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Physiological Quality of Soybean Seeds Stored in Different Periods in Dry Soil and Temperatures

Qualidade Fisiológica de Sementes de Soja Armazenadas em Diferentes Períodos em Solo Seco e Temperaturas

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Abstract

The soybean crop is one of the most economically important commodities in Brazil, and the correct way of sowing it is essential for successful production. Thus, the objective was to evaluate the physiological quality of soybean seeds subjected to different storage periods in dry soil and temperatures. The experimental design was completely randomized, in a 3x4 factorial scheme (temperatures: 15; 25 and 35ºC and seed storage periods in the soil: 0 (control); 7; 14 and 21 days) with four replications of 50 seeds. Physiological evaluations were based on emergence tests, length and dry mass of seedlings, in addition to digitalization of seedling images. It was verified that there was an interaction of the factors, thus interfering with the physiological quality of the seeds submitted to different temperatures and periods of storage in dry soil. It was concluded that the increase in the storage period of soybean seeds in dry soil, as well as the temperature, promoted a reduction in the percentage of emergence of normal seedlings. Implicating more heterogeneous results of seedling development, with image scanning being a positive way to assess the physiological quality of these seedlings.

Keywords: *Glycine max.* Seedling Evaluation. Seedling Scanning.

Resumo

A cultura da soja é uma das *commodities* de maior importância agroeconômica no Brasil, sendo fundamental a forma correta de sua semeadura para o sucesso da produção. Desde modo, o objetivo foi avaliar a qualidade fisiológica de sementes de soja submetidas a diferentes períodos de armazenamento em solo seco e temperaturas. O delineamento experimental foi conduzido inteiramente casualizado, no esquema fatorial 3x4 (temperaturas: 15; 25 e 35ºC e períodos de armazenamento da semente no solo: 0 (testemunha); 7; 14 e 21 dias) com quatro repetições de 50 sementes. As avaliações fisiológicas foram pelos testes de emergência, comprimento e massa seca de plântulas, além da digitalização das imagens das plântulas. Verificou-se que houve interação dos fatores, assim interferindo sobre a qualidade fisiológica das sementes submetidas a diferentes temperaturas e períodos de armazenamento em solo seco. Conclui-se que conforme o aumento do período de armazenamento das sementes de soja em solo seco, bem como da temperatura promoveu a redução da percentagem de emergência de plântulas normais. Implicando em resultados mais heterogêneos do desenvolvimento de plântulas, sendo a digitalização das imagens uma forma positiva para a avaliação da qualidade fisiológica dessas plântulas.

Palavras-chave: *Glycine max. Avaliação de Plântulas. Digitalização de Plântulas.*

1 Introduction

Brazil is an agricultural country par excellence, among commodities the cultivation of soy (*Glycine max* (L.) Merrill) stands out, which in the 2020/21 harvest occupied an area of 43.5 million hectares with a production of 152.7 million tons (CONAB, 2023). Since the success of this crop begins with the correct sowing, according to Peske (2020), part of the sowing of this crop in the country, approximately one million hectares, took place in the "dust", that is, in dry soil. Where this practice is intended to provide ideal conditions for plant development during the crop cycle. Thus, maximizing the efficiency of rain in regions where it regulates the growing season, in addition to enabling two crops in succession and as an operational strategy increasing the sowing period, which means a reduction in the restriction of machinery and labor, among other reasons.

The practice of sowing in dry soil consists of allocating the seed containing low soil moisture, in advance of rainfall, thus, the seeds are stored in the soil for a period waiting for moisture conditions to start the germination process. However, time and edaphoclimatic conditions are variable over the periods to which the seeds are exposed, which can cause different degrees of stress, mainly due to the lack of humidity and high temperatures. During the period of storage in dry soil, degradation of some proteins and reserve compounds may occur due to seed metabolism, consuming substrates used in respiration during germination (ABBADE; TAKAKI, 2014; FERNANDES *et al*., 2018; SHARMA *et al*., 2007).

The deterioration rate is directly associated with the period and environmental conditions that the seeds are exposed to during storage, with the variation in the water content of the seeds being an important factor that negatively affects their deterioration process (BERBERT *et al.*, 2008; SALINAS *et al.*, 2001). According to Ochandio *et al.* (2017), the increase in humidity and temperature during the storage period causes an increase in the respiratory rate. Interfering with the early consumption of reserves during this period, aiming at the hygroscopic and thermal balance of the seed-soil interaction.

Aiming at measuring this interaction, it is necessary to perform seedling emergence tests to verify the qualities of seeds submitted to storage conditions in dry soil. The tests can be performed adding a more detailed evaluation of the morphology with its classification, which can still be complemented with an important resource in the identification and classification of seedlings through the use of image scanning.

Scanning seedling images is a simple, accurate, low-cost, fast and easy-to-use tool that can complement other seed tests and evaluations. Some computational systems have already been used for this purpose, such as the Seed Vigor Imaging System (SVIS®) which evaluated the vigor of seeds through digitized images of seedlings, generating data on the length of the primary root, hypocotyl and entire seedling, root/hypocotyl ratios and indices of vigor and growth uniformity (SAKO *et al.*, 2001). The Automated Seed Vigor Analysis System (Vigor-S) is the result of a collaborative program between the University of São Paulo (USP/ESALQ) and Embrapa/ Agricultural Instrumentation (CNPDIA) (RODRIGUES *et al*., 2020) and the GroundEye developed by the company Tbit Technology and Systems (BRANDANI, 2017). These programs have the possibility of being adapted to countless cultures, as has already been proven by several researchers. Thus, the objective was to evaluate the physiological quality of soybean seeds subjected to different storage periods in dry soil and temperatures.

2 Material and Methods

The experiments were carried out in the Didactic and Research Laboratory with Seeds and in the greenhouse, both at the Department of Phytotechnics of the Federal University of Santa Maria (UFSM), located in Santa Maria, RS (29º43' S; 53º43' W and altitude of 95 m), in 2020. The climate in the region is humid subtropical (Cfa), according to the Köppen-Geiger classification, with average annual accumulated precipitation of 1,769 mm, average annual temperature close to 19.2 ºC and air humidity around 78.4% (ALVARES *et al.*, 2013).

In the laboratory, the initial characterization of the seed lots of the soybean cultivar Nidera (NA5909), harvested in the 2018/2019 season, these were evaluated by the following tests: mass of a thousand seeds, moisture content of the seeds and germination pattern test (GPT) by the methodology of Brazil (2009). For the GPT, four repetitions of 50 seeds were sown in a roll of paper, moistened with distilled water in a proportion of 2.5 times the mass of the dry paper. The rolls were kept in a B.O.D. (Box Organism Development), with a photoperiod of 24 h and a temperature of 25±2°C. The first count (FG) and germination (G) evaluations occurred at 5 and 8 DAS and the germination of normal seedlings at 8 DAS, with the results expressed in percentage. Together with this GPT test, the tests of length and dry mass of seedlings were performed with random selection of ten normal seedlings from each repetition, measuring the length of the hypocotyl and radicle with a millimeter ruler, and the determination of the dry mass occurred by drying of this material in a forced ventilation oven at 65±5º C for 48 h (NAKAGAWA, 1999). The batch was characterized with a mass of 1,000 seeds of 153.4 g; moisture content 9.5%, first count 69%, germination 88%, abnormal seedlings 11%, dead seeds 01%, hypocotyl length 4.0 cm, root length 8.43 cm, dry mass of hypocotyl 16.76 g and root dry mass 10.05 g.

The experiment was organized in a completely randomized design, in a 3x4 factorial scheme (temperatures and periods of seed storage in the soil) with four replications of 50 seeds. The physiological temperature ratings were 15; 25 and 35ºC and the seed storage periods in dry soil were: 0 (control); 7; 14 and 21 days.

The seeds were stored in dry soil for the periods and temperatures, in a B.O.D. type germinator, mentioned above. Sowing took place in plastic trays (2 L) at a depth of one centimeter. The soil used was Arenic Dystrophic Red Argisol (EMBRAPA, 2013), previously dried in an oven at 105ºC and sieved, fertilization and liming were carried out according to the soil analysis report (Table 1).

Table 1 - Soil analysis report Dystrophic Arenic Red Argisol.

Water pH 1:1	Ca	Mg	Al	H + Al	CTC effect.	Saturation (%)		SMP Index	Texture
	$cmol_c\ dm^{-3}$					Al	Bases		
5.7	6.6	2.0	0.0	4.4	9.0	0.0	67.4	6.0	3.0
% MO	% Clay	P-Mehlich	K	CTC pH7	K	Molar relations			
m/v		$mg\ dm^{-3}$	$cmol_c\ dm^{-3}$		$mg\ dm^{-3}$	Ca/Mg	(Ca+Mg)/K	$K/(Ca+Mg)^{1/2}$	
2.2	24.0	85.0	0.44	13.4	172.0	3.4	19.5	0.15	

Source: resource data.

Subsequently, for each storage period, all trays were irrigated with water, raising their humidity to 60% of the retention capacity (RC) following the methodology of Brasil (2009), with irrigations every 12 h. Next, they were placed in the germinator and maintained at a temperature of 25ºC and constant light for 24 hours, for five days, when the emerged seedling counts were performed and the normal, abnormal, and dead seedlings and the length and dry mass of seedlings were determined.

Physiological quality assessments were performed using the following tests:

a) Emergence test: performed on the fifth day after irrigation (DAI), with the seedlings evaluated under the soil surface and the result expressed as a percentage of normal seedlings emerged. Normal seedlings were removed from the trays, washed and dried on paper and classified as normal, abnormal (deformed or infected) and dead seeds, and the result expressed as a percentage (CARVALHO; SILVA; ABREU, 2011). The seedlings were also grouped under blue colored leaves and recorded with a smartphone camera with a resolution of 13 megapixel and the result expressed in the number of normal, abnormal and dead seedlings.

b) Seedling length: in the 5 DAI, ten normal seedlings were used randomly from each repetition, which were measured for root length, hypocotyl and total seedling length with the aid of a millimeter ruler, the result was expressed in centimeters per seedling (NAKAGAWA, 1999).

c) Dry mass of plants: ten normal seedlings were randomly used from each repetition and divided between hypocotyl and root, and then placed in paper bags and placed in an oven at 65±5ºC until constant mass was obtained. Then, the repetitions were measured on a 0.001 g precision scale. The mass obtained was divided by the number of normal seedlings, with the results expressed in milligrams per seedling (mg seedling^{-1}) of the hypocotyl, root and total dry mass (NAKAGAWA, 1999).

Data expressed as percentages were transformed into arcsine and analysis of variance (ANOVA) and comparison of means using the Scott-Knott test ($p<0.05$) were performed using the SISVAR statistical program (FERREIRA, 2014).

3 Results and Discussion

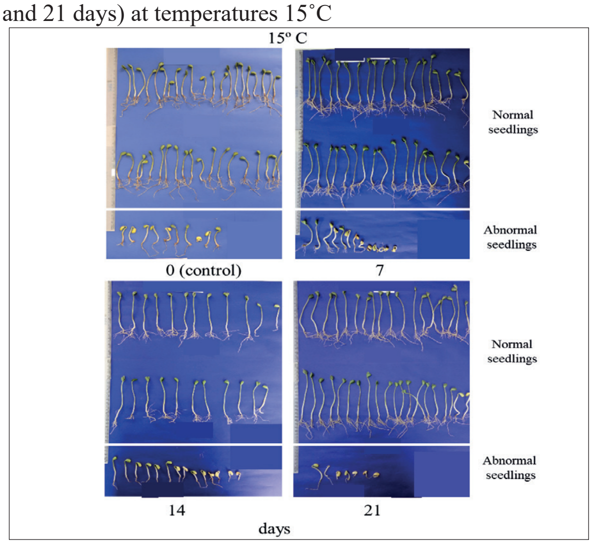
It was verified that the emergence of normal and abnormal seedlings, and dead soybean seeds submitted to different temperatures and periods of storage in dry soil (Table 2), in which at a temperature of 25ºC during storage in dry soil, a reduction was obtained in emergence and in normal seedlings after 7 days of storage and, related to this response, there was an increase in the number of dead seeds. These results can be clearly visualized with the digitized image of the seedlings after 7, 14 and 21 days of storage in dry soil and irrigation for five days in a germinator at a temperature of 25ºC (Figures 1, 2 and 3).

Table 2 – Emergence of normal seedlings, abnormal seedlings and dead seeds of soybean (*Glycine max* (L.) Merrill) submitted to different temperatures and periods of storage in dry soil

Storage[1]	15ºC	25ºC	35ºC	15ºC	25ºC	35ºC	15ºC	25ºC	35ºC
	Normal seedlings (%)			Anormal seedlings (%)			Dead seeds (%)		
0 dias	83 Aa*	83 Aa	83 Aa	16 Aa*	16 Aa	16 Aa	1 Ab*	1 Ab	1 Aa
7 dias	76 Aa	62 Bb	82 Aa	18 Aa	19 Aa	13 Aa	6 Bb	19 Aa	5 Bb
14 dias	56 Bb	69 Ab	80 Aa	20 Aa	21 Aa	15 Aa	24 Aa	10 Ba	6 Bb
21 dias	77 Aa	65 Ab	73 Aa	19 Aa	21 Aa	14 Aa	5 Ab	14 Aa	14 Aa
CV (%)	11.8			24.6			39.3		

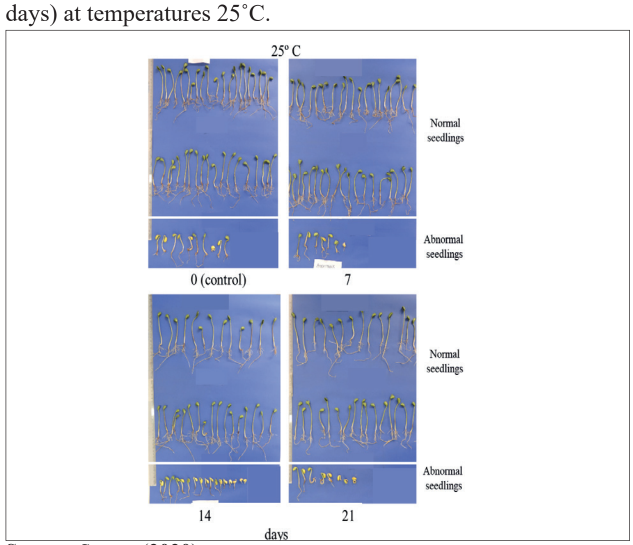
[1]Period of storage in dry soil in days. *Significant interaction between temperature x period of seed storage in the soil. Means not followed by the same uppercase letter differ in the row and lowercase letters in the column differ by the Scott Knott test ($p<0.05$). CV (%): coefficient of variation.
Source: resource data.

Figure 1 - Digitized images of seedling emergence (normal and abnormal) of soybean (*Glycine max* (L.) Merrill) after being stored in different periods in dry soil (0 (control); 7; 14 and 21 days) at temperatures 15˚C



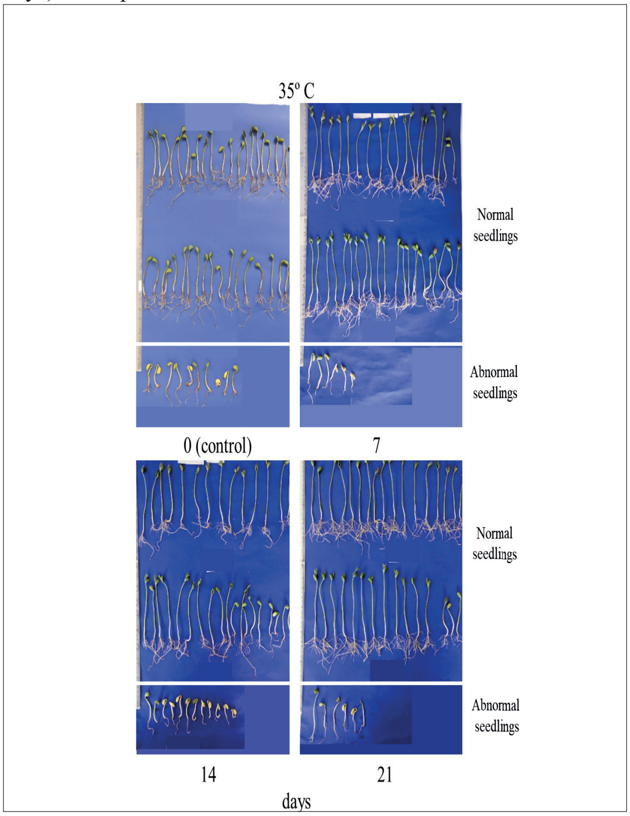
Source: Santos. (2020).

Figure 2 - Digitized images of seedling emergence (normal and abnormal) of soybean (*Glycine max* (L.) Merrill) after being stored in different periods in dry soil (0 (control); 7; 14 and 21 days) at temperatures 25˚C.



Source: Santos (**2020**).

Figure 3 - Digitized images of seedling emergence (normal and abnormal) of soybean (*Glycine max* (L.) Merrill) after being stored in different periods in dry soil (0 (control); 7; 14 and 21 days) at temperatures 35˚C



Source: Santos (2020).

Currently, some companies in the seed sector have been adopting emergency tests on benches with visual analysis and classification between normal and abnormal seedlings as pre and post control strategies in commercialization. Some automated systems could be adopted, such as the Seed Vigor Imaging System (SVIS), which according to Marcos Filho *et al.* (2009) and Wendt *et al.* (2017) is efficient to determine vigor in soybean seeds and, through the analysis of seedling images, it has greater capacity to predict the establishment of soybean seedlings in the field.

These systems do not have free use due to patent protection, however, the use of digitalization of seedling images would be important to create a database for later comparison of these results and/or complementation of the results similar to the present work.

At a temperature of 35 ºC during storage, it was observed that there was no significant reduction in the values of emergence of normal seedlings up to 21 days of storage in dry soil. It is known that respiratory activity can increase during storage at higher temperatures and, according to Voegele *et al.* (2012), the dry seed quickly resumes its metabolic activity and, consequently, the growth of cell expansion in the embryonic axis after rehydration. This situation can be visualized and confirmed in the respective images of the seedlings. It is still possible to observe a small variation in the number of abnormal and dead seedlings for this temperature in the different storage times in dry soil.

For Bewley (1997), one of the first changes during imbibition is the intensification of respiratory activity, which can be detected in minutes. Thus, it can be inferred that the greater intensity of metabolism due to the temperature of 35˚C during storage stimulated the seeds to germinate without damage to their physiological potential. Similarly, at a temperature of 15 ºC, up to 21 days of storage in dry soil, there was a small reduction in the values of emergence and normal seedlings. However, at this temperature, the results are more heterogeneous, as can be seen after 14 days of storage in dry soil. The valid assumption for a temperature of 35 ºC also fits this situation, where due to the lower temperature, they reduce the metabolic activity, and the seeds, when irrigated and exposed to a temperature of 25ºC, take a longer time to germinate to reactivate their metabolism and mobilize their reserves which affects its growth. So, soybean seeds when exposed to lower temperatures have slower and uneven emergence, in addition to presenting a reduction in growth.

According to Marcos Filho (2015) and Fernandes *et al.* (2018), temperature affects the speed, percentage and uniformity of germination, and the reduction in temperature influences the speed of imbibition and mobilization of reserves, causing a decrease in the speed of germination and reduction in seedling growth. Still by the same author, the performance of the seeds after sowing demonstrates whether the potential identified by the laboratory tests was appropriate and reached.

When analyzing the results of emergence and normal seedlings, regardless of the considered temperature and storage time in dry soil, there are differences in the percentages of emerged seedlings in relation to the number of seedlings that were interpreted as normal germination in the counts. This is due to the fact that in the count of emerged seedlings, only the structures exposed above the soil or substrate level are considered. However, the analysis of the entire plant is more reliable and accurate, as all abnormalities or normality of the seedling as a whole are visualized. Thus, the meticulous analysis of seedlings at the time of stand counts in a field is of great importance. Thus, the meticulous analysis of seedlings at the time of stand counts in a field is of great importance. Moreover, in fact, tools such as the removal and analysis of seedlings and their digitization into images are fundamental for understanding the physiological process at the time of germination of these seedlings and decision-making.

The results of abnormal seedlings did not show significant differences for the storage period and temperature factors, so we can say that regardless of the storage period in dry soil and the temperature during this period, it will not affect stresses in the seed that will cause abnormalities.

Table 3 presents the results of length and dry mass of seedlings. As can be seen, the seedlings that were stored at 35 ºC showed greater vigor and growth, being a response to the temperature stimulus during storage, since during germination all were submitted to a temperature of 25 ºC. with seedlings under different temperatures and different storage times in dry soil. Thus, it can be inferred that the temperature of 35ºC during storage increases pre-germination metabolism, which improves metabolic processes and mobilization of reserves in the germination and post-germination period. These results can be perceptible when analyzing the digitized images.

For root length of soybean seedlings, the temperature of 15ºC during storage showed the lowest lengths. As for hypocotyl length of soybean seedlings, the temperature of 25ºC was the worst treatment. Matthews *et al.* (2011), found in corn (*Zea mays* L.) lower temperatures cause a delay in radicle emission and an increase in the average germination time, due to the reduction of metabolic activity during germination.

Table 3 - Root and hypocotyl dry mass and length of soybean seedlings (*Glycine max* (L.) Merrill) submitted to different temperatures and periods of storage in dry soil.

Storage[1]	15ºC	25ºC	35ºC	15ºC	25ºC	35ºC
	Seedling radicle length (cm)			Seedling hypocotyl length (cm)		
0 days	8.1 Aa*	8.1 Ab	8.1 Ab	7.3 Ac*	7.3 Aa	7.3 Ac
7 days	8.8 Aa	8.5 Aa	9.3 Aa	10.4 Aa	6.3 Ba	11.1 Aa
14 days	6.7 Bb	8.9 Aa	8.8 Aa	8.2 Bb	6.9 Ca	9.8 Ab
21 days	8.4 Ba	7.8 Bb	9.3 Aa	8.5 Bb	7.2 Ca	10.0 Ab
CV (%)	6.7			7.7		
	Seedling root dry mass (mg pl^{-1})			Seedling hypocotyl dry mass(mg pl^{-1})		
0 days	14.1 Aa*	14.1 Aa	14.1 Aa	25.5 Ab*	25.5 Aa	25.5 Ab
7 days	11.5 Ab	9.7 Bd	12.0 Ab	30.4 Aa	22.8 Ba	30.1 Aa
14 days	9.6 Ab	11.3 Ac	10.6 Ab	30.5 Aa	23.8 Ba	28.8 Aa
21 days	10.2 Bb	12.3 Ab	11.0 Bb	28.5 Aa	24.6 Ba	28.2 Aa
CV (%)	5.6			9.4		

[1] Period of storage in dry soil in days. *Significant interaction between temperature x period of seed storage in the soil. Means not followed by the same uppercase letter differ in the row and lowercase letters in the column differ by the Scott Knott test (p<0.05). CV (%): coefficient of variation.
Source: resource data.

The temperature of 25 ˚C during storage showed the lowest hypocotyl mass of soybean seedlings, where there was an increase in dry mass from 7 days onwards for temperatures of 15ºC and 35 ºC. Root dry mass of soybean seedlings was higher at zero days of storage for all temperatures and decreased with storage.

The experiments were carried out in the laboratory and do not fully express the field conditions and their variations. However, the behavior of this species in the present study is interesting, because according to the results found, sowing in dry soil and at higher temperatures (around 35 ºC) is viable without compromising the performance of soybean seedlings with up to 21 days of storage in the soil. Such temperature conditions can be easily found in the Brazilian Midwest and in November and December in Rio Grande do Sul, months recommended for sowing in the State. However, sowing in dry soil associated with lower temperatures may result in uneven results and impair seed performance, characteristics found in September and October in RS.

4 Conclusions

The physiological quality of soybean seeds is negatively affected according to the extension of the storage period in the soil and the increase in temperature, by reducing the percentage of emergence of normal seedlings. Under these experimental conditions, the interaction of temperatures and periods of storage of the seed in the soil, averagely maintained the physiological quality of these seeds, however, it implies heterogeneous results in the development of seedlings.

The digitalization of the images positively complements the routine analysis in the evaluation of the physiological quality of soybean seedlings. The use of technologies and software that evaluate in an automated way can favor the use of this methodology for other studies and seed analysis laboratories.

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