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# Cooling of soybean seeds and physiological quality during storage<sup>1</sup>

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ABSTRACT - The aim of this study was to evaluate the physiological quality of soybean seeds submitted or not to dynamic cooling, and kept in cold storage (CS) and non-cold storage (NCS). A soybean seeds lot, variety M7639RR, was divided into two parts, and each one was subdivided into three parts that were subjected to one of these cooling procedure: seeds without cooling (SWC), dynamic cooling at 17 °C (SC17), and dynamic cooling at 13 °C (SC13). Seeds were kept in CS and NCS at 20 °C. Samples were collected at six times (0, 45, 90, 135, 180 and 225 days), and then submitted to germination, tetrazolium, accelerated aging and seedling emergence tests. Dynamic artificial cooling at 13 °C followed by maintenance at 20 °C ensured the preservation of physiological quality of soybean seeds for up to 225 days. The vigor of the soybean seeds subjected to dynamic cooling at 13 °C was better preserved than that of the non-cooled or of the cooled at 17 °C ones and kept in NCS for 225 days. CS at 20 °C contributed to the preservation of the physiological quality of the soybean seeds, regardless of whether or not they were submitted to the dynamic procedure.

Index terms: Glycine max, artificial cooling, germination, vigor

## Resfriamento de sementes de soja e qualidade fisiológica no armazenamento

RESUMO - O objetivo do trabalho foi avaliar a qualidade fisiológica de sementes de soja submetidas ou não ao resfriamento dinâmico e mantidas em armazém resfriado (AR) e não resfriado (ANR). Um lote de sementes de soja, variedade M7639RR, foi dividido em duas partes, que foram subdivididas em três partes, cada uma submetida a um procedimento de resfriamento: sementes não resfriadas (SNRE), resfriamento dinâmico a 17 °C (SRE17) e a 13 °C (SRE13). As sementes foram mantidas em um ANR e em AR a 20 °C. Foram coletadas amostras em seis momentos (0, 45, 90, 135, 180 e 225 dias), e as sementes foram submetidas aos testes de germinação, tetrazólio, envelhecimento acelerado e emergência de plântulas em canteiro. O resfriamento dinâmico artificial a 13 °C seguido de manutenção em AR a 20 °C assegurou a preservação da qualidade fisiológica de sementes de soja por até 225 dias. Sementes de soja submetidas ao resfriamento dinâmico a 13 °C preservam melhor o vigor, comparativamente às não resfriadas ou resfriadas a 17 °C e mantidas em ANR por 225 dias. O AR a 20 °C contribui para preservação da qualidade fisiológica da semente de soja, independentemente de a semente ser ou não submetida ao procedimento dinâmico.

Termos para indexação: Glycine max, resfriamento artificial, germinação, vigor.

## Introduction

Brazilian production of soybean (*Glycine max* (L.) Merril) is among the largest in the world, and it reached approximately 106 million tons in 2015/2016 harvest (CONAB, 2016). To keep the production of this commodity at this level or even higher, seeds are the most important inputs used by farmers. Through it, producers have access to the most recent

technologies available in the sector, thus it is essential that high quality seeds be produced and commercialized.

In tropical climate conditions, like important soybean producer regions in Brazil, the adequate storage is fundamental to preserve the physiological quality of soybean seeds (Zuchi et al., 2013). As said by Villela and Menezes (2009), the main goals of the storage are to preserve seeds viability and vigor from one harvest to another, and to reduce the deterioration

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speed. The deterioration process is inevitable, and can only be delayed (Cardoso et al., 2012).

The association between temperature and humidity influence the longevity of seeds during storage. Harrington (1959) affirmed that at this stage, for every decrease of 5,5 °C in temperature, the longevity doubles. According to Villela and Menezes (2009) and Demito and Afonso (2009), the reduction of seeds temperature is a viable technic to preserve their physiological quality, since it reduces the metabolism.

Therefore, artificial cooling of seeds is an important tool in controlling temperature and air relative humidity, which allows a reduction in the consumption of seeds reserve substances by pathogens or by their own respiration process. Artificial cooling of seeds is a technic of insufflation of air at 15 to 20 °C and relative humidity of 55 to 60% (Villela and Menezes, 2009). This process can be done during the bagging and/or during the storage period (Demito and Afonso, 2009). The technic revolutionized the storage of orthodox seeds, since it is possible to implement this system to large volumes of seeds.

Demito and Afonso (2009) observed that the physiological quality of artificially cooled soybean seeds was maintained for 140 days of storage, with a percentage of germination higher than the non-cooled ones. This result was attributed to the thermic stability of the seeds at the cooling temperature during storage.

When evaluating the physiological quality of three soybean cultivars submitted or not to dynamic cooling process, Zuchi et al. (2013) verified that the cooling only maintained the physiological quality of two of the cultivars. These authors suggested that more studies about artificial cooling of seeds are necessary. The aim of this work was to evaluate the physiological quality of soybean seeds submitted to dynamic cooling during storage.

#### **Material and Methods**

Location of the experiment and plant material

The experiment was installed at UBS Sementes Petrovina, located at BR 364 km 119, Pedra Preta – Mato Grosso, Brazil (latitude 16°50'28.3"S, longitude 54°03'35.4" W and altitude 734 meters), between March and October, 2014. A soybean seeds lot (1.8000 kg), cultivar of early cycle M7639RR, produced in 2013/2014 harvest, was used. Before packing, the lot was subdivided into three sublots to compose the cooling treatments of seeds. Each sublot was packed in six packs (big bags), made of interlaced polypropylene with 1,20 × 1,00 × 1,06 m dimensions and 1.000 kg capacity each.

Cooling of the seeds

At the moment of bagging, the first sublot was kept at room temperature of approximately 25 °C, and the other two sublots were put inside cooling boxes, and submitted to dynamic cooling by a CoolSeed cooler, model PCS 120, with cooling capacity of 363.636 kcal.h<sup>-1</sup> and potential of 35 tons per hour at 18 °C.

Two cooling boxes were used, with a static capacity of 15 tons each, to obtain the other sublots. The air was artificially chilled at 12 °C, conducted at low velocity inside ventilation tubes and insufflated through the mass of seeds. As it was passing, the cold air exchanged heat and moisture with the seeds, which had their temperature reduced over the time. At the moment that the mass of seeds of one of the boxes reached 17 °C, another sublot was packed. The third sublot was chilled and packed at 13 °C, using the same procedure previously described. Consequently, there were three cooling procedures: SC13 (seeds cooled at 13 °C at packing); SC17 (seeds cooled at 17 °C at packing) and SNC (seeds not cooled at packing).

Storage of seeds

The soybean seeds from each cooling procedure (SC13; SC17; SNC) were divided into two parts, each composed of three big bags that were kept in two warehouses for 225 days. One of the warehouses was cooled at 20 °C (CS), whereas the other was not refrigerated (NCS).

The warehouses had dimensions of  $06 \times 30 \times 154$  m, and static capacity of 27.720 m<sup>3</sup>. They were masonry built, with double-layered brick walls, cement floor and zinc roof covered by thermal coating, in order to reduce the heat flow from the outside. One of the warehouses had controlled temperature (CS), whereas the environmental conditions during storage remained uncontrolled on the other (NCS).

Monitoring of temperature and relative humidity in the warehouses

In both warehouses, the temperature (T °C) and the relative humidity (RH%) of the internal environment, and the temperature (T °C) of the seeds mass were monitored. A data logger DH 175 T2 was used to read the internal T °C and RH% of the warehouse every hour. To measure the temperature of the seed mass, a DH 175 T2 data logger was adapted with a probe, which remained inserted into the center of a big bag of each treatment during the entire storage period. The data were recorded in the memory of the data logger device and read every fifteen days.

Seeds sampling

The samples to evaluate the quality of the soybean seeds were obtained at 0, 45, 90, 135, 180 and 225 storage days, and three replications per treatment were collected.

In the laboratory, the samples were separated and identified according to company's standard procedures. Each sample was homogenized in a Boerner homogenizer, and the following analyses were carried out:

Germination test: It was performed in eight subreplications of fifty seeds per sample. They were placed on towel paper rolls, moistened with 2.5 times the dry paper mass, and then put inside germination chambers at constant temperature of 25 °C. The counts were performed five and eight days after sowing, so it was calculated the percentage of normal seedlings according to Brasil (2009).

Accelerated aging: It was performed in 200 seeds per replication, divided into four sub-replications of fifty seeds per sample. The seeds were placed in metal screens, which were fixed in transparent plastic boxes (11 × 11 × 3.5 cm) containing 40 mL of distilled water in the bottom. The boxes were kept in BOD incubator at 41 °C for 48 h. After this period, the paper roll germination test was carried out, as previously described for the germination test. At the end of the fifth day after sowing, the percentages of normal seedlings were computed.

Tetrazolium test: It was carried out in four replications of fifty seeds previously placed on towel paper, moistened with water equivalent to 2.5 times its weight. The treatments were kept in these conditions for 16 h at 25 °C. Afterwards, the seeds were deposited in plastic cups of 50 mL, and totally submerged in 0.08% tetrazolium salt solution. They remained at 40 °C for two hours, so the staining could occur and allow the data interpretation (Costa et al., 2007). The results for vigor and viability of the seeds were computed.

Emergence of seedlings in seedbed: It was performed in four sub-replications of fifty seeds per sample. The seeds were distributed equidistantly from one another in lines on the seedbed, inside furrows with 1.5 m long and 0.03 m deep, and a space of 0.05 m between them. Counts were performed eight days after sowing, and the percentages of emerging seedlings were computed.

Experimental design: The experiment was carried out in randomized blocks design, and subdivided plots, with a  $3 \times 2$  factorial in the plot. This means that there were three cooling procedures (SC13, SC17 and SNC) and two storage conditions (CS and NCS) in the plot. The storage periods (0, 45, 90, 135, 180 and 225 days) were the sub-plots. The data expressed in percentage were submitted to the transformation,

where x is the percentage value of each transformed variable. The analysis of variance (ANOVA) was done, in order to obtain the normality of the data and interpret them. In the case of significance, the data from cooling procedures and storage conditions were subjected to polynomial regression analysis.

#### Results and Discussion

The mean storage temperature in the unrefrigerated warehouse (NCS) was 24.6 °C, while it was 21.1 °C in the refrigerated one (CS) (Figure 1). In the CS, the temperature of the seed mass reached the equilibrium around the 23<sup>rd</sup> day, at an average of 23 °C; by the 50th, this value decreased to 21 °C (Figure 1A). Therefore, cooling at the moment of packing, either to 13 °C or 17 °C, was an alternative to maintain mild temperatures in the seed mass during the first days of storage, even when the external temperatures oscillated. In the treatments, there was an average reduction of 0.4 °C and 0.27 °C per day, respectively, until the equilibrium with the room temperature was reached. In the unrefrigerated warehouse (NCS), the average temperature was 24.6 °C (Figure 1B). There were fluctuations during the 225 days, and the maximum temperature got to 29 °C, while the minimum was around 10 °C. Despite these environmental oscillations, the seed mass of the treatments achieved equilibrium close to the 19th day and, from this point on, the NCS started to show the same behavior as the refrigerated storage (CS).

The relative humidity (RH) inside the CS oscillated between 60 and 68%, which indicates certain stability in this warehouse (Figure 2). Inside the NCS, the RH oscillated between 63% and 75%, until the 135<sup>th</sup> day. After this period, the RH started to decrease, and it reached 40% on the 165<sup>th</sup> day, and 24% on the 200<sup>th</sup> day. It remained at 50% by the end of the storage. In comparison to the NCS, the control of the temperature and the relative humidity in the CS allowed to maintain the seeds in more favorable conditions, thus guaranteeing a potential preservation of their longevity, and consequently a superior quality throughout the storage.

In the germination test, there was no significant interaction among the cooling procedures SC13, SC17 and SNC in each storage condition. However, there was a significant difference between the refrigerated (CS) and the non-refrigerated (NCS) environments, which was graphically expressed by polynomial regression, considering the mean of the treatments of each warehouse (Figure 3).

The seeds of all cooling procedure stored in the CS showed a less pronounced linear reduction of the physiological quality than those kept in the NCS. They began with 94% germination on the first day of storage, and ended with 91%

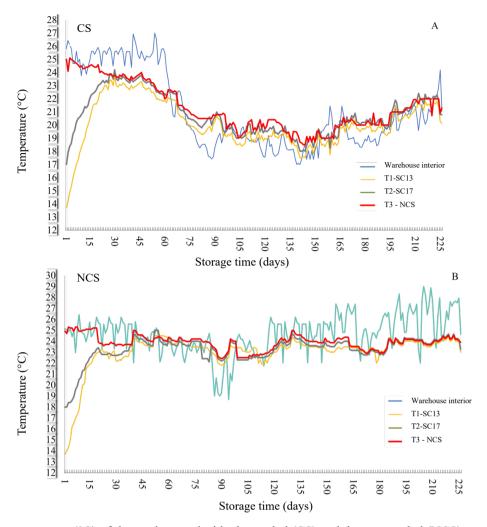


Figure 1. Daily temperature (°C) of the seeds mass inside the cooled (CS) and the non-cooled (NCS) warehouses, throughout 225 of storage. Seeds were submitted to the cooling procedures T1-SC13 (seeds cooled at 13 °C at bagging), T2-SC17 (seeds cooled at 17 °C at bagging) and T3-SNC (seeds not cooled at bagging).

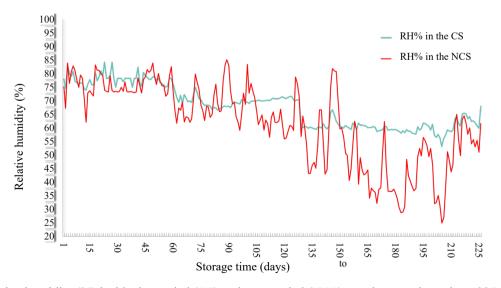


Figure 2. Relative humidity (%) inside the cooled (CS) and non-cooled (NCS) warehouses, throughout 225 days of storage.

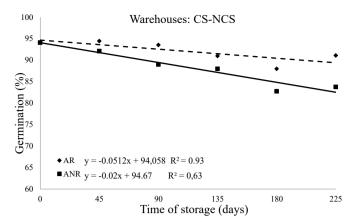


Figure 3. Germination of soybean seeds (%) throughout 225 days of storage, in the cooled storage (CS) and (B) non-cooled storage (NCS). It was considered the mean of the cooling procedures SC13 (seeds cooled at 13 °C at bagging), SC17 (seeds cooled at 17 °C at bagging) and SNC (seeds not cooled at bagging).

after 225 days, a decrease of three percentage points (pp) during storage. The seeds stored in the NCS also showed a linear decrease in germination, but it was more pronounced. The seeds began with 94% germination and, on the 225<sup>th</sup> day of storage, they reached 84% germination, a reduction of 10 pp during storage.

By the end of the storage period, the difference between the CS and the NCS reached seven pp. These results indicated that the preservation of soybean seeds quality was better in the CS. Therefore, the main goal of the storage was reached, which was the preservation of seeds viability (Villela and Menezes, 2009) through temperature control, which slowed down the deterioration (Cardoso et al., 2012). In this occasion, however, the cooling of the seeds at the moment of bagging did not brought any substantial benefit in relation to the non-cooling.

The analysis of viability evaluated by the tetrazolium test did not detect a marked variation among the cooling procedures. In both warehouses, refrigerated and non-refrigerated, the seeds submitted to the three cooling procedures behaved alike. The SC13 lot presented a quadratic adjustment, the SC17 a linear trend and the SNC showed no significant response.

The average viability reduction in the CS was four pp (Figure 4A), while it decreased seven pp in the NCS, in relation to the beginning of storage (Figure 4B). These results indicate once more that cooling soybean seeds during the storage contributes to maintain their physiologic quality. Despite these non-marked differences, negative effects in growing components may be noticed at the final culture stage. These, however, tend to

attenuate as the plant grow (Schuch et al., 2009).

According to the vigor determined by the tetrazolium test (Figure 5), the cooling procedures exhibited similar results for both refrigerated and non-refrigerated warehouses, with similar trend lines throughout the storage.

In the CS, the cooling procedures SC17 and SNC showed sharper reductions in vigor, expressed by quadratic functions, with a decline of 29 and 10 percentage points after 225 days of storage, respectively (Figure 5A). However, the cooling procedure SC13 presented a lower quality reduction, and the vigor had a maximum decrease of six percentage points. In this situation, the benefit of seed cooling at the moment of bagging is evident, emphasis given to the SC13 cooling procedure, which showed a superior seed performance in comparison to the SC17. These results agree with those found by Zuchi et al. (2013) and Demito and Afonso (2009), in which they observed that soybeans seeds subjected to cooling have maintained their physiological quality during storage for up to 120 and 140 days, respectively. It is worth noting that in all three cooling procedures and in the refrigerated warehouse,

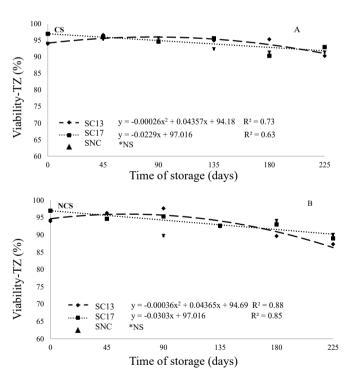


Figure 4. Viability of soybean seeds (%) according to the tetrazolium test, throughout 225 days of storage in (A) cooled storage (CS) and (B) non-cooled storage (NCS). Seeds were submitted to dynamic cooling in three procedures: SC13 (seeds cooled at 13 °C at bagging); SC17 (seeds cooled at 17 °C at bagging) and SNC (seeds not cooled at bagging).

seeds presented vigor above 80%, 135 days of storage later.

Despite the similar behavior in both warehouses, in the NCS the seeds of the three cooling processes presented a greater decrease in vigor (Figure 5B), compared to those stored in the CS. As for the comparisons among the procedures, it was verified that the seeds vigor decreased in all of them, and that in the SC17 procedure there was a 35-percentage points reduction. Nevertheless, in the SC13 and SNC procedures, the decrease was 14 pp throughout the storage period. When comparing the storage conditions, refrigerated and not, it is possible to confirm that the refrigerated warehouse kept the vigor of seeds at least five percentage points higher in all cooling procedures. This indicates that the CS favors the preservation of the physiological quality of soybean seeds.

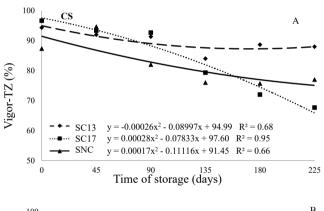
Therefore, it is worth emphasizing the importance of using greater vigor seeds, since low vigor plants will have less solar capture conditions. High vigor plants have more competitive capacity during the crop cycle, and can be identified as dominant plants in the population, in contrast

with the smaller plants, which have less capacity to gather resources (Cantarelli et al., 2015b)

The results of the accelerated aging test can be analyzed as another parameter of seeds physiological quality (Figure 6). In the CS, the seeds of all three cooling procedures showed responses that can be represented by quadratic trend curves. In this storage condition, there was an average vigor reduction of up to nine pp during the storage period (Figure 6).

In the NCS, there was difference among the cooling procedures. The SC13 showed a trend line similar to the CS, with 10-percentage points reduction in vigor during storage. However the decrease of vigor was more pronounced in the other procedures (Figure 6B). In the SC17, there was 18-pp difference between the initial vigor and its value after 225 days of storage. In the SNC procedure, the results varied, with a marked decline of 25 pp in vigor by the 180<sup>th</sup> day, and an overall 17-pp decrease, according to the last test performed at the end of the storage period (Figure 6B).

When the cooling procedures were compared under the



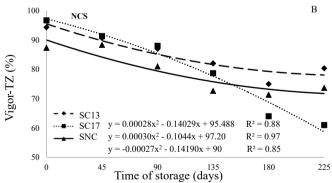
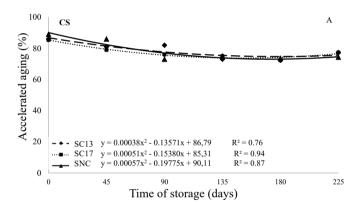


Figure 5. Vigor (%) of soybean seeds according to the tetrazolium test, throughout 225 days of storage in (A) cooled storage (CS) and (B) non-cooled storage (NCS). Seeds were submitted to dynamic cooling in three procedures: SC13 (seeds cooled at 13 °C at bagging), SC17 (seeds cooled at 17 °C at bagging) and SNC (seeds not cooled at bagging).



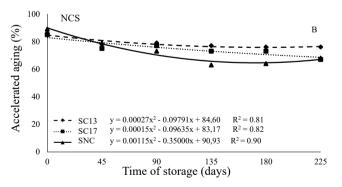
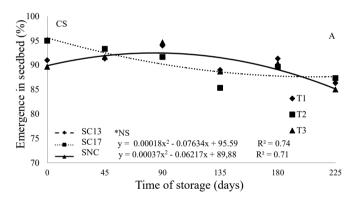


Figure 6. Accelerated aging (%) of soybean seeds throughout 225 days of storage in (A) cooled storage (CS) and (B) non-cooled storage (NCS). Seeds were submitted to dynamic cooling in three procedures: SC13 (seeds cooled at 13 °C at bagging), SC17 (seeds cooled at 17 °C at bagging), and SNC (seeds not cooled at bagging).

storage conditions, the CS stood out positively once again, with 10 pp more than the NCS, in the SC17 procedure. The accelerated aging test can be used to estimate seed longevity, which depends on the initial vigor and on the environment where they are stored (Baudet and Villela, 2012). In this work, seeds of all the cooling procedures presented satisfactory performance in the CS. However, in the NCS the reductions of vigor were more pronounced, which is why using artificial cooling throughout the storage period is recommended. The climate-controlled environment (20 °C) provides better conservation of the physiological quality of soybean seeds (Smaniotto et al., 2014). On the other hand, storage in an uncontrolled environment causes a greater reduction of the physiological potential of soybean seeds (Forti et al., 2010).

To confirm the results obtained in laboratory, the seeds were submitted to the emergence test in seedbeds (Figure 7). In the CS, the SC13 procedure did not show a significant trend curve, whereas the SC17 and the SNC procedures presented reductions in vigor, with a decrease of nine and five pp



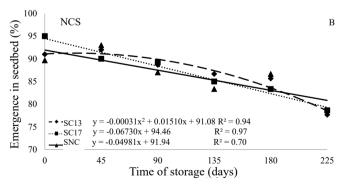


Figure 7. Emergence of seedlings in seedbeds (%) throughout 225 days of storage in (A) cooled storage (CS) and (B) non-cooled storage (NCS). Seeds were submitted to dynamic cooling in three procedures: SC13 (seeds cooled at 13 °C at bagging), SC17 (seeds cooled at 17 °C at bagging), and SNC (seeds not cooled at bagging).

throughout the storage, respectively (Figure 7A).

In the NCS, both SC17 and SNC procedures had similar performances, represented by linear equations that expressed a reduction in vigor of approximately 12 pp throughout the storage (Figure 7B). However, the seeds cooled at 13 °C (SC13) maintained the quality for up to 90 days, approximately, and then suffered a decline expressed by a quadratic trend curve.

A comparison between the two warehouses showed that the variation was significant. Seeds kept in the refrigerated warehouse had smaller reductions in vigor than those stored in the NCS, i. e., the CS contributed to maintain the physiological quality of seeds, according to the emergence test in seedbed.

The use of high-vigor seeds provides benefits to soybean crops, such as taller plants, larger stem diameter, and up to 25% gain in grain yield in comparison to the use of low-vigor seeds (Schuch et al., 2009). Therefore, less vigorous seeds may reduce grain yield, and one of the possible causes may be associated with a greater emergence inequality. According to Cantarelli et al. (2015a) and Cantarelli et al., (2015b), late emergent plants are subject to a higher degree of intraspecific competition, which reflects in a decrease of production.

A general analysis of the results allows stating that the combination of cooling at bagging and maintenance of the refrigeration in the warehouse during the entire storage preserves successfully the quality of soybean seeds.

## **Conclusions**

Artificial cooling at 13 °C at bagging, followed by storage in refrigerated warehouse at 20 °C, ensures the maintenance of soybean seeds physiological quality for 225 days. In non-refrigerated storage, the soybean seeds submitted to dynamic cooling at 13 °C at bagging show higher vigor for 225 days, in comparison to those not cooled or cooled at 17 °C. The refrigerated warehouse contributes to the physiological maintenance of soybean seeds, regardless of whether they are subjected to dynamic cooling or not.

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