Investigation of Vegetative Properties and Generative Production of the Potential Ornamental and Narrow Endemic Species *Verbascum yurtkuranianum* (Scrophulariaceae) for *Ex situ* Conservation

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Verbascum yurtkuranianum is a narrow endemic species occurring in a single location, the northern Bursa province (Turkey). It is an endangered and potentially ornamental plant. No conducted study on its life and biology, production, and aesthetic features is available. This study aimed to reveal its vegetative properties, seed characteristics, methods and requirements for seed germination, germination speed, and potential ornamental value so it can be conserved *ex situ* and produced. *Verbascum yurtkuranianum* has potential value as an ornamental plant regarding its aesthetic features as a flower. This study revealed that the total number of individuals in the species is 788. Without any treatment, 70.7% germination rate is achieved if the seeds are stored at 4 °C. The optimum germination temperature was from 15 to 20 °C (77.3% and 78.7%, respectively), and the photoperiod regulation for seed germination was 12/12 or 8/16 (light/dark) hours (74.7% and 76.0%, respectively). The most effective treatment to promote germination rate was found by implementation of 60 min ultrasonic waves (94.3%) or application of 120 min vacuum (95.3%). Germination occurred between 8 and 10 days. A parcel of *ex situ* conservation was constituted with the seedlings obtained from the germination studies.

Keywords: Verbascum yurtkuranianum; Endemic; Ex situ conservation; Seed germination; Ultrasonic wave; Vacuum; Ornamental plant

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INTRODUCTION

Today, the growing threats to biodiversity and ecosystems and the decline of certain species to the degree of extinction are considered the most severe problems of our time (Kırmızı *et al*. 2019). One of the primary conditions for conservation and sustainable use of biodiversity is securing endangered species and enhancing the protection endeavors to preserve remaining ecosystem fragments (Ganatsas *et al.* 2019; Locke *et al.* 2019). Pursuant to international agreements, protection of endemic and rare species is a requirement. Each signatory country of the Rio Convention is required to take their biological inventories and the necessary measures to conserve them (Ocak *et al*. 2017). In spite of these measures, endemic plants cannot be conserved sufficiently, and they are left endangered (Öztürk and Yiğit 2013). One of the ways to minimize the impacts of these dangers is being aware of the germination and dormancy behaviors of endemic species and conserving them accordingly *in situ* and *ex situ* (Kırmızı *et al.* 2019).

In situ and *ex situ* conservation of endemic and rare species requires comprehension of knowledge about the habitat and life biology of the plant (Ganatsas *et al.* 2019). This information constitutes critical subjects for conservation and sustainable use. To protect and reproduce endangered and endemic species, revealing the seed germination characteristics is one of the primary issues (García *et al*. 2012; Schnadelbach *et al.* 2016; Ganatsas *et al.* 2019). The knowledge of the germination period and the plant production rate heavily influences the fertility of production works. Therefore, it is critical to carry out the works for reproduction by being conversant with the germination tendencies of the plant (Cesur *et al.* 2017).

In this day and age, reducing the use, care costs, and water consumption of native species of a locality in ecological landscape design is considered to be the most important factor of sustainability, and this approach is becoming increasingly widespread (Brzuszek *et al*. 2010; Çetin and Mansuroğlu 2019). Making native species usable for commercial purposes requires successive long-term studies (Pollock and Biante 1996). The early stages of the study identify the characteristics of the species or genotype and determine the germination features (Mikkelsen 1987; Henting 1998).

The *V. yurtkuranianum* taxon, introduced to the scientific community in 2006, is a global endemic plant surviving with 788 individuals in a single locality. It exists in small groups in a 2 km^2 area (Kaynak *et al.* 2006). Because these spots are high-risk highway drainage channels, picnic sites, and natural gas pipeline routes precariously close to countryside dwellings, the risk level of being critically endangered (CR) according to IUCN criteria is extremely high (Çenil 2007; Erdoğan *et al.* 2011). The species is likely to lose more than 50% of its individuals in the next 10 years. Therefore, this species, which has potential for becoming an ornamental plant (due to its bright green, glabrous basal leaves, purple-violet corollas, and long flower life) is under immense pressure.

Many plant species manifest dormancy for different reasons and suspend germination until ready to grow seedlings (Baskin and Baskin 2014). Stratification and certain chemical treatments are known to terminate dormancy and enhance germination rates for seeds (El-Dengawy 2005; Jones *et al*. 2016; Peng *et al.* 2017). Except for using chemical substances, other practices are available for inserting water into the seed *via* vacuum or pressure to promote germination (Miyoshi and Mii 1998; Custódio *et al.* 2016; Katsalirou *et al.* 2019). Another treatment that began to be used to terminate dormancy in seeds and enhance germination percentage is ultrasonic wave implementation (Luo 2016; Nazari and Eteghadipour 2017; Wong *et al.* 2019).

Reproducing endemic and potential ornamental taxa by establishing the production methods – that benefit from them as ornamental plants on the condition that they are *ex situ* conserved and their sustainability is ensured – will provide multiple advantages regarding both the protection of biodiversity and transformation of natural resources into economic values. There are studies on the morphological characteristics of *V. yurtkuranianum* as one of these species (Kaynak *et al.* 2006; Çenil 2007; Erdoğan *et al.* 2011). However, no study of its ornamental plant features and *ex situ* conservation is available. This study aimed to establish the most ideal generative production methods for the global endemic species *V. yurtkuranianum* in a laboratory setting, to find a resource garden with the reproduced plants using the established methods for *ex situ* conservation, promotion, and cultivation works, and to determine the extent of the landscape value for the species.

EXPERIMENTAL

Materials

Verbascum yurtkuranianum seeds used in the study were collected from Ericek village (40^{o}19'06" N, 29^{o}15'52" E) of Bursa, Turkey (40^{o}11'40" N, 29^{o}03'14" E) (Fig. 1). There were three locations where the seeds were collected: location 1 (40^{o}18'56" N 29^{o}15'32" E, 681 m), location 2 (40^{o}18'56" N, 29^{o}16'22 E, 704 m), and location 3 (40^{o}18233" N, 29^{o}17'25" E, 739 m). Morphological measurements and physiological observations were made in three groups of the plants selected from these locations.

Seeds were collected on 24 July 2019 and 29 July 2020. They were put in a 4 ^{o}C freezer on September 1st. They were kept in the cold freezer until the experiments were conducted. In 2020, some seeds were put into room conditions (20 °C, 50% RH), for seed storage temperature tests.

Methods

Determining the ornamental plant potential, morphological and physiological features

The locality of the population was periodically visited between April and August to observe physiological and aesthetic features of the plant. Morphological, physiological, and life-biology-related observations and measurements were performed. This data also ascertains whether the species has potential ornamental plant value and provides insight regarding the aesthetic and functional characteristics of the plant for landscape use (Alp *et al.* 2020; Erken *et al.* 2021). Measurements were made in 2020 on 20 plants randomly selected from each population at three different locations (Fig 1.).

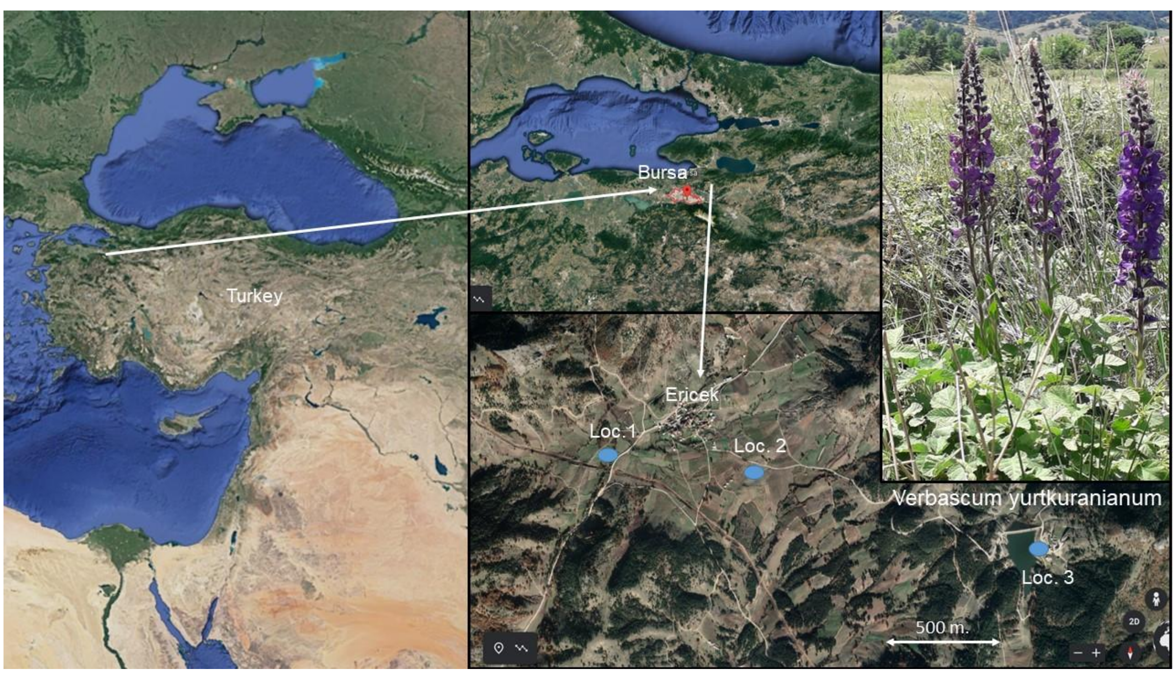

Fig. 1. Images indicating the locations of the *V. yurtkuranianum* where the plants were measured and the seeds were collected

Because the species is endemic and insufficient in the number of individuals, seeds were randomly collected in limited numbers to not damage the population pursuant to the protocol stated in Bacchetta *et al.* (2006). After collection, the seeds were air-dried in room conditions for 7 days. Twenty-five seed capsules were randomly selected to reveal the average seed count in a capsule, and the number of seeds considered healthy, alive, and fully mature was discovered after extraction. Some of the seeds were packed and stored in a 4 °C storage freezer (Ugur Cooling Inc., Co., Aydın, Turkey) and the other portion in room conditions (20 °C, 50% relative humidity (RH)) after they were extracted from their seed capsules and cleaned. Measurements and counts were performed regarding the physiological and morphological features of the seeds.

Germination tests

The germination tests were conducted in a Lovibond TC 140 G thermostatically controlled incubator (Liebherr, Dortmund, Germany). The glass Petri dished used were 100 mm x 20 mm. Before the treatments, the seeds were sterilized by washing three times with distilled water after they were steeped in ethanol 70% for 1 min. They were then soaked in sodium hypochlorite solution 20% (5.25% NaOCl, Koruma Cleaning Co., Kocaeli, Turkey) for 10 min. Petri dishes and blotting papers were used after they were sterilized at 100 °C for 30 min. Distilled water was used for preparation of the solutions and in Petri dishes. Two layers of blotting paper were placed at the bottoms of the Petri dishes, and these papers were dampened with 3 mL of purified water. Petri dishes were put on lids and wrapped in stretch film, and the germination tests were performed under a 12/12 light regime in the germination incubator with shelves set to 20 ± 0.5 °C. To prevent infection development in Petri dishes, a commercial fungicide (Sumiriz-T 60 WP) (SumiAgro Corp., Istanbul, Turkey) containing the agent of 30% tolclofos-methyl + 30% thiram was applied. Tests were performed in a 30-day period, and germinated seeds were counted at routine intervals (every 2 days). Seeds emerging with 2-mm radicles were considered to be germinated, counted, and removed from the Petri dish (Eser *et al.* 2005; ISTA 2013). The final germination percentage (FGP) was employed in germination research.

Determining the impact of seed storage temperature on germination

Because it was thought that the germination feature is lost if the seeds have been kept in room conditions (20 °C, 50% RH), the germination percentages of the seeds kept in room conditions (20 °C, 50% RH) and at 4 °C were sought through comparison experiments. To set up these experiments, the cleaned seeds were stored at 4 °C for 7 months, while some seeds were stored in room conditions for same time. The seeds collected 2019 and the experiment settings were established with seeds stored in two different conditions in April 2020.

Determining the optimum germination temperature

To find out the germination performances of seeds at different temperatures, germination experiments were performed at four different temperatures of 10, 15, 20, and 25 °C by fixing all the other conditions (12 h light/12 h dark and humidity 80%). Once the temperatures of 15 and 20 °C were determined more suitable for germination with these first experiments, the other germination studies were conducted at 20 °C. Experiments were set up with freshly harvested unstored seeds in August 2019.

Impact of light regime (photoperiodic treatments) on seed germination

Germination experiments were set up in environments of constant light, 16-h light/8-h dark, 12-h light/12-h dark, 8-h light/16-h dark, and constant dark to determine the photoperiodical reactions of *V. yurtkuranianum* seeds. The temperature was set to a fixed 20 °C in the experiments. Fifteen hundred lumen daylight-glow light bulbs were used as the light source. Experiments were established in April 2020 with seeds collected in 2019 and stored at 4 °C for 7 months.

Determining the impacts of ultrasonic wave and vacuum treatments on germination percentage and speed

The impact of 60 and 120 min vacuum and ultrasonic treatments on the germination percentage was investigated based on the literature reporting that ultrasonic wave and vacuum treatments speed up water absorption by seeds (Miyoshi and Mii 1998; CusTódio *et al.* 2016; Katsalirou *et al.* 2019). The seeds placed in small paper bags were laid on the plastic platform and put in the ultrasonic tank (Bandelin Sonorex Dıgıplus, Typ DL 510 H, Berlin, Germany). The seeds were exposed to 35 kHz ultrasonic wave treatment for periods of 60 and 120 min. During the implementation, the water temperature was set to a fixed 25 ± 2 °C. For vacuum treatment, the seeds in small cloth bags were laid on a platform and placed in the vacuum device (Binder, VD 23, Tuttlingen, Germany). They were held in the 27.3 °C vacuum device for periods of 60 and 120 min under 380 mm/Hg vacuum. Seeds kept in the same conditions and unexposed to vacuum were used as the control. To determine the germination speed as well as the final germination percentage (FGP), the period (in days) of the germination for 50% of the total number of germinated seeds was calculated. Experiments were established on 15 November 2019 with seeds collected in 2019 and stored at 4 °C for 2.5 months.

Determining the impacts of the cold-wet stratification, GA_3, ultrasonic wave, vacuum, and warm-water soak combinations on germination

To increase the rates of germination, combinations of the following experiments were performed: 30 °C warm-water soak for 48 h together with cold stratification; holding in 1000 ppm GA_3 solution (Merck, Darmstadt, Germany) for 24 h; and 60 and 120 min of ultrasonic wave and vacuum treatments. Seeds were stored in the 4 °C repository, and only wet stratification-applied seeds were used as the control (Hartman *et al.* 1990; Eser *et al*. 2005; Baskin and Baskin 2014). Wet-cold stratification was applied by storing the seeds placed in Petri dishes in small bags made from blotting paper in a 4 °C repository and kept in dark conditions for 12 weeks. Otherwise, the seeds were placed in damp perlite in these bags and stored in a 4 °C repository in dark conditions for 12 weeks. The 30 °C water soak treatment was implemented, placing the seeds in 300-cc lid jars topped with pure water and held in an incubator (30 °C) for 48 h.

The treatment of GA_3 solution was implemented by adding 50 cc GA_3 solution to the seeds placed in jars and kept in room conditions (20 °C) for 24 h (Hartman *et al.* 1990; Eser *et al.* 2005; Genç 2005). The seeds, which were stored for 3 months at 4 °C, were put into cold-wet folding on December 1 (2019). Folding was terminated on March 1 (2020) and then the seeds were put into an incubator for germination.

Founding the ex situ conservation parcel

The seeds germinated and were removed from the Petri dishes and were planted in turf environments in vials. The seedlings developing with continuous liveliness in vials were planted in the *ex situ* conservation field in outdoor conditions once they grew 2 to 3 small leaves.

Experimental design and data analyses

The experiments were established based on the Randomized Block Experimental Design. Three replications and 100 seeds for each replication were used. The study was conducted in the Bursa Technical University Laboratories (Bursa, Turkey) in 2019 and 2020. For the evaluation of the data acquired in the study, IBM SPSS Statistics 20 software (IBM Corp., Armonk, NY, USA) package program was employed. A T-test for the storage temperature data and one-way analysis of variance for other data was employed, and the operations between which differences were found were compared with a comparison test. Arc-sin data transformation was applied to the % germination rates acquired by counting.

RESULTS AND DISCUSSION

Ornamental plant potential, morphological, and physiological features of the plant

Table 1 exhibits the observation and measurement results regarding the life biology, morphological features, and ornamental plant characteristics of the species *V. yurtkuranianum* occurring in a single location (Fig. 2). The observations revealed that flower blooming takes place in May, June, and July. The branching, even though limited, in the plant proves that the plant can grow multiple peduncles if the removal of buds is practiced.

The percentage of the flowering plants in the plant population was 23.1%. The flowers start to bloom from the lower end of the peduncles, and new buds continue to grow at the top. Flowering continues at the top even if the first blooming flowers have gone to seed and have been capsulated. This feature ensures flower blooming for approximately 2 months and increases the aesthetic value of the plant. During the field work, the plant is picked and used as vase bouquets. Although it was discovered during the field observations that all the leaves were scorched and disappeared after July, the plant in the *ex situ* conservation garden founded in a culture medium preserved all its green parts and did not suffer from any damage at the below-zero temperatures with a minimum -11 °C for 20 h in the winter.

Table 1. Data on Plant Development and Life Biology of *V. yurtkuranianum*

Parameter	Time / Amount / Size
New leaf growth date of the plant	The first week of April
Start date of flower blooming of the species	Western and southern slopes the first week of May; Level areas the second and third weeks of May
The last flower-blooming time in the population	End of June
Average number of leaves per plant (quantity)	14.4
Leaf length (including the rachis) (cm)	18 to 21
Leaf blade length (cm)	13
Leaf width (cm)	6 to 8
Peduncle length (including the ear) (cm)	100.31
The length of the ear of flower (cm)	55.65
The number of corollas per ear (quantity)	63.79
Average branching per peduncle (quantity)	1.09

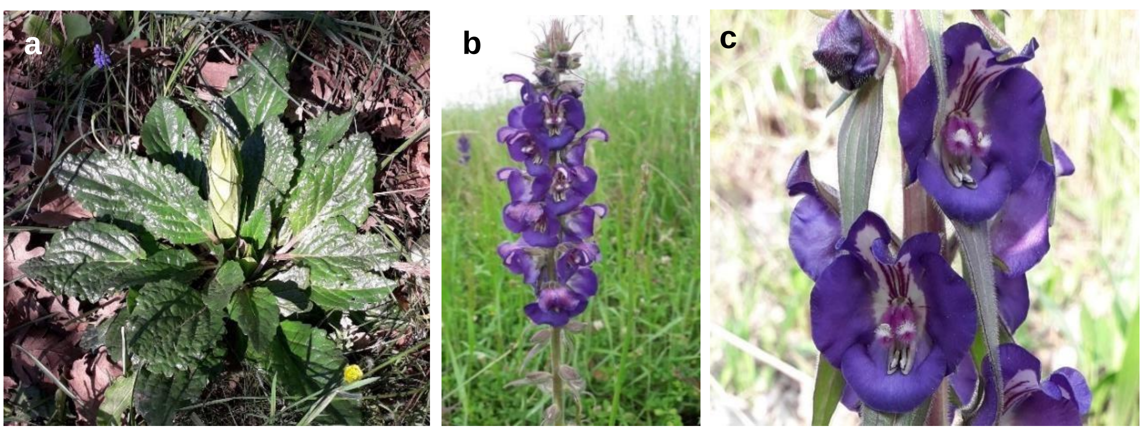

Fig. 2. General view of *V. yurtkuranianum* plants: a) rosette, b) inflorescence of plant, and c) flowers

The observations of *V. yurtkuranianum* revealed that the flower-bearing period of the population, one of the essential characteristics, is approximately 70 days. This period is quite a long time for single-peduncle *Verbascum* species. For example, in the study conducted on the multi-peduncle *V. thapsus* species by Ivanova and Valchev (2020), the flower-bearing period was determined as 72.1 to 91.4 days. *V. yurtkuranianum* represents a different leaf structure from other *Verbascum* species. Particularly, its hairless and bright green leaves increase the potential use of this species as an ornamental plant. Among the species of the genus *Verbascum* (Huber-Morath 1978), it is similar to the *V. bugilifolium* species in leaf structure and peduncles (Kaynak *et al.* 2006) and to the *V. weidemannianum* species with amaranth purple corolla in corolla structure (Senel *et al.* 2007), in spite of its difference in corolla color (blue). Unlike the *V. wiedemannianum*, it has hairless leaves and violet flowers, and it grows fewer pedicels per peduncle. Unlike the *V. bugilifolium*, the base color of *V. yurtkuranianum* corolla is blue.

Morphological features of the seed

The seed pods that develop on the plant are observed to start growing ripe from mid-July and dehiscing towards the end of July. It is noteworthy that because the flower blooming of the plant continues for a long time, ripening of the pods (and therefore the seeds) extends over a long period; moreover, the seeds in some pods dry out before ripening due to extremely hot climatic conditions. Pods that have dehisced and ripened recently and have not ripened yet exist concurrently on a plant. The pods must be harvested at the lower parts to collect seeds for production. The data obtained regarding the physiological and morphological features of the seeds are demonstrated in Table 2 and Fig. 3.

Table 2. Physiological and Morphological Features of *V. yurtkuranianum* seeds

Quality	Time / Amount / Unit
Seed ripening time	Mid-July
Seed throwing time	End of July
Average number of seeds in a pod (quantity)	136.96
Seed width (mm)	0.8
Seed length (mm)	1.2
Weight of 1000 seeds (g)	0.1899
The number of seeds in 1 g (quantity)	5265.9

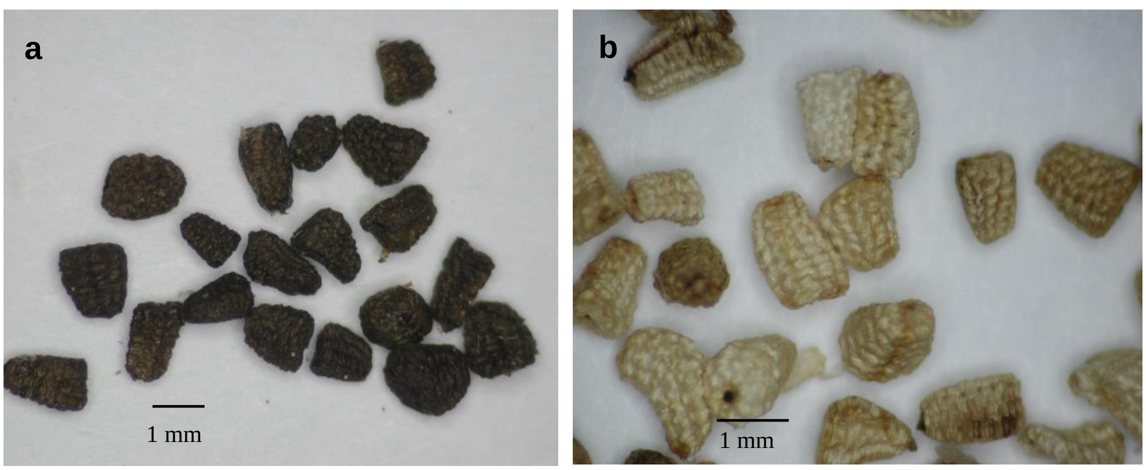

Fig. 3. Seeds of *V. yurtkuranianum*: a) natural seeds view and b) seeds view after sterilization (photos from a Irmeco stereomicroscope –Krüss Optronic, Hamburg, Germany)

Seed storage temperature

According to the results of the experiment's set up to determine the seed storage temperature, a higher and also statistically significant germination percentage was obtained from the seeds stored in 4 °C dry storage conditions (Table 3). Storing the seeds at 4 °C ensured germination of 25.7% more seeds. This expected result corresponds to the recommendations of Hong and Ellis (1996), De Vitis *et al*. (2020), and Palomeque *et al.* (2020) to store the seeds at a temperature between 0 and 5 °C for short-term storage (12 to 18 months).

Table 3. Impact of Storage Temperature on Germination Percentage of *V. yurtkuranianum* Seeds

Seed Storage Temperature	Mean Germination (%) ± SE
Storing at room temperature (20 °C, 50% RH)	45.00 ± 1.52 b*
Storing at 4 °C	70.67 ± 2.93 a

*: Within each column, means with the same letter are not significantly different ($p \leq 0.019$); SE: standard error

Optimum germination temperature

Different temperature treatments in the germination experiments conducted to determine the optimum germination temperature of the seeds were discovered to have a significant impact on seed germination (Table 4). No significant difference was observed at the temperatures of 15 to 20 °C for seed germination percentages. The 36.7% germination obtained in 10 °C germination environment revealed that this species can germinate in cool climates as well.

Table 4. Impact of Different Temperature Regimes on the Germination of *V. yurtkuranianum* Seeds

Temperature (°C)	Germination (%) ± SE
10	36.67 ± 7.5 b*
15	**77.33 ± 0.5 a**
20	**78.67 ± 1.7 a**
25	4.00 ± 1.8 c

*: Within each column, means with the same letter are not significantly different ($p = 0.05$); SE: standard error

According to the results acquired from the temperature experiments, the temperatures of 15 °C (77.3%) and 20 °C (78.7%) are more suitable for the germination of *V. yurtkuranianum* seeds. Şenel *et al.* (2007) determined that for *V. bithynicum* and *V. wiedemannianum* species and Ganatsas *et al.* (2019) for *V. dingleri* species the optimum seed germination temperature was 20 °C, as in the current study. In the study conducted on germination of the *V. dudleyanum*, *V. natolicum*, *V. serratifolium*, and *V. suworowianum* var. *suworowianum* species, the results of Isık *et al.* (2017) reflected the highest germination at a temperature, 22 °C, which is close to the results of the current study. In a study on nine taxa of *Verbascum* spp, Catara *et al.* (2016) produced good results in certain species at a 25 °C temperature environment in addition to 15 and 20 °C. However, in the current study, the germination percentage obtained at 25 °C (4.0%) decreased considerably while high-level germinations were provided similarly at 15 and 20 °C (77.3% and 78.7%). Leite and Takaki (2001) concluded that germination temperatures can vary based on species. Accordingly, Sarıbayır (2001) found the germination temperatures for the species *V. olympicum*, *V. bombyciferum*, and *V. prusianum* as 4 to 7 °C, which is distinctively different from the results of the current study. Seipel *et al.* (2015) provided high germination percentages at a temperature range between 20 and 35 °C in *V. thapsus* seeds collected from samples of different ecological conditions. In contrast, in the author's study, the germination percentage was reduced considerably at the temperature of 25 °C.

Even though Catara *et al*. (2016) reported better results in alternating temperature treatments compared to fixed temperature, the high germination percentages in the current study were obtained with 20 °C fixed temperature treatments. There are studies revealing that germination-enhancing effects of hormones are more significant at fixed temperatures between 20 and 25 °C (Chen *et al.* 2008) and high temperatures lead to deceleration of germination (Núñez and Calvo 2000; Shen *et al*. 2010). Alba *et al.* (2016) concluded that genetic differences developed regarding germination in natural and cultural forms of the species *V. thapsus*, this difference is more significant in natural populations and, therefore, natural populations of a species can germinate at different temperature ranges.

Impacts of light regime (photoperiodic treatments) on seed germination

According to the results acquired from the experiments to determine the response of seeds to photoperiodism, the light conditions of the germination environment were discovered to have a statistically significant impact on seed germination (Table 5). While the implementation of constant dark completely inhibited germination, the photoperiodic treatments of 12 / 12 and 8 / 16 light / dark were detected to be effective light regimes for *V. yurtkuranianum* seed germination.

Table 5. Impact of Different Photoperiodic Treatments on *V. yurtkuranianum* Seed Germination

Photoperiodic Treatments	Mean Germination (%) ± SE
24-h constant light	18.67 ± 1.58 c
16-h light / 8-h dark	64.00 ± 1.81 b
12-h light / 12-h dark	**74.67 ± 3.58 a**
8-h light / 16-h dark	**76.00 ± 2.02 a**
24-h constant dark	0.00 ± 0.00 d

*: Within each column, means with the same letter are not significantly different (p = 0.001); SE: standard error

Unlike the current study, Senel *et al.* (2007) found that to establish the seed germination protocols of *V. bithynicum* and *V. wiedemannianum*, a dark environment was the best treatment. Isık *et al.* (2017) acquired the best germination results for the *V. dudleyanum*, *V. natolicum*, *V. serratifolium*, *V. suworowianum*, *V. suworowianum*, and *V. orientale* species by applying the 8-h light/16-h dark periodic regime. In agreement with the current study, they achieved the best germination rate for the *V. wiedemannianum* species with the 16-h light/8-h dark photoperiodic regime, which falls into the secondary group in the current study. Senel *et al.* (2007) found that the effect of treatments of light, dark, and photoperiod can vary based on the species, and they should be established separately and meticulously for each species. Chanyenga *et al.* (2012), in contrast, reported that seeds of certain species can equally germinate both in light and dark conditions.

Impact of ultrasonic wave and vacuum treatments on germination

According to the results obtained from the vacuum (V) and ultrasonic wave (UW) treatments, the germination statistics of *V. yurtkuranianum* seeds were affected significantly ($P < 0.05$) and positively (Table 6). The best results were achieved from 60 min UW and 120 min V implementations. Because the 120 min UW implementation provided lower germination than the control implementation, it was determined that this period is too long for this treatment and affects germination negatively. Both 60 min UW and 120 min V implementation were observed to have a statistically same-level impact.

Table 6. Impact of Ultrasonic Wave and Vacuum Treatments on *V. yurtkuranianum* Seed Germination

Treatment	Mean Germination (%) ± SE	Germination Speed (T_{50}) (Day)
Control	85.33 ± 2.8 bc*	5.0
60 min Ultrasonic Wave	**94.33 ± 2.0 a**	5.0
120 min Ultrasonic Wave	83.33 ± 0.7 c	5.0
60 min Vacuum	91.00 ± 2.4 ab	5.3
120 min Vacuum	**95.33 ± 1.4 a**	5.0

*Within each column, means with the same letter are not significantly different (p = 0.05); SE: standard error

The 60 and 120 min V and 60 min UW implementations of UW and V treatments significantly increased germination percentages compared to the control. The fact that V treatments were more effective in implementation of both periods indicates that the V treatment had a wide impact. Implementing the UW doses for long periods affected germination negatively. A similar result was obtained in the study conducted by Mildažienė *et al.* (2019). The *in vitro* 200 Pa Vacuum (7 min) treatment in substrate for *Helianthus annus* seeds decreased the germination percentage compared to the control. In the study conducted on the impact of ultrasonic treatment time and temperature on sesame (*Sesamun indicum*) germination (Shekarı *et al.* 2015), the 10 and 20 min implementations in optimal temperature conditions decreased germination in 20 °C germination environments while the 25 °C treatments increased germination 2% compared to the control. In the study conducted by López and Vicient (2017), while the ultrasonic treatment was observed to be ineffective on new *Arabidopsis thaliana* seeds, it was found to increase germination in old seeds. There are papers reporting an increase in the germination of certain species *via* ultrasonic wave implementation (Goussous *et al.* 2010; Machikowa *et al.* 2013; Sharififar *et al.* 2015; Liu *et al.* 2016). Contrary to this literature, there are also papers reporting a decrease in germination (Goussous *et al.* 2010; Fateh *et al.* 2012), as indicated by the results of 120 min ultrasonic wave treatment acquired in the current study.

Germination speed of V. yurtkuranianum seeds

To establish the germination speed of *V. yurtkuranianum* seeds, the germination speed in vacuum and ultrasonic wave treatments were also determined. In the *V. yurtkuranianum* seeds on which the treatments of control, vacuum, and ultrasonic wave were performed, the germination speed started on the 2nd and 3rd day and substantially finalized within 10 days. Particularly on the 4th, 5th, and 6th days, a dense germination occurred. The control finalized on the 14th day in the 120 min UW and 60 min V treatments and 18th day in the 60 min UW and 120 min V treatments (Fig. 4).

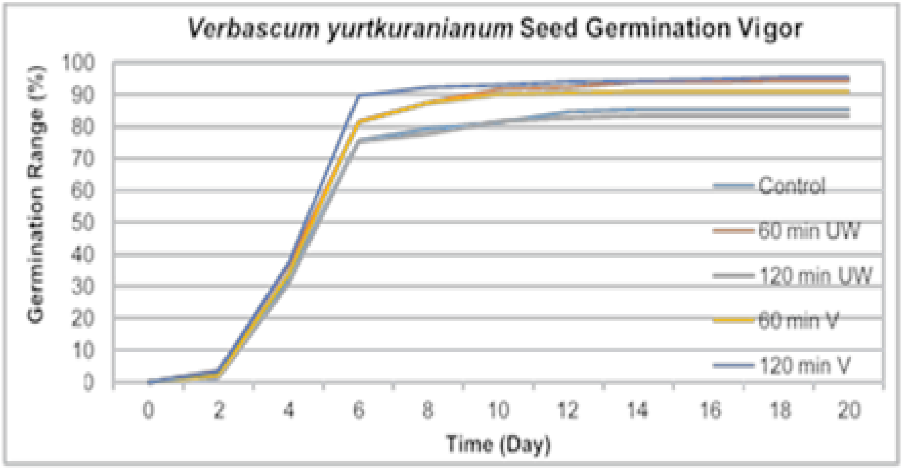

Fig. 4. The germination speed in the *V. yurtkuranianum* seeds on which ultrasonic wave and vacuum treatments were performed (seed germination trends)

It was observed in the experiments that germination was quite dense on the 3rd, 4th, 5th, and 6th day and substantially finalized between the 8th and 10th day, and the last germinations occurred on the 18th day. The period for the highest germination energy in the *V. thapsus* species was the 5th day by Ivanova and Valchev (2020), and the germination period for the *V. calycosum* species was established as 10.3 days by Hilooğlu *et al.* (2018). Hilooğlu and Sözen (2017) reported the germination speed index in the *V. alyssifolium* species as between 8.3 and 14.7 days, and the T_{50} germination index as 6.2, 7.5, 7.0, and 8.0 days in different treatments. This course took between 5.0 and 5.3 days in the current study. This difference between studies is presumed to be caused by the heterogeneity in the seeds obtained.

Impact of cold-wet stratification and GA_3, ultrasonic wave, vacuum, and warm water soak combinations on germination

The impacts of the 3-month cold-wet stratification treatments in Petri dishes and a perlite environment and the combinations of these with different treatments on *V. yurtkuranianum* seed germination varied. Lower germination percentages were acquired from all the wet stratification treatments in Petri dishes compared to the control. In contrast, higher germination percentages were achieved from the cold stratification treatment in a wet perlite environment (Table 7). Similar to the nonstratified temperature experiments, results of the statistically same group were obtained in the stratified seeds in 15 and 20 °C germination environments. The perlite used in the stratification environment was anticipated to promote the effect of stratification as it ensures a balanced humidity level.

Table 7. Impacts of Cold-wet Stratification in Petri Dishes and Perlite and their Combinations with Other Treatments on *V. yurtkuranianum* Seed Germination

Treatments	**Germination (%) ± SE**
Control	71.00 ± 3.0 b*
3-month cold-wet (4 °C) stratification in Petri dishes	25.67 ± 1.2 c
3-month cold-wet (4 °C) stratification in Petri dishes + 48-h soak in warm water (30 °C)	3.67 ± 2.5 d
3-month cold-wet (4 °C) stratification in Petri dishes + 24-h soak in 1000 ppm GA_3	0.33 ± 1.9 de
3-month cold-wet (4 °C) stratification in Petri dishes + 1-h ultrasonic wave treatment	24.33 ± 1.9 c
3-month cold-wet (4 °C) stratification in Petri dishes + 2-h ultrasonic wave treatment	0.00 ± 0.0 e
3-month cold-wet (4 °C) stratification in perlite + germination at 15 °C	**90.67 ± 5.4 a**
3-month cold-wet (4 °C) stratification in perlite + germination at 20 °C	**91.67 ± 4.8 a**

*: Within each column, means with the same letter are not significantly different (p = 0.05)

The best and statistically most significant level results acquired from the experiments of stratification treatments and their combination of different treatments were achieved in the 3-month cold-wet stratification in perlite. In the study conducted on the 6 taxa of *Verbascum- V. dudleyanum*, *V. anatolicum, V. serratifolium, V. wiedemannianum*, and *V. suworowianum* var. *suworowianum*, Işik *et al.* (2017) achieved the highest germination percentage in the treatments combined with a 10-day cold-wet pre-treatment. The highest germination rate in *V. orientale* was attained from the treatments combined with a 48-h cold-wet pretreatment. Even though the cold-wet stratifications stand out, as in the current study, the treatment periods seem different.

Hilooğlu *et al.* (2018) obtained a germination of 23.2% in *V. calycosum* seeds from the 7-day cold stratification treatment at 4 °C. Similarly, in the current study, a germination of 25.7% was obtained from the 3-month cold-wet stratification treatment in Petri dishes at the same temperature. These results present a value below the germination of the control implementation in the current study. In other words, cold stratification in Petri dishes affected germination negatively in the current study. Similar to these results, cold stratification treatments were reported to affect germination in the *V. nigrum*, *V. speciosum*, and *V. thapsus* species by Leo (2013) and the *V. alyssifolium* species by Hilooğlu and Sözen (2017). The germination rate reached 90.7% at 15 °C and 91.7% at 20 °C by applying stratification for 3 months in wet perlite.

Hilooğlu and Sözen (2017) achieved the best germination in the species *V. alyssifolium* with a mechanical stratification treatment. In another study, Hilooğlu *et al.* (2018) acquired germination percentages between 34.5% and 54.5% in *V. calycosum* seeds from mechanical abrasion treatment. Both of the studies reported that as the mechanical stratification treatments promoted germination, *V. alyssifolium* and *V. calycosum* seeds had physical dormancy due to their impermeable coats as well as to their physiological dormancy. However, in this study, a higher germination (91.7%) was achieved by extending the period of cold stratification without any process for eliminating the physical dormancy. Attaining a germination in the 90th percentile with only cold stratification treatment revealed, according to Baskin and Baskin (2014) and Wang *et al.* (2017), *V. yurtkuranianum* seeds have only physiological dormancy. Yang *et al.* (2020) reported elimination of physiological dormancy solely by cold stratification. This statement supports the findings of the current study.

Some of the study's worst results were obtained as 0.33% germination from the 24 h soak in the 1000 ppm GA_3 solution in addition to the cold-wet stratification treatment. Similar results were obtained by Şenel *et al.* (2007) in the *V. bithynicum* and *V. wiedemannianum* species. In contrast to the current study and Senel *et al.* (2007), Hilooğlu and Sözen (2017) concluded that there was an increase in germination percentages by significant numbers with GA_3 treatments in the species *V. alyssifolium* in comparison to the control.

Ganatsas *et al.* (2019) reported 32.0%, 26.7%, and 40.0% germinations from seeds of different sizes in the species *V. dingleri* without any treatments. In the current study, 85.3% and 71.0% germinations were obtained in the controls of both experiments in which no treatment was executed. These results constitute an advantage for the production and *ex situ* conservation works of the species *V. yurtkuranianum*.

Germination results were widely varied based on species and can be represented in germination studies conducted under the same conditions in different *Verbascum* species. While germination rates in the 0 to 1 percentile can be obtained by implementing all the treatments in certain species, a 96% germination rate can be achieved in certain species with the same treatments. These results reveal that the species of the genus *Verbascum* are affected in quite different ways from the treatments of light, temperature, photoperiod, and various dormancy elimination based on the species (Isık *et al.* 2017). Another reason to obtain different results in similar studies can be the germination-affecting storage process conditions in the waiting period between the harvesting and germination stages (Probert 2000). Considerable differences can arise regarding the germination characteristics of plant species based on genetic and environmental factors and even the environments utilized for germination testing (Elias *et al.* 2012; Baskin and Baskin 2014). It was recorded by some researchers that seed germination percentages can be affected even by the side of the plant bearing the fruit from which the seed was collected (Nielsen 1988; Copeland and McDonald 2001). Consequently, germination is a remarkably complex physiological process and can be affected by a wide range of factors (Gresta *et al*. 2010).

Ex situ conservation parcel

788 individuals of the locally endemic *V. Yurtkuranianum* species were counted in the wild. Its natural habitat is at great risk as it is close to the settlement. Therefore, *ex situ* conservation is required to protect the species. *V. yurtkuranianum ex situ* conservation parcel was established with the seedlings germinated during the experiments and developed in vials (Fig. 5). This parcel ensured protection of this endangered narrow endemic species as well as its natural distribution locality. The research related to this species will persevere in this parcel, and the plants and seeds from this parcel will be utilized for further studies. Thus, collecting seeds from the natural populations of this species will not be necessary at the next stage, even for scientific purposes. The studies to be conducted in this parcel will ensure cultivation of this species and its use as an ornamental plant.

Fig. 5. Producing *V. yurtkuranianum* seedlings and the status of the plants planted in the *ex situ* conservation area; a) seedlings view grown in viols, b) seedlings view planted in the garden, and c) close view of plants

CONCLUSIONS

Verbascum yurtkuranianum has high ornamental plant potential with its flower color, flower life and the number of flowers per plant.

Germination rates of 94.3% were obtained from 60 min ultrasonic wave and 95.3% from 120 min vacuum treatment. In addition, an acceptable level of germination (91.7%) was achieved treatment of 3 months cold-wet (4 °C) stratification in the perlite which does not require a technical equipment.

An *ex situ* conservation garden was established with the plants obtained during the study for this species, which only 788 individuals remained in the natural habitat. Future studies will continue with seeds obtained from this garden and no material will be collected from natural population.

The results revealed that generative production and *ex-situ* conservation are feasible for the *V. yurtkuranianum* species. The high germination rates obtained for this species is sufficient for fast production in the culture conditions.

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