

The cultivation environment influences the physiological quality of stored *Sonchus oleraceus* L. seeds

O ambiente de cultivo influencia na qualidade fisiológica de sementes de *Sonchus oleraceus* L. armazenadas

El ambiente de cultivo influye en la calidad fisiológica de las semillas de *Sonchus oleraceus* L. almacenadas

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Abstract

Understanding the responses of seeds to the detriment of the interaction of external factors is one of the challenges of research, especially with little explored species such as *Sonchus oleraceus* L. Thus, the objective was to investigate the effect of the cultivation system, storage time and temperature on the physiological potential of seeds of *Sonchus oleraceus* L. Seeds were used of two cultivation systems (organic and conventional) in three storage times (0, 90 and 180 days) and submitted at two temperatures (20 and 30 °C). For the evaluation of germination, the first count, germination percentage and germination speed index were determined, while root length and hypocotyl growth were determined for the evaluation of seedling growth. The first count was statistically higher than 180 days in both crops and temperatures. The seeds of conventional cultivation obtained higher germination percentage and germination

speed index at 30 °C. There was a reduction in root and hypocotyl growth at 180 days, especially at 20 °C. The cultivation environment influences the seed quality of *Sonchus oleraceus* L. during storage.

Keywords: Physiological quality; Sow thistle; Vigour.

Resumo

Compreender as respostas das sementes em detrimento da interação de fatores externos é um dos desafios das pesquisas, principalmente com espécies pouco exploradas como *Sonchus oleraceus* L. Assim, objetivou-se averiguar o efeito do sistema de cultivo, tempo de armazenamento e temperatura sobre o potencial fisiológico de sementes de *Sonchus oleraceus* L. Para isso, foram utilizadas sementes de *Sonchus oleraceus* L. de dois sistemas de cultivo (orgânico e convencional) em três tempos de armazenamento (0, 90 e 180 dias) e submetidas em duas temperaturas (20 e 30 °C). Para a avaliação da germinação foram determinadas a primeira contagem, porcentagem de germinação e índice de velocidade de germinação, enquanto para a avaliação do crescimento das plântulas determinou-se o comprimento radicular e o crescimento do hipocótilo. A Primeira contagem foi estatisticamente superior aos 180 dias em ambos os cultivos e temperaturas. As sementes do cultivo convencional obtiveram maior porcentagem de germinação e índice de velocidade de germinação na temperatura de 30 °C. Houve uma redução no crescimento radicular e do hipocótilo aos 180 dias sobretudo na temperatura de 20 °C. O ambiente de cultivo influencia na qualidade de sementes de *Sonchus oleraceus* L. durante o armazenamento.

Palavras-chave: Qualidade fisiológica; Serralha; Vigor.

Resumen

Comprender las respuestas de las semillas en detrimento de la interacción de factores externos es uno de los retos de la investigación, especialmente con especies poco exploradas como *Sonchus oleraceus* L. Así, el objetivo fue investigar el efecto del sistema de cultivo, el tiempo de almacenamiento y la temperatura sobre el potencial fisiológico de las semillas de *Sonchus oleraceus* L. Se utilizaron semillas sistemas de cultivo (ecológico y convencional) en tres tiempos de almacenamiento (0, 90 y 180 días) y sometidos a dos temperaturas (20 y 30 °C). Para la evaluación de la germinación, se determinó el primer recuento, el porcentaje de germinación y el índice de velocidad de germinación, mientras que la longitud de la raíz y el crecimiento del hipocótilo se determinaron para la evaluación del crecimiento de las plántulas. El primer recuento fue estadísticamente superior a 180 días tanto en cultivos como en temperaturas. Las semillas de cultivo convencional obtuvieron mayor porcentaje de germinación e índice de velocidad de germinación a 30 °C. Hubo una reducción en el crecimiento de raíces e hipocótilos a los 180 días, especialmente a 20 °C. El entorno de cultivo influye en la calidad de la semilla de *Sonchus oleraceus* L. durante el almacenamiento.

Palabras clave: Calidad fisiológica; Cerraja; Vigor.

1. Introduction

One of the greatest challenges today, due to climate change and population growth, is related to food production in a safe and sustainable way, preserving biodiversity and rescuing its food potential, such as unconventional vegetables. great potential for safety and diversification of diets in a more sustainable production system (Li & Siddique, 2018).

Sonchus oleraceus L. belonging to the Asteraceae family, is popularly known in Brazil as serralha and is considered an unconventional vegetable (HNC) appreciated in regional cuisines. Although its main importance in the country is as an invasive plant for large crops, it is among the most appreciated HNC for its nutritional and medicinal potential. It has high nutritional value, as a source of proteins, vitamins, minerals and essential amino acids (Viana et al., 2015; Al Juhaimi et al., 2017).

It is traditionally used in Brazilian folk medicine because it has several medicinal properties, and can be used as antioxidant, anti-inflammatory, antirheumatic and in the treatment of skin dyschromy, such as vitiligo (Vilela et al., 2009; Nonato et al., 2018). However, the lack of recognition of these species as a food source has contributed to the rapid disappearance of plants along with a wealth of traditional knowledge about their uses and cultivation (Souza et al., 2021).

An annual, herbaceous plant, with few branches and propagated by seeds, *Sonchus oleraceus* is a widely known invasive species that grows spontaneously in agricultural soils in almost all countries in the world, where it is considered a weed (Vieira & Barreto, 2006; Peerzada et al., 2019). Like other species of the Asteraceae family, the seeds of *Sonchus oleraceus* L. are of the achene type, easily dispersed by the wind, which can result in seed banks, due to their abundant production and dispersion (Sandoval et al., 2019).

Changes in both temperature and soil type can affect germination rates, induce dormancy and reduce seed vigor (Ali et al., 2020). The existence of a strong relationship between climatic variables and seed dormancy and germination indicates that predicted climate changes will inevitably affect the persistence of plant populations in different parts of the planet (Ooi, 2012).

The physiological quality of *Sonchus oleraceus* L. can be affected by the environmental conditions to which they are exposed, which may compromise the development of subsequent plants. Germination is an important process in the life cycle of plants and can affect the perpetuation of species. The viability of a seed can be modulated as a function of the domestication environment over time (Chauhan et al., 2006; Sandoval et al., 2019).

Thus, longevity, vigor and germination capacity are elements related to the biology of seeds essential to determine the seed as a species with high physiological quality. The longevity of a seed depends not only on endogenous factors intrinsic to its species, but also on factors (Araújo et al., 2022). The optimal temperature for germination varies between species, with exotic plants, such as *Sonchus oleraceus* L., whose studies are few and the responses incipient.

There are numerous reports on the seed responses of several Asteraceae species as a function of environmental factors alone. However, due to the complexity of relating more than two factors, studies that verify how the interaction of multiple factors affects the germination process are scarce. Thus, it becomes urgent to study the performance of species, especially in relation to seed production, as well as their storage capacity for a period of time (Chakraborty et al., 2020).

From this perspective and considering the nutritional potential of the species, the objective of this work was to evaluate the physiological quality of seeds of *Sonchus oleraceus* L. as a function of multiple factors.

2. Methodology

Seeds of *Sonchus oleraceus* L. were collected at the beginning of dispersal, in two growing environments (organic and conventional). The species was identified and an exsiccate is deposited in the Herbarium of the Agricultural Research Company of Minas Gerais (EPAMIG), Belo Horizonte, Minas Gerais, Brazil) under number 58897.

The experimental design used was completely randomized, where the treatments were arranged in a 3x2x2 factorial scheme, with four replications of 25 seeds. The treatments consisted of 3 storage times (0, 90 and 180 days), seeds from 2 crops (organic and conventional) and 2 temperatures (20 and 30 °C). A total of 1200 seeds were used, 400 of which were placed to germinate immediately after collection and 800 seeds were placed in plastic bags (polyethylene) and kept at a temperature of $\pm 10^{\circ}\text{C}$ for 90 and 180 days.

The seed germination test was performed by sowing in Gerbox-type boxes (on paper), using Germitest® paper as substrate, moistened 2.5 times its weight with distilled water. The seeds were kept in BOD (Biochemical Oxygen Demand) chambers, at two temperatures, 20 and 30 °C, under constant white light and a photoperiod of 16h light/8h dark, and the boxes were periodically rotated in the BOD chambers.

The physiological potential of seeds *Sonchus oleraceus* L. was estimated from the following tests: first count and germination test: percentage of seedlings germinated 4 days after sowing (%); germination speed index (GSI): following the formula of Maguire (1962) which corresponds to the number (n°) of germinated seeds or normal seedlings counted each day; germination percentage (G): according to Labouriau (1983), who verifies the number of seeds germinated from the number of seeds sown (%); hypocotyl and root growth: measurement of hypocotyl (mm) and radicle (cm) growth at the end of the experiment.

All statistical analyzes were performed using the R platform version 4.1.2. The data obtained were subjected to tests of homogeneity of variances (Levene and Bartlett, $p \geq 0.05$) and normality (Shapiro-Wilk, $p \geq 0.05$), using the “car” package

(Fox et al., 2019). The residuals that showed normality were submitted to an ANOVA analysis of variance (F test, $p \leq 0.05$) to verify the effect of the treatments and the means were compared by the Tukey test ($p \leq 0.05$), using the “ExpDes.pt” (Ferreira et al., 2021). To better approach the results, a cluster heatmap was built with the aid of the “ComplexHeatmap” package (Gu et al., 2016) and the grouping by the UPGMA (Unweighted Pair Group Method using Arithmetic averages) method.

3. Results and Discussion

There was a significant effect ($P \leq 0.05$) of the interaction between storage, temperature and cultivation system for all variables, except for Root Growth (RG) and Hypocotyl Growth (HG). An isolated effect of treatments for RG and HG was also observed.

For the first count (FC), there was a significant interaction between the treatments (Table 1). Seeds from conventional cultivation were statistically higher than those of organic cultivation at all storage times and temperatures. The seeds stored for 180 days obtained a higher percentage of normal seedlings in FC, while in the seeds stored for 0 and 90 days no differences were observed between them. Vigor is a parameter that can influence not only field performance, but also the storage potential of seeds (Silva et al., 2022).

First count is a parameter used as an indicator of vigor, as it demonstrates the percentage of normal seedlings in a given seed lot (Marcos Filho, 2015). It can be inferred that for this variable, there was a differentiation regarding the vigor of the seed lot, so that the seeds obtained from the conventional cultivation showed greater vigor than the organic ones, especially over time. The cultivation environment where the seeds are formed influences vigor and germination, due to the availability of nutrients to the mother plant during the seed maturation period (Araújo et al., 2022).

Table 1 - Breakdown of the mean test for the first germination count (FC) of *Sonchus oleraceus* L. at different storage times, temperature and cultivation.

Treatments	First Count (%)			
	Conventional		Organic	
Cultivation	20 °C	30 °C	20 °C	30 °C
Storage Time/Temperature				
0 days	7Bb ^a	44Ba ^a	0Bb ^b	10.5Ba ^b
90 days	7Bb ^a	44Ba ^a	0Bb ^b	10.5Ba ^b
180 days	58Ab ^a	83Aa ^a	16Ab ^b	53AA ^b

Means followed by different capital letters in the columns within storage time, lowercase in the lines within the temperature and superscripts in the lines between the types of cultures are differentiated from each other by the Tukey test ($P \leq 0.05$). Source: Authors (2022).

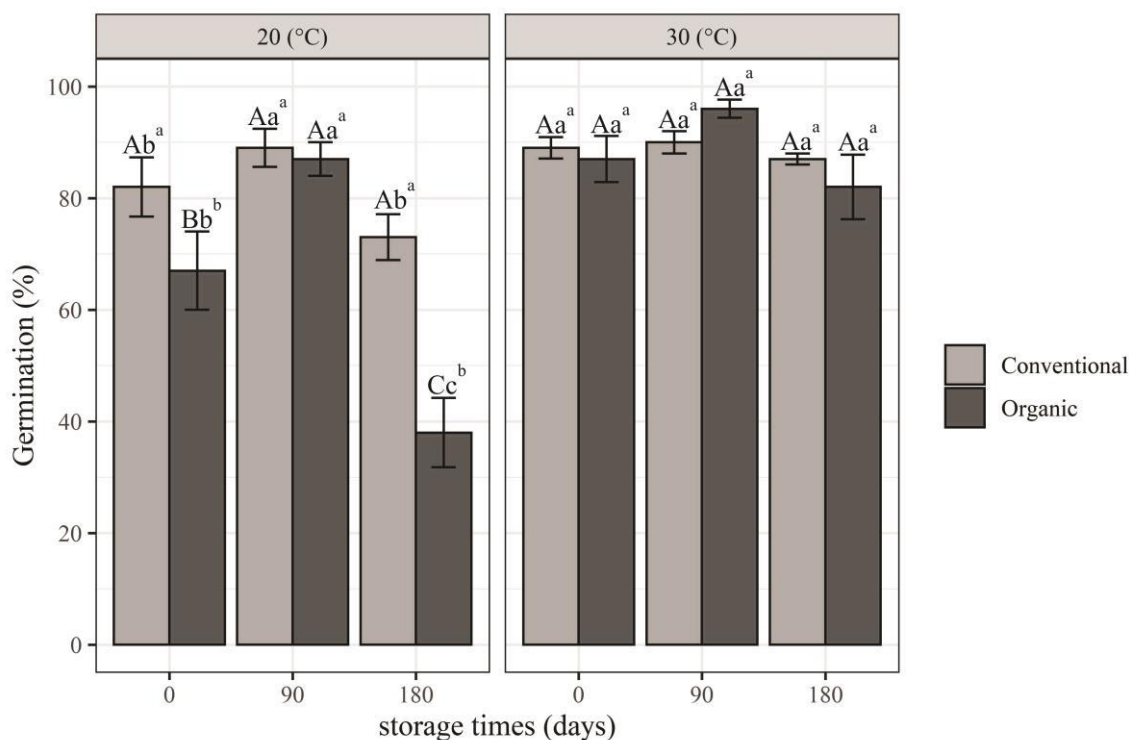
Possibly, the temperature of 20 °C influenced the low percentage of normal seedlings, because under these conditions there is a decrease in seed metabolism, delaying its initial development. At higher temperatures, as in the case studied (30 °C), there may be an increase in metabolic reactions and a faster displacement of the seed reserve tissue to the embryonic axis, accelerating the formation of normal seedlings (Araújo et al., 2022).

It can be observed that all seeds submitted to a temperature of 30 °C had a higher percentage of germination in FC. Depending on the species, some temperature ranges can affect germination. Temperature influences the translocation of reserves, and consequently, compromises the growth of the embryonic axis (Ndihokubwayo et al., 2016). During seed germination, high temperatures can delay or completely inhibit germination, depending on the species and the intensity of stress (Taiz et al., 2017). In contrast, *Sonchus oleraceus* L. showed germination potential at high temperatures, being an advantageous adaptive characteristic in conditions of fluctuating environments.

For the variable percentage of germination (G), differences and significant interaction between treatments can be

observed (Figure 1). Seeds from conventional cultivation did not differ from each other during storage times at both temperatures. On the other hand, the seeds obtained from organic cultivation showed significant differences between them, and at 90 days the percentage of germination was statistically higher, followed by 0 and 180 days with mean values of 91.5%; 77.0% and 66.0%, respectively.

Figure 1 - Average values of germination (%) of *Sonchus oleraceus* L. at different storage times, temperature and cultivation.



Means followed by different capital letters on the x axes within storage time, lowercase on the y axes within temperature and superscripts on the y axes between the types of cultivation are differentiated from each other by the Tukey test ($P \leq 0.05$). Source: Authors (2022).

It is possible to notice that although the germination percentage was high, the species shows different germination responses when submitted to adverse conditions. This may show that environmental conditions can affect seed development. The cultivation system may have directly influenced the point of physiological maturity of milkweed seeds, so that the maternal environment where *Sonchus oleraceus* L. plants developed may have influenced the variation of seed vigor (Finch-Savage & Bassel, 2015; Yuan & Wen, 2018).

Within the storage times, it was possible to verify a significant decrease in the germination percentage of the organic seeds stored for 180 days at 20 °C. Seeds from conventional cultivation were statistically superior to those from organic cultivation when exposed to a temperature of 20 °C during the germination process.

Regarding the temperatures within the storage times, it was observed that the temperature of 30 °C provided an improvement in the percentage of germination, especially when the seeds were stored for 180 days. Higher temperatures increase the speed of chemical reactions accelerating the germination process (Felix et al., 2020).

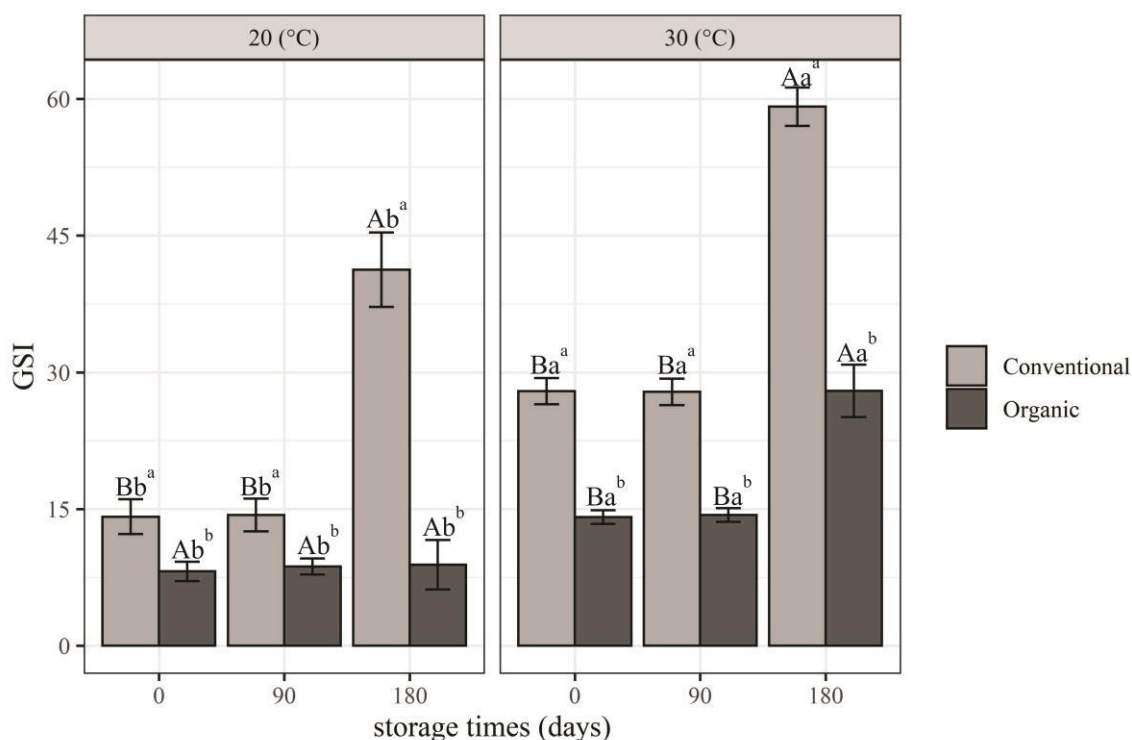
Newly harvested seeds from conventional cultivation had a higher germination rate than those from organic cultivation, when exposed to a temperature of 20 °C. When they were exposed to a temperature of 30 °C, there was no significant difference in any of the cultures. The mobilization of mother plant reserves can vary according to environmental factors, that is, the release and availability of nutrients varies with the cultivation system and this can directly influence seed

filling. In addition, lower temperatures can also slow down embryo growth (Hadi et al., 2014).

However, despite different responses, the high germination rate of the species, regardless of the factor, highlights the ability of milkweed to propagate, even in newly harvested seeds. The ability of *Sonchus oleraceus* to germinate in a wide range of temperatures at the time of maturity is probably one of the reasons why this species emerges throughout the year. Monemizadeh et al. (2021), studying the germinal responses of *Silybummarianum* (L.) Gaertn., found that the newly harvested seeds of the species did not germinate at constant temperatures or alternating in light and/or darkness. This can exemplify that the species of the Asteraceae family have a wide range of responses, making it difficult to understand the intrinsic characteristics of the family.

For Germination Speed Index (GSI), significant differences were observed between treatments, as well as between interactions. In all situations, seeds from conventional cultivation were significantly superior to those from organic (Figure 2).

Figure 2 - Mean values of Germination Speed Index (GSI) of *Sonchus oleraceus* L. at different storage times, temperature and cultivation.



Means followed by different capital letters on the x axes within storage time, lowercase on the y axes within temperature and superscripts on the y axes between the types of cultivation are differentiated from each other by the Tukey test ($P \leq 0.05$). Source: Authors (2022).

It can be seen in Figure 2 that seeds from conventional cultivation showed greater vigor regardless of temperature. The GSI of conventional seeds stored for 180 days showed an increase of 32.49% and 52.92% when compared to the storage times, respectively for temperatures of 20 °C and 30 °C. Germination speed is an intrinsic parameter, strongly associated with seed vigor and longevity. In this case, it is assumed that such seeds reached greater vigor and longevity for as long as the seeds have conserved their viability, possibly due to a greater accumulation of reserves (Sousa et al., 2021).

The temperature increase from 20 °C to 30 °C promoted an increase of 42.21%, 39.49% and 68.49% in GSI of seeds obtained from conventional cultivation, stored for 0, 90 and 180 days respectively. As for organic seeds, there was an increase in the order of 49.33%, 48.50% and 30.26% respectively for seeds stored for 0, 90 and 180 days. 30 °C obtained statistically

superior GSI at the temperature of 20 °C (Figure 2).

According to Gordin et al. (2014), the decrease in temperature tends to reduce the speed of seed germination, due to the effects on the speed of imbibition and mobilization of reserves. On the other hand, increasing the temperature up to a certain limit (optimal temperature) can make the germination process faster and more homogeneous (Silva et al., 2020).

It is noticed that the temperature of 30 °C, whose germination percentage was higher, the GSI was also higher. This may be associated with the fact that temperature is a factor that directly influences the biochemical reactions that occur in the germination process and, therefore, the speed of response was higher at higher temperature (Taiz et al., 2017).

For the variables hypocotyl growth (HG) and root growth (RG) there was a significant interaction between storage time and temperature, as shown in table 2. Regarding storage times, hypocotyl growth and root growth at 0 and 90 days were statistically higher than at 180 days. Seeds exposed to a temperature of 30 °C showed higher HG. The cultivation system did not affect hypocotyl growth, however it did affect root growth (Table 2).

Table 2 - Mean values of root growth (cm) and hypocotyl (mm) of *Sonchus oleraceus* L. as a function of different storage times, temperature and cultivation.

Treatments	Hypocotyl growth (mm)	Root growth (cm)
Storage time (S)		
0 days	41.63a	5.38a
90 days	40.88a	5.25a
180 days	10.63b	1.94b
F Test	23.72*	46.08*
Temperature (T)		
30 °C	23.25a	5.22a
20 °C	10.63b	3.15b
F Test	0.03*	38.76*
Cultivation (C)		
Conventional	31.54a	4.56a
Organic	30.08a	3.18b
F Test	0.26ns	5.03*

Means followed by different lowercase letters in the columns within each treatment are differentiated from each other by the Tukey test ($P \leq 0.05$). (*) significant at 5% probability and (ns) not significant, in relation to the F test. Source: Authors (2022).

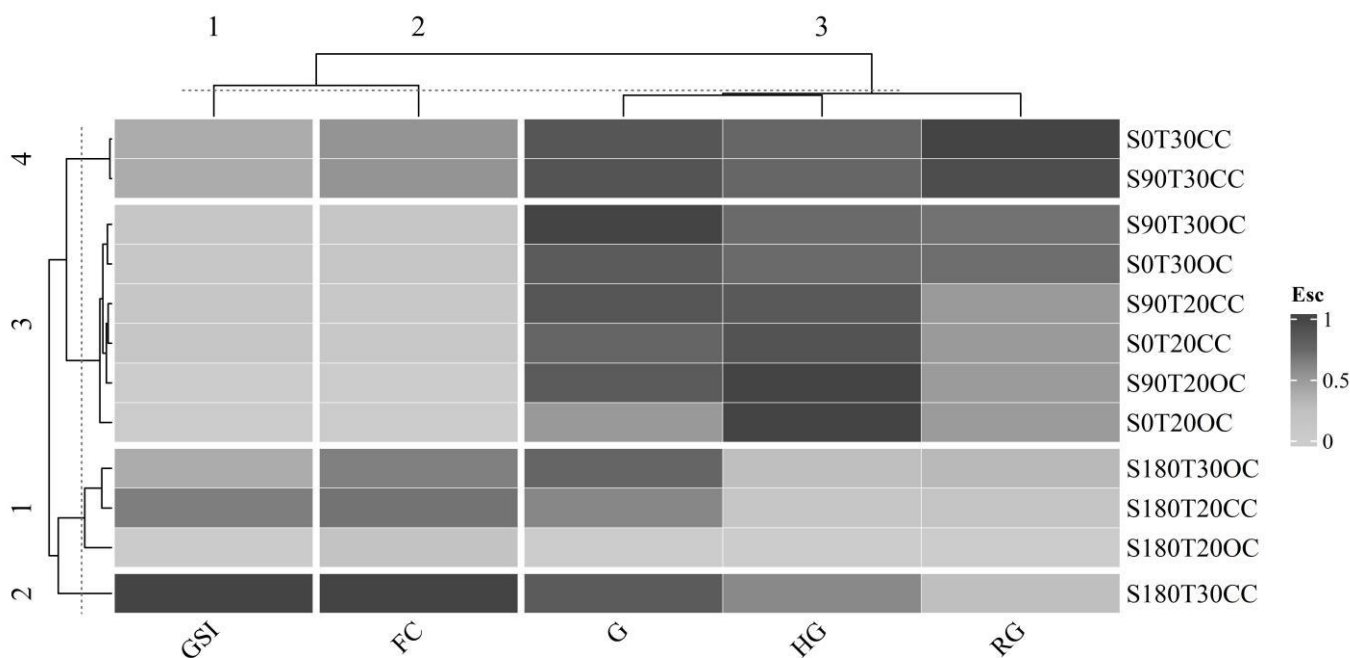
Differences found in HG and RG may be related to differences in seed vigor. Such a decrease observed at 180 days may be related to a reduction in the capacity to use and mobilize the reserves, which can lead to uneven growth of seedlings (Silva et al., 2022). Storage reduces seed vigor since its function is only of preservation by reducing metabolism and not of improving seed quality (Silva et al., 2019).

The RG when compared to HG corroborates results found in the literature (Gomaa et al., 2014; Silva et al., 2022). This can be explained by the fact that the radicle is more in contact with the substrate than the hypocotyl. On the other hand, a significant reduction in both hypocotyl growth and root growth can be seen in both cultures at 180 days. This decrease in seedling growth may be related to the supply of reserves in the seeds having been used in the germination process (Felix et al., 2020).

The formation of four clusters was detected by heat analysis (Figure 3), with a cophenetic correlation coefficient of 92%, demonstrating that there is a dissimilarity between the samples. The identification of groups is important to identify which condition can maintain the physiological quality of *Sonchus oleraceus* L seeds. Group 3 comprised half of the treatments, being characterized by most of the highest (G and HG) and lowest (FC and GSI) values for most traits, except for

RG. Furthermore, it was possible to observe in this group a greater similarity between the grouped samples (Figure 3).

Figure 3 - Heat map with Euclidean distance dendrogram by UPGMA method for physiological quality of *Sonchus oleraceus* L. seeds at different storage times, temperature and cultivation. The acronyms below the heat map represent the treatments and variables: S (Storage – 0, 90 and 180 days); T (Temperature 30 and 20 °C); CC (Conventional Cultivation); OC (Organic Cultivation); FC (First Count); G (Percentage of Germination); GSI (Germination Speed Index); RG (Root Growth); HG (Hypocotyl Growth).



Source: Authors (2022).

It is also possible to observe a grouping between variables that for FC and GSI the highest values were found in conventional seeds, under a temperature of 30 °C, at 180 days. For RG, the highest values were also observed in seeds of conventional cultivation at 30 °C at 0 days. The variables G and HG were the ones that obtained the highest values grouped in a cluster.

Seeds of *Sonchus oleraceus* L. have a high germination potential in a range of conditions, which shows their enormous potential for cultivation and, consequently, their use as food and medicinal plant in conditions of high temperatures. However, the physiological quality of the seeds was affected by the interaction between the cultivation system and temperature. In addition, the interaction between these factors may suggest that the species may present variations, indicating a sensitivity in maintaining the viability of its seeds over time (Chakraborty et al., 2020). Thus, more research is needed on the influence of environmental factors in order to maintain or minimize the loss of physiological quality of *Sonchus oleraceus* L.

4. Conclusion

The interaction between temperature and cultivation system influences the physiological quality of *Sonchus oleraceus* L. seeds during storage. Seeds from conventional cultivation had higher vigor at a temperature of 30 °C when stored for 180 days. *Sonchus oleraceus* L. have potential for cultivation at high temperatures.

The study highlights the importance of environmental factors and their influence on the physiological quality of

Sonchus oleraceus L. seeds during storage. New studies investigating the effects of climate change on the physiological quality of *Sonchus oleraceus* L. seeds may provide information that will help to mitigate the effect of climatic variations on this species over time.

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