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Physiological quality of second crop soybean seeds after drying and storage

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This work was carried out with the goal of determining the immediate and latent effects of different temperatures of drying air on the physiological quality of soybean seeds produced during the second crop. The seeds, which were collected close to their physiological maturity and had a moisture content of approximately 23% (w.b.), were exposed to different drying temperatures (40, 50, 60, 70, and 80°C) until their moisture content reached 12.5 ± 0.7% (w.b.). These were then stored in an environment with no temperature or humidity control for 180 days. The physiological quality was evaluated every 45 days thereafter by germination, first count, accelerated aging, modified cold, electrical conductivity, and tetrazolium chloride-based tests. From the results obtained, we conclude that a) an increase in the temperature of drying air influences the physiological quality of the soybean seeds produced during the second crop, and this effect is enhanced by the duration of storage; b) the viability and vigor are inversely related to both of these factors; and c) an air temperature of 40 °C can be recommended for drying second crop soybean seeds.

Key words: *Glycine max* L., viability, seed vigor, post-harvest.

INTRODUCTION

The attainment and maintenance of soybean seed quality has always been one of the main challenges of the production system (Marcos Filho, 2013). In this context the use of good quality seeds enables access to genetic advances, with quality assurance and localization technologies in different regions, ensuring higher productivity. Therefore, the soybean crop establishment with high quality seeds is of key importance (Pádua et al., 2014). However, given the rational use of new agricultural frontiers there is an increase demand for some changes towards the soybean seeds production systems.

The implementation of crops oriented toward the production of soybean seeds, once commonly limited to main crops, started to occur also in second crops (Albrecht et al., 2009). Thus, because the temperature and humidity levels vary through different stages of physiological maturity, as well as in the post-harvest phase, some questions arise. Namely, issues arose

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concerning the procedures used in harvesting, drying,and storage, given the likelihood that frosts can force an early harvest, and therefore essentially force the artificial drying of the crop. However, other scenarios also came up concerning the environmental conditions commonly found in these periods; as, for example, the possibility of storing seeds in an uncontrolled environment, although with temperatures below 25°C. This would reduce refrigeration costs while guaranteeing the quality of the seeds.

Drying essentially reduces the biological activity of the seeds, thus hindering the possibility of chemical and physical changes that could occur in the product during storage (Barrozo et al., 2014). This in turn aims to enable the storing of the product in a safe and effective manner until its commercialization. However, both operations could result in a reduction of the quality of the seeds, if not carried out properly. Drying can have an immediate damaging effect on the harvested product and storage might amplify such damages (Afonso Júnior and Corrêa, 2000; Deliberali et al., 2010; Schuh et al., 2011).

Regarding the damage that can result from these factors, we mainly highlight the detrimental effect on the seeds' physiological characteristics. In particular, seeds exposed to improper drying and storage show a reduction in germination and vigor (Resende et al., 2012; Faria et al., 2014; Rathinavel, 2014; Paraginski et al., 2015). Because of its high cost/benefit, even a single soy seed is of high value within a soybean production system (Sediyama et al., 2013). Therefore, it is important to understand the relationship between the quality of seeds and the factors that affect their performance - such as drying and storage. Thus, the goal of this work was to evaluate the immediate and latent effects of different drying air temperatures on the physiological quality of soybean seeds harvested from second crops.

MATERIALS AND METHODS

This study consisted of two phases. The first phase dealt with seeds production, occurring between January and May of 2014, at the São Lourenço's farm, located at 22°11′58.06″ S, 54°53′24.32″ W, and at 452 m of altitude, in the municipality of Dourados, MS. The second phase covered the drying, storage, and evaluation of the physiological qualities of the seeds, carried out at the Laboratory for Pre-processing and Storage of Agricultural Products, and the Laboratory of Physical Properties of Agricultural Products, both of which belong to the School of Agricultural Sciences of the Federal University of Grande Dourados (UFGD), also located in the municipality of Dourados, MS.

The soybean cultivar used to produce seeds was SYN 1059 RR (V-TOP), which was manually harvested at full physiological maturity, according to the specifications by Fehr and Caviness (1977), with moisture content of approximately 23 ± 0.5% (w.b.). After this phase, the seeds were subjected to drying process at temperatures of 40, 50, 60, 70, and 80 °C. The reduction in the mass of the product was monitored by a gravimetric method until a final moisture content of 12.5 ± 0.7% (w.b.) was achieved. In this process, the times required for each drying temperature to achieve the predetermined moisture content were 230, 160, 112, 75, and 57 minutes, respectively.

The drying took place in an experimental fixed-bed dryer, with a drying chamber of 0.80 m diameter and 1.0 m height. The experimental dryer used a series of electric resistors as a heating source, had a total power of 12 kW, and associated to this, an IBRAM centrifugal fan (model VSI-160) with a power of 0.75 kW. Temperature control was achieved using a universal Proportional-Integral-Derivative (PID) process controller (model N1200, Novus). The air flow rate used was 0.2 m³ s^{-1} m^{-2}, selected by a frequency inverter connected to the fan motor. The water reduction rate (WRR) of soybean seeds was determined by monitoring the drying process for each selected temperature used. This was determined using Equation 1, according to Corrêa et al. (2001), who defined WRR as the amount of water that a certain product loses per dry matter unit per unit of time:

$$\mathrm{WRR} = \frac{\mathrm{Mw_0} - \mathrm{Mw_i}}{\mathrm{MS}\,(\mathrm{t_i} - \mathrm{t_0})} \quad (1)$$

where, WRR: water reduction rate, in kg kg^{-1} h^{-1}; Mw_0: previous total water mass, in kg; Mw_i: current total water mass, in kg; MS: dry matter mass, in kg; t_0: total time for previous drying, in h; and t_i: total time for current drying, in h.

After drying, soybean seeds were conditioned through a drying process in non-hermetic metal containers. These, when closed, were kept in an environment with no temperature or relative humidity control for a period of 180 days. The physiological qualities of the seeds and their moisture content were evaluated and measured immediately after drying and every 45 days thereafter. The temperature and relative humidity of environmental air were recorded during the storage period by using two thermo-hygrometers installed next to the containers.

The quality of the seeds was determined by means of germination, first count, accelerated aging, modified cold, electrical conductivity, and tetrazolium chloride-based tests. A gravimetric method was employed to determine the moisture content levels. The test was performed in two repetitions, using an oven kept at 105 ± 3 °C for 24 h (Brasil, 2009). The germination and first count tests were performed concurrently, using four sub-samples of 50 seeds for each treatment, distributed on paper towels moistened with distilled water, in a volume equal to 2.5 times the mass of the dried paper used and then kept at 25 °C in a Mangelsdorf-type germinator. The analyses were carried out on the fifth and eighth days after the test. The percentage of normal seedlings on the fifth day was used for first count, while that on the eighth day was used for the germination test, according to the criteria established in Rules for Seeds Analyses (Brasil, 2009).

The accelerated aging test was carried out according to the methodology described by Marcos Filho (1999), for which 300 seeds for each treatment were distributed in a single uniform layer on stainless steel screens. These were then suspended inside a Gerbox type plastic box, containing 40 ml of distilled water at the bottom. The boxes were then closed and kept in a BOD chamber for 48 h at a controlled temperature of 41 °C. After this period, the moisture content of the seeds was determined, and a germination test (Brasil, 2009) was also performed, with the percentage of normal seedlings being computed on the fifth day after starting the test.

The cold modified test was performed according to methodology described by Barros et al. (1999). Four subsamples of 50 seeds from each treatment were used. The seeds were distributed on paper towels moistened with distilled water, in a volume equal to 3 times the dry paper. These rolls were kept in a BOD chamber for 5 days at a temperature of 10 °C. They were then transferred to a Mangelsdorf-type germinator, with a controlled temperature of 25 °C, where they stayed for an additional four days, after which the percentage of normal seedlings was computed.

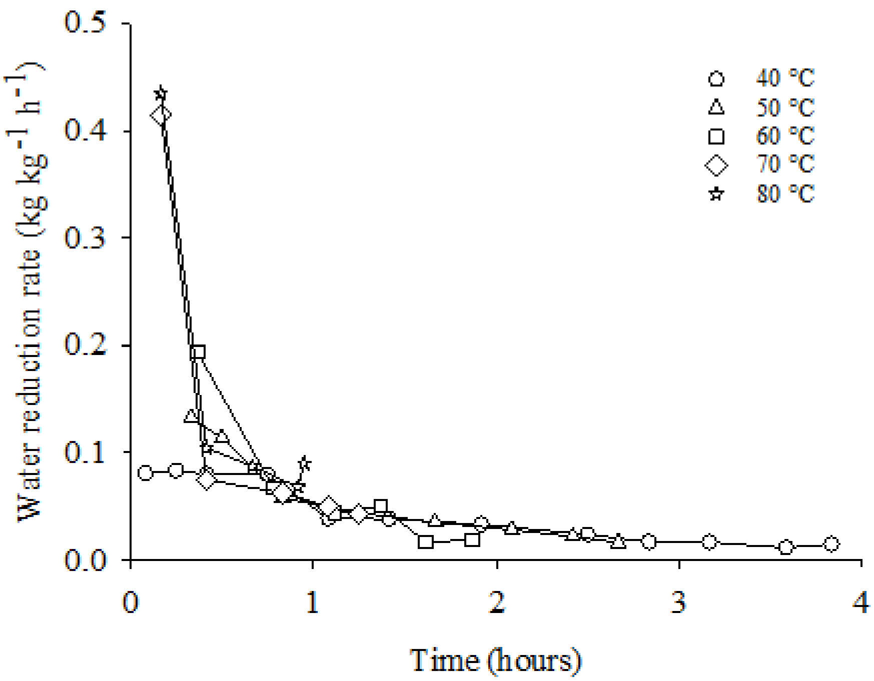


Figure 1. Water reduction rate for soybean seeds during the drying process at different temperatures.

In the electrical conductivity evaluation, four subsamples of 50 seeds for each treatment were weighed and left to soak in 75 ml distilled and deionized water for 24 h at 25°C, in 200 ml plastic cups. After this period, the electrical conductivity of the soaking solution was measured using the CG 1800 conductivity meter made by Gehaka. The results obtained from the readings, in µS cm^{-1}, were divided by the respective mass, with the final result being related to the test expressed in µS cm^{-1} g^{-1} (Vieira and Krzyzanowski, 1999).

The tetrazolium test, performed the following method described by Delouche (1962) and adapted by França Neto et al. (1999), made use of two subsamples of 50 seeds for each treatment. First the seeds were preconditioned for 16 h at 25°C in a wet towel moistened with distilled water, the volume of which corresponded to 2.5 times the mass of the dry paper. Then the seeds were taken through a coloring phase, where they were submerged in a tetrazolium chloride solution (0.075%) for 180 min, at a temperature of 40°C. At the end of that period, seeds were classified from 1 to 5 on their viability and from 1 to 3 on their vigor.

The experiment was carried out in 5 × 5 sub-divided plot arrangements, with five air-drying temperatures in each plot, and 5 different storage periods in each sub-plot, in a completely randomized design. A polynomial regression analysis was carried out to evaluate the latent effect of drying temperatures. The models were selected taking into consideration the magnitude of the coefficient of determination (R^2), the significance of the regression, by the F-test, and the phenomenon under study.

RESULTS AND DISCUSSION

Curves of water reduction rate obtained during the soybean seeds drying process showed that, according to the use of higher temperatures, such as 60, 70, and 80°C, this variable was clearly pronounced at the beginning of the process (Figure 1). However, over time this behavior became more homogenous between the drying treatments, since the water on the surface of the product was gradually substituted by a front of evaporation that moved into the interior of the same. Furthermore, due to the involvement of more complex mechanisms in water displacement to the interior or exterior of the seed, such as liquid diffusion and capillary action, the speed of the process decreased. Thus, the similarity in WRR among the drying treatments applied becomes evident 50 min after the start of the drying.

The variations in temperature and relative humidity throughout the experiment can be seen in Figure 2. Then, under these circumstances, the mean temperature observed during the storage period was of 21.4°C, where the maximum and minimum temperatures registered were 28.4 and 14.5°C, respectively. As for the relative humidity, the mean value observed was of 57.9%, the maximum was 83.4%, and the minimum was of 47.3%

It can be seen in Table 1 that, due to seed hygroscopicity, the moisture content might vary during storage, mainly due to the uncontrolled temperature and relative humidity conditions favoring phenomena such as sorption and desorption, as already observed by Tiecker Junior et al. (2014) and Bessa et al. (2015). Moreover, over the 180 days of storage, we also found that all of the batches analyzed showed increased water levels in evaluations carried out at 45 and 180 days of storage. This is likely because those reading dates followed

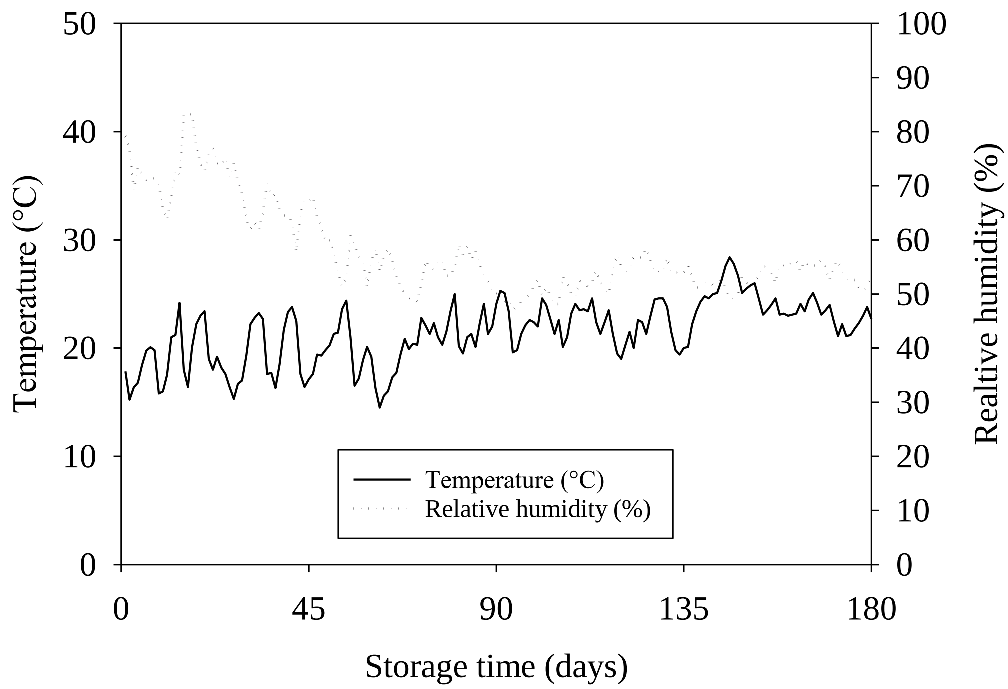


Figure 2. Daily mean of air relative humidity and temperature, during 180 days of storage of soybean seeds in a non-controlled environment.

Table 1. Mean moisture content (% w.b.) of soybean seeds after submitted to drying temperature and storage time.

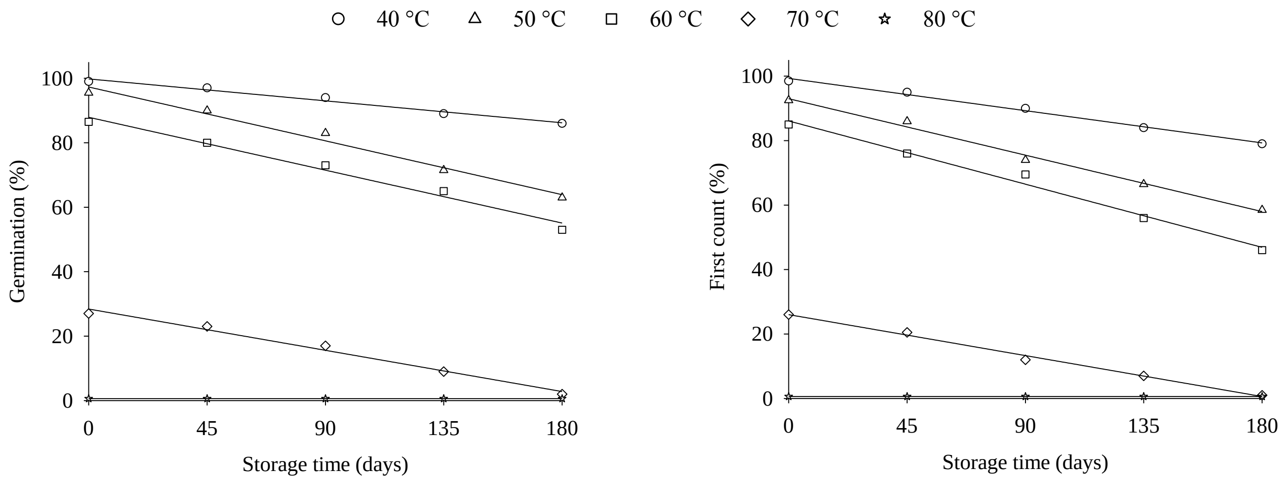
Storage time (days)	Drying temperature (°C)				
	40	50	60	70	80
0	13.2	12.7	12.4	12.5	12.0
45	13.4	12.8	12.4	12.6	12.3
90	12.6	12.1	12.0	11.8	11.4
135	11.1	10.6	10.6	10.5	10.2
180	11.3	11.0	10.8	10.8	10.4

periods of higher relative humidity, which would favor sorption.

Nevertheless, in general there was a reduction in moisture content in every batch evaluated (Table 1). This is probably due to the reduction in the values referring to the relative humidity or the atmosphere according to the weather (Figure 2) and also the centesimal composition of seeds, which for being aleuro-oleaginous tend to retain less water inside, since oily substances are less hydrophilic. Considering the germination and first count behavior against the tested factors, it can be observed that both variables had a lower percentage of normal seedlings. This negative effect was noticed immediately during the drying process and was caused by the increase in air temperature being linearly enhanced throughout the storage period (Figure 3).

The germination percentages were registered immediately after drying, for temperatures of 40, 50, 60, 70, and 80°C, which were of 100, 97, 88, 28, and 1%, respectively. It is worth noting the small difference between drying treatments at 40 and 50°C (Figure 3a). However, due to the latent behavior previously mentioned to occur, already in the evaluation carried out at 45 days the difference between the two treatments started to be clearer. The daily reduction in the percentage of normal seedlings caused at 50°C was equal to twice that favored by a temperature of 40°C, as assessed for temperatures of 60, 70, and 80°C.

In view of this behavior, at the end of the experiment the germination values registered for temperatures 40, 50, 60, 70, and 80°C were of 86, 64, 55, 3, and 1% (Figure 3a). Then, if adopted the parameters established in the standard guidelines number 45 instituted by Brasil (2013), which defines as lower limit for commercialization of soybean seeds 80% germination, only the batch of seeds dried at 40°C could be commercialized by the end



(a) (b)

$G_{40\,°C} = 99.8000 - 0.0756\ ST \quad (R^2 = 0.9729)$
$G_{50\,°C} = 97.3000 - 0.1856\ ST \quad (R^2 = 0.9784)$
$G_{60\,°C} = 87.9000 - 0.1822\ ST \quad (R^2 = 0.9774)$
$G_{70\,°C} = 28.4000 - 0.1422\ ST \quad (R^2 = 0.9820)$
$G_{80\,°C} = 0.6$

$FC_{40\,°C} = 99.3000 - 0.1111\ ST \quad (R^2 = 0.9905)$
$FC_{50\,°C} = 93.0000 - 0.1944\ ST \quad (R^2 = 0.9898)$
$FC_{60\,°C} = 86.1000 - 0.2178\ ST \quad (R^2 = 0.9841)$
$FC_{70\,°C} = 26.0000 - 0.1411\ ST \quad (R^2 = 0.9915)$
$FC_{80\,°C} = 0.6$

Figure 3. Results of the percentage of normal seedlings for germination test (a) and first count (b) of soybean seeds, as a function of drying temperature and storage time.

of the experiment. The product dried with a temperature of 50 and 60°C loses this germination level approximately 150 and 101 days after drying, while the others lose this level as early as during the drying process itself.

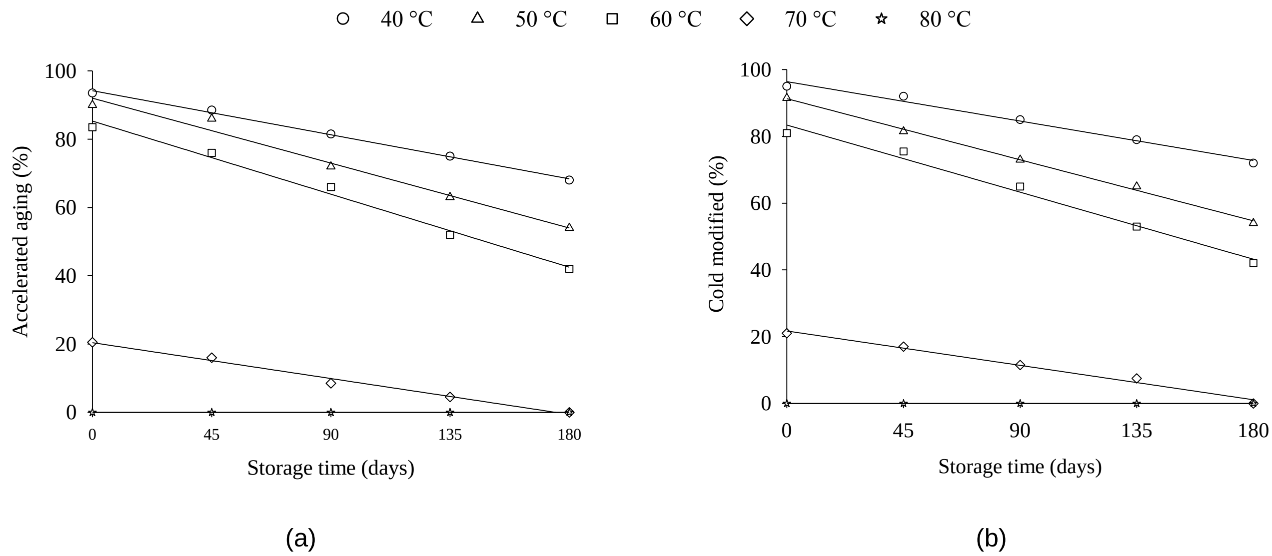
The first count test showed a behavior analogous to that of the germination test, but also showed other parameters beside the presence of normal seedlings at the fifth day. It also indirectly indicates the speed of the germination process, as already documented by Tillmann and Menezes (2012). Thus, since the results obtained are reduced when the air temperature and the duration of the storage period increases, it is possible to infer that germination speed was immediately reduced while latent. This is indicative of a decrease in seeds vigor and their respective storage potential (Figure 3b).

Ullmann et al. (2015), evaluating the effect of different drying temperatures on the physiological quality of sweet sorghum seeds, found similar results to those of this study. The authors found that the increase in drying air temperature results in a decrease of both germination and germination speed, expressed indirectly by the first count. As suggested by Afrakhteh et al. (2013), and also shown in this work, this fact results from the increase in the rate at which water is drained from the product, the WRR, which increased along with the drying temperature. This great temperature gradient between the inside and the periphery leads to the creation of ridges in the soft tissue and micro fissures in the cotyledon, and negatively affects the quality of the seed. In addition, as clarified by Mbofung et al. (2013) and observed in this study, this kind of situation can increase the susceptibility of the material to latent damages, or even increase its deterioration, thus reducing the longevity and physiological quality of the product itself.

The accelerated aging tests and the modified cold test also indicate an immediate and long-term reduction in the vigor of seeds with an increase in both of the factors (Figure 4). They also indicate a possible progressive increase of material’s susceptibility to adverse environmental conditions imposed by the tests, since all results assessed by these tests were lower than those assessed by germination and first count tests, which in turn were conducted under conditions theoretically ideal for the crop (Figure 3).

Immediately after drying, for example, the accelerated aging test revealed normal seedling levels of 94, 92, 85, and 20% for drying processes of 40, 50, 60, and 70°C (Figure 4a). As for the modified cold test, the results for these same temperatures tested were 96, 91, 83, and 22% of normal seedlings (Figure 4b), indicating the deleterious effects of temperatures above 40°C on seed vigor.

Although the results obtained by Menezes et al. (2012) refer to rice culture, a species belonging to another family and with different physical and chemical structures from soybean, they corroborate the results of this study. The



$AA_{40\,°C} = 94.2000 - 0.1433\ ST \quad (R^2 = 0.9959)$
$AA_{50\,°C} = 92.0000 - 0.2111\ ST \quad (R^2 = 0.9746)$
$AA_{60\,°C} = 85.3000 - 0.2378\ ST \quad (R^2 = 0.9870)$
$AA_{70\,°C} = 20.4000 - 0.1167\ ST \quad (R^2 = 0.9853)$
$AA_{80\,°C} = 0$

$CM_{40\,°C} = 96.4000 - 0.1311\ ST \quad (R^2 = 0.9807)$
$CM_{50\,°C} = 91.3000 - 0.2033\ ST \quad (R^2 = 0.9964)$
$CM_{60\,°C} = 83.4000 - 0.2233\ ST \quad (R^2 = 0.9808)$
$CM_{70\,°C} = 21.7000 - 0.1144\ ST \quad (R^2 = 0.9828)$
$CM_{80\,°C} = 0$

Figure 4. Results of the percentage of normal seedlings for tests of accelerated aging (a) and modified cold (b) of soybean seeds, according to the drying temperature and storage time.

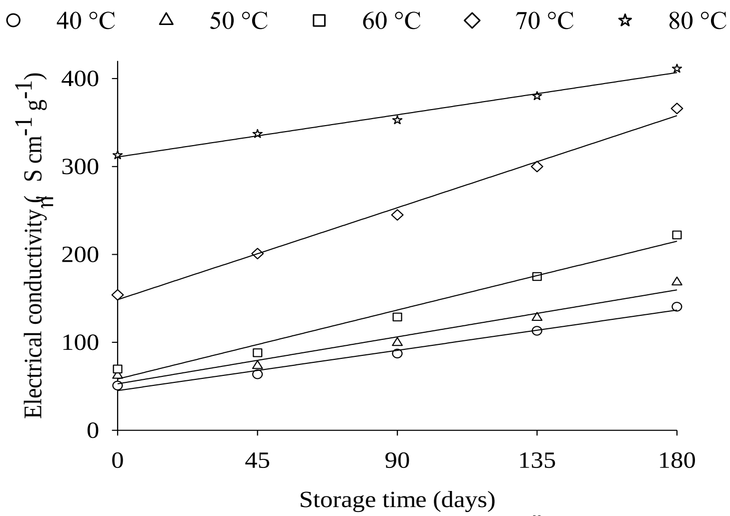
authors also found that the susceptibility of seeds to adverse environmental conditions, such as high temperatures, relative humidity, and cold, is greater in seeds subjected to higher drying temperatures. This fact is explained by the authors as the result of an increased number of cracks found in seeds dried at higher temperatures.

The electrical conductivity test confirms that the increased drying temperature is a determining factor in the reduction of the physiological quality of the seeds, as well in the worsening of conditions in the course of the storage. The results obtained by this test immediately following drying increased and, over time, continued to show a linear growth over time for every drying treatment (Figure 5).

In addition, this test provides information about the integrity of the material, especially concerning its cellular membranes (Marcos Filho, 2015). The results obtained allow hypothesizing that the increase in both factors, besides causing a physical disorder in the tegumentary tissue of the seeds in the surface of the cotyledons, might have resulted in a sort of metabolic disorder that might have led to severe leaching of a series of nutrients essential for germination and the development of vigorous seedlings. According to Menezes et al. (2012), the electrical conductivity test is very effective in identifying damage resulting from drying, since the values obtained by the test typically correlate directly with the number of cracks on the seeds and the temperatures used in drying. Increasing the drying temperature to impair tegumentary and cellular integrity favors solutes leaching, since cell membranes do not efficiently perform their function as selective barriers in first stages of the soaking process. This eventually increases the conductivity values of the solution (Resende et al., 2012).

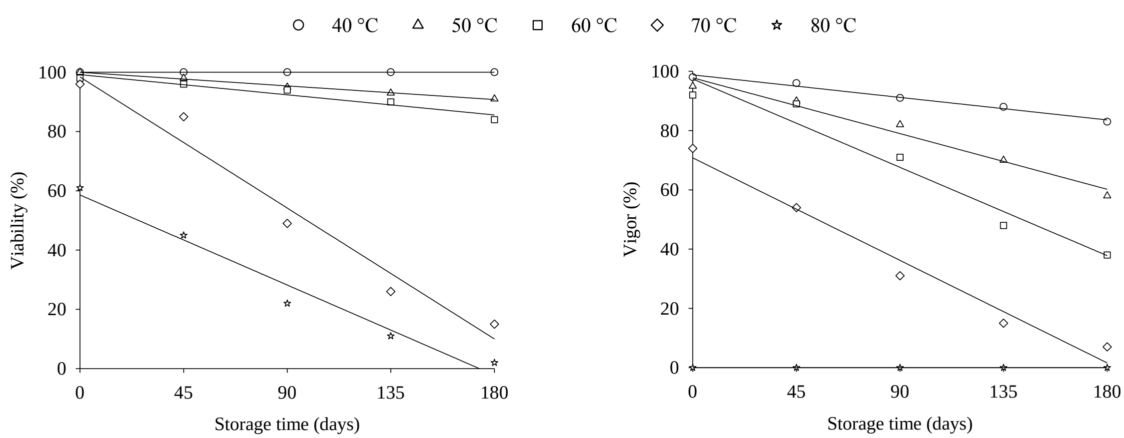
A series of results similar to those found in the electrical conductivity test were found using other crops sensitive to temperatures higher than 40 °C, as bean seeds (Faroni et al., 2006), jatropha (Ullmann et al., 2010), sweet sorghum (Ullmann et al., 2015) and for adzuki bean seeds (Resende et al., 2012). The tetrazolium test also demonstrated a negative effect caused by the increase in air temperature and, subsequently, the amplification of this effect during storage. However, only during storage was it possible to check the difference between the drying treatment, since for viability only the batch of seeds dried at 80°C was immediately inferior to the others, and for the vigor the batches dried at 70 and 80°C showed similar behavior (Figure 6).

According to the results obtained, there was a growing negative tendency correlated with an increase in drying temperature. This shows how much the interference in the initial quality of a seed batch might negatively affect its storage potential and latent maintenance necessary for its physiological quality (Figure 6).



$EC_{40\ °C} = 45{,}1447 + 0{,}5082\ ST$ $(R^2 = 0{,}9799)$
$EC_{50\ °C} = 52{,}8178 + 0{,}5932\ ST$ $(R^2 = 0{,}9506)$
$EC_{60\ °C} = 58{,}3232 + 0{,}8707\ ST$ $(R^2 = 0{,}9719)$
$EC_{70\ °C} = 148{,}5115 + 1{,}1625\ ST$ $(R^2 = 0{,}9906)$
$EC_{80\ °C} = 310{,}8511 + 0{,}5329\ ST$ $(R^2 = 0{,}9833)$

Figure 5. Results of electrical conductivity of the soaking solution of soybean seeds, according to drying temperature and storage time.



(a) (b)

$VIA_{40\ °C} = 100.0000$
$VIA_{50\ °C} = 100.0000 - 0.0511\ ST$ $(R^2 = 0.9925)$
$VIA_{60\ °C} = 99.2000 - 0.0756\ ST$ $(R^2 = 0.9177)$
$VIA_{70\ °C} = 98.4000 - 0.4911\ ST$ $(R^2 = 0.9550)$
$VIA_{80\ °C} = 58.6000 - 0.3378\ ST$ $(R^2 = 0.9617)$

$VIG_{40\ °C} = 98.8000 - 0.0844\ ST$ $(R^2 = 0.9782)$
$VIG_{50\ °C} = 97.8000 - 0.2089\ ST$ $(R^2 = 0.9642)$
$VIG_{60\ °C} = 97.4000 - 0.3311\ ST$ $(R^2 = 0.9397)$
$VIG_{70\ °C} = 70.8000 - 0.3844\ ST$ $(R^2 = 0.9645)$
$VIG_{80\ °C} = 0$

Figure 6. Results of the percentages of viable seeds (a) and vigor (b) in the context of the tetrazolium chloride test, according to the drying temperature and storage time.

Afonso Júnior and Corrêa (2000) obtained similar results as those found in this study when they evaluatedthe physiological quality of bean seeds. The authors verified that when they imposed a drying temperature of 40°C, both immediately drying and after 180 days of storage, the viability and vigor of seeds harvested with approximately 25% moisture content was mostly unaltered. However, when the imposed temperatures were higher than this, for example 50°C, especially during storage, the same parameters were reduced since the latent effect resulting from the increased drying temperature was characterized by favoring the action of degenerating problems and, subsequently, having a negative impact on the physiological quality of the harvested product.

Conclusion

The increase in the drying temperature influences the physiological quality of soybean seeds produced by a second crop, and this effect is enhanced by the storage period. In the range from 40 to 80°C, an air drying temperature of 40°C should be recommended to dry soybean seeds produced in a second crop. In this way, the germination, viability, and vigor are all diminished with the increase in drying temperature, as well as with storage time of up to 180 days.

Conflict of Interests

The authors have not declared any conflict of interests.

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