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OPTIMAL GERMINATION METHODS, ORNAMENTAL PLANT FEATURES, AND EX SITU CONSERVATION OF ENDEMIC *CAMPANULA GRANDIS* FISCH & C.A. MEY

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Highlights

- Endemic *Campanula grandis* plant demonstrates ornamental plant potential.
- Two-year growth performance in natural and cultural conditions was investigated.
- *Campanula grandis* needs ex-situ conservation and cultivation.
- Germination rates of seeds can be improved by treatments.
- Germination speed of seeds can also be improved by GA_3 treatments.
- Ideal temperature for germination as 20 °C for the best germination rate.

Abstract. This study aimed to investigate the ornamental utilization, two-year growth performance in natural habitat and cultural conditions, generative production methods, and morphologic and phenologic plant properties of *Campanula grandis*, which is an endemic species demonstrating ornamental plant potential and needs ex-situ conservation and cultivation. In addition, the germination and growth performance of seeds collected in the new season was compared with seeds that were kept for one year. Seeds stored dry for 3 months at 4 °C and then treated with 200 ppm GA_3 exhibited the highest germination percentage (76%). Furthermore, GA_3 treated seeds had best germination speed, shortening the germination time. This study also identified the ideal temperature for germination as 20 °C for the best germination rate. It was revealed that the number of flowers on the plant in natural habitat was 48 while in cultural conditions it was found as 165 flowers in biennial plants. The seedlings were planted in an *ex-situ* collection garden established for placing the *Campanula grandis* species under conservation. The *Campanula grandis* species has the potential for use as an ornamental plant for landscaping applications due to its lengthy flowering period, vigorous second-year stem growth, and numerous, enormous, flamboyant, blue-purple, and bell-shaped flowers.

Keywords: *Campanula grandis*, endemic plant, floriculture, native plant, ornamental plant, seed germination.

Introduction

The genus *Campanula* L., containing approximately 420 species, is one of the largest genera in the subfamily Campanuloideae (Mehrvarz & Kashi, 2015). *Campanula* L. is recognized at warm regions, especially in the Mediterranean, Middle East, Greece, and the Balkan Peninsula, as being rich in species and have attracted much attention from botanists (Liveri et al., 2019). In Turkey, however, the genus is represented by 6 subgenera and 125 species that fall within those subgenera, half of which are endemic (Khansari et al., 2012; Yıldırım & Şenol, 2014). They may be annual, biennial, or perennial.

Some *Campanula* species are suitable for outdoor ornamental plantings, while others may be used as cut flowers (Bosma & Dole, 2002; Steiner, 2005). In their research employing natural plants, including *Campanula* species, Bretzel et al. (2009) reported that the utilization of *Campanula* species is feasible in grassy areas and rich soils for landscaping and to benefit from their ornamental plant properties. According to Gülbağ (2017), some natural species of the *Campanula* genus will soon find a position for

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themselves in the ornamental plant industry due to their high adaptability, strikingly colored flowers, and lengthy flowering vegetation, along with other comparable properties. Advanced evaluations of the naturally grown *Campanula* species and the identification of novel varieties will contribute to product diversity in the ornamental plant industry.

C. grandis is defined as an upright and simple-stemmed plant species that extends from 40 to 100 cm in length. The literature also states that the plant has dentated leaves and non-pedicelled blooms emerging from the axils of the leaves. The flower petals of the plant are broadly bell-shaped, naked, and blue in color, according to the same literature (Doğan, 2022).

It is vital to conduct species-specific germination studies and to ensure the sustainability of production (Baskin & Baskin, 2020; León-Lobos et al., 2020) due to the need to conduct *ex situ* conservation in endemic species (Kırmızı et al., 2011), to provide seed regeneration in gene banks, restoring their natural habitats (Hay & Probert, 2013; Baskin & Baskin, 2020), and to perform cultivation studies among wild and endemic species. For large-scale production, however, it is critical to discover proper germination techniques to overcome the existing germination barriers in the seeds of the mentioned species and elevate their current germination rates (Kadis & Georghiou, 2010; Kırmızı et al., 2019). Utilizing natural populations as a sustainable material source required for seed germination research is not a rational or prudent strategy (Pérez & Chumana, 2020). Therefore, it is necessary to generate genetic resources and *ex situ* conservation gardens while conducting cultivation studies with plants that serve as the material resource for research.

In line with the mentioned information, this study analyzed the endemic *C. grandis* species, a member of the *Campanula* genus that is classified as least concern (LC) according to IUCN criteria data (Tunçkol et al., 2020; Yaman et al., 2020; Çelik & Eker, 2020). Various treatments were used to break the *C. grandis* seed dormancy under laboratory conditions, and the treatments' effects on the seed germination rate and speed (T_{50}) were thoroughly examined. Furthermore, the growth of the species in the cultivation environment was monitored, and its landscaping potential and aesthetic features suitable for plant designs were identified.

1. Materials and methods

1.1. Materials

The primary material of this study was plants (Figure 1a), flowers (Figure 1b) and seeds (Figure 1c) of the endemic *C. grandis* species, of which the natural population extends on the stony slopes by the roadside between the town Teşvikiye of Yalova province and the Delmece Plateau Natural Park (Figure 3a, 3b). The study was carried out in Yalova province's climate in 2019, 2020, and 2021 (Figure 2). Seeds were collected on 27 July 2019, and Figure 3c depicts the location of the seed population. Germination studies were conducted in the Research Laboratory of Yalova University Yalova Vocational School. The seedlings generated by the germination studies were grown in an unheated research greenhouse. A research garden established at the location illustrated in Figure 3b was chosen as the study field to assess the adaptation skill of *C. grandis* to the cultivation conditions, to monitor its vegetative and aesthetic properties throughout its development stages, and to establish an *ex situ* collection garden. Table 1 lists the soil characteristics of the natural population from which the seeds were collected and the research garden where the plants were monitored throughout their development.

a) b) c)

Figure 1. a) Typical examples of *C. grandis* in the cultural conditions and view of the plant in *ex-situ* conservation garden, b) general view of the flowers, c) seeds of *C. grandis*

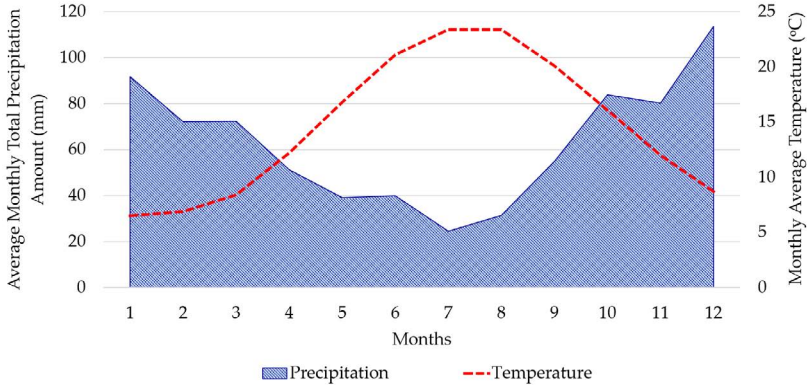


Figure 2. Climatic data on the distribution of temperature and precipitation in Yalova province (Meteorology General Directorate, 2021)

Figure 3. a) The location where the work is carried out, Turkey, b) Yalova and Çınarcık research garden, c) The location between Teşvikiye and Delmece Plateau Nature Park that seeds collected

Table 1. Soil characteristics of samples from Mount Taz (natural population) and Çınarcık Research Garden

Soil Characteristics		pH	EC_{25} (µmhos/cm)	$CaCO_3$ (%)	Organic Matter (%)	Available Phosphorus (ppm)	Extractable Potassium (ppm)	Clay (%)	Sand (%)	Silt (%)
Locations	Taz mountain	5.23	774	0.39	4.57	179	370	27.96	37.97	34.07
	Çınarcık research garden	7.63	547	4.11	2.69	20	190	36	47	17

1.2. Methods

1.2.1. Adaptation studies related to cultivation conditions and the vegetative and aesthetic properties of the species

This phase of the study aimed to compare the adaptation skills, biological cycles, and developmental performances of plant groups developed naturally in the population and those planted in the soil and pots in the research garden. For this purpose, the natural population and the research garden were visited routinely between April 1 and August 15 to collect visual observations and data measurements, as indicated in Table 2. Afterward, the acquired data were compared with the measurements and observations of *C. grandis* plants grown in the soil and pots in the research garden. They were also monitored for their development. Ten plants were selected randomly in each growing medium to make measurements and observations, and three groups were formed, totaling 30 plants utilized for data collection.

The seedlings produced from the seeds under laboratory conditions were initially nurtured in 104 viols with peat until they developed 2–3 true leaves. Then, they were transferred to 8.1×8.1×8.9 cm square peat pots when they generated 6–7 true leaves. The date of transfer of the seedlings into the viols was 14 November 2019. Finally, they were transplanted into the soil environment in the research garden at 30×30 cm spacing on 28 March 2020. Round production Pots 16×13.5 cm in size containing soil as described in Table 1 were used for potting. The experiment aimed to analyze the species' growth performance in both pots and soil environments. Studies to identify whether the plant has ornamental aesthetic value for landscape designs were taken into account at this stage as a primary objective.

The seeds collected from the natural population on 27 July 2019 were cleaned and left to air dry in an open field. After a drying period of approximately 10 days, the seed evaluation process began on 8 August 2019.

Observations and measurements of the seed sizes (width, length), 1000-kernel weight, number of seeds per (1) gram, seed ripening date, capsule cracking date, and seed shedding date took place during the field studies in the natural population.

1.2.2. Seed germination tests

Disposable plastic Petri dishes 100×20 mm in size were used for germination tests. The Petri dishes and blotting papers used for germination tests were sterilized at 100 °C for 30 minutes before usage. The seeds used in the germination tests were sterilized by soaking in 70% ethanol for 1 minute and in 20% solution (commercial brand) containing 5.25% sodium hypochlorite for 10 minutes before placing in Petri dishes. Then, they were rinsed twice with distilled water after the sterilization process and put in Petri dishes on moist blotting papers on the bottom. The seeds were also treated with a commercial fungicide (2.5 mL/L dose) branded Maxim XL 035 FS, for which the active ingredient is 25 g/L Fludioxonil + 10 g/L Metalaxyl-M, to eliminate potential disease factors. The results were evaluated based on the percentage of total germinated seeds (FGP) and the number of days in which 50% of the total number of seeds germinated was calculated as the germination speed (T_{50}). The SWGC-450 Programmable Plant Growth Chamber was used in the germination tests. Various applications were made to break dormancy in the *C. grandis* seeds. For instance, while temperature effects (15 °C, 20 °C, and 25 °C) on germination were tested on a particular seed group, other pretreatments were applied to another group to break dormancy (storing at 4 °C, cold-wet stratification, gibberellic acid, and their combinations). Different pretreatments were used under 20±0.5 °C temperature, 70% humidity, and 12/12-hour (light/dark) photoperiods.

To increase the germination percentage, which is very low in taxon's seeds; treatments were made as soaking seeds to GA_3 solution for 24 hours, and/or storing at 4 °C for 3 months, and/or cold-wet stratification for 3 months or their combinations. 100, 200, 300 ppm GA_3 doses were applied as soaking solution for 24 hours. Doses of 200, 400 and 600 ppm GA_3 were applied after 3 months of dry storage at 4 °C. Another seed group were subjected to 3 months of cold- wet stratification after 3 months of dry storage at 4 °C, and then 200, 400 and 600 ppm GA_3 treatments were made.

After assessing the treatment method with the best results among the different germination pre-treatments,

Table 2. Descriptions of the measuring and counting locations at the stage of plant counting and measurement

Plant Properties Measured and Counted	
Plant height	The height measured from the soil to the tip of the flowering section (cm)
Pedicel length	The length measured from the first bloom to the last bloom appearing on the shoot (cm)
Number of leaves	The total number of leaves on the plant counted from the soil to the top (unit)
Number of flowers	The total number of flowers on the plant (unit)
Flower width	The widest part of the flower measured in mm between two petal tips (mm)
Flower length	The longest part of the flower measured in mm between the flower receptacle attached to the pedicel and the most distant point of the petals
First blooming time	The date the first flower bloomed
Last blooming time	The date the last flower deformed

it was applied to the one-year-old (2020 seeds) and new-season seeds harvested in 2021. The seeds harvested in 2020 had been preserved in glass jars for one year under 4 °C storage conditions; the lids were not placed on the jars, but they were covered with a permeable fabric.

The seed was deemed to have germinated when the radicles elongated from the seed shell by 2 mm. A 30-day monitoring period was set, and seeds were examined once every two days (Eser et al., 2005; International Seed Testing Association, 2013). Before exposing them to applications, the seeds were placed in paper bags and kept in dry storage. The seeds exposed to cold-wet stratification previously were kept in moist perlite in paper bags. The GA_3 treatments, were performed by soaking the seed-filled packages in GA_3 solution for 24 hours. Then, these packages were opened, and the seeds were placed properly in Petri dishes.

The following phase of the project involved the establishment of an ex-situ collection garden as described above. With the intention of using them for recovery purposes, the plants grown throughout the research period that were not utilized in the research garden or ex situ collection garden studies were transferred and transplanted on 28 March 2020, into the habitat where the natural population is located.

1.2.3. Experimental design and data analysis

All the experimental designs were based on the Randomized Block Experimental Design. Germination tests were designed as four replications, using 50 seeds for each. A total of 30 plants, as 10 replications, were subjected to measurements and counts for the assessment of the ornamental plant properties in populations and the cultivation conditions. The data were analyzed using the statistical program IBM SPSS Statistics Base 22.0 (IBM Corp., Armonk, NY, USA). Duncan's multiple comparison test was used to compare various outcomes using a one-way analysis of variance. After counting the seeds germinated, the arc-sin data transformation application was used to evaluate the percentage (%) germination rates.

2. Results

Assessment of Campanula grandis for its adaptation to cultivation conditions and its vegetative and aesthetic properties

Table 3 illustrates the findings from the monitoring experiments to assess the adaptation ability and potential of the *C. grandis* species as an ornamental plant under cultivation conditions. Observations of the plants under cultivation conditions revealed a modest decrease in the number of flowers at the end of the first year as compared to the plants in the natural population. Accordingly, the number of plant flowers in the natural population was 48.33; however, the counts were 8 and 8.33 in plants grown in pots and soil under cultivation conditions, respectively. While the plant height was measured as 94 cm in the natural population, it was 64.66 cm and 80.66 cm among the plants grown in pots and soil under cultivation conditions at the end of the first year. However, the number of leaves, plant height, pedicel length, and number of flowers were measured as 57.66, 137.66 cm, 113.33 cm, and 165, respectively, at the end of the second year in plants grown in the soil under cultivation conditions, suggesting significant increases in these parameters. There were also other significant findings regarding flower width and length. As a result, the measurements of the flower width were 36.24 mm and 36.2 mm in the pots and soil under cultivation conditions, respectively. The flower length was 28.01 mm in the natural population, 26.47 mm in pots, and 27.1 mm in the soil cultivation conditions in the first season. However, the flower width and length were 41.82 mm and 28.88 mm, respectively, in soil conditions in the second season. The blooming period

Table 3. Properties of *C. grandis* related to its plant and flower structures in the natural population, cultivation conditions in pots, and cultivation conditions in soil

Growing Conditions	Natural Population (Aged plant)	Cultivation Conditions–Pots (Annual plant)	Cultivation Conditions–Soil (Annual plant)	Cultivation Conditions–Soil (Biannual plant)
Number of Leaves (unit)	25.33±2.65 b	14.66±2.33 b	18.66±0.88 b	57.66±5.80 a*
Plant Height (cm)	94.00±13.87 b	64.66±3.17 b	80.66±2.33 b	137.66±4.54 a
Pedicel Length (cm)	48.00±8.33 b	13.00±3.79 b	22.33±1.20 b	113.33±4.77 a
Number of Flowers (unit)	48.33±11.67 b	8.00±1.00 c	8.33±0.88 c	165.00±10.54 a
Flower Width (mm)	52.21±4.05 a	36.24±0.35 b	36.20±0.90 b	41.82±2.51 b
Flower Length (mm)	28.01±1.38	26.47±0.64	27.10±0.20	28.88±2.37 ns**
First Blooming Date	5.06.2020	28.05.2020	30.05.2020	20.05.2021
Last Flower Fading Date	10.07.2020	02.07.2020	06.07.2020	25.06.2021
Blooming Period (day)	35.00±1.55	35.00±3.21	36.60±6.64	36.00±2.52 ns

Note: * No statistically significant difference between values marked by the same letter in the same line. **ns = not significant.

beginning from the date of the first blooming flower to the date of the last deforming flower was approximately a 1–1.5-month interval for both research years.

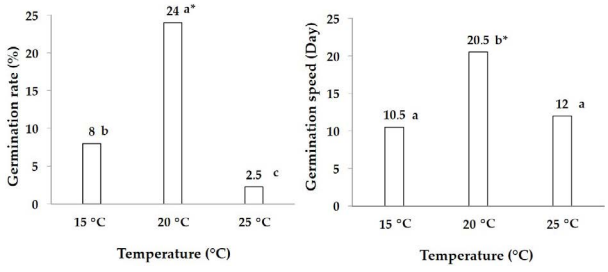
Seed properties of C. grandis

According to the measurements, counts and observations; *C. grandis* seeds was elliptical in shape and light brown in color (Figure 1c). There was an average of 13 888.89 seeds per gram. The seed size was measured as 0.79×0.57 mm. The 1000-kernel weight was 0.072 g.

According to the findings the fruit ripening, capsule cracking, and seed shedding stages of *C. grandis* happen concurrently for plants in the natural population and those under cultivation conditions. These data indicate that the period following the first week of July (10–20 July) is the fruit ripening date of *C. grandis* species. Capsule cracking date 21 July–10 August and seed shedding date is after 15 August. Given that these dates may vary by one to three weeks due to climatic variations in some years, plants should be monitored beginning from the first days of July, and seeds should be collected just before the capsule cracking stage, depending on the conditions of that year.

Effects of different temperatures on the germination rate and speed (T_{50}) of C. grandis seeds

Concerning the effects of different temperatures on the germination of *C. grandis* seeds, the best result was obtained at a temperature of 20 °C, with a 24.00% germination rate; this was categorized as the first group (Figure 4). The rate identified at 20 °C was significantly higher than the two other rates. This finding suggests that 20 °C is the optimum temperature for the germination of *C. grandis* seeds under a 12/12 h light/dark photoperiod. However, the second-best result was obtained at 15 °C, with an 8.00% germination rate, while 25 °C provided the worst germination rate, at 2.5%, and was placed as the final group. In terms of germination speed (T_{50}), 15 °C and 25 °C were categorized into the first group, with 10.50 and 12.00 days, respectively, and they shortened the germination period to 10 days. However, 20 °C remained in the second group with an average of 20.5 days, translating into a much slower germination speed (T_{50}) (Figure 4).



Note: No statistically significant difference ($p \leq 0.05$) between values marked by the same letterin the same figure.

Figure 4. Effects of different temperatures on the germination rate and germination speed (T_{50}) of *C. grandis* seeds

Effects of different GA_3 doses, dry storage, stratification, and their combinations on the germination rate and speed (T_{50}) of C. grandis seeds

Considering the effects of different GA_3 doses and stratifications on the germination of *C. grandis* seeds, the best result was achieved as a 76.00% germination rate by the combination of storing at 4 °C for 3 months + soaking in 200 ppm GA_3 solution for 24 hours (Table 4). This was followed by germination rates of 66.50% and 66.00% using the following combinations: storing at 4 °C for 3 months + soaking in 400 ppm GA_3 solution for 24 hours or soaking in 600 ppm GA_3 solution for 24 hours. Thus, these stratification combinations were categorized into the second group. There was no statistically significant difference between 400 and 600 ppm GA_3 doses. The group in which a 300 ppm GA_3 dose was applied solely was classified in the same category as the control group–storing at 4 °C for 3 months–indicating that these treatments did not differ statistically and that storing at 4 °C arguably served a similar purpose to the 300 ppm GA_3 dose. The treatment combination of storing at 4 °C for 3 months + wet stratification for 3 months + 0 ppm GA_3 dose (control) produced the lowest germination rate, at 17.00%. This finding was followed by germination rates of 34.50%, 30.50%, and 31.50%, respectively, for the control and GA_3 groups with 600 and 400 ppm treated with wet stratification. These findings suggest that cold-wet stratification is ineffective in promoting seed germination in *C. grandis* species.

In terms of germination speed (T_{50}), a result of 8.75 days was obtained for 100 ppm and 300 ppm GA_3 doses (for both doses), and 10 days was obtained for 200 ppm GA_3 doses; these produced the best results (Table 4). However, the worst germination speed (T_{50}) was attained from the dry storage (control), with germination taking 19.50 days; as a result, it was categorized into the last group statistically (Table 4).

Table 5 provides the analysis of variance results demonstrating the effects of seed origin (natural population or cultivation conditions) and seed storage (one year or none) on the seed germination rate and speed (T_{50}) in *C. grandis*.

The analysis of variance results indicates that seed storage for a year after harvesting and seed origin negatively impacted the germination rate of the *C. grandis* species. There was 20.83% more germination among the seeds collected from the plants grown under cultivation conditions than among those gathered from the natural population. It is expected that the germination rate of the seeds obtained from these plants will be higher than the seeds obtained from the natural habitat due to the good maintenance supply in the plants under culture conditions (Pizza et al., 2021). Even storing the seeds under ideal conditions reduced the germination rate of the harvested seeds. Seed origin and storing the harvested seeds for one year did not affect the seed germination rate statistically. The interaction between seed storage and seed origin was found to be statistically insignificant for both the seed germination rate and speed (T_{50}) variables.

Table 4. Effects of different GA_3 doses, dry storage, stratification, and their combinations on the germination of *C. grandis* seeds

Applications			Average Germination Rate (%) ± SE	Average Germination Speed (T_{50}) (day) ± SE
GA_3 (ppm)	Pretreatments			
	Storing 4 °C Duration (S)	Storing 4 °C Duration with Cold Wet Stratification (CWS)		
0 (Control 1)		–	34.50±0.76 def*	10.00±0.71 ab
100		–	35.50±1.01 de	**8.75±0.25 a**
200		–	38.50±0.28 cd	10.00±0.71 ab
300		–	44.00±0.55 c	**8.75±0.25 a**
0 (Control 2)	3 months	–	42.50±0.60 c	19.50±0.87 e
200	3 months	–	**76.00±1.99 a**	11.75±0.75 bc
400	3 months	–	66.00±0.49 b	11.75±0.47 bc
600	3 months	–	66.50±0.91 b	15.00±1.00 d
0 (Control 3)	3 months	3 months	17.00±0.88 g	14.00±0.00 cd
200	3 months	3 months	42.00±0.47 c	14.75±1.31 d
400	3 months	3 months	31.50±0.59 ef	15.50±0.86 d
600	3 months	3 months	30.50±0.78 f	16.00±1.08 d
p-value			p ≤ 0.05	p ≤ 0.05

Note: * Statistical difference (≤0.05) between the means marked by different letters in the same column.

Table 5. Effects of seed origin and storage on seed germination rate and speed (T_{50}) in *C. grandis*

	Origin	Storage		Average (Origin)**
		Seed stored for one year (2020)	Harvest Seed–No storage (2021)	
Germination Rate (%)	Natural Population	50.67±4.95***	71.00±1.17	**60.84±6.63 b***
	Cultivation Conditions	74.67±3.07	88.67±4.34	81.67±4.30 a
	Average Storage Period (seed age)**	**62.67**±6.90 **b***	**79.84±3.84 a**	storage* origin***
Germination Speed (T_{50}) (days)	Natural Population	11.00±0.58***	9.67±0.33	**10.34±0.42*****
	Cultivation Conditions	11.33±0.88	10.33±0.88	10.83±0.60
	Average Storage Period (seed age)***	11.17±0.48	10.00±0.45	storage* origin***

Note: * Statistical difference (p ≤ 0.05) between the means marked by different letters in the same line or column. ** Statistically significant difference (significant, 0.008). *** Statistically insignificant (insignificant, p ≤ 0.05).

3. Discussion

An evaluation of the literature related to the plant properties of *C. grandis* species, the development of which was observed within the scope of this project, revealed that the *C. grandis* species blooming in June–July are more durable, according to Clausen (1975) and Clausen (1976). However, this study observed that May–June was the ideal period for flowering. In parallel with the researcher's findings, this study also measured approximately 30–40 days of the flowering period. Additionally, Clausen (1976) reported that the plant possessed enormous flowers (5 cm). According to our measurements, although the flower size did not reach such a size in the first and second seasons, there was a substantial increase in flower size in the second season under cultivation conditions. Furthermore, Doğan (2022), Clausen (1975), and Clausen (1976) reported that the plant grows at least 0.5 m tall and might even reach up to 1 m high. However, the measurements in this study demonstrated that the plant height exceeded these limits.

The findings of this study allow us to conclude that the plant properties of the *C. grandis* species are maintained without any modifications under cultivation conditions. Furthermore, the plants grown under cultivation conditions were more flamboyant in the second season than those in the natural population. In the light of the

study findings, it is possible to contend that once the plant completes its development in the second year, it becomes highly flamboyant (an increase in the number of blooms, in particular, is noteworthy in this regard) and fully acclimates to the cultivation conditions outside its natural environment. On the other hand, the plant taxon appears to be regarded as an attractive ornamental plant if it is grown in soil under cultivation conditions. Perhaps this outcome may be explained by the fact that the maintenance opportunities under cultivation conditions are improved relative to those in the natural environment.

Tikhonova et al. (1991) reported a 0.04–0.21 g average 1000-kernel weight when measuring the seed sizes of nine different *Campanula* species. Gülbağ (2016), however, reported the weights for this parameter as 0.26360 mg for *C. alliariifolia*, 0.1675 mg for *C. betulifolia*, 0.2915 mg for *C. glomerata* L. subsp. *hispida*, 0.0299 mg for *C. lactiflora*, and 0.4455 mg for *C. latifolia*. Mehrvarz and Kashi (2015) identified the seed size of *C. stevenii* subsp. *stevenii* (0.77 – 0.92 × 0.23 – 0.4 mm).

Kobakhidze and Barblishvili (2017) measured the 1000-kernel weight of *C. armazica* seeds as 0.005 g. Shetler and Morin (1986) stated that the seed color in *Campanula* species ranges from buff (rosewood) to chestnut brown. Similarly, Alçıtepe (2016) reported that the seed color of *C. davisii* can be yellowish-brown, deep brown, or light brown. The seed color reported in the current study remains within the average limits and is consistent with the literature. However, it would be appropriate to underline that seed sizes may vary among *Campanula* species. Kobakhidze and Barblishvili (2017) stated that *C. armazica* seeds mature in July. Our findings for *C. grandis* were similar.

The best result was obtained at a temperature of 20 °C with a germination rate of 24.00% when taking into account the effects of different temperatures on the germination statistics of the *C. grandis* seeds. However, when it came to germination speed (T_{50}), 10 °C and 25 °C gave the best results of 10.50 and 12.00 days, respectively, and were categorized in the first group statistically. The germination speed was 20.50 days at a 20 °C temperature. Similarly, Galloway (2001) obtained a germination speed in the range of 10–20 days in the mountain and river populations of the seeds of *C. americana*. It is thought that the germination speed of 20.5 days at 20 °C in our study is due to the variation and difference in the seeds used. According to Galié et al. (2019), an average germination speed (27.9 days) was determined with *Campanula garganica* subsp. *garganica* seeds which was close to the speed we obtained in our study.

With results similar to those of the current study, Gülbağ and Özzambak (2017) underlined that the best germination rate in *C. glomerata* L. subsp. *hispida* (Wtasek) Hayek was obtained at a 20 °C temperature with a 12/12 photoperiod and constant dark treatments. Accordingly, our study results concur with those of Gülbağ (2016), who proposed a 20 °C constant temperature to obtain the highest germination rate and speed for the *C. alliariifolia* and *C. lactiflora* species. Blionis and Vokou (2005) reported the ideal temperature as 15 °C for several species based on their germination studies conducted among nine different *Campanula* species. Ægisdóttir et al. (2007) found a high germination rate of up to 78.8% in *C. thyrsoides* species under 12/12-hour light and 20/10 °C (day/night). The germination rates of *C. tomentosa* Lam. and *C. vardariana* Bocquet were also high, according to Güvensen (2013). However, the germination rate for the *C. grandis* species was not that high in the current study.

Considering the effects of different GA_3 doses and stratification treatments on the germination of *C. grandis* seeds, the best germination rate was calculated as 76.00% with the treatment of cold storage + 200 ppm GA_3 dose. In terms of germination speed (T_{50}), however, the applications of 100 and 300 ppm GA_3 doses, giving a result of 8.75 days, and 200 ppm GA_3 dose, giving a result of 10.00 days, yielded the best results. These findings concluded that stratification had no significant impact–indeed, it may be even detrimental–on the germination of *C. grandis* seeds. This finding concurs with that of Baskin and Baskin (1984), who underlined that wet stratification is unnecessary for seed germination in *C. americana*. Our data are also in parallel with those of Frattaroli et al. (2013), who claimed that cold-wet stratification significantly reduced the germination rate in *C. fragilis* subsp *cavolinii* seeds. Bevenuti et al. (2016) found that cold storage at 4 °C was the most efficient way to boost the seed germination rate in the *C. versicolor* Andrews species. Our findings also demonstrate a positive response in the germination rate to cold storage treatment (storing) at 4 °C in all groups. It is safe to say that storage at 4 °C for 3 months might replace the 300 ppm GA_3 treatment. Additionally, the GA_3 doses and storage treatment increased the germination rate compared to the control. Consistent with these results, Subaşı et al. (2012) stated that different GA_3 doses and cold treatments were significantly effective on the germination rate of *C. teucrioides* seeds. Gülbağ and Özzambak (2017) also reported that the best results in terms of germination rate were obtained with 750 $mg^{*}L^{-1}$ and 1000 $mg^{*}L^{-1}$ GA_3 treatments in *C. glomerata* L. subsp. *hispida* (Wtasek) Hayek when analyzing the effects of GA_3 doses and stratification treatments on seeds. Similarly, Gülbağ and Özzambak (2018) recommended 1000 $mg^{*}L^{-1}$ GA_3 and the October–March season for early and high seed germination in *C. glomerata* L. subsp. *hispida* (Witasek) Hayek under unheated greenhouse conditions. Subaşı and Güvensen (2014) revealed that a 500 ppm GA_3 dose under dark conditions was efficacious in the germination of *C. tomentosa* seeds, and a 250 ppm GA_3 dose under the same conditions was successful in *C. vardariana*. However, Güvensen (2013) reported that different GA_3 doses applied to *C. tomentosa* Lam. and *C. vardariana* Bocquet seeds had no discernible impact on the germination rate. Similarly, Koutsovoulou et al. (2014) indicated that GA_3 treatments effectively improved germination under dark conditions.

With regard to the average germination speed (T_{50}), the current study revealed that seeds germinated faster

in the groups that were not stored and where only GA_3 was applied. Consequently, it is safe to say that storing at 4 °C delayed the germination speed (T_{50}) proportionally as compared to the seed treated with 100, 200, and 300 ppm GA_3 doses without a storing process. However, wet stratification with some GA_3 treatments, for instance, a 600 ppm dose, doubled the delay. This conclusion is consistent with that of Frattaroli et al. (2013), who reported that stratification caused 100% more delay in the seed germination of *C. fragilis* subsp. *Cavoliniid*. In this context, Güvensen (2013) also stated that GA_3 treatments sped up the germination process.

Conclusions

The *Campanula grandis* species has the potential for use as an ornamental plant due to its lengthy flowering period, vigorous second-year stem growth, and numerous, enormous, flamboyant, large-in-size, blue-purple, and bell-shaped flowers. Thus, it is an excellent choice for landscaping applications with such attractive flowers.

On the other side, storage for 3 months at 4 °C and GA_3 applications were determined to be effective in obtaining a higher seed germination rate. We suggest a 200 ppm GA_3 dose for this purpose. Furthermore, the application where GA_3 was the only treatment accelerated the germination speed (T_{50}), shortening the germination time. Finally, the combination of storing at 4 °C for 3 months + soaking in 200 ppm GA_3 solution for 24 hours was the best practice for seed germination, giving a result of 76%.

This study also identified the ideal temperature as 20 °C for the best germination rate applied together with a 12/12 h light/dark photoperiod regime.

From the study findings, we conclude that seeds stored for a year demonstrated lower germination rates, and seeds collected from plants maintained under cultivation conditions had a higher germination rate than those taken from natural populations. It was also discovered that seed storage and seed origin did not affect the germination speed (T_{50}).

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Author contributions

Conceptualization, G. Y. and K. E.; methodology, G. Y. and K. E.; software, G. Y.; validation, G. Y., K. E; formal analysis, K. E.; investigation, G. Y.; resources, G. Y.; data curation, K. E.; writing–original draft preparation, G. Y.; writing–review and editing, G. Y. and K. E; visualization, K. E.; supervision, G. Y.; project administration, G. Y; funding acquisition, G. Y. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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