Abstract
The Brazilian Midwest is responsible for 54.3% of the country's soybean production. Adequate storage technologies are essential to maintain physical, physiological, and sanitary seed qualities while also minimizing deterioration processes and consequent germination and vigor declines. In this context, the aim of the present study was to evaluate the physiological potential and physical qualities of soybean seeds stored under different environmental conditions and storage bag depths. Assays were carried out in September 2021 employing Foco 74i77 RSF IPRO cultivar seeds stored from April to August 2021 under three conditions, as follows: chilled at an average temperature of ≤20 °C, at an average temperature of ≤25 °C using a with blanket, and in without blanket warehouse structure at an average temperature of ≥25 °C with no with blanket. A completely randomized 3x3 factorial experimental design was applied, comprising three storage environments and three bag positions (top, middle, and bottom), with three replication each. Physical, physiological, and biochemical tests were performed on the stored seeds. The findings indicate better seed preservation in the chilled environment or when using a with blanket, with significant differences noted for seeds stored the middle of the storage bag. Therefore, soybean seed storage in a chilled environment or using a with blanket aids in slowing down the seed deterioration processes, preserving physiological quality and vigor compared to a conventional storage environment. Additionally, the quality of soybean seeds stored under these conditions in the middle of storage bags is maintained.

Keywords: glycine max., storage environments, seed position, deterioration, post-harvest management.

Resumo
A região Centro-Oeste do Brasil representa 54,3% da produção nacional de soja. A busca por tecnologia de armazenamento adequada é primordial para manter as qualidades físicas, fisiológicas e sanitárias, além de minimizar o processo de deterioração e consequente declínio na germinação e vigor de sementes mantidas em ambientes inadequados. Portanto, objetivou-se avaliar qualidade de sementes de soja armazenadas em diferentes ambientes e profundidade no bag quanto ao potencial fisiológico e qualidade física. Os ensaios foram realizados com sementes da cultivar Foco 74i77 RSF IPRO, armazenadas nos meses de abril a agosto de 2021 e início dos testes em setembro. As sementes foram armazenadas em ambiente refrigerado com temperatura média ≤20 °C; manta térmica, temperatura média ≤25 °C; ambiente sem manta, estrutura padrão do armazém e temperatura média ≥25 °C. O delineamento experimental foi inteiramente casualizado com fatorial 3x3, 3 ambientes de armazenamento e 3 posições no bag, dividido em partes superior, meio e inferior, com 3 repetições. Nas sementes foram realizados testes físicos, fisiológicos e químicos. Os resultados obtidos para as avaliações físicas, fisiológicas e químicas, demonstraram melhor preservação das sementes nos ambientes refrigerado ou com manta térmica. Para profundidade, foi verificada significância nas sementes na porção média do bag. Este estudo evidencia que o armazenamento de sementes de soja em ambiente refrigerado ou revestido com manta térmica, auxilia no desaceleramento do processo de deterioração das sementes, conservando a qualidade fisiológica e vigor das sementes quando comparadas ao ambiente convencional. Assim como, as sementes de soja armazenadas em ambiente refrigerado ou revestido com manta térmica e na profundidade média no bag, mantêm a qualidade.

Palavras-chave: glycine max., ambienta de armazenamento, posição das sementes, deterioração, manejo pós-colheita.
1. Introduction

Soybean cultivation is the main Brazilian agribusiness commodity, with a national production of 125.5 million tons obtained in the 2021/2022 harvest and approximately 3.7 million tons destined for seed production (ABRASEM, 2022), making Brazil the main soy producer worldwide (CONAB, 2022). The Brazilian Midwest is responsible for 54.3% of the national soybean production, with the main producers, located in the states of Mato Grosso, Goiás, Paraná, and Rio Grande do Sul, responsible for about 52.3% of this total (CONAB, 2022). This expressive soybean production is associated with the development of new seed technologies, increasing seed quality control, and improved storage and treatment methods (Marcos Filho, 2015; Krzyzanowski et al., 2018).

In most production areas, a 6 to 7 month-interval is established between the soybean harvest and new crops, requiring seeds be stored in adequate conditions to reduce quality losses. Seed integrity during the storage process is directly affected by relative humidity (RH) and temperature conditions, which must be controlled (Radha et al., 2014; Marcos Filho, 2013). Seed quality loss takes place, in fact, very quickly when seeds are stored under high water content (WC), RH and temperature conditions (Chichir et al., 2017). Mass decreases are also noted due to lipid peroxidation, altering the germination process and compromising seed vigor and longevity (Vitis et al., 2020), resulting in reduced productivity (Schuch et al., 2009; Smaniotto et al., 2014). In view of this, producers face several challenges in maintaining seed quality during storage periods.

When stored, soybean seeds are placed in large cubic flexible polypropylene bags highly resistant to tearing. When full of seeds, these bags can support up to 3,000 kg, depending on the packaging design. The bag handles are designed to be placed around the forks of a forklift for easy transportation. Bags can be customized according to the needs and demands of each business, whether for storage or transport. Other bag elements, such as capacity, load, size and loading and unloading features, are customizable (Embetec, 2022). Despite the versatility of these storage containers, no studies assessing the physiological quality of seeds stored at different storage bag depths have been conducted to date.

The seed market has become increasingly demanding, seeking high quality, vigorous seeds that fulfill their functions and become high-yielding plants (Cardoso et al., 2012; Ferreira et al., 2015). The search for first-rate storage technologies has become an important option to maintain physical and physiological seed qualities while at the same time minimizing seed deterioration processes and consequent germination and vigor declines (Cardoso et al., 2012). Due to concerns regarding adverse storage condition seed quality effects considering environmental exposure factors, such as uncontrolled temperature or RH, from harvest to sowing in tropical regions (Coradi et al., 2020), chilled storage environments have been pointed out as a solution to reduce negative soybean seed effects and maintain seed quality (Ferreira et al., 2017; Coradi et al., 2020).

Considering current soybean seed storage technologies, this study postulates that controlled temperature and RH environments along with seed bag positioning are able to maintain physiological soybean seed quality, resulting in greater storage efficiency. Thus, the aim of the present study was to evaluate the physiological potential and physical quality of soybean seeds stored under three conditions, one natural environment, another chilled and the last covered with a with blanket, at different storage bag depths.

2. Material and Methods

2.1. Experimental assays

All experiments were conducted at the Federal Institute of Education, Science and Technology – IF Goiano (Rio Verde Campus) Seed Laboratory with Foco 74i77 RSF IPRO cultivar seeds. This cultivar exhibits indeterminate growth with a cycle ranging from 100 to 118 days. Assays were carried out in September 2021 employing seeds stored from April to August 2021.

2.2. Experimental design

A completely randomized 3x3 factorial scheme experimental design was applied, comprising three storage environments (natural, chilled environment and covered with a with blanket) and three bag depths (top, middle and bottom), with three repetitions each.

2.3. Storage environments

The soybean seed storage environments comprised a concrete structure with a polyethylene coating on the inside and an aluminum roof. Each environment presented different structure, temperature and RH specifications, as follows:

- Chilled environment: This structure is cooled through diesel compressors, maintaining an average temperature ≤20 °C. Environment with a thermal blanket: This structure is coated with a with blanket containing two layers of reflective aluminum on the bottom and a reinforcement layer on the inside, maintaining an average temperature ≤25 °C.

- Environment without a thermal blanket: This is a without blanket warehouse structure, without any type of cooling or coating system, maintaining an average ≥25 °C.

2.4. Seed sampling

Seed samplings from each storage environment and bag depths (Figure 1A) were carried out with the aid of a composite caliper (Figure 1B) with an alternating opening system that begins seed collection from the bottom, followed by the middle, and, finally, the top. The holes were sealed with tape for sample collection (300 - 500 g) according to depth, with a 0.46 m variation. Samples were collected from randomly selected bags at three levels (Figure 1), comprising three bags per treatment. The samples from the three bags from each treatment were homogenized prior to the analyses.
2.5. Physical tests

2.5.1. Thousand-seed weight (P1000):

This test consisted in weighing four repetitions comprising eight 100-seed subsamples from each treatment on a precision scale (AY220, Marte, Santa Rita do Sapucaí – MG – Brazil). The Thousand-seed weight was then calculated, expressed in grams (Brasil, 2009).

2.5.2. Water Content (WC):

The WC was determined applying the oven method, drying two subsamples comprising 50 seeds from each treatment at 105 ± 3 °C for 24 hours followed by weighing on a precision scale (AY220, Marte, Santa Rita do Sapucaí, MG, Brazil). The results were expressed as percentages on a wet basis (Brasil, 2009).

2.6. Physiological tests

2.6.1. Germination test

A total of 200 seeds comprising four 50-seed replications were distributed over two Germitest® sheets and covered with a paper sheet moistened with distilled water at 2.5 times the dry weight of the paper. The rolls were then assembled, placed in a germination chamber (TE 402, TECNAL, Brazil), and maintained at a constant temperature of 25°C. Assessments took place in two counts, the first carried out on the fifth day and the final, on the eighth day. Normal seedlings were considered as germinated seeds according to Brasil (2009). The results were expressed as mean percentages based on the number of normal seedlings.

2.6.2. Accelerated aging test

This test was carried out using transparent acrylic plastic boxes (11.0 x 11.0 x 3.5), containing 40 mL of water, where a total of 400 seeds were distributed in a single uniform layer on an aluminum screen. The boxes were kept in a germination chamber (TE 4013, TECNAL, Brazil), at 41 °C for 48 h. After the aging period, four 50-seed subsamples were submitted to the germination test, following the methodology described in section 2.6.2. Seed WC was also determined, to verify test condition uniformity, with values expressed as percentages (Tunes et al., 2012).

2.6.3. Germination speed index (GSI) and emergence speed index (ESI)

The number of seeds presenting root protrusions was counted daily for the germination test. Emerged seedlings with a formed aerial portion were then counted in the sand emergence test until germination/emergence stabilization. Both indices were calculated according to Maguire (1962).

2.6.4. Seedling sand emergence

This test was carried out in beds containing washed sand, comprising four 50-seed replications, arranged in two 25-seed rows. Sprinklers were used to guarantee greenhouse bed humidity (HADAR 7110, 50 L h⁻¹ flow rate) programmed to activate four times a day for 10 minutes. Emerging seedlings with both cotyledons exposed on the soil surface were counted.

2.6.5. Seedling length

A total of 10 seedlings per treatment replicate were used in the germination and emergence tests. Total seedling and root lengths were measured with a ruler and the results expressed in centimeters (Nakagawa, 1999).

2.6.6. Dry mass

The same seedlings measured in the seedling length evaluations were dried in an air circulation oven for 24 h at 65°C and weighed on a precision scale (AY220, Marte, Santa Rita do Sapucaí, MG, Brazil). The results were expressed as grams seedling⁻¹ (Nakagawa, 1999).

2.7. Biochemical assays

2.7.1. Electric conductivity (EC)

Conducted according to Vieira and Krzyzannowski (1999). Briefly, four replicates of 50 previously weighed seeds were immersed in 75 mL of distilled water for 24 hours at a constant temperature of 25 °C. The EC determinations were performed using a Portable Microprocessed Conductivity Meter (CG 1400, GEHAKA, Brazil). The readings were divided by the initial mass of the seeds and the results were expressed as µS.cm⁻¹ g⁻¹ of seeds.

2.7.2. Potassium leaching

The potassium leaching test was performed at the same time as the EC determinations employing a flame photometer (910, ANALYSER, Brazil), multiplying, the readings by the volume of analyzed distilled water (K mL⁻¹) divided by the mass of the sample (g). The results were expressed as ppm (µg g⁻¹) of seeds (Alves and Sá, 2010).

2.7.3. Tetrazolium test

This test was carried out with two 50-seed subsamples conditioned on paper towels moistened with water at 2.5 times the weight of the dry paper at 25°C, for 16 hours.
The seeds were then stained using 2,3,5-triphenyl-tetrazolium chloride solution at 0.075% (w/v), at 40 °C in the dark for 2.5 h. After staining, the seeds were washed under running water and classified according to vigor and viability, as described by França Neto et al. (1998).

2.8. Statistical analyses

The data were submitted to an analysis of variance (ANOVA) followed by the Tukey test when statistical differences were detected (p < 0.05). The Sisvar software was used for all statistical analyses (Ferreira, 2011).

3. Results

Ambient temperature and RH inside the three different storage environments

Room temperature ranged from 20 to 30 °C during the seed storage period (Figure 2), while RH ranged ~33% to 73%. Temperature and RH where the most stable in the chilled environment, averaging 20 °C and 60% RH, while the other environments exhibited unstable parameters, averaging 26 °C and 46% RH in the without blanket storage environment and 25 °C and 52% RH when using the with blanket.

3.1. Physical soybean seed characteristics and Accelerated Aging Test

The parameters used to evaluate the P1000 and WC of soybean seeds stored under the three different environmental conditions exhibited significant interactions (p < 0.05). Seed bag depth, on the other hand, displayed no significant factor interactions (Table 1).

The WC means in the without blanket environment decreased 5% compared to the chilled environment, while no difference was observed between the chilled environment and the with blanket environment. The P1000 for the chilled environment was about 18% higher than that of the other environments.

Concerning the accelerated aging test, seeds stored in the chilled environment exhibited the highest WC (8.80%), while the lowest WC (7.51%) was observed in without blanket environment, comprising a 14% difference. Concerning first count (FC) results, the germination rates of seeds stored in the chilled and with blanket environments were 81.50% and 89.66% respectively, while seeds stored in the without blanket environment exhibited a 5% germination rate reduction (77.16%).

3.2. Physiological tests

An ANOVA significance (p < 0.05) was detecting concerning environment and seed FC, germination (G), Total length (TL), Root length (RL) and dry mass (DM), while FC and DM were significantly different concerning bag depth (Table 2). The interaction data between the factors differed between the physiological analyses when compared to the environment factor, with significance noted for the FC, TL and RL variables (Table 2).

The germination test results used to assess physiological soybean seed quality concerning the three studied storage environments and bag depths are displayed Table 3. Concerning FC, without blanket environment presented the lowest germination percentage (84.5%), while the other two exhibited germination percentages of over 90%

Germination concerning FC was different between storage environments and bag depths, which may be associated to increased seed deterioration (Table 3). The final germination percentage was higher than 80% in the chilled and heating blanker environments, whereas seeds stored in the without blanket environment exhibited decreased germination rates by 9.58%. No significant difference concerning seed bag depth was observed (Table 3).

Total (Figure 3B) and root (Figure 3C) seedling lengths were different among the three storage environments, with the highest length means observed in the chilled environment, following by the with blanket environment and, finally, the without blanket environment (Table 3).

![Figure 2. Temperature (°C) and relative humidity (RH %) values in the without blanket, chilled and with blanket environments assessed herein. The bars represent temperature and the lines, RH.](image)

**Table 1.** Water content (WC), thousand-seed weight (P1000) and first accelerated aging count (FAAC) of soybean seeds stored under different storage environmental conditions and storage bag depths. Data are expressed as means (±SE).

<table>
<thead>
<tr>
<th>Environment</th>
<th>WC</th>
<th>P1000</th>
<th>Accelerated aging test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/kg)</td>
<td>(g)</td>
<td>WC</td>
</tr>
<tr>
<td>Chilled</td>
<td>8.77 ± 0.08a</td>
<td>174.92 ± 0.47a</td>
<td>8.80 ± 0.66a</td>
</tr>
<tr>
<td>With blanket</td>
<td>8.61 ± 0.05a</td>
<td>142.59 ± 0.34c</td>
<td>8.38 ± 0.83a</td>
</tr>
<tr>
<td>Without blanket</td>
<td>8.30 ± 0.08b</td>
<td>149.61 ± 0.18b</td>
<td>7.51 ± 0.81b</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same lowercase letter for each studied factor do not differ from each other by the Tukey test at p < 0.05.
Table 2. Analysis of variance results for the seed germination test, first count (FC), germination (G), germination speed index (GSI), mean germination time (MGT), total length (TL), root length (CR) and dry mass (MS) according to soybean storage environment (A), bag depth (P), and their interactions (A x P) in the germination test conducted in a Biochemical Oxygen Demand chamber.

<table>
<thead>
<tr>
<th>VS</th>
<th>DF</th>
<th>Germination</th>
<th>FC</th>
<th>G</th>
<th>GSI</th>
<th>MGT</th>
<th>TL</th>
<th>RL</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment (A)</td>
<td>2</td>
<td>&lt;0.00*</td>
<td>0.00</td>
<td>&lt;0.20*</td>
<td>&lt;0.91*</td>
<td>&lt;0.04*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td></td>
</tr>
<tr>
<td>Depth (P)</td>
<td>2</td>
<td>&lt;0.00*</td>
<td>&lt;0.09</td>
<td>&lt;0.03*</td>
<td>&lt;0.35*</td>
<td>&lt;0.19*</td>
<td>&lt;0.11*</td>
<td>&lt;0.00*</td>
<td></td>
</tr>
<tr>
<td>A x P</td>
<td>4</td>
<td>&lt;0.00*</td>
<td>&lt;0.82*</td>
<td>&lt;0.17*</td>
<td>&lt;0.58*</td>
<td>&lt;0.0*</td>
<td>&lt;0.00*</td>
<td>&lt;0.70*</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>-</td>
<td>2.85</td>
<td>8.02</td>
<td>3.04</td>
<td>2.95</td>
<td>7.58</td>
<td>10.91</td>
<td>5.86</td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>-</td>
<td>89.44</td>
<td>81.88</td>
<td>29.11</td>
<td>2.15</td>
<td>14.506</td>
<td>7.52</td>
<td>1.095</td>
<td></td>
</tr>
</tbody>
</table>

*: significant at p < 0.05 by the F test. ns: non-significant. VS: variation source; DF: Degrees of freedom; CV: coefficient of variation.

Table 3. Mean (±SE) germination test results for first count (FC), germination (G), germination speed index (GSI), mean germination time (MGT), total length (TL), root length (RL) and dry mass (DM) for the three different storage environments and soybean seed bag depths assessed in the present study.

<table>
<thead>
<tr>
<th></th>
<th>FC (%)</th>
<th>G</th>
<th>GSI</th>
<th>MGT</th>
<th>TL (cm)</th>
<th>RL (cm)</th>
<th>DM (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chilled</td>
<td>91.83±1.59a</td>
<td>84.00±1.63a</td>
<td>28.92±0.28a</td>
<td>2.15±0.02a</td>
<td>15.00±0.24a</td>
<td>8.29±0.21a</td>
<td>1.08±0.03b</td>
</tr>
<tr>
<td>With blanket</td>
<td>92.00±1.33a</td>
<td>89.33±1.50a</td>
<td>29.50±0.37a</td>
<td>2.14±0.01a</td>
<td>14.66±0.41ab</td>
<td>7.47±0.33ab</td>
<td>0.89±0.02c</td>
</tr>
<tr>
<td>Without blanket</td>
<td>84.50±1.88b</td>
<td>72.33±2.33b</td>
<td>28.92±0.18a</td>
<td>2.15±0.02a</td>
<td>13.85±0.42b</td>
<td>6.81±0.33b</td>
<td>1.30±0.02a</td>
</tr>
<tr>
<td>Top</td>
<td>88.50±1.52b</td>
<td>79.66±2.53a</td>
<td>28.57±0.25a</td>
<td>2.15±0.01a</td>
<td>14.12±0.31a</td>
<td>7.83±0.23a</td>
<td>1.06±0.05b</td>
</tr>
<tr>
<td>Middle</td>
<td>94.50±0.78a</td>
<td>85.33±2.31a</td>
<td>29.13±0.27a</td>
<td>2.13±0.01a</td>
<td>14.43±0.32a</td>
<td>7.12±0.30a</td>
<td>1.06±0.06b</td>
</tr>
<tr>
<td>Bottom</td>
<td>85.33±2.05c</td>
<td>80.66±3.15a</td>
<td>29.65±0.31a</td>
<td>2.17±0.02a</td>
<td>14.95±0.49a</td>
<td>7.62±0.44a</td>
<td>1.16±0.06a</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same lowercase letter for each studied factor do not differ from each other by the Tukey test at p < 0.05.

Figure 3. First germination count (A), total (B) and root (C) seedling lengths in the soybean seed germination test for the “Foco 74777” cultivar under different storage environments and bag depths. Lowercase letters in the same depth differ between environments, uppercase letters in the same environments are different between bag depths by the Tukey test at p < 0.05.
No significant difference concerning seed bag depth was observed (Table 3).

The seeds sampled from the middle of the storage bags exhibited a 94% germination rate at FC, slightly higher than at the top and bottom (88.55% and 85.33%, respectively). The observed germination rates were higher than 80% when assessing both environment and bag depth (Figure 3A).

Regarding the sand emergence test (Table 4), the ANOVA test indicated significance (p < 0.05) for all variables in all storage environments and bag depths, while interactions between factors indicated significance for the DM variable (Figure 4).

Concerning emergence test (Table 5), FC values indicate an >80% emergence in all storage environments, while an average emergence rate of 72% was noted for the without blanket environment, and the chilled and with blanket environments exhibited higher averages than the without blanket environment. No differences for FC, E, GSI, MGT, TL and RL were observed regarding bag depth.

The GSI result (Table 5), confirms the aforementioned results, in which the chilled and heating blanket environments presented the best means, similar to the MGT, guaranteeing emergence uniformity at 4 days. Concerning TL and RL, seeds stored in the with blanket environment performed better compared to the other environments.

Seedling DMs were lower in the chilled and with blanket environments, (Table 5), with a 19% decrease.

**Table 4.** Analysis of variance results for the sand emergence test with regard to first count (FC), total emergence (E), germination speed index (GSI), mean germination time (MGT), total length (TL), root length (RL) and dry mass (DM) according to storage environments (A), bag depth (P), and their interactions (A x P) in the germination test conducted in a Biochemical Oxygen Demand chamber and in the sand emergence test conducted in a greenhouse.

<table>
<thead>
<tr>
<th>VS</th>
<th>DF</th>
<th>FC</th>
<th>E</th>
<th>GSI</th>
<th>MGT</th>
<th>TL</th>
<th>RL</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment (A)</td>
<td>2</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
<td>&lt;0.00*</td>
</tr>
<tr>
<td>Depth (P)</td>
<td>2</td>
<td>&lt;0.16ns</td>
<td>&lt;0.09ns</td>
<td>&lt;0.42ns</td>
<td>&lt;0.28ns</td>
<td>&lt;0.74ns</td>
<td>&lt;0.56ns</td>
<td>&lt;0.00*</td>
</tr>
<tr>
<td>A x P</td>
<td>4</td>
<td>&lt;0.75ns</td>
<td>&lt;0.82ns</td>
<td>&lt;0.34ns</td>
<td>&lt;0.08ns</td>
<td>&lt;0.83ns</td>
<td>&lt;0.70ns</td>
<td>&lt;0.00*</td>
</tr>
<tr>
<td>CV (%)</td>
<td>-</td>
<td>4.19</td>
<td>8.02</td>
<td>3.99</td>
<td>1.72</td>
<td>6.05</td>
<td>9.08</td>
<td>4.70</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>88.33</td>
<td>81.88</td>
<td>11.55</td>
<td>4.10</td>
<td>21.00</td>
<td>13.13</td>
<td>4.70</td>
</tr>
</tbody>
</table>

ns: not significant. ** and *: significant at 1% and 5%, respectively, by the F test. VS: source of variation; DF: Degrees of freedom: CV: coefficient of variation.

**Table 5.** Mean (±SE) sand emergence results concerning first count (FC), total emergency (E), germination speed index (ESI), mean germination time (MGT), total length (TL), root length (RL) and dry mass (DM) for the three different storage environments and soybean seed bag depths assessed in the present study.

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>E</th>
<th>ESI</th>
<th>MGT</th>
<th>TL</th>
<th>RL</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chilled</td>
<td>$91.66±0.59a$</td>
<td>$84.00±1.63a$</td>
<td>11.75 ± 0.09a</td>
<td>4.08 ± 0.02a</td>
<td>20.77 ± 0.28b</td>
<td>12.45 ± 0.29b</td>
<td>1.09 ± 0.02b</td>
</tr>
<tr>
<td>With blanket</td>
<td>$89.66±1.01a$</td>
<td>$89.33±1.50a$</td>
<td>12.10 ± 0.16a</td>
<td>4.27 ± 0.02a</td>
<td>23.91 ± 0.47a</td>
<td>15.39 ± 0.43a</td>
<td>1.09 ± 0.01b</td>
</tr>
<tr>
<td>Without blanket</td>
<td>$83.83±1.34b$</td>
<td>$72.33±2.33b$</td>
<td>10.82 ± 0.14b</td>
<td>3.95 ± 0.02b</td>
<td>18.31 ± 0.22c</td>
<td>11.56 ± 0.18b</td>
<td>1.36 ± 0.03a</td>
</tr>
<tr>
<td>Top</td>
<td>$89.83±1.47a$</td>
<td>$79.66±2.53a$</td>
<td>11.56 ± 0.25a</td>
<td>4.13 ± 0.06a</td>
<td>21.14 ± 0.81a</td>
<td>13.27 ± 0.70a</td>
<td>1.13 ± 0.03b</td>
</tr>
<tr>
<td>Middle</td>
<td>$86.83±1.47a$</td>
<td>$85.33±2.31a$</td>
<td>11.43 ± 0.22a</td>
<td>4.10 ± 0.5a</td>
<td>21.08 ± 0.70a</td>
<td>13.31 ± 0.49a</td>
<td>1.21 ± 0.05a</td>
</tr>
<tr>
<td>Bottom</td>
<td>$88.50±1.38a$</td>
<td>$80.66±3.15a$</td>
<td>11.68 ± 0.13a</td>
<td>4.08 ± 0.03a</td>
<td>20.77 ± 0.82a</td>
<td>12.83 ± 0.59a</td>
<td>1.20 ± 0.05a</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same lowercase letter for each studied factor do not differ from each other by the Tukey test at p < 0.05.
when compared to the without blanket environment. Concerning seed bag depth, seeds stored at the top presented the lowest DM accumulation when compared to the middle and bottom (1.21 and 1.20 g, respectively). Thus, a significant interaction between environmental factors and bag depth was observed at a 5% probability level for DM, noting that seeds stored in the chilled and with blanket environments exhibited less DM accumulation regardless of bag depth (Figure 4).

3.3. Biochemical assays

Differences concerning EC and potassium leaching means were observed in seeds stored under different environmental conditions, while no differences were noted concerning bag depths (p < 0.05).

Electrical conductivity was higher in seeds stored in the without blanket environment, while seeds stored in the chilled and with blanket environment displayed 11.27% and 17.80% decreases, respectively (Figure 3A). The greater leaching of potassium ions observed in seeds stored in the without blanket environment and in the chilled environment averaged 255.013 µg k/g and 232.594 µg k/g, respectively, demonstrating that potassium ion leaching is high in non-chilled environments, due to higher internal temperatures during storage (Figure 5B).

The tetrazolium interaction test between environment and storage bag depth was not significant for vigor ($F_{2,245}$ and $F_{2,373}$ respectively) or viability ($F_{2,166}$ and $F_{2,347}$ respectively) (Table 6). The vigor and viability of soybean seeds stored in the controlled environments ranging from 15 to 25°C maintained vigor of ≥69%, while seeds stored in without blanket environment exhibited a 6.76% decrease in this variable (Table 6). The overall mean seed viability was of 89%, with no difference between treatments.

Table 6. Mean (±SE) tetrazolium (TZ) vigor and viability of soybean seeds stored under different storage environments and bag depths. CV: coefficient of variation.

<table>
<thead>
<tr>
<th></th>
<th>TZ – Vigor</th>
<th>TZ – Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chilled</td>
<td>69.00±4.25a</td>
<td>90.33±0.61a</td>
</tr>
<tr>
<td>With blanket</td>
<td>74.66±3.17a</td>
<td>90.33±1.89a</td>
</tr>
<tr>
<td>Without blanket</td>
<td>64.33±2.85a</td>
<td>86.66±0.67a</td>
</tr>
<tr>
<td><strong>Bag depth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>68.33±3.63a</td>
<td>88.66±1.12a</td>
</tr>
<tr>
<td>Middle</td>
<td>67.00±4.81a</td>
<td>90.33±1.41a</td>
</tr>
<tr>
<td>Bottom</td>
<td>72.66±2.67a</td>
<td>88.33±1.58a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>12.20</td>
<td>3.41</td>
</tr>
<tr>
<td>Means</td>
<td>69.33</td>
<td>89.11</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same lowercase letter, for each factor studied, do not differ from each other by the Tukey test at p < 0.05. Data are expressed are means ± without blanket error of four replicates.

4. Discussion

Seed bag depth can negatively influence seed quality, accelerating seed deterioration processes for top or bottom seeds. This may be associated to the opening metering valves located at the bottom and/or top of the bags. Thus, storage environments and bag depths can directly influence soybean seed quality.

Concerning the chilled environment, seeds positioned at a depth of 70 cm, in the middle of the storage bags, receive less oxygen, located further away from the discharge valves on the top and bottom, consequently reducing metabolic seed rates and deterioration processes. Gaseous exchanges with atmospheric air, in addition to the intergranular air that oscillates due to the steam pressure inside the bags, which are impermeable, both take place during the storage period. These factors can influence seed quality and consequently, germination. This finding is of paramount importance and provides a basis for verifying the quality of the seed storage packages used in the seed industry (Aguiar et al., 2012).

Regarding the percentage of germination in the FAAC, increased germination rates were noted for seeds sampled from the middle bag depth in the chilled environment, while decreased rates were noted for top and bottom seeds, again, probably due to proximity to the bag discharge valves. The presence of oxygen and high respiration rates during storage both promote biochemical reactions that contribute to efficient seed germination processes, although...
also contributing to accelerated deterioration processes, affecting one of the main enzymes, malate dehydrogenase (MDH), responsible for Krebs cycle regulation (Dode et al., 2013; Taiz et al., 2016).

Soybean seeds stored in the chilled environment or covered with a with blanket lose less water when compared to the without blanket environment, with a 5.35% variation. Similar results have been reported for soybean seeds stored in an air-conditioned environment at 20 ºC (Smaniotto et al., 2014) and non-air-conditioned environments at 26 ºC (Conceição et al., 2016). Herein, concerning P1000, only the chilled environment resulted in greater seed weight, due to the fact that the other studied environments undergo RH variations, while chilled environment undergoes less water losses due to the stoppage metabolism, not consuming seed reserves. The temperature increases noted in the non-acclimatized environment directly interferes with RH (%), leading to consumption of seed reserves, and consequent weight losses. Seed storage in stable environments, thus, contributes to seed viability and vigor maintenance, while decreased seed viability times are noted in low humidity storage conditions. In this sense, Coradi et al. (2020) observed decreased seed weights during the storage period regardless of storage environment, indicating several management factors that may interfere in seed maintenance and conservation time.

High storage environment temperatures directly influence the respiratory activities of seeds and their associated microorganisms, causing physiological quality losses (Carvalho and Nakagawa, 2012). Therefore, Kafer et al. (2019) indicate that seeds exhibit less emergence potential when stored in a conventional warehouse compared to controlled environments, as seeds may lose moisture at room temperature, compromising seed quality.

The biochemical assays conducted herein indicate that storing soybean seeds in conventional environments leads to higher moisture loss. This causes seeds to consume their reserves, as this is associated to cell membrane organization. Thus, deteriorated seeds undergo high level of cell membrane degeneration and, therefore, low vigor (Ferreira et al., 2016), with higher concentrations of leached ions noted than in controlled temperature and RH (%) environments, which reduce seed deterioration during the storage period (Smaniotto et al., 2014; Neve et al., 2016; Coradi et al., 2020). Concerning potassium leaching, higher potassium leaching values were observed in seed stored in the without blanket environment (255.013 µg k/g) compared to the chilled environment (232.594 µg k/g), corroborating Fessel et al. (2010), who demonstrated high potassium ion leaching regardless of storage temperature.

The tetrazolium test indicated no difference for both vigor and viability. This may be due to the batch classification test used by the sowing equipment, when seeds arrive for processing. Seed quality preservation is associated with temperature and humidity control offered by storage systems, whether mechanical or physical, which aids in delaying soybean seed deterioration processes. Rocha et al. (2017) and Coradi et al. (2020) both demonstrated that seed storage in climate-controlled environments preserves vigor, with temperature and time as the main conservation factors. Therefore, temperature and RH can directly influence biochemical processes, increasing the speed of deterioration processes, causing protein denaturation and, consequently, decreased cell membrane integrity (Carvalho and Nakagawa, 2012).

Seed germination capacity is reduced over time in non-chilled environments (Han et al., 2014; Carvalho et al., 2016; Hartmann Filho et al., 2016; Neve et al., 2016). Thus, temperature-controlled environments comprise a viable alternative for seed conservation for longer periods of time without significant seed quality losses (Smaniotto et al., 2014; Ferreira et al., 2017). The findings reported herein indicate that soybean seeds stored in a chilled or with blanket environment minimizes temperature and RH increases, maintaining seed germination rates within the established limits of ≥ 80% according to the Rule for Analysis of Seeds (RAS) (Brasil, 2009).

Physiological soybean seed assessments indicate seed characteristic maintenance under chilled conditions, demonstrating the importance of storage under controlled temperature and humidity conditions. Soybean seeds are hygroscopic, cotyledonous and oleaginous seeds that lose vigor after reaching their maturation point, which is accelerated when seeds are stored under adverse conditions (Carvalho and Nakagawa, 2012). This was confirmed by Zuffo et al. (2017), who investigated the physiological and sanitary quality of soybean seeds stored in a non-air-conditioned environment, leading to significantly reduced WC, also due to the lower permeability of storage packages and hygroscopic equilibrium.

5. Conclusion

The storage of soybean seeds in chilled environments or covered with a with blanket at medium storage bag depth aids in maintaining physical and physiological seed quality when compared to the without blanket environments. Seed storage bag depth can interfere in physiological seed quality, indicating that this issue must be further analyzed by the seed industry.

Acknowledgements

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