# Seed Propagation, Adaptation to Cultivation Conditions, Determination of Ornamental Plant Properties, and *Ex Situ* Conservation of the Endemic Species *Centaurea hermannii* F. Hermann

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This study focused on seed propagation and *ex situ* conservation of the endemic species *Centaurea hermannii* F. Hermann. Plant properties such as vegetation cycle, adaptation to the cultivation environment, morphological characteristics of the plants and seeds, and esthetic properties as an ornamental variety were investigated. The percentage of viability of the seeds, as well as the effects of different seed germination methods when applied to the germination speed, were also explored. *C. hermannii* specimens planted in soil adapted quickly under field conditions and flowered over a period of two months, with a bright-orange color and glossy blossoms. In addition, the plants displayed all the type-specific botanical and esthetic properties, as well as provided value as an ornamental plant. An *ex situ* conservation area was created with the plants collected. To determine the best germination conditions for *C. hermannii* seeds, various methods were explored. Among these, a combination of 200 ppm of $GA_3$ treatment during a three-month storage period at 4 °C, followed by cold-wet stratification at 4 °C for three months, produced the best results in terms of mean percentage germination (70.5 %) and mean germination speed (3 days).

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## INTRODUCTION

Destruction of the natural environment, a rapid increase in the global population, unsustainable use of plant resources, and growing pollution in many different parts of the world have led to the threat of extinction of a large number of plant species by the end of the century (López-Pujol *et al.* 2006; Okay *et al*. 2011; Convention on Biological Diversity (CBD) 2018; Kırmızı *et al.* 2019). Therefore, supporting biodiversity has become an essential factor in conservation and sustainability. In addition, the recognition that biodiversity is a source of wealth has grown in the vast majority of countries around the world (Avcı 2008). Efforts to protect biodiversity and adopt sustainable measures have begun to attract the world’s attention (Carwardine *et al.* 2018; Locke *et al*. 2019). The Rio convention, on biological diversity, encourages all parties to the convention to recognize biological diversity and to take necessary measures to protect it (Ocak *et al*. 2017). Therefore, a variety of *in situ* and *ex situ* conservation methods have been explored to ensure the sustainability of biodiversity and to protect species in danger of extinction

(Bürün 2021).

Propagating plants from seeds is an inexpensive and practical method for *ex situ* conservation of endangered plant species. One of the principal issues in the effective protection of endangered species is to adopt the necessary measures for the reproduction of these species (Okay *et al.* 2011). In addition to being a crucial propagation stage of seeds, germination is the first step in the vegetative phase of plants and is the priority concern for the recovery and sustainability of endangered species (Schnadelbach *et al.* 2016; Ganatsas *et al.* 2019; Kırmızı 2019; Erken 2021). Knowledge of both the germination percentage and germination period for existing plant seeds is directly related to the success of propagation techniques. Thus, determination of the most appropriate methods, in addition to specifying the germination properties of individual plant species, is critical for carrying out research in this area (Cesur *et al.* 2017; Uskutoğlu and Şenkal 2019).

The successful propagation of most plants requires the use of appropriate methods in the right environments. In this regard, natural plant-based designs have become increasingly popular in many countries, instead of using costly and unsustainable plans that do not adapt to a region's ecology, climate, or natural structure. Governments agree on various regulations to encourage the utilization of natural plant species (Sağlam and Önder 2018). Natural plants are a target market for arboriculture producers and commercial dealers due to their environmental benefits (Anderson *et al.* 2021). However, the use of natural plants in horticultural designs is only possible if the properties of the individual species and their production methods are well known (Mikkelsen 1986; Von Henting 1998). According to Noroozi *et al.* (2019), endemism is an important criterion for the conservation of biodiversity both at the national and global level. Therefore, using endemic species as natural plants in horticultural designs makes leads to attractive results.

The genus *Centaurea*, which includes various producible and usable endemic species, is also remarkable. Turkey is the main center of species diversity for the *Centaurea* L.; the *Centaurea* genus is the third largest genus in Turkey. There are 217 species (146 endemic), 36 sub-species (22 endemic), and 28 varieties (16 endemic), and its percent endemism is 66.8% (Wagenitz 1975; Özhatay *et al.* 2011; Bona 2013, 2014).

*Centaurea hermannii* is a Euxine endemic species distributed in the northwest region of Turkey (Gürdal and Özhatay 2011). Bilz *et al.* (2011) noted that *C. hermannii* is an endemic species in Turkey and Bulgaria. While Ozhatay and Keskin (2007) and Eroglu *et al.* (2014) reported that the species is found only on the Çatalca Peninsula and Ömerli basin in Istanbul, Turkey. Uğurtaş *et al.* (2014) also located the species in the Delmece plateau of Yalova province. In general, the *Centaurea hermannii* F. Hermann is a simple, perennial, and vertical-growing herbaceous plant with a height of 30 to 60 cm. Its flowers are yellow-orange in color, and it flowers from June to July. *Centaurea hermannii* is an endemic species and grows in scrubs (maquis) and oak forests at altitudes of 100 to 500 meters (Yaltırık and Efe 1989; Akkemik 2017).

Özdemir and Ulus (2018) reported that some *Centaurea* species, such as *Centaurea consanguinea*, *Centaurea hermannii*, and *Centaurea kilaea*, are highly suitable for pollination yards, which are critical in supporting the decreasing population of pollinators, especially in urban areas, and for supporting urban biodiversity. Furthermore, Yankova-Tsvetkova *et al.* (2018) found that the distributions of some species of the genus Centaurea are considerably restricted and are represented by a single population. In this case, if there is habitat damage and population fragmentation, the species will be in danger of extinction. Its seeds could be infested with insects, and the percent germination of endemic species reduced, limiting their ability to regenerate, spread, and reproduce. However, Tel *et al.*

(2019) suggested that the species *C. hermannii* possesses the right characteristics to become a potential ornamental plant.

The issues mentioned above make research on propagation necessary the genus *Centaurea*. This study discussed the endemic *C. hermannii* taxon, which is among the endangered (EN) species based on International Union for Conservation of Nature (IUCN) (2014) data. This study concentrated on determining the most appropriate method for propagation of *C. hermannii* from seeds under incubation conditions. The plants resulting from this process were planted either in pots or in soil in the research yard and monitored. Attempts were made to determine the species' landscape characteristics, and the adaption potential of the species outside its natural population was assessed. However, the ultimate objective of this study was to cultivate, promote, and create a collection yard for *ex situ* conservation of *C. hermannii*.

## EXPERIMENTAL

### Materials

The seeds of *C. hermannii* F. Hermann, which constituted the parent material for this study, were collected from a natural population of plants in Delmece in the province of Yalova on 25 July 2019 (Fig. 1). The study was carried out under the climate conditions of Yalova province in 2019, 2020, and 2021 (Fig. 2). The laboratory, greenhouse, and field studies were performed in the following locations: seed germination studies and seedling growth stages were made at Yalova Vocational High School Research and Application Laboratory and Greenhouse facilities at Yalova University; and observations and measurement of the seedlings, which were planted in pots and in soil, were carried out in the Çınarcık research yard (Fig. 1). The soil characteristics of both Delmece and the research yard in Çınarcık, where plant cultivation studies were conducted, are provided in Table 1.

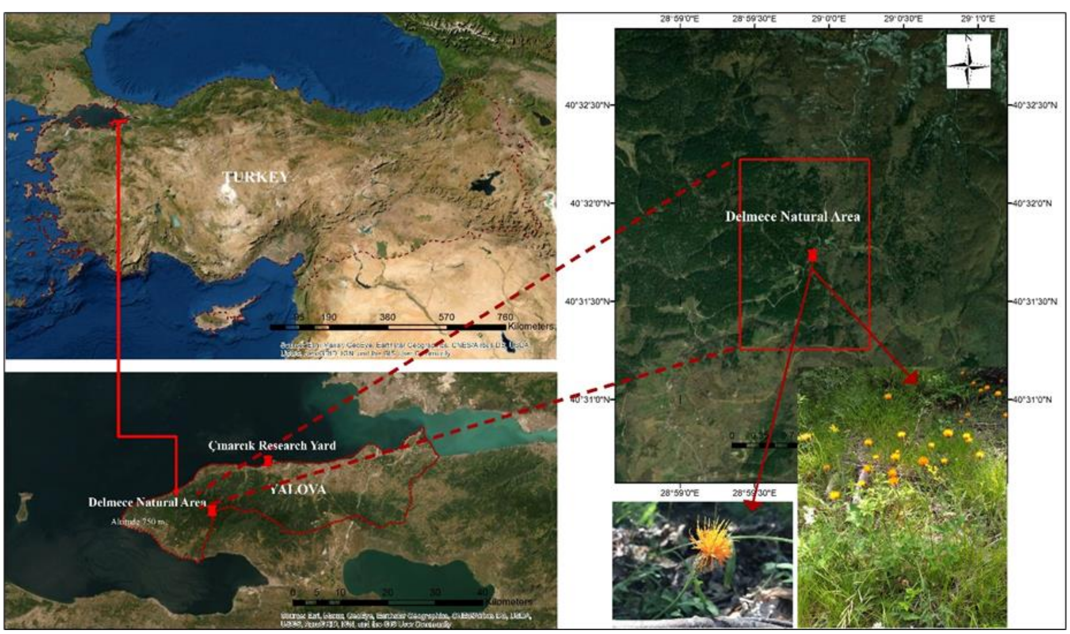


**Fig. 1.** Delmece natural area; general appearance of *C. hermannii* in the natural population; and Cınarcık research yard, Yalova

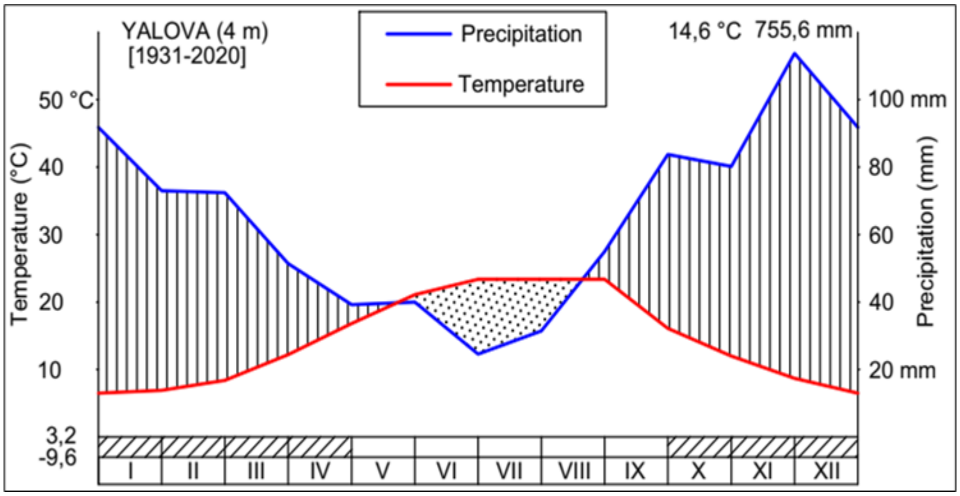


**Fig. 2.** Climate diagram showing the relationship between temperature and precipitation in Yalova province (Walter *et al.* 1975)

**Table 1.** Soil Characteristics of Delmece Natural Area and Çınarcık Research Yard

| Soil Characteristics | Locations | |
|---|---|---|
| | Delmece natural area | Çınarcık research yard |
| Saturation % | 57 | 46 |
| pH | 5.75 | 7.63 |
| $EC_{25}$ (µmhos/cm) | 117 | 547 |
| $CaCO_3$ (%) | 0.20 | 4.11 |
| Organic Matter (%) | 7.40 | 2.69 |
| Available Phosphorus (ppm) | 6 | 20 |
| Extractable Potassium (ppm) | 160 | 190 |
| Clay (%) | 34 | 36 |
| Sand (%) | 33 | 47 |
| Silt (%) | 33 | 17 |

## Methods

*Determination of morphological and physiological characteristics and ornamental plant properties*

Some of the seedlings were planted in the soil (60 plants) of the research yard, while others were planted in pots (60 plants) to evaluate the physiological and esthetic properties of the plants and to determine their adaptability to the cultivation conditions. Between April and August, the plants in both the Çınarcık research yard and Delmece (natural population) were monitored, and the necessary measurements and observations of the morphological characteristics (number of main shoods, main shoods length, number of side shoots, *etc*.) of the plant were recorded. In total, 3 viols with 104 compartments were used for all measurements, observations and *ex situ* conservation and 312 seedlings were planted.

104 seedlings tallied after the germination tests were planted in 104 vials containing peat (10 November 2019) and grown in unheated greenhouse conditions (average 14/9 °C day/ night temperature 70% RH and 3 times daily 10 s. mistpropagation application) in order to carry out observations and measurements on the seedlings placed into pots. Seedlings were transplanted into 8x8 cm square peat pots, depending on their stage of development (*i.e.*, with 2 to 3 leaves). On 16 March 2020, the growing seedlings (with 6–7 leaves) were transferred to 16 x 13.5 cm pots filled with soil, the composition of which

is listed in Table 1 (Çınarcık research yard) and the morphological characteristics of the plants (number of main shoods, main hoods length, number of side shoots, *etc*.) monitored appropriately. A similar method was also used to monitor the seedlings planted in the soil. The seedlings grown in 8x8 cm square pots were planted at 30 x 30 cm intervals in the yard soil (Çınarcık research yard), the composition of which is provided in Table 1 and monitored accordingly.

All observations, measurements, and evaluations of the plants' morphological and physiological characteristics, and as well as their life cycle, were made in the natural population in Delmece, and in the seedlings planted in pots and in soil in the Çınarcık research yard. These data also generated preliminary information on the potential ornamental plant value of this species. Similarly, it was also possible to obtain information about the esthetic and functional properties of the plant for its potential use in landscape designs; To determine morphological characteristics, three groups of 10 plants each (30 plants in total), were set out randomly, and the following parameters and measuring/counting methods were carried out on the different species:

- Number of main shoots: The number of above-ground main shoots on the plant (number).
- Main shoot length: The distance from the ground to the most extreme flower on the shoot (cm).
- Number of side shoots: The number of side shoots emerging from the plant's main branches (number).
- Average side-shoot length: The distance between the place on the main shoot where the side shoot emerges and the flower at its most extreme point (cm).
- Number of buds: The total number of buds on the plant (number).
- Main and side-shoot widths: The width of the most bulging part of the bud in millimeters. The buds were measured just before they developed color.
- Bud height: The measurement from end to end of the longest portion of the bud in millimeters.
- First blooming date: The date on which the first flower on the plant opened.
- Last blooming date: The date when the last flower on the plant was completely shriveled.

Seeds were collected from the location with care to avoid harm to the endemic plant population. Using the method specified in Bacchetta *et al.* (2006), seeds were picked randomly and in minimum amounts. The collected seeds were cleaned and dried in a shaded place with an average temperature of 20 to 21 °C, where air flow was provided by ventilation devices. The study began on 5 August 2019 after the seeds had been left to dry for approximately ten days. The following measurements and observations were performed to determine the morphological and physiological characteristics of the seeds: seed size (width, height), 1000 kernel weight, the average number of seeds in 1 gram, seed maturation date, capsule splitting date, and seed shedding date. In addition, a seed viability test was conducted.

*Seed viability test*

Viability tests were carried out on 8 August 2019. Initially, the seeds were separated into three groups, each containing 20 seeds. Subsequently, the seeds were maintained in water at room temperature for 24 h, and then they were removed (from the water). After cutting 1/3 of the seeds, they were kept in 1% tetrazolium (2,3,5 Triphenyl tetrazolium chloride) (Merck, Darmstadt, Germany) solution at 30 °C for 24 h. Following these procedures, the seed boll was peeled. Based on their degrees of staining and physical observations, the seeds were classified as follows: viable (completely stained), semi-viable (less coloration or colorless patches on the seed), or nonviable (no coloration) (Moore 1985; Peters 2000).

*Germination tests*

Germination tests were made using 100x20 mm glass Petri dishes in a germination cabinet (Programmable Plant Growth Chamber SWGC-450; Daihan Scientific, Seoul, Korea). Before being placed into Petri dishes, the seeds were sterilized in 70% ethanol (Soltek, Turkey) for 1 min, after which they were treated for 10 min with a commercially available 20% solution containing 5.25% sodium-hypochlorite (BRTR Chemistry, İzmir, Turkey). Following sterilization, distilled water was used to purify the seeds. Petri dishes and blotting papers were sterilized for 30 min at 100 °C before use. Seeds were gently placed into Petri dishes on moist blotting papers in such a way that they did not contact each other. To prevent disease, a commercial fungicide containing the active ingredients Fludioxonil (25 g/L) + Metalaxyl-M (10 g/L) (Maxim XL 035 FS, Syngenta, Gaillon, France) was applied to the seeds in the Petri dishes at the level of 2.5 mL/L. Parafilm was used to cover Petri dishes with closed lids. When analyzing the germination studies, the final germination percentage (FGP) of the seeds was taken into consideration. In situations where light/dark conditions and temperatures were not specified, the 12/12-hour photoperiod and a temperature of 20±0.5 °C was assumed. A seed was considered to have germinated when a two-millimeter radicle had emerged from the seed shell. Counts were taken every two days, and radication was monitored for 30 days (Eser *et al.* 2005; ISTA 2013).

The conditions listed in Table 2 were used to determine the germination performance under light/dark conditions at different temperatures. Seeds were kept at a temperature of 10, 15, 20, or 25 °C and under light conditions of either constant light or constant dark. The research was carried out on 15 August 2019, and the light source had an intensity of 3400 lumens.

**Table 2.** Temperature, Light Applications, and Time Periods

| Conditions | Period |
|---|---|
| 10 °C constant light | 30 days |
| 10 °C constant dark | 30 days |
| 15 °C constant light | 30 days |
| 15 °C constant dark | 30 days |
| 20 °C constant light | 30 days |
| 20 °C constant dark | 30 days |
| 25 °C constant light | 30 days |

As shown in Table 3, different conditions of temperature and light were used to determine the effects of three months storage at 4° C, cold-wet stratification, applications of Gibberellic acid ($GA_3$) (Merck, Darmstadt, Germany), and the effects of these combinations on germination. The seeds were kept in paper bags and moist perlite for cold-wet stratification at 4 °C for three months, respectively. The $GA_3$ treatments were carried out by soaking the seeds in a 200, 400, or 600 ppm $GA_3$ solution for 24 h. After applying the relevant variables of temperature and light, the seeds were exposed to germination tests in Petri dishes in a climate cabinet under the following conditions: 70% humidity and 20 ± 0.5º C temperature under a 12/12 light regime.

**Table 3.** Applications of Storage (4 °C), Cold-Wet Stratification, and Their Combinations with Gibberellic Acid ($GA_3$) and Dates

| Applications | Period |
|---|---|
| Cold-wet stratification | 3.08.2019–2.11.2020 |
| Cold-wet stratification (4 °C and three months) + 24-h soak in ppm $GA_3$ (200, 400, or 600 ppm) | 6.08.2019–5.11.2020 |
| Storage for 3 months at 4 °C | 5.08.2019–4.11.2019 |
| Storage for 3 months at 4 °C + 24-h soak in $GA_3$ (200, 400, or 600 ppm) | 6.08.2019–5.11.2019 |
| Storage for 3 months at 4 °C + cold-wet stratification (4 °C, 3 months) | 5.08.2019–4.02.2020 |
| Storage for 3 months at 4 °C + cold-wet stratification (4 °C, 3 months + 24-h soak in $GA_3$ (200, 400, or 600 ppm) | 6.08.2019–5.02.2020 |

100 plants were reserved to create an *ex situ* conservation yard. On 18 March 2020, plants with sufficient growth (*i.e.*, with 2 to 3 leaves) in 8x8 cm square pots were planted in the *ex situ* conservation yard at 30x30 cm intervals. On 16 March 2020, the extra plants that were not used in the collection yard, were planted within their natural populations for recovery.

*Experimental design and data analysis*

Experiments were designed by the Randomized Block Experimental Design with four replications, with each replication containing 50 seeds. Data obtained this experiment were analyzed with the IBM SPSS Statistics Base 22.0 (IBM Corp., Armonk, NY, USA) statistical program. The data were subjected to a one-way analysis of variance (ANOVA), with Duncan’s multiple comparison test applied to the treatments that were identified differentially. Germination percentages (%) were determined after counting and were subjected to the application of arc-sine data transformation.

## RESULTS AND DISCUSSION

### Plant, Flower, and Seed Properties of *Centaurea hermannii*

Table 4 shows the flower and plant features of *C. hermannii* plants grown in natural areas, in pots, and in the soil. Observations and measurements of the plants in the research yard showed a loss in the number of buds, in both pots and in the soil, at the end of the first year only. The fact that the plants were only one year old and had not yet completed their two-year development could explain this observation. Indeed, significant increases in all properties were observed among the plants cultivated in the field during the second vegetation period. Despite differences in soil structure, the plants grown in the soil fully adapted to the conditions in the field and developed their full ornamental plant properties, especially in their second year, compared to those plants in the natural area. During the two-year period, buds generated a succession of flowers, one after the other, and the plants remained in bloom for about two months. In terms of the flowering period, the present observations are in line with those of Akkemik (2017), who noted that *C. hermannii* blooms in June-July. In terms of landscape value, however, the most significant characteristic of *C. hermannii* is undoubtedly its bright orange flowers, a feature that increases the esthetic properties of the plant. Özdemir and Ulus (2018) defined *C. hermannii* as an endemic plant that grows naturally in Istanbul and in pollinated yards. Tel *et al*. (2019) reported that *C. hermannii* 'is a potential candidate for growing in parks and gardens, given its ornamental, orange-colored flowers and suitable height.' In this work, *C. hermannii* was observed to be a reliable biennial ornamental plant that would flourish in parks and gardens. It should also be noted that, depending on the development of the plant, the attractiveness of *C. hermannii* continues to increase, especially in the second year.

**Table 4.** Properties Related to the Structure of Flowers and Plants Grown in a Natural Population (Delmece), in Pots, and in Soil (Çınarcık research yard)

| Growing Conditions | Natural Population (Delmece) (2-year plant) | Çınarcık Research Yard | | |
|---|---|---|---|---|
| | | Pot (1-year plant) | Soil (1-year plant) | Soil (2-year plant) |
| Number of Main Shoot (number) | 10.83 | 4.33 | 6.66 | 9.43 |
| Main Shoot Length (cm) | 62.28 | 40.46 | 52.00 | 70.50 |
| Number of Side-Shoots (number) | 20.83 | 2.66 | 1.53 | 19.43 |
| Average Side-Shoot Length (cm) | 15.62 | 11.50 | 18.33 | 24.12 |
| Number of Buds (number/plant) | 18.66 | 5.12 | 5.23 | 28.04 |
| Bud Height (mm) (main shoot) | 21.32 | 20.27 | 22.30 | 24.05 |
| Bud Width (mm) (main shoot) | 16.02 | 12.75 | 14.53 | 17.20 |
| Bud Height (mm) (side shoot) | 15.61 | 13.02 | 11.97 | 13.01 |
| Bud Width (mm) (side shoot) | 12.86 | 13.78 | 15.04 | 16.41 |
| First Blooming Date | 15.05.2020 | 05.05.2020 | 07.05.2020 | 10.05.2021 |
| Last Blooming Date | 18.07.2020 | 08.07.2020 | 03.07.2020 | 08.07.2021 |

## Morphological Characteristics of the Seeds

*C. hermannii* seeds are yellowish-brown in color and slightly tapered at the tip. In the direction of the tip section, the color noticeably changes to yellow. At the top of the seed, villiform structures are arranged in a brush form.

Table 5 summarizes the data obtained from measuring, counting, and weighing of *C. hermannii* seeds. On average, each gram contained 114.69 seeds. Average seed height and width was found to be 5.27 and 2.37 millimeters, respectively. The net viability percentage of seeds was identified as 72.2% in the tests performed. In addition, the seed viability percentage of *C. hermannii* was observed to be low in the year the study was conducted. The high proportion of semi-viable seeds suggested that there could have been damage from insects, harm due to physical impacts, and seed maturation issues. However, the net non-viable seed percentage was considered to be within acceptable limits (Fig. 3).

**Table 5.** Seed Properties of *Centaurea hermannii*

| Seed Characteristics | Size/ Quantity /Percent |
|---|---|
| Seed Height (mm) | 5.27 |
| Seed Width (mm) | 2.37 |
| 1000 kernel weight (g) | 8.7192 |
| The average number of seeds in 1 g | 114.69 |
| Non-viable seeds (%) | 2.22 |
| Semi-viable seeds (%) | 25.56 |
| Viable seeds (%) | 72.22 |


**Fig. 3.** Appearance of viable, semi-viable and non-viable seeds in *Centaurea hermannii*

The average seed height and width were 5.27 and 2.37 millimeters, respectively. Several studies, including those by Gürdal and Özhatay (2010), Eroğlu *et al.* (2014), and Tel *et al.* (2019) reported 4 to 5 mm of seed height of *C. hermannii*. These results also support the measurements found in this study. In addition, there was 72.2% seed viability percentage in *C. hermannii*. This value was reported differently among *Centaurea* species. For instance, Ozel *et al.* (2006) reported as 85% in *C. tchihatcheffii* species, Kurt and Erdağ (2009) as 98% in *C. zeybekii* species, Emek and Erdağ (2012) as 80% in *Rhaponticoides mykalea* species, Atasagun and Aksoy (2018) as 82% in *C. amaena* species, and Yankova-Tsvetkova *et al.* (2018) as 17.5% in *C. achtarovii* species, respectively. Therefore, the seed viability percentages could vary significantly among the species. In addition, due to environmental conditions such as climate, soil, and insect damage, variations might even occur among seeds of the same species.

In this study, it was observed that the fruits started to ripen proximately 60 days after the first blooming in the culture medium, the seed capsules began to crack after 60-70 days, and the seeds were shed to a large extent after 75 days (Table 6). However, in the natural area, the fruits began to ripen 60 days after the first blooming the fruit began to ripen, and the capsules split approximately 65 to 70 days later. Furthermore, both in the cultivation environment and natural area, the capsules were observed to be shedding their seeds quickly after reaching the maturation stage. The findings suggest that 70 to 80 days after the first blooming are the most suitable period for collecting *C. hermannii* seeds.

**Table 6.** Periods of Fruit Ripening, Capsule Splitting, and Seed Shedding of the Plants Grown in the Natural Population (Delmece), in Pots, and in Soil (Çınarcık research yard)

| Growing Conditions | Fruit Ripening Time | Capsule Splitting Time | Seed Shedding Time |
|---|---|---|---|
| Delmece Naturel Area | 2nd week of July | 3rd week of July | 20–25 July |
| Cınarcık Research Yard - in Pot | 1st week of July | 2nd week of July | 15–20 July |
| Cınarcık Research Yard - in Soil | 1st week of July | 2nd week of July | 15–20 July |

## Determination of Seed Germination Performances at Different Temperatures and Light/Dark Conditions

*Germination Percentage*

When only the percent germination is taken into account while evaluating the effects of different temperature and light applications on the seed germination of *C. hermannii*, there was no statistical difference among the following applications: 8.00% percent germination at 10 ℃ and in dark condition, 7.00%, 8.00%, and 8.00% in the full-light application and at 10, 15, and 20 ℃, respectively; and all four applications were classified in the first group. Although some applications produced partially positive results, it would be plausible to say that the germination percentages for all applications remained at a significantly low level when three different temperatures and two constant light conditions were taken into consideration (Table 7).

Various studies in the literature reported germination temperatures and light conditions used in different *Centaurea* species. For instance, while Abbasian *et al.* (2017) proposed a 20 ℃ temperature for optimum germination of *C. balsamita*, Türkoğlu *et al.* (2009) suggested that 15 ℃ was suitable for three different species: *C. balsamita, C. virgata*, and *C. iberica*. However, Albert *et al*. (2002) stated that the highest germination percentage in *C. pinnata* was observed at a constant temperature range of 15 to 20 ℃, and a constant temperature of 15 ℃ generated a significant effect on the germination percentage when compared to the variable temperature regime of 15/25 ℃. Similarly, Pitcairn *et al.* (2002) determined that 20 ℃ was the only constant temperature, and the varying temperature regime of 15/25 ℃ further supported the optimum germination rate for *C. calcitrapa*. Therefore, studies carried out with varying *Centaurea* species also supported this study results.

Zare *et al.* (2020) suggested that a suitable germination temperature was 25 °C for *C. bruguierana* species. In this study, while the emerging opinion that constant light for *C. hermannii* seeds has a negative effect on the percentage and speed of germination, Valletta *et al.* (2016) reported that *C. cineraria* subsp. *circae* had the highest germination percentage at a temperature of 20/10 °C and a 12/12 photoperiod application (67.5%). Similarly, Uysal *et al*. (2006) identified that 16/8 h of light/dark at 25 °C produced better results than 8/16 h of light/dark applications in *C. tomentella*. Furthermore, Nosratti *et al*. (2017a) reported that in *C. iberica*, the highest germination percentage was recorded at varying temperatures of 15/25 °C and 16/8 h of light/dark conditions. They also stated that light had a stimulating effect on seeds when compared to constant dark conditions. They further determined in *C. cyanus* that light at high temperatures positively affected the germination percentage; however, low temperatures negatively affected it. Therefore, these reports supported the findings that full-light resulted in a significant difference in the germination percentage at 15 to 20 °C.

In this study, when the germination percentage was considered, light had no effect at 10 °C; however, it positively affected germination at 15 and 20 °C, and the full-light application additionally provided better results on the percentage of germination. However, further increments in temperature reversed the situation, as the temperature reached 25 °C. This result suggests that higher temperatures above a certain level reduces the light effect on the percentage of germination in *C. hermannii*. In *C. diffusa* Lam 20 °C and 20 to 30 °C dark, and 20 to 30 °C light/dark conditions had a lesser effect on percentage of germination when compared to 15 °C and dark conditions (Buhler and Hoffman 1999; Türkoglu *et al.* 2009). Demirel *et al.* (2017) also reported that *Centaurea* sp. plants had a long vegetation period. Nosratti *et al.* (2017b) reported that while the best germination percentage was observed at 25 °C in *C. balsamita* taxon, the light and dark conditions had similar effects on seed germination of *C. balsamita*. Noting that the responses of different *Centaurea* species to the light period were not comparable, Köse and Yücel (2015) monitored varying photoperiod conditions and germination percentages at 25 °C in four different *Centaurea* taxa. Accordingly, *C. aphrodisea* and *C. luschaniana* were not affected by any light conditions, and *C. amaena* increased its germination percentage with an increased exposure time. However, the percentage of germination of *C. lycia* decreased with low light exposure.

*Germination speed*

Table 4 shows the germination speed of *C. hermannii* seeds under various temperature and light conditions. In terms of germination speed, the best results were obtained from 20.00 days of full-light at 15 °C 19.33 days of full-dark at 20 °C, and 26.00 days of full-dark at 25 °C. However 10 °C was found to delay the germination speed both in dark and light conditions. Hence, it can be speculated that the constant-dark condition may result in a significant slowdown in the germination percentage at 10 °C. When the temperature was raised to 15 to 20 °C, an increase in germination speed was also observed. Similarly, Turkoglu *et al.* (2009) stated that the maximum germination speed in *C. balsamina* was observed at 15 °C. However, when the study results were evaluated collectively, the constant light and temperature combinations did not provide the expected outcomes in germination speed.

**Table 7.** Effects of Different Temperature and Light Conditions on the Seed Germination of *Centaurea hermannii*

| Applications | Mean Germination (%) ± SE | Germination Speed (T50) (Day) |
|---|---|---|
| 10 °C constant light | 7.00 ±0.64 a* | 39.00 ±0.58 d |
| 10 °C constant dark | **8.00 ±0.61 a** | 53.33 ±0.88 e |
| 15 °C constant light | **8.00 ±0.34 a** | **20.00 ±0.00 a** |
| 15 °C constant dark | 5.33 ±0.42 b | 32.33 ±1.20 c |
| 20 °C constant light | **8.00 ±0.61 a** | 22.00 ±0.58 ab |
| 20 °C constant dark | 2.67 ±0.61 c | **19.33 ±0.33 a** |
| 25 °C constant light | 2.00 ±0.61 c | 24.33 ±1.45 bc |
| 25 °C constant dark | 4.67 ±0.88 b | 26.00 ±1.66 bc |

*Within each column, mean values denoted with the same letter are not significantly different ($p \leq 0.001$); SE: standard error.

## Determination of the Effects of Three Months of Storage at 4 °C, Cold-Wet Stratification, Gibberellic Acid ($GA_3$) or their Combinations on Germination

*Percentage of germination*

When the effects of three months storage at 4 °C, cold-wet stratification, and $GA_3$ treatments were evaluated on the germination of *C. hermannii* seeds (Table 8), the best result in terms of percentage of germination was recorded as 70.5% in the applications of three months storage at 4 °C + cold-wet stratification + 200 ppm $GA_3$. It was noteworthy that among the applications, storage at 4 °C for three months without $GA_3$ treatment + cold-wet stratification resulted in a low percentage of germination. In other applications, it seemed that the $GA_3$ treatment tolerated the negative effect of cold-wet stratification.

*Germination speed*

In terms of germination speed, storage at 4 °C for three months + cold-wet stratification + 200 ppm $GA_3$ treatment yielded the best results. The considerable difference in germination speeds between the groups with and without cold-wet stratification indicated that cold-wet stratification increased germination speed.

Considering the data specified in the literature for both parameters, Okay and Günöz (2009), Okay *et al*. (2011), and Okay and Demir (2021) noted that stratification for 120 to 150 days and application of 100 ppm $GA_3$ before planting increased the seed germination percentage in *C. tchihatcheffii*. Similarly, Eddleman and Romo (1988) reported that cold-wet stratification shortened the seed germination period; however, it increased total germination in *C. maculosa* Lam. Saba *et al*. (2017) indicated that the $GA_3$ treatment in seeds increases germination in *C. balsamita* Lam. In his germination study with *C. diffusa* and *C. maculosa*, Nolan (1989) also stated that the $GA_3$ is a robust stimulant, and some seeds in a dormant state treated with cold at 3 °C successfully germinated at 25 °C. However, Luna *et al*. (2008) indicated that cold stratification did not result in a significant effect on seed germination of *C. ornata* and *C. pinae*. According to Aghilian *et al*. (2014), a six-day precooling treatment at 4 °C had no influence on the germination process in *C. cyanus*. As a result, depending on the species, responses of *Centaurea* seeds to various applications could provide notably different results.

Elias *et al.* (2012) and Baskin and Baskin (2014) reported that germination qualities of plant species could vary greatly based on genetic and environmental factors, as well as the media used in germination tests. Some researchers suggested that seed germination percentages could be altered depending on the fruit the seed came from or where the seeds were collected from the plant (Nielsen 1987; Copeland and McDonald 2001). Another explanation for disparities in results across studies could be differences in seed storage conditions, which could alter germination during the time between seed harvest and germination (Probert 2000). Therefore, Gresta *et al.* (2010) noted that germination is a complex physiological process that could be affected by many factors.

**Table 8.** Effects of Storage for Three Months at 4 °C, Cold-Wet Stratification, and $GA_3$ Treatments on Germination of *Centaurea hermannii* Seeds

| Applications | Mean Germination (%) ± SE | Germination Speed (T50) (Day) |
|---|---|---|
| Storage for 3 months at 4 °C | 41.00 ±0.24 d* | 28.25 ±1.18 e |
| Storage for 3 months at 4 °C + 24-h soaking in $GA_3$ 200 ppm | 51.00 ±0.78 c | 20.25 ±0.75 d |
| Storage for 3 months at 4 °C + 24-h soaking in $GA_3$ 400 ppm | 58.00 ±1.26 b | 15.00 ±0.71 c |
| Storage for 3 months at 4 °C + 24-h soaking in $GA_3$ 600 ppm | 53.00 ±1.37 c | 27.75 ±1.03 e |
| Storage for 3 months at 4 °C + 3 months cold-wet (4 °C) stratification | 23.00 ±0.67 e | 8.00 ±0.58 b |
| Storage for 3 months at 4 °C + 3 months cold-wet (4°C) stratification + 24 h soaking in $GA_3$ 200 ppm | **70.50 ±1.07 a** | **3.00 ±0.41 a** |
| Storage for 3 months at 4 °C + 3 months cold-wet (4 °C) stratification + 24- h soaking in $GA_3$ 400 ppm | 50.50 ±1.09 c | 7.25 ±0.25 b |
| Storage for 3 months at 4 °C + 3 months cold-wet (4 °C) stratification + 2 -h soaking in $GA_3$ 600 ppm | 43.50 ±0.86 d | 7.50 ±0.50 b |

*Within each column, mean values denoted with the same letter are not significantly different ($p \leq 0.001$); SE: standard error. All applications were at 12/12 photoperiod and 20 °C constant temperature

*Creation of an ex situ conservation yard*

During the study, the seeds that germinated were removed from the Petri dishes and planted in vials in the peat medium. The seedlings grown in vials up to 2–3 leaflets were re-planted in 8x8 cm square pots containing peat, and then, they were transferred to the *ex situ* conservation yard in outdoor conditions at the beginning of March (Fig. 4).

*Recovering plants to the natural population*

The redundant plants, which were not used in the collection yard, were transferred to their natural populations in mid-March (Fig. 4).

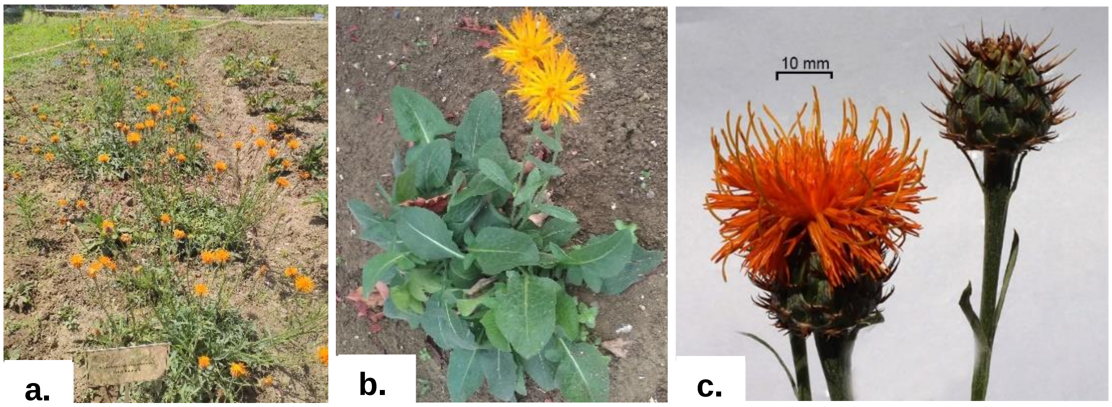


**Fig. 4.** a) The *ex situ* conservation yard built by cultivated *C. hermannii;* b) the appearance of the plant grown in the soil; c) the appearance of the plant flower

## CONCLUSIONS

1. Based on the results of this study, *C. hermannii* is suitable in both ecological and landscape designs. In addition, it was determined that the plants are able to adapt to the cultured conditions without any difficulty, and that it even grows more spectacular as it adapts to the area. This finding suggests that *C. hermannii* could be used as a potential ornamental plant in landscape designs.
2. For the plant's generative production, temperature and light requirements for germination were specified in detail. Cold-wet stratification and $GA_3$ treatments 200 ppm of $GA_3$ treatment during a three-month storage period at 4 °C, followed by cold-wet stratification at 4 °C for three months resulted in high germination percentages. In addition, it was observed that cold-wet stratification could shorten the germination time when combined with $GA_3$ treatment.
3. An *ex situ* conservation yard for *C. hermannii* was built with the plants gathered from the study. The results of the study showed that the *ex situ* conservation could be achieved for *C. hermannii* by seed production.
4. When needed, the plants could be grown easily under cultured conditions and used to restore damaged natural areas. The high percentages of germination of this species would enable rapid production of this species under cultured conditions.

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