 2020).

Shoot and radicle length

The average shoot (LS) and radicle (RL) lengths were measured in normal seedlings obtained from the germination test, using a millimeter-graduated ruler.

Emergence

For each treatment, 200 pure soybean seeds were taken from the working sample and divided into four subsamples of 50 seeds. The seeds were sown in plastic boxes containing sand moistened with distilled water at an amount equivalent to 50 % of the substrate weight. The boxes were kept in a protected laboratory environment at room temperature for 15 days. Normal seedlings were counted to determine the percentage of emergence (%E) (Sá *et al.*, 2011). A seedling was considered emerged when its cotyledons were above the soil level.

Emergence speed index (ESI)

Seedlings emerging from the substrate were monitored and counted daily to determine the emergence speed index (ESI). Daily observations began immediately after the experiment was set up, recording the number of newly emerged seedlings each day until a stable count was reached (Rodrigues *et al.*, 2020).

Statistical analysis

For statistical evaluation, the data were first subjected to the Shapiro-Wilk test to verify normality. After fulfilling the test requirements, analysis of variance (ANOVA) was performed using the F-test ($p < 0.05$), and treatment means were compared using the Scott-Knott test at the 5 % significance level. In addition, principal component analysis (PCA) was carried out to assess the contribution of each treatment factor, using the RStudio software (R Core Team, 2015).

Results and discussion

Statistical differences were observed in electrical conductivity after both 3 and 24 hours of soaking. After 3 hours, the soybean cultivar 8579RSF IPRO exhibited the lowest electrical conductivity, with values approximately 57.37 % lower than those of cultivars 84I86RSF IPRO, GH 8383 RR, and DM80I79RSF IPRO, which showed the highest conductivity. After 24 hours, the cultivar 84I86RSF IPRO again presented the highest electrical conductivity values (Table 1).

It is also noteworthy that the cultivars 84I86RSF IPRO and 8579RSF IPRO showed the highest contribution to the principal components, as illustrated in Figure 1.

The electrical conductivity test is based on the principle that, as deterioration progresses, cellular constituents are leached from seeds immersed in water due to the loss of cell membrane integrity (Vieira *et al.*, 2004). Thus, low electrical conductivity values are associated with higher germination performance. This relationship was evident in the cultivar 8579RSF IPRO, which showed the lowest electrical conductivity (12.91) and the highest percentage of normal seedlings (85.00 %). These results highlight the distinct responses of soybean cultivars to long-term storage and reinforce the importance of selecting cultivars with greater physiological stability for extended storage periods.

A significant difference was observed in the concentration of Ca in the solution after 24 hours. The cultivar 84I86RSF IPRO showed the highest Ca concentration (203.19 mg kg^{-1}), a value 83.61 % higher than that of the cultivar M 8644 IPRO (33.30 mg kg^{-1}), which presented the lowest concentration (Table 1). Calcium has low mobility within plant tissues because it plays a structural role, contributing to the adhesion

Table 1. Mean values of electrical conductivity after 3 and 24 hours (EC3h and EC24h) and concentrations of Ca, Mg and K in the solution

	EC3h	EC24h	Ca mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}
84I86RSF IPRO	30.29a	246.99a	203.19a	410.99a	7322.05a
8579RSF IPRO	12.91c	158.47b	77.33c	240.88b	4703.15c
81I81RSF IPRO	19.35b	172.84b	188.34a	174.82c	6922.14a
GH 8383 RR	27.64a	146.62b	100.25c	54.95d	5301.81b
80I82RSF IPRO	19.13b	159.19b	138.91b	355.63a	7039.91a
DM80I79RSF IPRO	30.20a	136.29b	93.46c	148.44c	4272.25c
M 8644 IPRO	21.63b	157.48b	33.30d	56.24d	5003.77b
p-value	0.0001**	0.0008**	0.0001**	0.0001**	0.0001**
OA	23.02	168.27	119.25	205.99	5795.01
CV%	9.50	17.60	15.24	24.24	7.51

*The Scott-Knott test was applied at the 5 % significance level. Means followed by the same letter do not differ statistically. OA = overall average, CV = coefficient of variation.

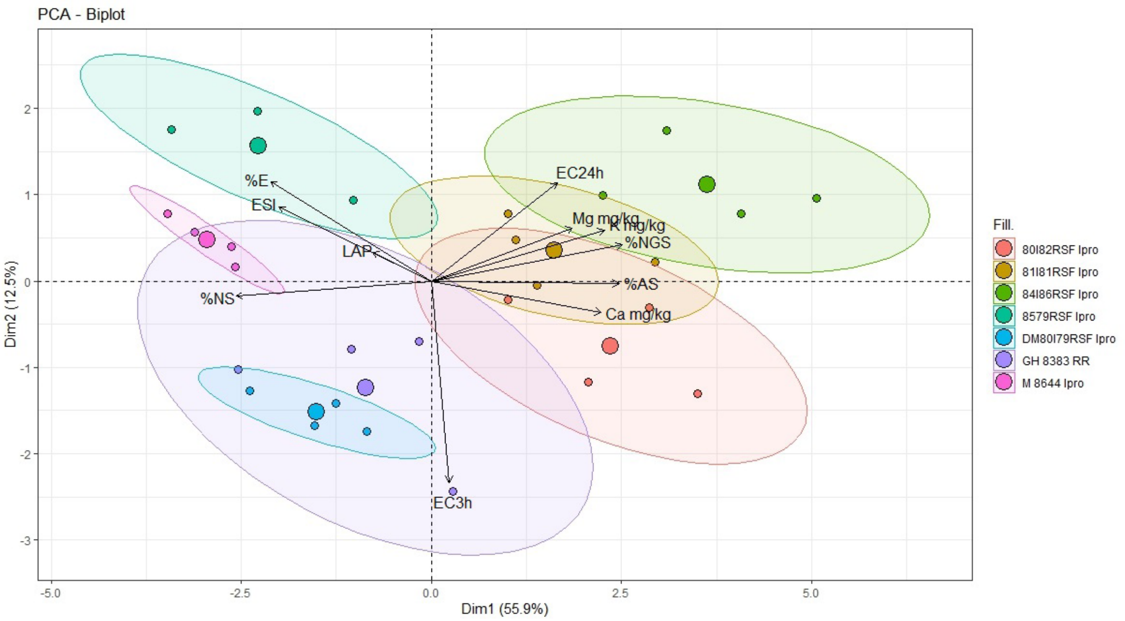

Figure 1. Biplot of the principal component analysis (PCA) showing the contributions of soybean cultivars to the response variables.

of cellulose molecules during cell wall formation. Therefore, high Ca release into the solution indicates marked degradation of seed cellular structures (Taiz *et al.*, 2017).

A significant difference in the concentration of Mg in the solution was also observed after 24 hours. The cultivar 84I86RSF IPRO showed the highest Mg concentration (410.99 mg kg^{-1}), a value approximately 86.62 % higher than those of the cultivars GH 8383 RR (54.95 mg kg^{-1}) and M 8644 IPRO (56.24 mg kg^{-1}), which presented the lowest averages (Table 1). A statistical difference was likewise detected for the concentration of K, with 84I86RSF IPRO again exhibiting the highest mean value, approximately 41.65 % higher than that of cultivar DM80I79RSF IPRO, which showed the lowest K concentration (Table 1).

Unlike calcium, potassium and magnesium are highly mobile in plant tissues, including storage and reproductive organs, because they participate in signaling processes, osmotic regulation, and membrane permeability. Therefore, seed degradation may have increased the solubility and leaching of these elements, resulting in higher concentrations in the soaking solution (Taiz *et al.*, 2017; Rezende *et al.*, 2020).

A significant difference was also observed for the percentage of normal seedlings (%NS). The cultivar 84I86RSF IPRO showed the lowest mean value, which was approximately 31.57 % lower compared to the cultivars M 8644 IPRO, 8579RSF IPRO, DM80I79RSF IPRO, and GH 8383 RR, all of which exhibited higher averages (Table 2).

The cultivar 84I86 RSF IPRO showed a higher release of nutrients into the solution; consequently, these reserves were no longer available during germination. In contrast, cultivars that retained their nutrient reserves demonstrated superior germination performance and produced healthier seedlings. Nutrients stored in the seeds are essential during the initial days after emergence, as they sustain the seedling before photosynthetic structures become fully functional. Variations in the concentration of these reserves can therefore compromise early vegetative development (Taiz *et al*., 2017). Thus, seed production and multiplication companies, as well as soybean growers, must give careful attention to environmental conditions (humidity and temperature) and to long-term storage periods for soybean cultivars.

A significant difference was observed in the percentage of abnormal seedlings (%PA) (Table 2). The cultivar 84I86RSF IPRO showed the highest percentage of abnormal seedlings, a value approximately 57.69 % higher than that of the cultivar 8579RSF IPRO, which presented the lowest average. Likewise, for the percentage of non-germinated seeds (%NGS), the cultivar 84I86RSF IPRO again stood out, showing a value about 80 % higher than that of the cultivar M 8644 IPRO, which had the lowest average. As expected, there was an inverse relationship between the percentages of normal and abnormal seedlings. Cultivars 80I82RSF IPRO and 81I81RSF IPRO contributed more strongly to the percentage of abnormal seedlings (%PA), whereas the cultivar GH 8383 RR contributed most to the percentage of normal seedlings (%PN) (Figure 1).

Similar results were reported by Dalgado *et al*. (2019), who observed that, during the storage of soybean seeds, longer storage periods led to reduced physiological quality, characterized by an increase in abnormal seedlings and a decrease in germination. These findings are consistent with those of Dan *et al*. (2011), who found that germination rates decline as storage time increases.

The cultivar M 8644 IPRO presented the highest emergence speed index (20.04), a value 28.59 % higher than that of the cultivar 84I86RSF IPRO, which showed the lowest average value (Table 2). This difference may be explained by intrinsic and environmental factors. According to Cunha *et al*. (2009), high temperatures can affect the germination process even after storage, as exposure to extreme temperatures may lead to denaturation of enzymes (such as amylases and proteases) involved in reserve mobilization. This enzymatic impairment prevents the degradation of cotyledon reserves, reducing their availability to the embryo and consequently slowing emergence.

A significant difference was observed in the percentage of emergence (E%). The cultivar M 8644 IPRO presented the highest mean value, which was 24.11 % higher in relation to cultivar 84I86RSF IPRO, which showed the lowest mean (Table 2).

No statistically significant differences were observed in the mean values of shoot length (SL) and root length (RL) of seedlings originating from seeds of soybean cultivars stored under refrigeration for five years. The overall mean shoot length was 7.58 cm (p = 0.8062ns; CV = 20.20 %), and the overall mean root length was 8.08 cm (p = 0.5080ns; CV = 16.27 %). These results indicate that the seedlings displayed similar initial development among cultivars; therefore, the physiological differences observed in the seeds did not translate into differences in early seedling development.

Table 2. Mean values of normal seedlings (%NS), abnormal seedlings (%AS), non-germinated seeds (%NGS), emergence speed index (ESI), and emergence percentage (%E) of soybean cultivars stored long-term under refrigeration

	%NS	%AS	%NGS	ESI	%E
84I86RSF IPRO	58.50c	26.00a	15.50a	14.31b	64.50b
8579RSF IPRO	85.00a	11.00b	4.00c	19.56a	84.50a
81I81RSF IPRO	67.50b	21.00a	11.50b	16.37b	71.00b
GH 8383 RR	78.00a	17.00b	5.00c	16.85b	72.00b
80I82RSF IPRO	66.50b	21.50a	12.00b	16.00b	68.50b
DM80I79RSF IPRO	81.50a	13.50b	5.00c	17.20b	72.50b
M 8644 IPRO	85.50a	11.50b	3.00c	20.04a	85.00a
p-value	0.0001**	0.0001**	0.0001**	0.0204*	0.0054**
OA	74.64	17.35	8.00	17.19	74.00
CV%	7.22	22.66	24.70	12.97	10.16

*The Scott-Knott test was applied at the 5 % significance level. Means followed by the same letter do not differ statistically. OA = overall average, CV = coefficient of variation.

The seed storage process is fundamental to maintaining seed vigor and can influence subsequent seedling development. Pereira *et al.* (2021) reported that the physiological potential of soybean seeds is affected by the combined effects of phytosanitary treatments and storage duration, which together reduce seed quality and vigor. Furthermore, seeds subjected to storage and phytosanitary treatments showed higher catalase (CAT) activity, whereas the control group exhibited greater superoxide dismutase (SOD) activity, which indicated distinct physiological responses depending on the storage conditions (Çelik and Kenanoğlu, 2023). Corbineau (2024) also observed that storage begins to compromise seed vigor after the fifth month, although seeds may still maintain high germination percentages.

The different responses presented by the cultivars in the evaluated tests can be attributed to their genetic quality, which is associated with intrinsic factors that determine physiological and biochemical characteristics (Sá *et al.*, 2011). The cultivars in this study may also have exhibited different responses to the tests due to differences in seed lot moisture, as the lots originated from different production fields. Due to the morphological and chemical characteristics of soybean seeds, they are highly sensitive to environmental factors that change throughout long-term storage (Sun and Gong, 2024). Therefore, further studies are needed to better understand the physiological responses of seeds stored under different conditions and for extended periods.

Conclusions

The results of this study demonstrate that long-term refrigerated storage affects soybean cultivars differently. The cultivar 84I86RSF IPRO showed the lowest physiological quality, with higher electrical conductivity values, greater leaching of calcium, magnesium, and potassium, and lower percentages of normal seedlings and emergence. In contrast, the cultivar M 8644 IPRO exhibited the best performance across vigor-related variables, including higher germination, emergence, and emergence speed.

Long-term storage (five years) promoted increased leaching of Ca, Mg, and K, indicating deterioration of membrane integrity and degradation of cotyledonary reserves. These nutrient losses, particularly in cultivars more sensitive to storage, directly compromised seed vigor. Electrical conductivity proved to be an effective indicator for detecting these physiological changes, showing strong correspondence with vigor parameters under prolonged refrigerated storage.

The absence of differences in shoot and root length suggests that, although seed vigor varied among cultivars, early seedling growth was not significantly affected in a uniform laboratory environment. However, the reduction in physiological quality observed in some cultivars highlights the importance of considering genetic factors, seed lot moisture, and environmental conditions during storage.

Overall, the findings reinforce the need for careful selection of cultivars intended for long-term storage and emphasize the importance of maintaining appropriate temperature and humidity conditions. Further studies are recommended to evaluate the physiological responses of soybean seeds under different storage environments and for extended periods, as well as to investigate the biochemical mechanisms involved in cultivar-specific tolerance to storage.

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