PRODUCTION AND ORNAMENTAL PLANT PROPERTIES OF ENDEMIC *RHAPONTICOIDES WAGENITZIANA* (BANCHEVA & KIT TAN) M. V. AGAB. & GREUTER

YÜCEL, G.[1*] – ERKEN, K.[2]

[1]*Yalova University, Yalova Vocational High School, Landscape and Ornamental Plants Program, Yalova 77200, Turkey*

[2]*Bursa Technical University, Faculty of Forestry, Department of Landscape Architecture, Bursa 16310, Turkey*

[*]*Corresponding author*
e-mail: gul.yucel@yalova.edu.tr

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Abstract. This study focused on *Rhaponticoides wagenitziana*, including an assessment of its plant and seed properties, adaptation to cultivated conditions, aesthetic and functional properties as an ornamental plant, and seed viability rates. The study was conducted in Yalova/Turkey between 2019 and 2021. Dry storage (3, 5, and 8 months at 4 °C), cold-wet stratification (2 and 3 months at 4 °C), gibberellic acid (GA_3) treatments (200, 400, 600, 1000, 1500, and 2000 mg/L), and various combinations of these treatments on seed germination were also investigated. During the vegetation period, this green-leaved species displayed purple flowers for about two months in the summer and demonstrated no difficulties in adapting to cultivated conditions, where it retained its aesthetic and functional properties. Thus, it was concluded that this species could be utilised as an ornamental plant in herbal designs. Among seed treatments, 600 and 1000 mg/L GA_3, following storage at 4 °C for three months, yielded the best germination rates: 48.25% and 52.75%, respectively. Given this species' problematic germination, the results on breaking dormancy were quite successful compared to those reported in previous studies. Furthermore, after storage at 4 °C for three months, treatments of 1000 and 1500 mg/L GA_3, or 200 and 600 mg/L GA_3 with cold-wet stratification, accelerated germination speed, reducing it from 18 days in the T_{50} control group to 5.8 and 6 days, respectively. A collection yard was developed using the plants grown in the study, and the species was placed under ex-situ conservation.
Keywords: *ex-situ conservation, gibberellic acid, ornamental plant, Rhaponticoides wagenitziana, seed germination, stratification*

Introduction

Plants used in scientific studies, and which are grown in accordance with the principles of sustainability, should be described in terms of their proper placement and appropriate use. For instance, plant designs that utilize natural species contribute to the long-term sustainability of landscaping (Russo and Cirella, 2018). Natural plant species adapted to their original climatic conditions and the surrounding flora have the capacity to provide ideal alternatives to traditional plant designs due to their lower water consumption, ease of maintenance, and low cost (Alam et al., 2017). Karimian et al. (2017), Hopkins and Al-Yahyai (2015) suggested that natural plants adapted to adverse soil and climatic conditions should ultimately be incorporated into landscape designs to prevent risks to the environment.

Natural plants also constitute a valuable resource for seedling producers and sellers since they provide ecological enhancement to ecosystems (Anderson et al., 2021). As a result, introducing novel and natural ornamental plants into commercial production, and mastery of the production process, is a prerequisite for developing the commercial

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production of ornamental plants (Karlović, 2007). Increasing the seed germination rate is also needed to achieve large-scale production to meet the demands of the market (Khadis and Georghiou, 2010; Kırmızı et al., 2019) and is crucial for restoring natural habitats (Baskin and Baskin, 2020). Species richness in this field undoubtedly provides a primary foundation for landscape design. The endemic nature of the plant species used in plant designs adds greater significance and interest to subject. Noroozi et al. (2019) noted that endemism plays a highly critical role at global, national, and regional levels in the conservation and sustainability of biodiversity.

A garden established to preserve the species will constitute an ex-situ conservation area. In this context, the *Centaurea* genus, for which Anatolia is recognised as the principal region in terms of species diversity and includes several endemic species, is of particular interest. The genus *Centaurea* is recognized as the third largest genus among the flora of Turkey. It includes 217 species (146 endemic), 36 subspecies (22 endemic), and 28 varieties (16 endemic) in total, with a 66.8% endemism rate (Ozhatay et al., 2011; Bona, 2013, 2014). The distribution of some species of the *Centaurea* genus is severely restricted, and in some cases solely represented by a single population. The risk of extinction for certain species is very high due to increasing habitat damage and population fragmentation. In addition, the effects of insect damage, dormancy, and environmental conditions limit the dispersal, reproduction, and regeneration of the seeds of these species (Yankova-Tsvetkova, 2018).

In view of the current situation, conducting research to support seed production has become necessary for endemic species of the genus *Centaurea*. In particular, the present study investigated the endemic *R. wagenitziana* (Synonym: *Centaurea wagenitziana*) taxon. According to IUCN (2014) data, *R. wagenitziana* is listed among species that are critically endangered (CR). The species occurs naturally in the eastern Balkans, and in the vicinity of Istanbul-Aydos Mountain (Tan, 2009), and Taz Mountain in Yalova, Turkey (Uğurtas et al., 2014). The present study examined the morphological and phenological vegetative characteristics and seed properties of this species. Various treatments were applied to the seeds under laboratory conditions to identify the most suitable germination method and germination rates. The plants were subsequently transplanted into the soil of the research yard, and their growth was monitored for two consecutive years. An attempt was made to determine whether this species displayed substantial ornamental properties, and its potential for cultivation and adaptation. Finally, a collection yard was established to provide resources for ex-situ conservation, cultivation, and promotion of the species.

Materials and methods

Material

Rhaponticoides wagenitziana (Bancheva & Kit Tan) M.V.Agab. & Greuter (Synonym: *Centaurea wagenitziana*) and its seeds constituted the main material in the study. Seeds were collected from Taz Mountain (40º32’44” N 28º55’01” E, 922 m) in Yalova/Turkey on 28 July, 2019 (*Fig. 1A,B,C*), and field studies was conducted under the climatic conditions of Çınarcık between 2019 and 2021 (*Fig. 2*). The research was conducted in two different locations of Yalova (Yalova and Çınarcık) in Turkey. The seed germination and seedling growth stages were carried out at the Yalova Vocational High School Research and Application Laboratory (40º38’40’N” 29º152’55” E, 12 m) and at the Greenhouse facilities of Yalova University (*Fig. 1B*). After the plants were

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transplanted into the soil, the Çınarcık Research Yard (ÇRY) (40º38'32" N 29º06'21" E, 21 m) was employed for plant measurements and observations and to verify their adaptative features (*Fig. 1B*). The soil characteristics of Taz Mountain and Çınarcık Research Yard are provided in *Table 1*.

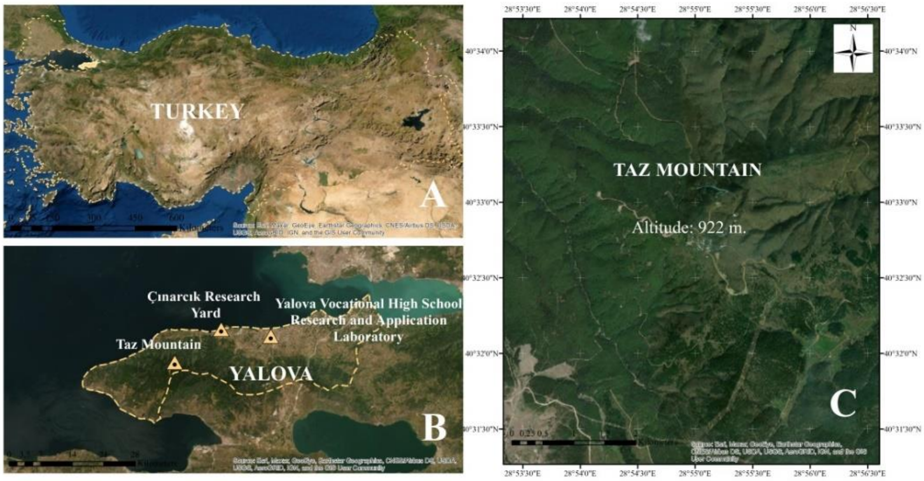

Figure 1. *Geographical locations of R. wagenitziana. Research yard location in Turkey (A), in Yalova and Çınarcık (B), natural population in Taz Mountain (C)*

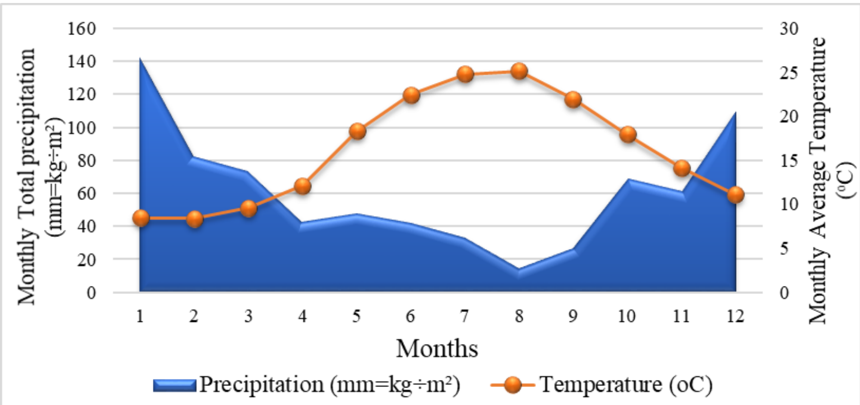

Figure 2. *Climatic data for Cinarcik with the distributions of temperature and precipitation (Yalova office of General Directorate of Meteorology, 2021; Url, 1)*

Table 1. *Soil Characteristics of Taz Mountain and Çınarcık Research Yard*

Soil Characteristics	Locations	
	Taz Mountain	**Çınarcık Research Yard**
pH	5.23	7.63
EC25 (µmhos/cm)	774	547
$CaCO_3$ (%)	0.39	4.11
Organic Matter (%)	4.57	2.69
Available Phosphorus (ppm)	179	20
Extractable Potassium (ppm)	370	190
Clay (%)	27.96	36
Sand (%)	37.97	47
Silt (%)	34.07	17

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Method

Determination of ornamental and seed properties and morphological and phenological characteristics of the species

The seedlings of *R. wagenitziana* were grown in the research yard to observe their aesthetic properties and phenological characteristics and to test their adaptability to cultivated conditions. The natural population in the Taz Mountain, and the ÇRY areas was visited once every fifteen days during the vegetation period (from April to August) for monitoring the plants.

Monitoring data and measurements were collected during the field trips. The seedlings produced after the germination tests provided the plant samples for monitoring in the research yard. Initially, the seedlings counted in the Petri dishes were transferred into 104 viols containing peat (10 November 2019) for this purpose. The seedlings were nurtured in an unheated greenhouse and then transplanted into 8x8 cm square pots filled with peat according to their developmental stage (i.e., 2-3 leaves per seedling). When the plants generated 6-7 leaves on 16 March 2020, they were transplanted into the soil of the ÇRY, the characteristics of which are listed in *Table 1*. Finally, were monitored throughout their vegetation period.

Data on morphological and phenelogical characteristics were measured and observed from the naturally grown plants at Taz Mountain and from the soil-grown plants at the ÇRY. The biological cycle of the species was also monitored. Overall, these results provided initial clues (based on the observed data) about the potential ornamental value of this species. Furthermore, these observations provided preliminary data on the species' functional and aesthetic plant properties, which are of great importance in plant designs. For the morphological observations in natural population, three groups of 10 plants each, for a total of 30 plants, were randomly selected. Counting and measuring procedures, as detailed below, were carried out on the plant groups under observation (*Table 2*). Sixty plants were also grown in pots to test their development performance under cultivation conditions and measurements and counts were made on 10x3 of these plants. Total 30 (10x3) plants were used for experiments from the many plants planted in the ground for *ex-situ* conservation. Plants were randomly selected.

Table 2. *Criteria for plant counting and measurement procedures*

	Plant Properties (criteria)
Peduncle length	The length measured from the soil surface to the first flower appearing on the plant (cm)
Number of main shoots	Number of the main shoots on the plant (unit)
Number of side shoots	Number of side shoots on the main shoots of the plant (unit)
Number of buds on main shoots	Number of buds on the main shoots (unit)
Number of buds on side shoots	Number of buds on the side shoots (unit)
Flower width	The size of the widest part of the flower (mm)
Flower length	The size of the longest part of the flower (mm)
First blooming date	The date the first flower bloomed in population
Last flower date of death	The date the last flower deformed in population
Fruit ripening date	the date the first fruits begin to ripen in population
Seed shedding date	the date the first seeds begin to shedding in population

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During the seed collection process, the utmost care was taken to avoid harming the endemic plant population of the area; as a result, only a small number of seeds were randomly collected. The seeds delivered to the study area were cleaned, sorted, and air-dried by laying them horizontally in a shaded area. The study began on 6 August, 2019 after the 10-day air-drying process. In addition, the phenological and morphological parameters of the seeds were identified, including measurements and observations of seed size (width, height), seed maturation date, number of seeds per gram (1 g), 1000-kernel weight, capsule-cracking period, and seed-shedding date. In addition to these procedures, a viability test was conducted to determine seed viability. For seed dimensional analysis, 10 groups seeds were arranged with include 10 seeds, and dimension of the seed were calculated with 0.01 mm precision. Thousand seed weights were calculated with 8 replications (100x8) according to The International Seed Testing Association (ISTA) (2013).

Seed viability test

Viability tests were conducted on 9 August 2019. Seeds were divided into three replications, each containing 20 seeds, and were soaked in water for 24 hours at room temperature. After removing the seeds from the water, 1/3 were cut and kept in 1% tetrazolium (2,3,5-triphenyl tetrazolium chloride-Merck, Darmstadt, Germany) solution at 30 °C for 24 hours. The seed bolls were subsequently peeled, and their physical characteristics assessed. Seeds were classified as non-viable (no coloration), semi-viable (less coloration or with colorless patches), and viable (completely stained) based on the degree of staining (Moore, 1985; Peters, 2000).

Germination tests under laboratory conditions

A germination cabinet (Programmable Plant Growth Chamber SWGC-450-Daihan Scientific, Seoul, Korea) was used for the seed germination tests. Petri dishes measuring 100x20 mm were used for the experiments. Seeds were soaked in 70% ethanol (Soltek-Turkey) for one minute (1 min) before placing them into the Petri dishes. Immediately afterward, they were kept for 10 minutes in a commercially available 20% solution containing 5.25% sodium hypochlorite (BRTR Chemical, Izmir, Turkey). All seeds were sterilized in this manner, and subsequently rinsed twice with distilled water. All glass Petri dishes and blotting papers used in the study were sterilized at 100 °C for 30 minutes before use. In addition, the Petri dish lids were coated with parafilm to limit air permeability. Experiments were carried out with 4 replications and 50 seeds in each of them. To eliminate potential disease factors, 2.5 mL/L of maxim XL 035 FS a commercial fungicide with the active ingredients of Fludioxonil (25 g/L) + Metalaxyl-M (10 g/L) (Syngenta, Gaillon, France), was applied to the seeds. When evaluating the results of the germination studies, the percentage of the final germinated seeds (FGP) was taken into account. Experiments were designed and carried out under a 12/12-hour light/dark photoperiod regimen at a temperature of 20±0.5 °C. Counts were taken every two days, and radication was monitored for 30 days, with the two-millimeter radicle emerging from the seed shell considered as the measure for seed germination (ISTA, 2013; Eser et al., 2005). The effects of various treatments (i.e., dry storage at 4 °C for three months, cold-wet stratification, gibberellic acid (GA_3 - Merck, Darmstadt, Germany - treatments, or their combinations) on seed germination were investigated, and the details are presented in *Table 3*. The seeds were kept in either paper storage bags at 4 °C for 3, 5, 8 months for cold dry storage or in moist perlite at 4 °C for 2, 3 months for cold-wet stratification. The

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GA_3 treatments were applied by soaking the seeds in either 200, 400, 600, 1000, 1500, or 2000 mg/L GA_3 solution for 24 hours. The seeds for control applications were stored under uncontrolled conditions until the first sowing date of 4 January 2020.

Table 3. *Pre-treatments and application times of seeds (storage at 4 °C, cold-wet stratification, gibberellic acid)*

Treat. Nmb.	Treatments	Execution Date
T1	Control	4.01.2020
T2	Storage at 4 °C for 3 months	5.08.2019-4.11.2019
T3	Storage at 4 °C for 5 months	3.08.2019-4.01.2020
T4	Storage at 4 °C for 8 months + cold-wet stratification at 4 °C for 2 months	6.08.2019-7.06.2020
T5	Storage at 4 °C for 3 months + cold-wet stratification at 4 °C for 3 months	5.08.2019-4.02.2020
T6	Storage at 4 °C for 3 months + GA_3 treatments (200 mg/L)	6.08.2019-5.11.2019
T7	Storage at 4 °C for 3 months + GA_3 treatments (400 mg/L)	
T8	Storage at 4 °C for 3 months + GA_3 treatments (600 mg/L)	
T9	Storage at 4 °C for 3 months + GA_3 treatments (1000 mg/L)	
T10	Storage at 4 °C for 3 months + GA_3 treatments (1500 mg/L)	
T11	Storage at 4 °C for 3 months + GA_3 treatments (2000 mg/L)	
T12	Storage at 4 °C for 3 months + cold-wet stratification at 4 °C for 3 months + GA_3 treatments (200 mg/L)	4.08.2019-6.02.2020
T13	Storage at 4 °C for 3 months + cold-wet stratification at 4 °C for 3 months + GA_3 treatments (400 mg/L)	
T14	Storage at 4 °C for 3 months +cold-wet stratification at 4 °C for 3 months + GA_3 treatments (600 mg/L)	

All Petri dishes containing seeds were kept in a germination cabinet with a temperature of 20 °C and under a 12/12-hour light/dark cycle for germination testing following the treatments. For the purpose of generating an *ex-situ* collection yard, the seedlings were transferred into previously prepared 8x8 cm square pots and transplanted in the *ex-situ* collection yard in inter-rows of 30x30 cm on 18 March 2020. The plants in the collection yard that were not intended for research purposes were transferred and re-planted in natural areas on 26 March 2020, with the aim of reintroducing them into the natural population.

Experimental design and data analyses

Experiments were designed using the randomized block experimental design. The experimental data were analyzed using the IBM SPSS Statistics Base 22.0 statistical program. The data were subjected to a one-way analysis of variance (ANOVA), and Duncan's multiple comparison test was used to compare the differences between means. The percentage (%) germination rates determined after seed counting were subjected to the arcsine data transformation application.

Results

Plant characteristic and seed properties of Rhaponticoides wagenitziana

Table 4 shows the measurement results of the plant properties of *R. wagenitziana*. In the first year, all plant properties observed in the research yard underperformed compared to those assessed in the species' natural habitat. In the second year, however, those disparities were offset, and the assessed plant properties in the research yard remained within the acceptable limits compared to those in the natural habitat. Furthermore, since plants in the natural habitat are perennial, vegetative plant properties under cultivated conditions are likely to develop and express their characteristics more effectively in the following years. No measurable significant difference in flower size among the plants in the research yard during the first and second years was observed, which is a strong indicator that the species has an ornamental value. Therefore, the study findings showed that this plant species has a high level of adaptability could perfectly adjust to the conditions in which it is grown (*Fig. 3*).

Table 4. *Comparison of plant properties of R. wagenitziana grown in its natural habitat and under cultivated conditions*

	Natural Area	Cultural Condition		
	Taz Mountain	Cinarcık Research Yard		
Measurements	**In Natural Polulation Old Plants Mean ± SD**	**In Pots (1st Year Plant)Mean±SD**	**In Field (1st Year Plant) Mean±SD**	**In Field (2nd Year Plant) Mean±SD**
Peduncle length (cm)	79.00±10.38	28.36±7.89	60.33±4.93	54.4±4.54
Number of main shoots (unit)	3.66±0.45	1.25±0.25	1.11±0.09	1.5±0.52
Number of side shoots (unit)	12.00±3.70	6.19±1.05	5.25±1.15	10.6±2.99
Number of buds on main shoots (unit)	4.33±0.57	2.22±0.19	2.13±1.26	2.8±0.31
Number of buds on side shoots (unit)	32.00±2.79	11.66±4.04	16.66±5.85	22.71±3.06
Flower width (mm)	31.25±5.13	22.69±2.13	34.02±11.49	35.04±3.67
Flower length (mm)	16.21±2.69	17.56±2.16	19.08±1.06	21.70±2.94
First blooming date	14.06.2020	06.06.2022	05.06.2020	08.06.2021
Last flower date of death	28.07.2020	20.07.2022	18.07.2020	24.07.2021
Blooming duration of the population (day)	44	44	43	45
Fruit ripening date	The second week of July	The first week of July		
Seed shedding date	Between 20 and 25 July	Between 15 and 20 July		

Table 4 provides information on the dates of the fruit ripening and seed shedding of *R. wagenitziana* in Taz Mountain locations. The fruit ripening, and seed shedding stages in *R. wagenitziana* occurred at approximately one-week intervals in natural populations and cultivated plants, respectively. Therefore, according to these data, the time best suited for growing *R. wagenitziana* was the third week of July in the natural environment and the second week of July under cultivated conditions.

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Figure 3. *The appearances flower (A) and leaves (B) of R. wagenitziana grown under cultivated conditions*

The seeds of the *R. wagenitziana* are yellowish-beige in color, and the seed boll is glossy and slightly tapering at the tip of the base. The color gets lighter as in the direction of the seed tip. The stiff, hair-like structures (pappus) are arranged in a brush structure at the top of the seed. These structures serve as propellers and play a significant role in transporting seeds over great distances (*Fig. 4*).

Figure 4. *The appearance of R. wagenitziana seeds*

The length and width of the *R. wagenitziana* seeds were 7.61 mm and 3.76 mm, respectively (*Table 5*). The 1000-kernel weight and the average number of seeds in one gram (1 g) was 21.5801 and 46.34, respectively. The seed viability rate of *R. wagenitziana* in this study was 61.32% (*Table 5*).

During the year the research for the present study was conducted, the seed viability rate was low in *R. wagenitziana* due to empty seeds or undeveloped embryos. Similarly, insect damage was widespread among the seeds of this species. Similar damages among seeds collected in the same region were also observed in the years excluded from the present research. A significant percentage of semi-viable seeds (18.25%) measured in the same period suggested that there could be concerns with regard to insect damage, physical abnormalities, or seed maturation. Consequently, the non-viable seed rate (20.43%) was also high. It appeared that seed size and seed viability rates varied significantly among

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species. Changes in seed quality might occur over the years due to environmental factors such as insect damage, climate, soil, and even among seeds of the same species.

Table 5. *Selected morphological and biological characteristics and viability test results of R. wagenitziana seeds*

Seed Characteristics	Size/ Quantity /Percent (Mean±SD)
Seed length (mm)	7.61 ± 1.09
Seed width (mm)	3.76 ± 0.60
1000 kernel weight (g)	21.5801 ± 0.27
The average number of seeds in 1 g (piece)	46.34 ± 0.20
Viable seed rate (%)	61.32 ± 3.57
Semi-viable seed rate (%)	18.25 ± 3.02
Non-viable seed rate (%)	20.43 ± 2.13

Effect of pre-treatments on seed germination of Rhaponticoides wagenitziana

The effects of storage, stratification, and GA_3 treatments at different periods on the germination of *R. wagenitziana* seeds are shown in *Figure 5.* Statistically, the best germination rate for *R. wagenitziana* seeds was determined to be 48.25% and 52.75% from 600 mg/L and 1000 mg/L GA_3 treatments, respectively. when analyzing the effect of GA_3 treatment at various periods and at a temperature of 4 °C While there was no significant difference in germination rates between treatment with 200 mg/L and 400 mg/L GA_3 and storage at 4 °C for three months. in our study, 600 and 1000 mg/L GA_3 treatments after storage at 4 °C for 3 months, resulted in the best germination rates (*Fig. 5*). However, the germination rate decreased following GA_3 treatment with 1500 mg/L. Such a result showed that doses of GA_3 higher than 1000 mg/L had a limited impact on germination rate. The cold-wet stratification at 4 °C applied after three months of storage at 4 °C also resulted in a positive germination rate compared to that of the control seeds. However, this effect was comparable to three months of storage at 4 °C. This result suggests that the seeds did not require any cold-wet stratification following storage at 4 °C (*Fig. 5*).

Among the storage treatments, decreases in germination rate were observed when cold storage exceeded three-months. The dramatic differences observed with 5 and 8 months of storage compared to 3 months could be explained by the negative effects of prolonging the storage pre-treatments. The current study demonstrated that combining cold storage and cold-wet stratification with GA_3 treatments resulted in a higher germination rate (*Fig. 5*).

The use of 1000 mg/L GA_3 treatment after cold storage accelerated the germination speed, reducing it from T_{50}=18 days to an of T_{50}=5.8 days. Statistically, the treatments of 1000 mg/L and 1500 mg/L GA_3 after cold storage, and 200 mg/L and 600 mg/L GA_3 after three months of cold-wet stratification following the cold storage pre-treatment yielded the best results in germination speed (*Fig. 6*). The 600 mg/L GA_3 treatment after cold storage without cold-wet stratification was regarded as the most feasible and recommended combination since these combinations were classified in the same group, statistically.

However, there was substantial variation in germination rates between three- and five-month storage treatments, of 11 days and 18 days, respectively. Such a result revealed

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that increments in storage time had a positive and stimulating effect on germination speed, which was in contrast to its negative impact on germination rate (*Fig. 5* and *Fig. 6*).

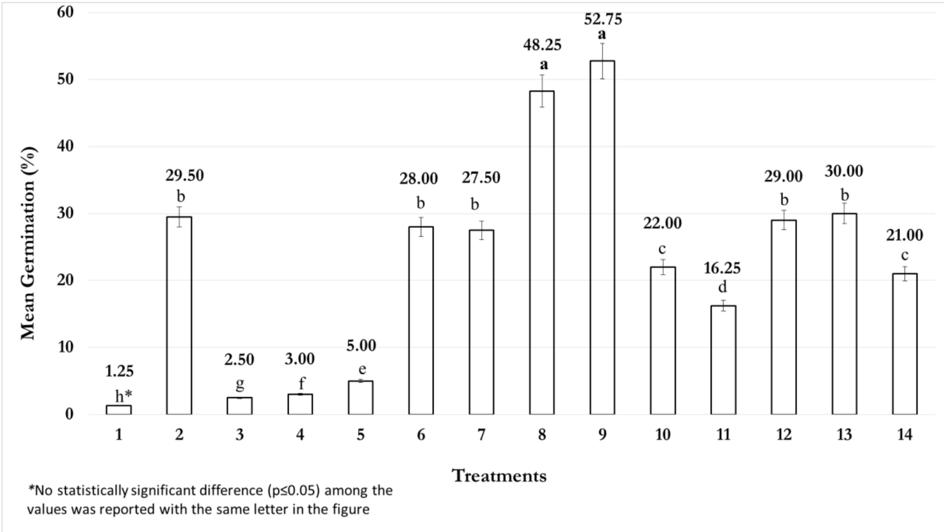

Figure 5. *The effects of storage, cold-wet stratification, and GA_3 treatments on seed germination of R. wagenitziana. T1-Control, T2-Storage 3 months, T3-Storage 5 months, T4-Storage 8 months + stratification 2 months, T5-Storage 3 months + stratification 3 months, T6-Storage 3 months + GA_3 (200 mg/L), T7-Storage 3 months + GA_3 (400 mg/L), T8-Storage 3 months + GA_3 (600 mg/L), T9-Storage 3 months + GA_3 (1000 mg/L), T10-Storage 3 months + GA_3 (1500 mg/L), T11-Storage 3 months + GA_3 (2000 mg/L), T12-Storage 3 months + stratification 3 months + GA_3 (200 mg/L), T13-Storage 3 months + stratification 3 months + GA_3 (400 mg/L), T14-Storage 3 months + stratification 3 months + GA_3 (600 mg/L)*

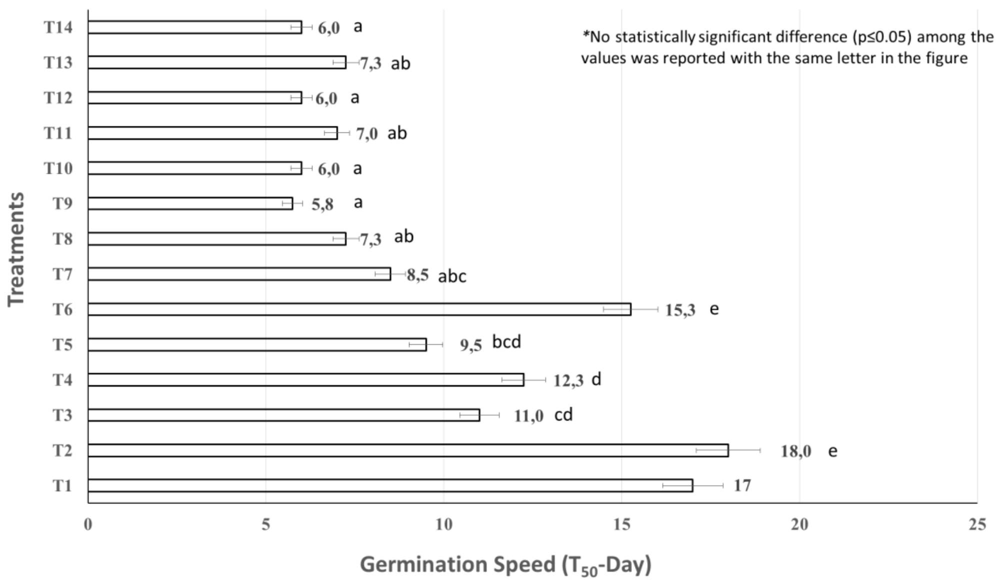

Figure 6. *The effects of storage, cold-wet stratification, and GA_3 treatments on germination speed of R. wagenitziana*

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Establishment of an ex-situ collection yard

Some seedlings were transplanted into the ex-situ collection yard during the research period. The seedlings grown in peat medium in viols of up to 2-3 leaflets were re-planted to 8x8 cm square pots. Subsequently, the grown seedlings were transferred to the ex-situ conservation yard in the second week of March. Excess plants not used in the study were re-planted in the natural population during the last week of March.

Discussion

The literature on the plants and seeds of *R. wagenitziana* is limited; therefore, studies on the related *Centaurea* species were also used to evaluate the validity of the results of the present study. Tan et al. (2009) reported that *R. wagenitziana* began to flower in mid-June and persisted until mid-August, with the fruits remaining on the plant until October. However, the present study found that although the seeds remained on the plant for a considerable amount of time, both in the natural habitat and in the research yard, they shedding near the end of July. The present study also discovered that flowering in both the research and cultivated areas occurred intensively in June and July. Based on these findings and observations, we determined that *R. wagenitziana* was a suitable choice for ecological landscape designs due to its attractive amethyst (lilac)-colored flowers that last for about one and a half months during the summer, a perennial nature that eliminates the need for yearly renewal, and a high degree of adaptability.

When the results of this study compared to Tan et al. (2009) who states that the germination rate is low (20%) under *R. wagenitziana*'s laboratory conditions, reveals that the applications made are effective in breaking dormancy. In terms of breaking dormancy, Saba et al. (2017) reported that 100 ppm GA_3 treatments increased seed germination rate more than 0, 25, 50 and 75 ppm in *Centaurea balsamita*. Luna et al. (2008) reported that stratification had no significant impact on the seed germination rate in their research on *C. ornata* and *C. pinae*, a finding that supports our own results. Similarly, Aghilian et al. (2014) found that the pre-cooling stage was ineffective in *C. cyanus* seed germination. Okay and Gunoz (2009), Okay et al. (2011), and Okay and Demir (2021) found that stratification for 120-150 days and 1000 mg/L GA_3 treatment prior to seed-sowing improved the germination rate of *C. tchihatcheffii*. Our findings showed that the stratification process improved the germination rate, but storage at 4 °C had no effect on *R. wagenitziana* seeds.

In this study it was determined that *R. wagenitziana* seeds are longer than that of other *Centaurea* species. For example, Eroglu et al. (2014), Tel et al. (2019), and Yucel (2022) reported that the seed size for *C. hermannii* was approximately 4-5 mm. The 1000-kernel weight and the average number of seeds in one gram (1 g) measurements also varied across previous reports in the literature. For example, Nosratti et al. (2017) reported a 1000-kernel weight of three grams (3 g) on *C. iberica* seeds, and Ebadi et al. (2014) reported a value of 2.318 g in *Centaurea depressa* species. Numerous seed viability testing results on different *Centaurea* species have been reported in the literature. For instance, Atasagun and Aksoy (2018) found an 82% seed viability rate in *C. amaena* species while Ozel et al. (2006) reported a rate of 85% in *C. tchihatcheffii*, Emek and Erdag (2012) a rate of 80% in *Rhaponticoides mykalea*. Yankova-Tsvetkova et al. (2018) identified a rate of 17.5% in *C. achtarovii*.

In this study, similar data with the literature have been reported. Eddleman and Romo (1988) found that cold-wet stratification reduced the germination speed in *C. maculosa*

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seeds. Nolan (1989) confirmed these findings and reported that GA_3 was a potent stimulant in his germination study with *C. maculosa* and *C. diffusa* and that some dormant seeds stored at 3 °C successfully germinated in a shorter time at 25 °C. In the light of these findings, seed reactions of species from the *Centaurea* genus to various pre-treatments may differ considerably. Indeed, Elias et al. (2012) and Baskin and Baskin (2014) reported that environmental and genetic factors, and even the germination media utilised, could have a wide range of effects on seed germination.

Conclusions

Overall, *R. wagenitziana* has potential as an ornamental plant with its extensive green-leaved appearance that covers the soil surface perfectly, aesthetic display, long-lasting purple (lilac) flowers that are rarely found in nature during the summer period, and a substantial adaptative capacity for cultivated conditions, while retaining its aesthetic and functional properties. When grown under cultivated conditions, this species generates on average about 10.6 sub-branches on a 54.4 cm peduncle in the second year. Almost 25.51 flowers bloom at the size of 3.5x2.0 cm on a single plant. The flowers bloom and remain on the plants for 45 days between the second week of June and the last week of July.

When evaluating *R. wagenitziana* in terms of the speed and germination rate of its seeds, an overall 52.75% germination rate was observed, with a germination speed of 5.8 days (T_{50}) using the combination of the 1000 mg/L GA_3 treatment after three months of seed storage at 4 °C. Extended storage periods, such as the three-month cold storage mentioned above, had a negative effect on the germination rate. In contrast, any increment in the storage period resulted in a positive and stimulating effect on germination speed. Furthermore, the combination of some selected pre-treatments resulted in both a higher germination speed and rate.

In the present study, special consideration was given to the use of seeds, seedlings, and plants of *R. wagenitziana*. For this purpose, a collection yard was established to ensure the *ex-situ* conservation and sustainable use of the plant materials. Seedlings were grown from germinated seeds and transplanted to the established *ex-situ* collection yard during the research period. In addition, the excess plants not used in the study were re-planted in the natural population and successfully maintained. Future research and cultivation studies will be conducted by utilizing the seeds and plants from the *ex-situ* yard to design further research.

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REFERENCES

[1] Aghilian, S., Khajeh-Hosseini, M., Anvarkhah, S. (2014): Evaluation of seed dormancy in forty medicinal plant species. – International Journal of Agriculture and Crop Sciences 7(10): 760-768.

[2] Alam, H., Khattak, J. Z. K., Ppoyil, S. B. T., Kurup, S. S., Ksiksi, T. S. (2017): Landscaping with native plants in the UAE: A review. – Emirates Journal of Food and Agriculture 29(10): 729-741.

[3] Anderson, A. G., Messer, I., Langellotto, G. A. (2021): Gardeners' perceptions of northwestern us native plants are ınfluenced by ecological ınformation and garden group affiliation. – HortTechnology 1: 1-14. doi: 10.21273/HORTTECH04770-20.
[4] Atasagun, B., Aksoy, A. (2018): Autecology and conservation biology of *Centaurea amaena* (Asteraceae). – Journal of Animal and Plant Sciences 28(1): 208-214.
[5] Baskin, C. C., Baskin, J. M. (2014): Seeds: ecology, biogeography, and evolution of dormancy and germination. – 2nd ed. Elsevier/Academic Press, San Diego.
[6] Baskin, C. C., Baskin, J. M. (2020): Breaking seed dormancy during dry storage: a useful tool or major problem for successful restoration via direct seeding? – Plants 9(5): 636.
[7] Bona, M. (2013): An overview to Centaurea s.1. (Asteraceae) based on herbarium specimens of ISTE. – Journal of the Faculty of Pharmacy of İstanbul Üniversity 43(2): 121-137.
[8] Bona, M. (2014): New floristic records of Centaurea S.l. (Asteraceae) for 27 squares in the flora of Turkey. – Hacettepe J. Biol. & Chem. 42(3): 331-335.
[9] Ebadi, S. Z., Golzardi, F., Vaziritabar, Y., Vaziritabar, Y., Ebadi, M. S. (2014): Environmental maternal effects on drought and salinity tolarance of Iranian knapweed (*Centaurea depressa* M. Bieb.) at germination and seedling growth stage. – Indian Journal of Fundamental and Applied Life Sciences 4(3): 157-166. http://www.cibtech.org/jls.htm.
[10] Eddleman, L. E., Romo, J. T. (1988): Spotted knapweed germination response to stratification, temperature, and water stress. – Canadian Journal of Botany 66(4): 653-657.
[11] Elias, S. G., Copeland, L. O., McDonald, M. B. (2012): Seed testing-principles and practices. – Michigan State University Press, East Lansing, MI, USA.
[12] Emek, Y., Erdağ, B. (2012): Researchs on in vitro seed germination of the critically endangered endemic plant *Rhaponticoides mykalea*. – Journal of Nevsehir University Institue of Science 1(2): 46-59.
[13] Eroğlu, H. K., Ozyigit, I. I., Altay, V., Yarcı, C. (2014): Autecological characteristics of *Centaurea hermannii* F. Herm. An endemic species from Turkey. – Bulgarian Journal of Agricultural Science 20(1): 183-187.
[14] Eser, B., Saygılı, H., Gökçöl, A., İlker, E. (2005): Tohum bilimi ve teknolojisi [Seed science and technology]. – Cilt I-II, Ege Üniversitesi Tohum Teknolojisi Uygulama ve Araştırma Merkezi Yayın No: 3, İzmir, 908 p.
[15] General Directorate of Meteorology (2021): https://mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=YALOVA. – Erişim Tarihi 24.12.2021.
[16] Hopkins, E., Al-Yahyai, R. (2015): Landscaping with native plants in Oman. – In VIII International Symposium on New Ornamental Crops and XII International Protea Research Symposium. ISHS Acta Horticulturae 1097: 181-192.
[17] ISTA (International Seed Testing Association) (2013): International Rules for Seed Testing. – Bassedorf, Switzerland.
[18] Karimian, Z., Samiei, L., Nabati, J. (2017): Evaluation of drought resistance in *Nitraria schoberi* as a native plant by irrigation intervals for applying in arid urban landscape. – Acta Scientiarum Polonorum Hortorum Cultus 16(6): 77-84.
[19] Karlović, K. (2007): Introduction of ornamental native plants into commercial production in Croatia. – In VI International Symposium on New Floricultural Crops. ISHS Acta Horticulturae 813: 107-112.
[20] Khadis, C., Georghiou, K. (2010): Seed dispersal and germination behaviour of three threatened endemic labiates of Cyprus. – Plant Species Biology 25: 77-84.
[21] Kırmızı, S., Güleryüz, G., Arslan, H. (2019): Germination responses to GA_3 and short-time chilling of three endemic species: *Tripleurospermum pichleri*, *Cirsium leucopsis* and *Senecio olympicus* (Asteraceae). – Plant Species Biology 26(1): 51-57. doi: 10.1111/j.1442-1984.2010.00302.x.
[22] Luna, B., Perez, B., Cespedes, B., Moreno, J. M. (2008): Effect of cold exposure on seed 58 plant species comprising several groups from a mid-mountain Mediterranean area. – Écoscience 15(4): 478-484.

[23] Moore, R. P. (ed.) (1985): Handbook on tetrazolium testing. – International Seed Testing Association, Zurich, Switzerland.

[24] Nolan, D. G. (1989): Seed germination characteristics of *Centaurea diffusa* and *C. maculosa*. – B.Sc., The University of British Columbia. A Thesis Submitted in Partial Fulfillment of the Requirments for the Degree of Master of Science.

[25] Noroozi, J., Talebi, A., Doostmohammadi, M., Manafzadeh, S., Asgarpour, Z., Schneeweiss, G. M. (2019): Endemic diversity and distribution of the Iranian vascular flora across phytogeographical regions, biodiversity hotspots and areas of endemism. – Scientific Reports 9(1): 1-12.

[26] Nosratti, I., Abbasi, R., Bagheri, A., Bromandan, P. (2017): Seed germination and seedling emergence of Iberian starthistle (*Centaurea iberica*). – Weed Biology and Management 17(3): 144-149.

[27] Okay, Y., Günöz, A. (2009): The effects of some applications on seed germination of endemic *Centaurea tchihatcheffii* Fisch. et Mey. in Gölbaşı Province. – Journal of Agricultural Sciences 15(2): 119-126.

[28] Okay, Y., Günöz, A., Khawar, K. M. (2011): Effect of cold stratification pretreatment and ph level on germination of *Centaurea tchihatcheffii* Fisch. et Mey. seeds. – African Journal of Biotechnology 10(9): 1545-1549.

[29] Okay, Y., Demir, K. (2021): *Centaurea tchihatcheffii* Fisch. and Mey: An endangered endemic species found around Golbasi district of Ankara province, Turkey. – Advanced Journal of Environmental Science and Technology 12(1): 001-006.

[30] Özel, C. A., Khawar, K. M. (2006): Factors affecting in vitro plant regeneration of the critically endangered Mediterranean knapweed (*Centaurea tchihatcheffii* Fisch et. Mey). – The Science of Nature 93(10): 511-517.

[31] Özhatay, F. N., Kültür, Ş., Gürdal, M. B. (2011): Check-list of additional taxa to the supplement Flora of Turkey V. – Turkish Journal of Botany 35(5): 589-624.

[32] Peters, J. (2000): Tetrazolium testing handbook: contribution no. 29 to the handbook on seed testing. – Association of Official Seed Analysts, Inc., Lincoln, NE, USA.

[33] Russo, A., Cirella, G. T. (2018): Modern compact cities: how much greenery do we need? – Int. J. Environ. Res. Public Health 15(10): 2180. https://doi.org. /10.3390/ijerph15102180.

[34] Saba, Y., Hassan, A., Iraj, N., Sohbat, B. (2017): Evaluation the effects of different factors on the seed germination and dormancy-breaking of knapweed (*Centaurea balsamita* Lam). – Iranian Journal of Weed Science 13(1): 89-96.

[35] Tan, K., Bancheva, S., Vural, M., Strid, A. (2009): *Centaurea wagenitziana* (Asteraceae: Centaureinae), a new species from the Eastern Balkans. – Phytologia Balcanica 15(1): 51-58.

[36] Tel, M., Özhatay, F. N., Keskin, M. (2019): İstanbul ili - Çatalca Peygamber Çiçeği (*Centaurea hermannii*) tür eylem planı [Istanbul province - Çatalca Peygamber flower (*Centaurea hermannii*) species action plan. – AK-TEL Mühendislik.

[37] Uğurtaş, İ. H., Kaynak, G., Yılmaz, Ö., Kankılıç, T., Tuncalı, T., Emiroğlu, O., Çakır, D. T., Güvenç, Ş., Varlı, S. V., Çekici, T., Yıldırım, B., Aksu, S., Başkurt, S., Arslan, M. (2014): Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Yalova Şube Müdürlüğü Ulusal Biyolojik Çeşitlilik Envanter ve Izleme Sonuç Raporu [Yalova Office of General Directorate of Nature Conservation and National Parks, National Biodiversity Inventory and Monitoring Project Result Report]. – Orman ve Su İşleri Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü, [General Directorate of Nature Conservation and National Parks]. Yalova-Turkey.

[38] Yankova-Tsvetkova, E., Ilieva, I., Stanilova, M., Stoyanov, S., Sidjimova, B. (2018): Reproductive biology of the endangered Bulgarian endemic *Centaurea achtarovii* (Asteraceae). – Biologia (Bratislava) 73(12): 1163-1175.

[39] Yucel, G. (2022): Seed propagation, adaptation to cultivation conditions, determination of ornamental plant properties, and ex situ conservation of the endemic species Centaurea hermannii F. Hermann. – BioResources 17(1): 616-633.