Research Article

Changes in the physiological potential of sunn hemp seeds during storage[1]

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ABSTRACT

Sunn hemp (*Crotalaria spectabilis*) is widely used for soil correction and cover, but it is still necessary to know the characteristics that influence the storage of its seeds. This study aimed to evaluate the physiological quality of sunn hemp seeds under uncontrolled environmental conditions and in acclimatization chamber. A completely randomized experimental design was used, with four replicates, in subplots evaluated in different periods. Freshly harvested seeds and seeds at 90, 180 and 270 days of storage were evaluated for germination, first germination count, germination speed index, seedling emergence in the field, accelerated aging, cold test and seedling growth. The environment and storage time significantly influenced the seed germination and vigor. The seeds showed a high physiological performance for both the environmental conditions, maintaining the ability to produce normal seedlings up to approximately 150 days of storage, deteriorating afterwards, although without losing their germination capacity up to 270 days of storage.

KEYWORDS: *Crotalaria spectabilis*, seed dormancy, seed vigor.

RESUMO

Mudanças no potencial fisiológico de sementes de crotalária durante o armazenamento

A crotalária (*Crotalaria spectabilis*) é amplamente utilizada na correção e cobertura do solo, mas ainda é necessário determinar as características que influenciam no armazenamento de suas sementes. Objetivou-se avaliar a qualidade fisiológica de sementes de crotalária durante o armazenamento, em condições de ambiente não controlado e em câmara climatizada. O delineamento experimental foi o inteiramente casualizado, com quatro repetições, em subparcelas divididas no tempo. As avaliações foram realizadas em sementes recém-colhidas e aos 90, 180 e 270 dias de armazenamento, por meio de germinação, primeira contagem de germinação, índice de velocidade de germinação, emergência de plântulas a campo, envelhecimento acelerado, teste de frio e crescimento de plântulas. O ambiente e o tempo de armazenamento influenciaram significativamente na germinação e vigor das sementes. As sementes apresentaram elevado desempenho fisiológico nas duas condições de ambiente, mantendo a capacidade de produzir plântulas normais até por volta de 150 dias de armazenamento, deteriorando-se posteriormente, porém, sem perder a capacidade de germinação até 270 dias de armazenamento.

PALAVRAS-CHAVE: *Crotalaria spectabilis*, dormência de sementes, vigor de sementes.

INTRODUCTION

In agricultural cropping systems, *Crotalaria spectabilis* stands out within the *Crotalaria* genus for its high nutrient cycling capacity (Mauad et al. 2019), competitive ability against weeds (Erasmo et al. 2004, São Miguel et al. 2018), biological nitrogen fixation and antagonistic action against nematodes (Wang et al. 2002). However, for the adequate establishment of the sunn hemp crop, and in order to ensure the performance of its functions, it is essential to use high-quality seeds with fast and uniform germination.

In this context, storage is one of the steps after seed harvest that may affect the seed quality. Every seed begins to deteriorate from the point of physiological maturity onwards, but deterioration is more intense under suboptimal storage conditions, such as under high temperature and relative humidity (Waterworth et al. 2019). Thus, seed deterioration may be delayed with the adoption of adequate storage technologies.

Although there are reports in the literature on the effects of seed aging, sunn hemp seeds may show a different behavior during storage. In a study by Egley (1979), only 9 % of the freshly harvested

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seeds of *C. spectabilis* imbibed water, and after three months of storage at 23 ºC, 24 % of the seeds imbibed water, indicating that the seed coat becomes more permeable after storage. According to Araújo et al. (2018), during the storage of *C. juncea* seeds, the number of hard seeds decreases and seed germination shows a linear increase up to 240 days of storage. On the other hand, Maina et al. (2018) reported that the environment significantly influences the germination speed and germination rate of *C. ochroleuca* seeds, which show higher results after three months of storage at room temperature, when compared to a freezing environment (-2 ºC).

This species is still in the introductory phase, and, in order to increase its use in agricultural systems, it is necessary to know the characteristics that influence the storage of its seeds.

In order to elucidate the physiological changes that may occur with *Crotalaria spectabilis* seeds during storage, this study evaluated the physiological quality of *C. spectabilis* seeds stored under two environmental conditions for 270 days.

MATERIAL AND METHODS

The *Crotalaria spectabilis* seeds used in this study were produced at the experimental farm of the Universidade Federal da Grande Dourados, in Dourados, Mato Grosso do Sul state (22º13'16"S, 54º48'02"W and altitude of 430 m), in 2019, in a Dystrophic Red Latosol (Santos et al. 2018), or clayey Rhodic Ferralsol (FAO 2006). The climate of the region is classified as Cwa, according to the Köppen classification. The daily data of maximum and minimum temperatures and rainfall during the seed production are shown in Figure 1.

Before conducting the experiment, soil samples (0-20 cm) from the production area were collected for chemical analysis, and the results are shown in Table 1. There was no need to correct the soil fertility.

The sowing took place in March 2019, on corn crop residues, using a seeder (Jumil®, model 2680 TD). The plots consisted of eight rows, spaced 40 cm apart, with density of 20 seeds m^{-1}. During the seed production, treatments necessary for crop development were applied. The mechanized seed harvest took place in August 2019, using a harvester with a platform for soybean. The borders of each experimental area were excluded.

The seed lots consisted of 700 g of visually mature and whole seeds with uniform size packed in Kraft paper bags (27 x 19 x 31 cm). After packing, the seed lots were stored under two conditions:

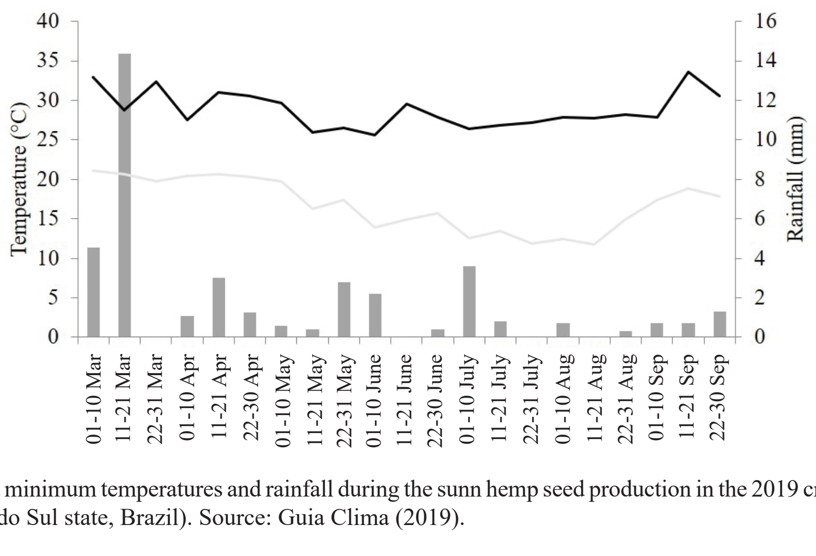

Figure 1. Maximum and minimum temperatures and rainfall during the sunn hemp seed production in the 2019 crop season (Dourados, Mato Grosso do Sul state, Brazil). Source: Guia Clima (2019).

Table 1. Soil chemical characteristics (0-20 cm) before sowing the *Crotalaria spectabilis* seeds.

pH	P resin	H + AL	K^+	Ca^{2+}	Mg^{2+}	V
	mg dm^{-3}	cmol$_c$ dm^{-3}				%
5.8	12.8	5.6	1.53	5.34	1.87	58

uncontrolled condition, regarding temperature and relative humidity (mean temperature of 25 ºC, with minimum of 18 ºC and maximum of 30 ºC, and mean relative humidity of 60 % during 270 days, with minimum of 38 % and maximum of 81 %), but protected from light and ventilation; and acclimatization chamber, with temperature of 15 ºC and relative humidity of 45 %.

The seeds were packed according to each period and evaluated at the beginning (control) and every 90 days up to 270 days of storage. The water content was determined by the oven drying method at ± 105 ºC, for 24 hours (Brasil 2009), with four replicates containing 2 g of seeds. The seeds physiological changes before and during storage were evaluated using the following tests:

Germination: carried out using Germitest® paper towel rolls moistened with distilled water at the ratio of 2.5 times the weight of the dry paper. The germination rolls were placed in a B.O.D chamber, with alternating temperature of 20-30 ºC and four replicates of 50 seeds, remaining in the chamber for 10 days. The results were expressed as percentage of normal seedlings (Brasil 2009);

Germination speed: the number of normal seedlings was counted daily (Brasil 2009) in the germination test, and the formula proposed by Maguire (1962) was applied. The results were expressed as an index;

First count: conducted together with the germination test. The percentages of normal seedlings were recorded at the fourth day after sowing, with results expressed as percentage (Brasil 2009);

Seedling emergence in the field: the test was carried out in a greenhouse, in trays with soil (Dystrophic Red Latosol), consisting of four replicates of 50 seeds. The seedlings that presented a fully expanded first leaf pair above the ground were recorded and the results were expressed as percentage (Nakagawa 1999);

Accelerated aging: four replicates of 50 seeds for each treatment were placed in a single layer on the surface of a stainless-steel screen and placed inside plastic Gerbox boxes containing 40 mL of distilled water at the bottom. The boxes with seeds were placed in a germination chamber regulated at 42 ºC, for a period of 48 hours. After this period, the seeds were submitted to the germination test, according to the methodology previously described. The evaluations were carried out at four days after sowing, with results expressed as percentage of normal seedlings (Marcos Filho 1999);

Cold test: four replicates of 50 seeds arranged on paper towels, according to the germination test previously described, were used. The paper rolls with seeds were maintained in a B.O.D chamber regulated at a constant temperature of 10 ºC, for five days. Then, the seeds were transferred to a germination chamber regulated at 20-30 ºC, where they remained for another five days. The results were expressed as percentage of normal seedlings (Barros et al. 1999);

Seedling length: conducted with four replicates of 20 seeds for each treatment. The seeds were placed on the upper third of Germitest® paper (Nakagawa 1999) and maintained in a B.O.D chamber at alternating temperatures of 20-30 ºC. The shoot and root measurements were conducted at four days after sowing, using a millimeter ruler. The shoot length was determined as the region between the apex of the seedling and the root insertion, while the root length was determined as the region between the root node insertion and the root tip. The results were expressed in centimeters (cm);

Seedling dry matter: determined from the normal seedlings resulting from the seedling length test. The seedlings were divided into shoots and roots, placed in paper bags and dried in an oven with forced air circulation at 40 ºC, for 48 hours (adapted from Nakagawa 1999). After this period, the samples were removed from the oven and weighted to determine the dry mass. The results were expressed as g seedling^{-1}.

A completely randomized split-plot experimental design was used, with evaluations performed at 0, 90, 180 and 270 days of storage, with four replicates. The data were submitted to analysis of variance and, in case of significance, the storage periods were submitted to regression analysis and the environments were compared by the t test, both at 5 % of probability, using the Sisvar® software (Ferreira 2019).

RESULTS AND DISCUSSION

The sunn hemp seeds presented 9.7 % of initial water content and average water content of 11.6 and 12.4 % after 270 days of storage under the uncontrolled environment and in the acclimatized chamber, respectively. Although there was an increase in the water content of the seeds during storage, these values are within the limits considered safe for seeds of this species at temperatures of 15 and 25 ºC (Granella et al. 2020).

The interaction between environments and storage periods was significant ($p \leq 0.05$), with adjustments of quadratic polynomial models for first germination count, germination, seedling emergence in the field, cold test, accelerated aging test and root dry matter (Figure 2). These results indicate that the dynamics of germination and vigor of the sunn hemp seeds depend on temperature conditions and conditioning time.

The seeds stored under the uncontrolled environment showed a maximum estimated germination of 85 %, after 77 days of storage. In the acclimatized chamber, the seeds showed a maximum germination of 84 %, after 18 days of storage (Figure 2A). The stored seeds showed a maximum first count of 78 % germination at 194 days of storage and 62 % germination at 136 days, respectively under the uncontrolled environment and for the acclimatized chamber (Figure 2B). During the early stages of storage, changes in seed germination do not occur or are difficult to detect (Fleming et al. 2019, Batista et al. 2021). The results of seed germination indicate that, although the evaluated storage environments are favorable to obtain seeds with high germination

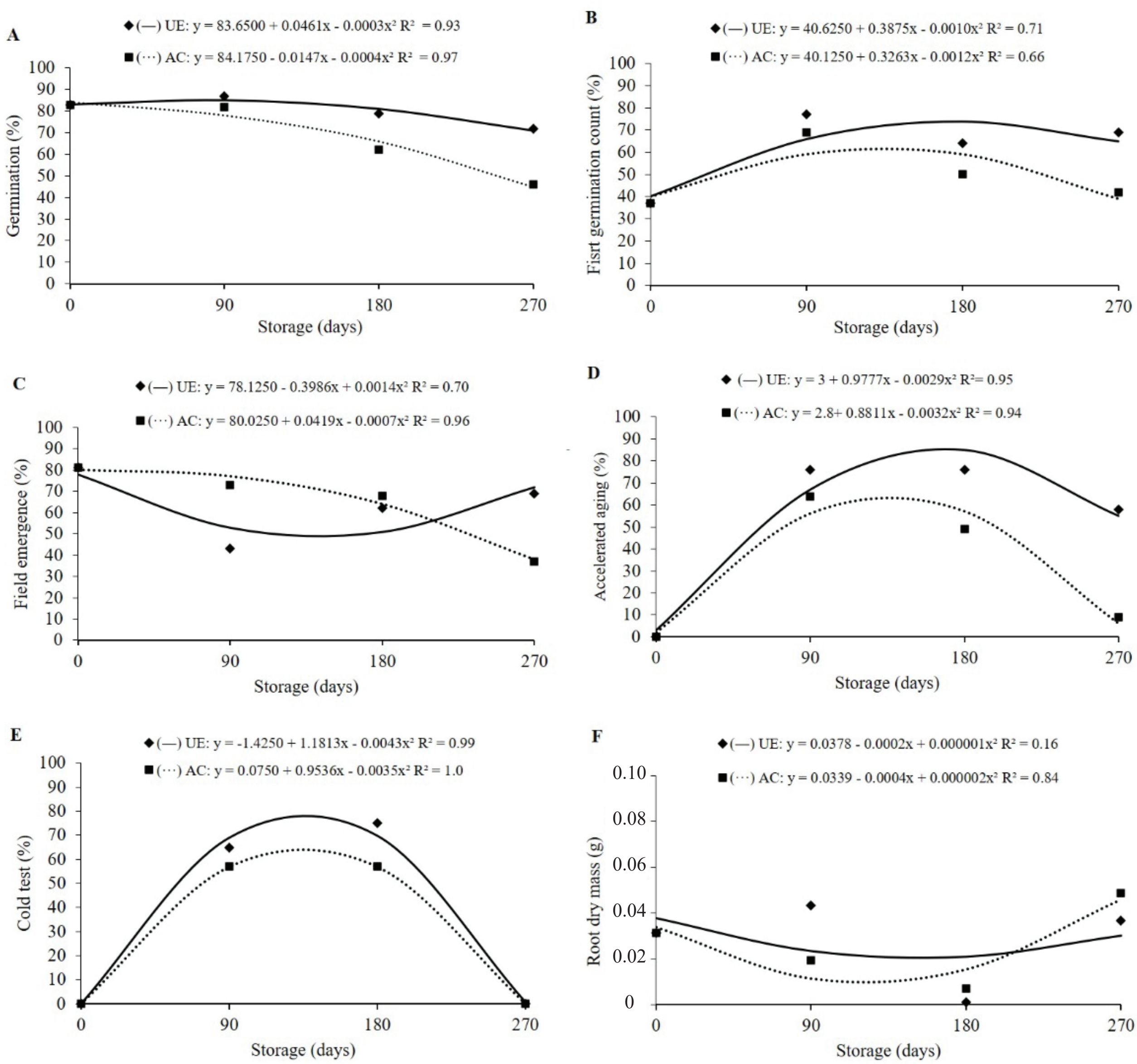

Figure 2. Germination (A), first germination count (B), field emergence (C), accelerated aging test (D), cold test (E) and root dry mass (F) of *Crotalaria spectabilis* seeds submitted to storage. (–) UE: uncontrolled environment; (···) AC: acclimatized chamber.

percentages, the sunn hemp seeds may be stored for longer in an environment with an average temperature of 25 ºC, still being suitable to be commercialized. The official recommendation of an adequate germination percentage for sunn hemp is 60 % (Brasil 2008). It is worth noting that, according to the adjusted equation, the *C. spectabilis* seeds stored under the uncontrolled environment for up to 180 days still showed an average germination of 82 % (Figure 1).

The stored seeds showed a minimum emergence in the field of 50 % at 142 days of storage under the uncontrolled environment, and a maximum emergence of 81 % at 30 days of storage in the acclimatized chamber (Figure 3C). Positive results of seed storage for both environments were also observed for seed vigor, which was determined by the accelerated aging and cold tests (Figures 2D and 2E, respectively). In the accelerated aging test, a maximum result of 85 % at 168 days of storage and of 90 % at 138 days of storage were observed for the uncontrolled environment and the acclimatized chamber, respectively (Figure 2D). In the cold test, the stored seeds showed a maximum estimated result of 80 % at 137 days of storage under the uncontrolled environment and of 65 % at 136 days in the acclimatized chamber (Figure 2E).

The similar results in both environments indicate that the physiological performance of seeds during storage was satisfactory, as they maintained the ability to produce normal seedlings. The results obtained in the present research corroborate those observed for other crotalaria species submitted to storage. *C. brevidens* seeds showed germination above 90 %, even after storage for up to two years at room temperature (Abukutsa-Onyago 2016). The vigor of *C. juncea* seeds harvested at different periods increased after eight months of storage in a controlled environment (temperature of 20 ºC and relative humidity of 50 %), what the authors attributed to the overcoming of seed dormancy, and not to the physiological potential of the seed lot (Araújo et al. 2018).

Seedlings from stored seeds of *C. spectabilis* presented minimum results of 0.0278 g and 0.0139 g for root dry matter at 100 days of storage, when stored under the uncontrolled environment and in the acclimatized chamber, respectively (Figure 2F).

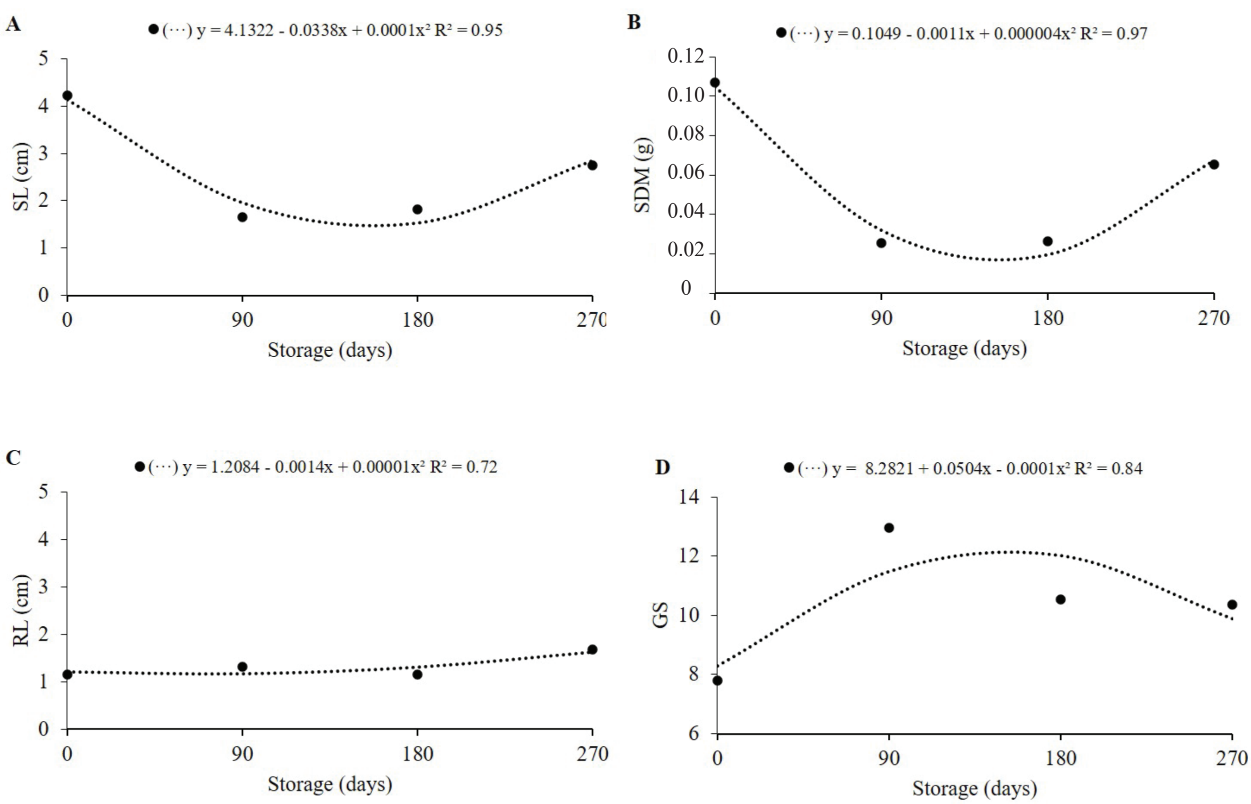

Figure 3. Shoot length (SL; A), shoot dry mass (SDM; B), root length (RL; C) and germination speed (GS; D) of *Crotalaria spectabilis* seeds submitted to storage.

The isolated effect of storage periods was significant ($p \leq 0.05$) for shoot length and dry matter and root length and germination speed, with adjustments of quadratic polynomial models (Figure 3). The isolated effect of storage environments was significant ($p \leq 0.05$) only for seed germination speed (Figure 4).

The minimum result for shoot length was 1.3 cm at 169 days (Figure 3A), while the minimum result for shoot dry matter was 0.0292 g at 137 days of storage (Figure 3B), since these characteristics are correlated. The result for minimum root length was 1.2 cm at 70 days of storage (Figure 3C). Although the seeds stored for up to approximately 150 days showed a high aptitude for seedling formation (Figure 2), the seedling growth characteristics indicate that the translocation capacity of reserves to the embryonic axis was negatively affected (Figures 3A, 3B and 3C). In this context, although the changes observed during the storage of *C. spectabilis* seeds indicate the overcoming of dormancy, the physiological changes also involved a reduction in the translocation capacity of reserves for seedling growth. Genetic analysis has shown that dormancy status and storage potential are negatively correlated, and seeds with deep dormancy have a low storage potential and vice-versa, what consists of alternative mechanisms for the species conservation (Nguyen et al. 2012, Nguyen et al. 2015, Sano et al. 2016).

Regarding germination speed, the seeds showed a maximum result from 15 to 252 days of storage (Figure 3D), while the seeds stored under the uncontrolled environment showed a greater germination speed, when compared to seeds stored in the acclimatized chamber (Figure 4). According to Baskin & Baskin (2020), the overcoming of seed dormancy during storage generally occurs faster in environments with high temperatures than in environments with low temperatures. The degradation of abscisic acid or the increase in the sensitivity of seeds to endogenous gibberellins during storage (Pritchard 2020) is conditioned by temperature and may favor seed germination.

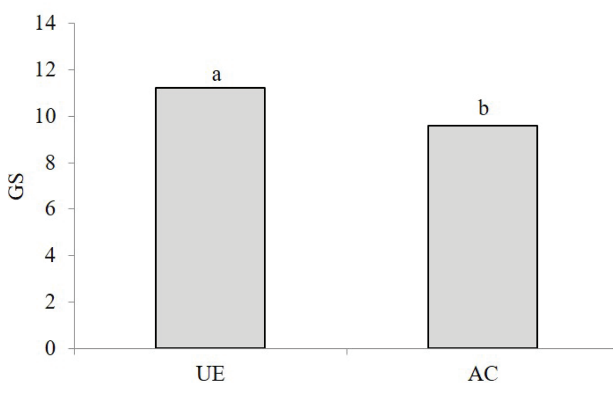

Figure 4. Germination speed (GS) of *Crotalaria spectabilis* seeds stored under uncontrolled environment (UE) and acclimatized chamber (AC). Equal letters do not differ from each other by the t test at 5 % of probability.

According to the results obtained in the tested conditions, the *C. spectabilis* seeds are initially not dormant (80 % germination, on average), although there is a gradual increase in seed germination and vigor up to approximately 150 days of storage in the cold and dry chamber or under the uncontrolled environment. Then, the seeds gradually deteriorate, but do not lose their ability to germinate up to 270 days of storage.

Taking into consideration that storage conditions may change the dormancy status of seeds, the obtained results indicate that studies on the adoption of technologies for storage of sunn hemp seeds should take into consideration the dynamics of the dormancy phenomenon in the evaluation of vigor indices, even in seed lots with high initial quality.

CONCLUSIONS

1. *Crotalaria spectabilis* seeds may be stored under uncontrolled environment (temperature of 25 ºC and relative humidity of 60 %, on average) and in acclimatized chamber (15 ºC; 45 %) without affecting their physiological quality;
2. After 150 days of storage, there is a reduction in germination and seed vigor, regardless of the storage environment. However, the seeds are still suitable for commercialization up to 180 days of storage.

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