

Study of storage on seed germination of *Chresta sphaerocephala* DC. – Asteraceae

Estudo do armazenamento na germinação de sementes de *Chresta sphaerocephala* DC. – Asteraceae

Estudio del almacenamiento en la germinación de semillas de *Chresta sphaerocephala* DC. –

Asteraceae

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Abstract

High-altitude fields have been subjected to an intense anthropization process. Due to this, studies on germination, seed storage and propagation of species that compose this plant formation become fundamental. Among such species is the *Chresta sphaerocephala* DC., Asteraceae. The purpose of this work was to study different temperatures for germination and storage of this species. Four germination temperatures (15°C, 20°C, 25°C and 30°C) and two storage temperatures (-5°C and +5°C) were tested. In order to assess seed vigor, the germination speed index (GSI) and average seed germination time were calculated. ANOVA and Tukey's test were performed at a 5% significance level. In addition, a morphological difference was observed in the analyzed *Chresta sphaerocephala* seeds, with these being classified as C1 (small seeds), C2 (long thin seeds), C3 (thicker seeds) and C4 (dark colored seeds). As for the storage temperature, 5°C was shown to be the best. When correlated with the germination temperature, 20°C resulted in a statistically higher number of germinated seeds.

Keywords: Seed analysis; High-altitude fields; Southern Minas Gerais.

Resumo

Os campos de altitude vêm sofrendo intenso processo de antropização, desta forma, tornam-se fundamentais os estudos sobre germinação, armazenamento de sementes e propagação das espécies que compõem tal formação vegetal. Entre estas, destaca-se *Chresta sphaerocephala* DC., Asteraceae. O objetivo deste trabalho foi estudar diferentes temperaturas para germinação e armazenamento desta espécie. Foram testadas quatro temperaturas de germinação (15°C, 20°C, 25°C e 30°C) e duas de armazenamento (-5°C e +5°C). Afim de avaliar o vigor das sementes foi calculado o índice de velocidade germinação (IVG) e o tempo médio de germinação das sementes. Fez-se ANOVA e o teste do Tukey ao nível de 5% de significância. Além disso, percebeu-se diferença morfológica nas sementes de *Chresta sphaerocephala* analisadas, sendo as mesmas classificadas como C1 (sementes pequenas), C2 (sementes finas e compridas), C3 (sementes mais espessas) e C4 (sementes de coloração escura). Quanto à temperatura de armazenamento, 5°C mostrou-se como a melhor. Ao correlacionar com a temperatura de germinação, 20°C resultou num número estatisticamente superior de sementes germinadas.

Palavras-chave: Análise de sementes; Campos de altitude; Sul de Minas Gerais.

Resumen

Los campos de altura han estado experimentando un intenso proceso de antropización, por lo que los estudios sobre germinación, almacenamiento de semillas y propagación de las especies que componen dicha formación vegetal se han vuelto fundamentales. Entre estos, destaca *Chresta sphaerocephala* DC., Asteraceae. El objetivo de este trabajo fue estudiar diferentes temperaturas para la germinación y almacenamiento de esta especie. Se probaron cuatro temperaturas de germinación (15 ° C, 20 ° C, 25 ° C y 30 ° C) y dos temperaturas de almacenamiento (-5 ° C y + 5 ° C). Para evaluar el vigor de la semilla se calculó el índice de velocidad de germinación (IVG) y el tiempo promedio de germinación de la semilla. El ANOVA y la prueba de Tukey se realizaron al 5% de nivel de significancia. Además, se notó una diferencia morfológica en las semillas de *Chresta sphaerocephala* analizadas, siendo las mismas clasificadas como C1 (semillas pequeñas), C2 (semillas largas y delgadas), C3 (semillas más gruesas) y C4 (semillas de color oscuro). En cuanto a la

temperatura de almacenamiento, 5 ° C resultó ser la mejor. Cuando se correlacionó con la temperatura de germinación, 20 ° C resultó en un número estadísticamente mayor de semillas germinadas.

Palabras clave: Análisis de semillas; Campos de altitud; Sur de Minas Gerais.

1. Introduction

High-altitude fields are fragile ecosystems, classified as a phytophysiology of the Atlantic Forest biome. They are susceptible to the elements and are associated with major degradations due to human action.

The Poços de Caldas Municipality has an average altitude of 1.186 m (Pereira & Fontes, 2009), is located in the Atlantic Forest biome and has high-altitude fields as one of its native plant formations. It is located in the microregion of the south of the State of Minas Gerais, being one of the most important socioeconomic hubs of the region, and is connected to the industrial, agricultural and mining sectors (Pereira & Fontes, 2009).

The Poços de Caldas Plateau has degraded areas caused by years of bauxite mining. After the exploitation process occurs, in these areas, there is usually eucalyptus cultivation so as to obtain profits in the revegetation process after the mining activity. Among eighteen works analyzed by Almeida et al. (2019), five of them used eucalyptus in the process of recovering degraded areas, in addition, the same author concludes that the use of species from the Asteraceae family promoted a greater capacity for vegetation cover.

For native areas, not anthropized before the mining activity, the law requests that recovery is done using the typical vegetation of high-altitude fields, that is, herbaceous and shrub species.

In general, there are insufficient studies on germination conditions, seed dormancy and forms of propagation. To date, high-altitude field species *Hippeastrum vittatum* (L'Hér) Herb., *Southbya organensis* Herzog, *Chaptalia hermogenis* M.D. Moraes, *Berberis campos-portoi* Brade, *Gaylussacia pruinosa* Loes, *Gaylussacia retivenia* Sleumer, *Sinningia cochlearis* (Hook.) Chautems, *Oxalis arachnoidea* Progel, *Achetaria caparaoense* (Brade) V.C. Souza, *Aristida constricta* Longhi-Wagner, *Galium rubidiflorum* Dempster, *Xyris hatschbachii* L.B.SM. & Downs, *Xyris rigida* Kunth classified as "critically endangered", i.e. they run an extremely high risk of extinction in nature (CNFLORA, 2013), are not mentioned in CONAMA Resolution No. 423/2010 (Brasil, 2010), only the *Tillandsia reclinata* E. Pereira & Martinelli, *Agrostis ramboi* Parodi, *Valeriana organensis* Gardner species are mentioned in the Resolution, which provides basic parameters for identifying and analyzing the primary vegetation and the successional stages of the secondary vegetation at high-altitude fields, associated with or encompassed by the Atlantic Forest

This study is expected to contribute to identifying the most suitable temperature for germinating and storing *Chresta sphaerocephala* DC. seeds, promoting their propagation and use in the recomposition of high-altitude fields. Moreover, studies on germination are necessary to ensure the production of healthy seedlings, for use in conservation projects and recovery of areas and species, and several factors can influence the germination process, such as temperature, light, humidity, among others (Dantas et al., 2021; Löbler et al., 2016).

2. Methodology

2.1 Seed collection and storage

In this study, the term cypsela was considered a synonym of seed, in accordance with Cury, Novembre and Gloria (2010). In addition, the *Chresta sphaerocephala* seeds showed varied morphologies, and thus were classified as C1 (small seeds), C2 (long thin seeds), C3 (thicker seeds) and C4 (dark colored seeds).

The *Chresta sphaerocephala* seeds were collected at high-altitude fields in the municipality of Poços de Caldas, located in the west and south. Collections were done in September, 2017. Afterwards, the cypselae were stored at the Poços de Caldas

Botanical Garden at 5°C and -5°C temperatures for eight months.

2.2 Procedures

Initially, the moisture content of the seeds was obtained through the 105°C±3°C Greenhouse Method during 24h (Brasil, 2009). Next, 1600 *Chresta sphaerocephala* seeds were selected to conduct the germination experiment, with 800 seeds stored at 5°C and 800 stored at -5°C.

For both storage temperatures, the procedures described in this essay were performed in the same manner. Hands, tools and workbench were disinfected with 70°GL ethyl alcohol to prevent contaminations. The chosen substrate was square filter paper, being placed in gerbox type germination boxes (Godinho, Mantovani-Alvarenga & Faria, 2011).

A beneficiation treatment had to be applied, removing the feathery papus from the cypselae with the aid of scissors (Velten & Garcia, 2005). After, the seeds were disinfected by being placed in plastic containers with 50 seeds each, soaking in bleach with 2.5% active chlorine percentage for five minutes and then rinsed with the aid of a sieve, washing them under running water (Botezelli, Davide & Malavasi, 2000). The gerboxes were washed with water and detergent, and then disinfected with bleach with 2.5% active chloride percentage and rinsed under running water. The filter papers were sterilized in a Panasonic brand, Style NN-ST654W model, 900 W power microwave, four units at a time, during one minute.

Each set of 800 seeds was divided into four groups of 200 seeds, referring to the four different germination temperatures: 15°C, 20°C, 25°C and 30°C. The 200 seeds were divided into four repetitions of 50 seeds each (Brasil, 2009), identified with their access number, storage temperature (ST), germination temperature (GT) and start date and repetition number (R1, R2, R3, R4). The seeds were placed in the gerboxes with the filter paper, in a 7x7 matrix plus one seed placed on the side. This placement allowed for a more suitable distance between the seeds, in addition to making it easier to count them.

Each set was put in BOD germinators, eight gerboxes per germination temperature, for both storage temperatures. In addition, the incubator drawers were filled with distilled water so that their internal humidity was maintained (Cury et al., 2010; Godinho et al., 2011). The seeds were monitored daily, in order to count the germinated seeds and moisten the filter paper, when required. Seeds that showed a minimum protrusion of 1mm of radicle were considered germinated.

2.3 Analyses

The moisture percentage of the seeds was calculated by applying the following expression:

$$U = \frac{P1-P2}{P1} \times 100 \quad (1)$$

Where,

U is the moisture percentage of the sample, P1 the weight of moist seeds, P2 the weight of dry seeds.

As the test was conducted in triplicate, the moisture percentage values were averaged for each storage temperature.

The germination speed index (IVG) is used to determine relative seed vigor and analyzes the speed of seedling emergence in the germination process. To calculate the IVG, the method proposed by Maguire in 1962 was used (Lopes, 1990).

$$IVG = \frac{NP_1}{ND_1} + \frac{NP_2}{ND_2} + \frac{NP_3}{ND_3} \dots \frac{NP_n}{ND_n} \quad (2)$$

Where,

$NP_1, NP_2, NP_3 \dots NP_n$ = number of emerged seedlings in the first, second, ..., and last counting day;

$ND_1, ND_2, ND_3 \dots ND_n$ = number of days elapsed from sowing to the first, second, and last count.

To calculate the average germination time (TMG), the following formula was used (Cetnarski Filho & Carvalho, 2009; Walters, 1998).

$$TMG = \frac{\sum (t*n)}{\sum n} \quad (3)$$

Where, n = number of germinated seeds and t = germination time in days. Unit: days.

And to calculate the average germination speed (VMG) (Cetnarski Filho & Carvalho, 2009):

$$VMG = \frac{1}{TMG} \quad (4)$$

Where, TMG = mean germination time in days. Unit: $days^{-1}$.

For the statistical analyses of the experiment, four repetitions were considered for each set of the experiments. The germination data in percentage were transformed using the formula $\sqrt{\frac{x}{100}}$. For data analysis, the Analysis of Variance (ANOVA) was used with two factors, namely: germination temperature and storage temperature. If the ANOVA was significant to one of the factors, the Tukey's test was conducted to detect where this difference was. As described by Ferraz et al. (2020), all tests were conducted using the 5% significance level and Sisvar 5.6 software (Ferreira, 2010).

3. Results and Discussion

3.1 Germination and morphology of *Chresta sphaerocephala* seeds

Among the 1600 *Chresta sphaerocephala* seeds analyzed, 1,304 seeds did not germinate, 105 seeds died, mainly due to fungi, during observations, and only 191 seeds germinated, totaling a germination rate of 14.5% (Table 1). This low germination rate can be explained due to the requirement of specific environmental conditions demanded by the species (Godinho et al., 2011).

Table 1. Amount of *Chresta sphaerocephala* seeds that did not germinate, that did germinate, and that died, described in details, for each storage and germination temperature.

Storage Temperature	Non-Germinated Seeds	Germinated Seeds	Dead Seeds	Germination Temperature
+5°C	149	37	14	15°C
	157	37	6	20°C
	154	32	14	25°C
	180	9	11	30°C
-5°C	155	17	28	15°C
	173	14	13	20°C
	168	27	5	25°C
	168	18	14	30°C

Source: Authors.

According to Velten and Garcia (2005), low germination happens frequently in the Asteraceae family, and this is a reflection of the high production of seeds without embryos or with non-viable embryos and low percentages of fertile achenes.

When checking the 191 germinated seeds, it was found that seeds from the C4 group were not healthy seeds and had a dark color on the cypsela. This color may be attributed to some type of fungus in the endocarp, as 59 germinated seeds, according

to the 1mm radicle protrusion, began showing signs of fungus attack; the 4g/L solution Amstar fungicide was applied, but it was not proven effective with regard to the seeds in group C4, which showed softening and death. Another factor noted was that the seeds stored at the -5°C temperature had greater amounts of seeds with this dark color, i.e. the low temperature may have influenced this process.

Analyzing the C2 seed group, these were not viable as there was no germination of these seeds. These may have insufficient amounts of reserves or, in accordance with the classification of Maluf & Wizenier (1998), seeds of the *Eupatorium vauthierianum* DC. species – also Asteraceae, that did not germinate and remained intact were considered dormant, thus, considered dormant according to this classification.

Lastly, the G3 seed group was proven to be viable, as most of the germinated seeds were from this group. Among the 132 seeds, only 94 germinated seeds appeared to be healthy and with at least one pair of leaves. A reminder that the C1 group was not tested in this study.

Among the 38 seeds that germinated, but did not appear to be healthy, a few anomalies found can be mentioned. Firstly, some seeds that showed the 1mm radicle protrusion were not able to develop, and this protrusion was detached from the seed. Additionally, the comparative study by Cury et al. (2010) also observed that 0.6% of *Chresta sphaerocephala* seeds had embryo malformation.

Within the C3 group, it was noticed that some germinated seeds had, instead of a pair of leaves, a trio of leaves or even just one leaf; the latter proved to be non-viable. In addition, some of those seeds mentioned above did not have good radicle development, with the radicle being too small, and then the first pair of leaves or trio of leaves already appeared, so that the radicle did not support it, and this resulted in a separation of the leaves.

Pereira, Von Pinho, Oliveira and Kikuti (2002) proposed that seed germination along with the elongation of roots and stems could be negatively impacted according to growth inhibitors, phenolic chemicals, with such chemicals being present in the seed integuments. Martinotto et al. (2007) correlated the presence of these phenolic chemicals with the malformation of seedlings of the *Eugenia dysenterica* DC. species, as after removing their integuments, the seeds germinated more evenly and quickly, but when they were germinated *in vitro* with the presence of the integument, they had a high occurrence of malformed seedlings.

3.2 Germination potential of *Chresta sphaerocephala* seeds

With the germination values obtained, the germination speed index (IVG), the average germination time and the average germination speed for both storage temperatures were calculated (Tables 2, 3 and 4). According to the values described in Table 2, the storage temperature +5°C had the highest IVG values

Table 2. Germination speed index (%) and average germination time values for *Chresta sphaerocephala* seeds, for the four germination temperatures analyzed in relation to the two storage temperatures.

Storage Temperature	Germination Temperature	Germination Speed Index (% germination)
+5°C	15°C	9.31
	20°C	11.13
	25°C	10.54
	30°C	5.95
-5°C	15°C	6.00
	20°C	6.68
	25°C	8.39
	30°C	6.04

Source: Authors.

Table 3. Mean germination time (days) of *Chresta sphaerocephala* seeds submitted to different temperatures.

Storage Temperature	Germination Temperature (°C)			
	15	20	25	30
+5°C	1,75a2	1,32a1a2	0,97a1a2	0,47a1
-5°C	0,99 a1	0,52 a1	0,67 a1	0,40 a1
C.V = 57,40%				

In each line, means followed by the same number do not differ according to Tukey's test ($P > 0.05$); CV: coefficient of variation. Source: Authors.

The mean germination time was influenced according to storage temperature. For seeds stored at +5°C, the TMG values decrease with increasing temperature, but this same behavior was not observed for the storage temperature -5°C. In addition, the mean germination time values do not differ for the germination temperatures 20°C and 25°C, but for the temperatures of 15°C and 30°C, both differ from each other and in relation to the other temperatures, with the shortest average germination time being obtained by the germination temperature of 30°C.

Table 4. Average germination speed (day⁻¹) of *Chresta sphaerocephala* seeds submitted to different temperatures.

Storage Temperature	Germination Temperature (°C)			
	15	20	25	30
+5°C	0,70a1	0,92a1	1,64a1	3,27a1
-5°C	1,33a1	2,10a1	1,52a1	1,70a1
C.V = 81,40%				

In each line, means followed by the same number do not differ according to Tukey's test ($P > 0.05$); CV: coefficient of variation. Source: Authors.

The mean germination rate does not differ for storage temperatures and temperatures for germination.

After, the data mentioned in Table 2 were analyzed by the ANOVA. The P-Value column was observed, which shows the probability of obtaining a statistical test value higher or lower than 0.05, which is the significance level of the study (Ferreira

& Patino, 2015). Thus, within the interaction between the storage temperature and germination temperature and only the germination temperature, there was no significance (Table 5).

Table 5. Analysis of Variance of the germination speed index as a function of the storage temperature, germination temperature and the relation between both. DF: degree of freedom; MS: mean squares.

Causes of Variation	DF	MS	P-Value
Storage Temperature	1	48.2452	0.0272*
Germination Temperature	3	18.9919	0.1170 ^{NS}
Storage T. x Germination T.	3	7.5237	0.4740 ^{NS}
Error	24	8.7239	-

NS-non-significant; *-significant to 5% probability. Source: Authors.

As the interaction between both factors was not significant, it may be suggested that the levels of one factor do not interfere with the levels of the other factor. Only the storage temperature showed significance, as the P-Value is lower than 0.05. The second statistical treatment, Tukey's test, was conducted to make it possible to know which levels were actually differentiating from the rest. As the factor only has two temperature levels, the one that has a higher IVG average will be considered the better temperature. Thus, the most suitable storage temperature was +5°C, it had the highest IVG average (Table 6).

Table 6. Percentage averages of the germination speed index of *Chresta sphaerocephala* seeds, for the -5°C and 5°C storage temperatures and for the 15°C, 20°C, 25°C and 30°C germination temperatures, using the Tukey's Test.

Storage Temperature	Average IVG (%)	Germination Temperature	Average GSI (%)
-5°C	6.79 a1	15°C	5.99 a1
+5°C	9.23 a2	20°C	7.65 a1
		25°C	8.90 a1
		30°C	9.47 a1
C.V. (%) = 36.89			

In each column, averages followed by the same number do not differ from each other, by Tukey's test ($P > 0.05$); C.V.: coefficient of variation. Source: Authors.

According to Cury et al. (2010), the best germination temperatures for *Chresta sphaerocephala* seeds are in the 20-35°C range, after being stored for 62 days in a refrigerated chamber at 20°C. Compared to the results obtained in this research, this temperature range proved to be efficient for seeds that were store at -5°C for a period of 8 months.

As there was no significant interaction between the germination temperature and storage temperature, any combination between the levels of these two factors can be used for seed germination. For long storage periods, according to the results obtained in this study, the most suitable temperature for storing *Chresta sphaerocephala* seeds is + 5°C. For the other analyzed factor, the germination temperature, statistically, the 15°C, 20°C, 25°C and 30°C temperatures did not differ from each other.

It is worth highlighting that, for another species of the Asteraceae family, *Eupatorium vauthierianum* DC., the 20°C temperature was the best for germination (Maluf & Wizenier, 1998). The same author, Maluf (1993), studied the effect of temperature and light on the germination of seeds of two populations of *Vernonia polyanthes* (Asteraceae), and for this species

the most efficient germination temperature was also 20°C. A similar result was described by Cássero, Pastorini & Souza (2018) when studying a species of the Asteraceae family. Despite the 20°C germination temperature statistically not influencing the GSI values, it had the second highest number of germinated seeds per day. A possible indication of correlation of the 20°C germination temperature with tropical herbaceous species of the Asteraceae family is therefore suggested.

The study by Gomes & Fernandes (2002), with *Baccharis dracunculifolia*, found the best germination index and fastest germination values in the 15°C and 20°C temperatures. This fact was observed in the *Chresta sphaerocephala* seeds, considering that, at the same germination temperatures tested, they produced the highest values of germinated seeds per day.

In a study by Cury et al. (2010), 1500 *Chresta sphaerocephala* seeds were stored at a 20°C temperature for a period of 67 days, and 1456 seeds did not germinate. In the present study, 1600 seeds were selected at two storage temperatures for a period longer than 67 days, and similarly there were 1304 non-germinated *Chresta sphaerocephala* seeds. Thus, it can be said that, based on the drop in seed viability, *Chresta sphaerocephala* does not support any of the storage conditions, this low germinability is also associated with the Asteraceae family (Cássero, Pastorini & Souza, 2018). Another factor that may be influencing the results of post-storage germination of *Chresta sphaerocephala* seeds could be their different morphologies.

4. Conclusion

The germination temperature matches the temperatures found at high-altitude fields, as the average temperature in Poços de Caldas is 17°C, which promotes the germination of *Chresta sphaerocephala* seeds. It is suggested that *Chresta sphaerocephala* seeds require more studies, especially on the physiology of their different morphologies, further studies on their genetics, and comparisons of germination rates of different groups C1, C2, C3 and C4. By aggregating this information, the germination rate of the species can be increased, thus contributing to the production of a higher number of seedlings and their use in recovering high-altitude field areas.

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