

## EFFECT OF LIGHT, TEMPERATURE AND DIFFERENT PRETREATMENTS ON SEED GERMINATION OF *GENTIANA BOISSIERI* SCHOTT ET KOTSCHY EX BOISS. (GENTIANACEAE) AN ENDEMIC TO TURKEY

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### Abstract

This study was undertaken with the aim of determining the germination characteristics of *Gentiana boissieri* (Gentianaceae), an endemic species. The effects of light, temperature and different pretreatments on the germination of seeds collected from nature were investigated. Two different experiments were run to determine germination characteristics of seeds and the final germination percentage (FGP), mean germination time (MGT) and germination index (GI). The first experiment involved twenty different treatments including soaking in water, soaking in gibberellic acid (GA<sub>3</sub>), dry stratification, moist cold stratification, moist warm stratification + moist cold stratification, moist cold stratification + soaking in GA<sub>3</sub> and control. The germination test was carried out at +20°C in dark. The highest final germination percentages were obtained from the treatments of soaking in 500, 750, 1000 ppm GA<sub>3</sub>, soaking in 250, 500 ppm GA<sub>3</sub> + 4 weeks of moist cold stratification (89.00; 95.00; 93.50; 91.33; 94.00%, respectively). In the control group with no pretreatment, the final germination percentage of seeds was found to be 13.50%. In the second experiment, seeds treated with 750 ppm GA<sub>3</sub> pretreatment, providing highest germination rate in the first experiment, were germinated under dark and light conditions (12/12 h; dark/light) at 15, 20, 25 and 10/20°C. At the end of 28 days under four different temperatures, it was found that light significantly increased the final germination percentage and the highest final germination percentages were found at 15 and 20°C (87.00; 89.50%, respectively).

**Key words:** *Gentiana boissieri*, Gentianaceae, Final germination percentage, MGT, GI

### Introduction

The family *Gentianaceae* contains about 100 genera and over 1800 species widely distributed in all continents except Antarctica. *Gentiana* L., comprising about 400 species, is the largest genus in this family. This genus has nearly worldwide distribution, but is absent from low-altitudes of tropical regions and all but northernmost Africa and eastern Australia (Pringle, 2014).

The majority of *Gentiana* species have medicinal and ornamental uses. As a result, they have been uprooted from their natural setting for many years (Kohlein, 1991). The perennial herbaceous plant *G. boissieri* (Fig. 1) is an endemic species and distribution is limited to Bolkar Mountains in Niğde, Turkey (Davis, 1978; Anon., 2014). The species is listed in the VU class (vulnerable: under high threat in the medium term) according to the Red Data Book of Turkish Plants (Ekim *et al.*, 2000).

The seed germination studies of endemic species are extremely important in order to preserve the genetic variability of the population and decrease the risk of extinction due to low population levels (Fenner & Thompson, 2005). In many species of the *Gentiana* genus, the embryo is poorly developed and some species require prolonged chilling in order to germinate (Nikolaeva *et al.*, 1985; Kohlein, 1991). The seeds of the *Gentianaceae* family are in the morphophysiological dormancy class. In other words, the seed embryo is not fully developed and is in a dormant stage. To break the seed dormancy, application of GA<sub>3</sub> and combinations of warm+cold stratification are applied (Baskin & Baskin,

2004b). *Gentianaceae* family seeds have “Axillary Linear Embryo” structure. These embryos are rudimentary. Gibberellic acid increases the rate of embryo development. Also the seed coat may limit oxygen entry. Seed coat permeability may increase when the seeds are exposed to light (Ellis *et al.*, 1985). However, Atwater (1980) and Kohlein (1991) stated that the *Gentiana* seeds require darkness for germination.

The purpose of this study therefore was to show the effect of temperature, light and different pre-treatments on seed germination of endemic *G. boissieri*.

### Materials and Methods

This study was carried out during 2013 and 2014 at the Atatürk Horticultural Central Research Institute in Yalova, Turkey.

**Seed collection:** Seeds of *Gentiana boissieri* Schott Et Kotschy Ex Boiss., were collected from the native population on Bolkar Mountain (Turkey) in the alpine zone at an altitude of 2650 m in September 2013. After drying and cleaning, the collected seeds (Fig. 2) were stored at +4°C until pretreatment.

**Sterilization:** The seeds were surface-sterilized with 70% ethyl alcohol for one minute and washed with sterile distilled water three times before the start of experiments. All materials used (filter papers, forceps, flasks) were sterilized in an autoclave at +121°C for 30 minutes before the experiments.



Fig. 1. A general view of *Gentiana boissieri* plant in nature.



Fig. 2. A general view of the *Gentiana boissieri* seeds.

### Experiment-1

In the first experiment, twenty different treatments were tested to determine the best pretreatment and to increase the germination percentage of seeds. These treatments were:

- Control,
- soaking in water at +20°C and +65°C for 24 hours (Water20 and Water65),
- soaking in 100, 250, 500, 750 and 1000 ppm gibberellic acid (GA<sub>3</sub>) for 24 hours (GA100, GA250, GA500, GA750 and GA1000),
- dry stratification at +20°C until the test time (Dry20),
- dry stratification at +90, -10 and -20°C for 5 minutes (Dry 90, Dry-10 and Dry-20),
- moist cold stratification at +4°C for 2, 4 and 8 weeks (Cold2, Cold4 and Cold8),
- moist warm stratification at +20°C for 2 weeks + moist cold stratification at +4°C for 4 and 8 weeks (Warm2+Cold4 and Warm2+Cold8),
- soaking in 100, 250 and 500 ppm GA<sub>3</sub> for 24 hours + moist cold stratification at +4°C for 4 weeks (GA100+Cold4, GA250+Cold4 and GA500+Cold4).

Pretreated and control group seeds were incubated at +20°C in the dark for 28 days.

### Experiment-2

In the second experiment the purpose was to determine the effect of temperature and light on seed germination. Before the experiment the seeds were soaked in 750 ppm GA<sub>3</sub> for 24 hours which was predetermined as the best treatment in the first experiment. Pretreated seeds were sown in petri dishes and placed in incubator at 15, 20, 25 and 10/20°C (12/12 h); under dark and light

conditions (12/12 h; dark/light) for 28 days. Lighting was provided from a white light source ( $16 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ ). Darkness treatment was completed by wrapping petri dishes with aluminum foil.

**Germination tests:** All experiments were carried out with four replications of 50 seeds each for all treatments. Seeds were placed on double-layered filter paper with 2.5 ml sterile distilled water in 6 cm sterile petri dishes under aseptic conditions in a laminar flow cabinet. Petri dishes were wrapped with a plastic paraffin film to avoid moisture loss. Germinated seeds were counted every day until the 28th day (Ellis *et al.*, 1985; Anon., 2011; Zecchinelli, 2011) and seeds were considered germinated when their radicle length was least 2 mm (Zecchinelli, 2011). At the end of all the experiments, final germination percentage (FGP), mean germination time (MGT) and germination index (GI) were calculated as follows:  $\text{MGT} = \frac{\sum(n_1 \times d_1)}{\sum n}$ ,  $\text{GI} = \frac{\sum(n_1/d_1)}{n}$  where  $n$  is the total number of germinated seeds at the end of the germination test,  $d_1$  is the number of days counted during the germination period, and  $n_1$  is the number of germinated seeds on day  $d_1$  (Anon., 1983).

**Statistical analysis:** The germination percentage data were transformed to arcsine square root. The data were analyzed using ANOVA according to the randomized complete parcel design for all experiments. After analysis of variance, treatment means were tested by the least significant differences test (LSD) at significance level  $p < 0.05$ . All statistical analyses were carried out using statistical analysis software (JMP 7.0).

## Result and Discussion

### Experiment-1

The results of the germination characteristics of different pretreated and control group seeds are given in Table 1. When all treatments are investigated, the highest final germination percentages were obtained with GA750, GA500+Cold4, GA1000, GA250+Cold4 and GA500 pretreatments (95.00; 94.00; 93.50; 91.33; 89.00%, respectively) while the highest germination index values were obtained from GA1000, GA750, GA500+Cold4 and GA250+Cold4 pretreatments (8.52; 8.46; 8.40; 8.28, respectively). The earliest mean germination time was observed for the pretreatments with highest germination index values (5.72; 5.98; 5.99; 5.83 days, respectively). It was seen that the most significant effect on seed germination for all investigated parameters was from the soaking in GA<sub>3</sub> and moist cold stratification pretreatments. In studies of other species of *Gentiana* (Atwater, 1980; Ellis *et al.*, 1985; Kohlein, 1991; Grubisic *et al.*, 1995; Kery *et al.*, 2000; Erken & Kaleci, 2010; Yang *et al.*, 2011; Millaku *et al.*, 2012), used soaking in GA<sub>3</sub> and cold stratification pretreatments to break the dormancy of the seeds and found effects similar to our study.

In the control group without any pre-treatment, final germination percentage was 13.50%, mean germination time was 15.06 days and germination index value was 0.48. Different authors have stated that *Gentiana* seeds show dormancy and that germination has very low rates without pretreatment (Atwater, 1980; Ellis *et al.*, 1985;

Kohlein, 1991). The studies carried out by Arslan & Yılmaz (1989) and Erken & Kaleci (2010) on *G. lutea*; and by Grubisic *et al.* (1995) on *G. cruciata*, control group seeds are reported to show no germination. Hesse *et al.* (2007), Jevdjovic & Maletic (2007) and Lorite *et al.* (2007) have reported that germination rate in *G. lutea* control group seeds was as; 0.40%, 29.00% and 0.30% respectively, besides Morgan *et al.* (1997) obtained 2.00% germination rate in *G. corymbifera*.

All soaking in water and dry stratification pretreatments gave negative results for final germination percentage compared to the control group. Some studies on *G. lutea* species has shown that there is no germination from soaking in water and dry stratification pretreatments used by Erken & Kaleci (2010) and from dry stratification pretreatment by Arslan & Yılmaz (1989). While Jevdjovic & Maletic (2007) obtained 29% germination rate from the control group seeds in *G. lutea* were exposed to dry stratification at +4°C for 90 days and germination rate was increased to 43.15%.

In moist cold stratification pretreatments comparing the 2-week stratification process with the control group, a significant positive effect was not seen on the basis of final germination percentage (14.00%) and germination index value (0.68). However, there was a positive effect according to mean germination time (11.65 days) and earlier germination was observed. When the stratification process increased from 2 weeks to 4 weeks, final germination percentage (56.66%) and germination index value (4.72) increased to a significant degree, also mean germination time (6.37 days) was reduced to a significant degree. When the stratification process increased from 4 weeks to 8 weeks, a significant positive effect was not seen for all investigated parameters. Kohlein (1991) stated that to break the dormancy of *Gentiana* seeds and stimulate germination, the seeds need moist stratification at 0-5°C for least 5-6 weeks, while Ellis *et al.* (1985) and Atwater (1980) stated that seeds needed moist stratification at +5°C for 8 weeks. In our study we obtained results parallel to these findings. However, a study by Erken and Kaleci (2010) mentions that these prerequisites are not fully sufficient to break the dormancy of *G. lutea* seeds and that they require at least 4 months of cold moist stratification. Jevdjovic & Maletic (2007) in a study with 29% germination rate of control group seeds from *G. lutea* found that seeds subjected to moist stratification at +4°C for 3 months increased the germination rate to 64.25%.

Moist warm stratification for 2 weeks + moist cold stratification for 4 weeks pretreatment (FGP: 18.00%; MGT: 8.15 days; GI: 1.22) had a significant reduction in final germination percentage and germination index value compared to results from the moist cold stratification for 4 weeks pretreatment (FGP: 56.66%; MGT: 6.37 days; GI: 4.77), while according to mean germination time the seeds were found to germinate later. Leaving the seeds in moist warm stratification before moist cold stratification did not show a positive effect on seed germination. Baskin and Baskin (2004b) stated that for seeds with morphophysiological dormancy warm+cold stratification treatments may show a positive effect on seed germination. However in our study, warm+cold stratification treatments did not show a positive effect compared to just cold stratification treatments.

**Table 1. Final germination percentage (FGP), mean germination time (MGT) and germination index (GI) of different pretreated and control group seeds in *Gentiana boissieri*.**

Treatments		FGP (%)		MGT (day)		GI	
1.	Water20	7.50	i	13.83	c	0.28	hi
2.	Water65	0.00	k	ng <sup>x</sup>		ng <sup>x</sup>	
3.	GA100	72.00	d	8.18	e	5.17	d
4.	GA250	80.50	cd	6.92	f	6.61	c
5.	GA500	89.00	abc	6.39	fg	7.56	b
6.	GA750	95.00	a	5.98	gh	8.46	a
7.	GA1000	93.50	ab	5.72	h	8.52	a
8.	Dry20	6.50	i	12.25	d	0.29	hi
9.	Dry90	0.00	k	ng <sup>x</sup>		ng <sup>x</sup>	
10.	Dry-10	1.50	jk	15.00	ab	0.05	i
11.	Dry-20	3.50	j	14.00	bc	0.15	hi
12.	Cold2	14.00	gh	11.65	d	0.68	gh
13.	Cold4	56.66	e	6.37	fg	4.72	de
14.	Cold8	53.00	ef	6.94	f	4.15	ef
15.	Warm2 + Cold4	18.00	g	8.15	e	1.22	g
16.	Warm2 + Cold8	46.66	f	6.99	f	3.54	f
17.	GA100 + Cold4	85.00	bc	6.22	gh	7.48	b
18.	GA250 + Cold4	91.33	ab	5.83	gh	8.28	a
19.	GA500 + Cold4	94.00	ab	5.99	gh	8.40	a
20.	Control	13.50	h	15.06	a	0.48	hi

Means with same letter in the same column are not significantly different at  $p < 0.05$

<sup>x</sup>No germination

**Table 2. Final germination percentage (FGP), mean germination time (MGT) and germination index (GI) at 15, 20, 25 and 10/20°C in *Gentiana boissieri* seeds under the dark and light conditions.**

		15°C		20°C		25°C		10/20°C		Average	
FGP (%)	Dark	85.00		88.50		71.50		81.50		81.63	b
	Light	89.00		90.50		84.00		83.50		86.75	a
	Average	87.00	a	89.50	a	77.75	c	82.50	b		
MGT (day)	Dark	8.27	b	6.29	d	7.56	c	9.21	a	7.83	
	Light	8.18	bc	5.95	d	6.06	d	8.75	ab	7.24	
	Average	8.23		6.12		6.81		8.98			
GI	Dark	5.87		7.74		7.06		4.99		6.42	b
	Light	5.91		8.15		8.07		5.12		6.81	a
	Average	5.89	b	7.95	a	7.57	a	5.06	c		

Means with same letter in the same column or line on FGP and GI; in the table on MGT are not significantly different at  $p < 0.05$

When the pretreatments involving soaking in GA<sub>3</sub> were investigated, in terms of all parameters all ppm doses produced positive results compared to the control group. When the GA<sub>3</sub> dose increased from 100 ppm to 500 ppm, a significant increase was observed in terms of final germination percentage (100 ppm: 72.00%; 500 ppm: 89.00%). When the dose increased from 500 ppm to 1000 ppm, there was no statistically significant difference identified in terms of final germination percentage. In terms of mean germination time and germination index value while a positive effect was observed when GA<sub>3</sub> dose increased from 100 ppm to 750 ppm, the increase from 750 ppm to 1000 ppm did not show a statistically significant difference. Atwater (1980) and Ellis *et al.* (1985) reported that application of 400-800 ppm GA<sub>3</sub> may have a significantly positive effect on the germination of *Gentiana* seeds. Similar studies on other species of *Gentiana* have reported that, as the applied GA<sub>3</sub> dose rose

to a certain upper limit, the germination rate generally rose too while mean germination time was reduced (Grubisic *et al.*, 1995; Morgan *et al.*, 1997; Kery *et al.*, 2000; Petroval *et al.*, 2006; Erken & Kaleci, 2010; Yang *et al.*, 2011; Garcia *et al.*, 2012).

When the results on the combination treatments of soaking in GA<sub>3</sub> + moist cold stratification for 4 weeks are examined, positive results have been recorded for all parameters compared to just soaking in GA<sub>3</sub> or just moist cold stratification treatments. While final germination percentages of 72.00%, 80.50%, and 89.00% were obtained from soaking in 100, 250, and 500 ppm GA<sub>3</sub>, respectively, final germination percentages of 85.00%, 91.33%, and 94.00% were obtained from the combination of soaking in 100, 250 and 500 ppm GA<sub>3</sub> + moist cold stratification for 4 weeks, respectively. A study on *G. lutea* by Millaku *et al.* (2012) reports that more positive results have been recorded from GA<sub>3</sub> + moist cold stratification combinations which support our findings as well.

## Experiment-2

The results of the effects of different temperatures (15, 20, 25 and 10/20 °C) on germination characteristics under dark and light conditions are given in Table 2. When the FGP parameter is investigated, the highest final germination rate was obtained from temperatures of 15 and 20°C (average 87.00; 89.50%, respectively) both under dark and light conditions. We also determined that light has a significant effect on seed germination. The seeds germinating in light had higher final germination percentage under all temperature conditions compared to seeds left to germinate in the dark.

While the first germination at 25°C began on the 3rd day, at 15 and 20°C it began on the 4th day and at alternate temperatures of 10/20°C it began on the 5th day. According to mean germination time, a statistical interaction between temperature and light/dark factors was observed. The earliest mean germination time was found at 20°C under dark, at 20°C and 25°C in light conditions (6.29; 5.95 and 6.06 days, respectively).

When GI parameters are investigated, the highest germination index value was obtained at temperatures of 20 and 25°C (average 7.95; 7.57, respectively) under both dark and light conditions. The germination of seeds in light had higher germination index values at all temperatures compared to seeds left in dark.

Atwater (1980) and Kohlein (1991) report that *Gentiana* seeds prefer dark conditions for germination, while Ellis *et al.* (1985) found that the seed coat of *Gentiana* seeds inhibited the entry of oxygen, the oxygen permeability increased when seeds were exposed to light and thus light may have a positive effect on seed germination. Grubisic *et al.* (1995) pretreated seeds of *G. cruciata* with GA<sub>3</sub> and obtained 82% germination rate in dark, while under light conditions germination rate was 95%. In our study, as stated by Ellis *et al.* (1985) and Grubisic *et al.* (1995), we found light had a positive effect on seed germination. However, Yang *et al.* (2011) in a study of germination of *G. rigencens* seeds; state that light lowered the germination rate. When all results are evaluated with the literature, it may be said that effect of light on germination of *Gentiana* seeds shows differences depending on species.

For germination of *Gentiana* seeds Ellis *et al.* (1985) stated that the most appropriate temperatures were 15°C, 20°C, 10/30°C and 20/30°C; while Atwater (1980) found 15°C was best. In our study similar results were obtained. Highest germination rate was found at 16°C for *G. cruciata* seeds by Grubisic *et al.* (1995), at 25°C for *Gentiana rigencens* seeds by Yang *et al.* (2011) and at 15/6°C for *Gentiana quinquefolia* (L.) seeds by Baskin & Baskin (2004a).

## Conclusions

In conclusion, for high rates of seed germination of the endemic taxon *G. boissieri*, firstly the dormancy must be fully broken. For this purpose, different alternatives may be applied to the seeds. In general, the most successful

germination results were obtained from exposure to 500-1000 ppm GA<sub>3</sub> for 24 hours or from moist cold stratification at +4°C for 4 weeks. A combination of both applications may provide better results. Warm moist stratification applied before cold moist stratification or more than 4 weeks of cold moist stratification did not show a more positive effect on seed germination. Seeds should be germinated at 15-20°C and in 12/12 h darkness/light conditions. If seeds are planted in soil, they should be planted near the surface in order to allow exposure to light.

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