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A Study on the Propagation of Snowflake (*Leucojum aestivum* L.) as an Endangered Species by Seeds and Forced Bulbs

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Abstract

Snowflake is an ornamental-medicinal plant species native to the North of Iran. The production of this plant outside its natural habitat was studied in two separate experiments including (i) Seed propagation by the seeds primed with a temperature of 4 ºC, 24 ºC, or 27 ºC, gibberellic acid (GA) at a rate of 50 or 100 mg L-1, and KNO3 at a rate of 0.1% or 0.2 % and (ii) Propagation by the bulbs forced at a temperature of 4 ºC, 24 ºC, or 27 ºC for 8 weeks, 4 ºC for 4 weeks + 27 ºC for 4 weeks, or 27ºC for 4 weeks + 4ºC for 4 weeks. The results of seed priming showed that the seeds treated with 4ºC or 27ºC did not germinate. The best treatment for increasing germination percentage, germination rate, seedling length, and seedling length vigor was 100 mg L-1 GA. Among the treatments that led to seed germination, the lowest values of the recorded traits were related to the seeds treated with 24 ºC and 0.2 % KNO3. The results of bulb forcing revealed that the treatment of the dormant bulbs with 4 ºC for 8 weeks was not appropriate for their flowering. The highest number of flowers (2.66 flowers), flower diameter (21.70 mm), days from bulb planting to flowering termination (149.5 days), and leaf number (6 leaves) were related to the bulbs treated with 27 ºC for 8 weeks, but this treatment had the longest time to bud emergence (117.5 days), so it is not a good treatment for bulb forcing although it performed the best in terms of flower and leaf number, flower diameter, and the number of days from bulb planting to flowering termination. The shortest time to bud emergence (78.3 days) was obtained from the bulbs treated with 27 ºC for 4 weeks + 4 ºC for 4 weeks. So, this is recommended for snowflake forcing.

Keywords: Irrigation, Ornamental plant, Seasonal flowers, Water stress.

INTRODUCTION

Snowflake (*Leucojum aestivum* L.) from the family Amaryllidaceae is native to southern Europe, Turkey, Caucasus, Balkan, and northern Iran (Davis, 1984; Çiçek *et al*., 2007). The genus *Leucojum* has 10-12 species, but *L. aestivum* is the only species found in the lowland plains on the coast of the Caspian Sea in the North of Iran (Padasht Dehkaei *et al*., 2011). *L. aestivum* is used as a cut, potted, or garden flower in horticulture (Sandler Ziv *et al*., 2011). In addition to its ornamental value, the bulbs and leaves of this plant species contain several medicinally valuable alkaloids, e.g., galantamine, which are of significance in the pharmaceutical industry. Indeed, *L. aestivum* is presently the commercial source of galantamine in the world (Çiçek *et al*., 2007; Paroloa *et al*., 2011). Despite the economic, medicinal, and ornamental value of snowflakes, their extensive collection from natural habitats and the degradation of these habitats throughout the world have posed this species to the risk of distinction (Çiçek *et al*., 2007; Georgieva *et al*., 2007). Obviously, by cultivating this plant outside its natural habitats, its medicinal and ornamental potential can be used, thereby effectively contributing to efforts to prevent its excessive collection from natural habitats and its extinction.

Snowflakes can be propagated by both seed and bulb cultivation. Its seed propagation, however, fails in normal conditions because it needs breaking seed dormancy and providing favorable conditions to germinate. In addition, since the flowering phase of snowflakes produced from seed propagation would need five years to start, this propagation method is less appealing (Çiçek *et al*., 2007; Kahraman and Akçal, 2016). There is little information available about the dormancy and germination of snowflake seeds. Given that seed priming reduces the time needed for germination and seedling emergence (Cornea Cipcigan *et al*., 2020), the present research investigates how snowflake seed germination is affected by temperature as the most important environmental factor influencing germination (Roberts, 1988; Khosh-Khui, 2013), gibberellic acid as the most effective hormone for the control and acceleration of germination (Mayer and Poljakoff Mayber, 1982), and potassium nitrate as an effective chemical in eliminating seed dormancy and increasing germination rate (Khan *et al*., 1999). Roberts (1988) reports that temperature affects germination percentage and rate by controlling seed dormancy. Kumar *et al*. (2011) suggest that over- and under-optimal temperatures reduce germination rate and percentage. In general, different plant species have different thermal requirements for germination. The seeds of any plant require a minimum temperature for germination above which the higher the temperature is, the higher the germination percentage will be (Alebrahim *et al*., 2011).

Gibberellic acid (GA) is a growth hormone that is influential on breaking physiological dormancy, thermal dormancy, and photodormancy and stimulates the germination of seeds that have a dormant embryo (Shengzuo *et al*., 2006). There are reports about the positive effect of GA on increasing antioxidant activities, reducing senescence (Rashidi, 2020), neutralizing the effect of abscisic acid, and breaking seed dormancy (Nadjafi *et al*., 2006). Cornea Cipcigan *et al*. (2020) reported that seed priming of different cyclamen cultivars with GA increased germination percentage and seedling growth.

Potassium nitrate (KNO_3) is a chemical widely used to accelerate germination (Gashi *et al*., 2012). This chemical affects auxin biosynthesis, thereby triggering the growth of embryos and increasing germination rate and percentage (Khan *et al*., 1999). Bahmani *et al*. (2016) reported that the treatment of *Capparis cartilaginea* seeds with KNO_3 accelerated their germination. They found that by penetrating the embryos, KNO_3 stimulated metabolic activities and induced germination. In a study on the germination of *Salsola rigida* as influenced by GA (100 or 300 mg L^{-1}) and KNO_3 (0.1 or 0.2%), Tavili *et al*. (2009) revealed that the treatments increased germination percentage. The highest positive impact on germination was related to the treatment of 0.2% KNO_3 and the second highest to the treatment of 300 mg L^{-1} GA. These researchers ascribed the positive effect of GA and KNO_3 on germination to the effect of these compounds on creating a balance in seed hormone ratios and reducing such inhibitors as abscisic acid.

The common way of propagating geophytes, e.g., snowflakes, is by the means of underground storage organs. Post-harvest storage conditions (temperature, light, and humidity) and bulb dormancy play a key role in the growth and flowering of geophytes. Among the environmental parameters, the temperature is the main factor that controls dormancy, vegetative and reproductive growth, and off-season production of geophytes (de Hertogh and Le Nard, 1993). Researchers argue that the storage of dormant bulbs at an optimal temperature can physiologically advance the initiation of their reproductive phase (de Hertogh and Le Nard, 1993). It has also been reported that depending on the plant species, the storage of dormant bulbs at an optimal temperature and at specific time intervals can significantly affect germination, plant growth (Dhua *et al*., 1987), and different developmental stages of the flowers (de Hertogh and Le Nard, 1993).

The temperature requirement for growth and flowering vary among plant species. It has been reported that low temperatures during the bulb dormancy period reduce vegetative growth and cause the production of weak and/or low-quality plants by prolonging the dormancy period and retarding/inhibiting the physiological maturity of the bulbs (Glover, 2007; Ladan Moghaddam, 2017). However, some geophytes need a period of low temperature to break dormancy and show desirable growth and flowering. The storage of these species at low temperatures will advance their growth and flowering (Rietveld *et al*., 2000; du Toit *et al*., 2004; Roh and Hong, 2007). We have little information about forcing and the effect of storage temperature on the vegetative and reproductive growth of snowflakes. Sandler Ziv *et al*. (2011) reported that the storage of snowflake bulbs at 9ºC for 2 weeks influenced their vegetative and reproductive growth positively. Dole (2003) showed that the application of 0-9ºC for 15-18 weeks was appropriate for breaking snowflake dormancy.

As was already expressed, despite the ornamental and medicinal value of snowflakes and the fact that it is at the risk of extinction, we have little understanding of its sexual and asexual propagation. So, the present study aimed to shed light on the effects of temperature, gibberellic acid, and potassium nitrate on snowflake seed germination and the effects of different temperatures on its vegetative propagation and forcing.

MATERIALS AND METHODS

Experiment 1: Seed priming

The mature seeds of snowflake were collected from its natural habitat in the south of the Anzali Lagoon (Long. 49°38’48’’ E., Lat. 37°18’16’’ N.) in Guilan, Iran. In mid-May of 2018, mature seed capsules were collected from intact maternal stands of snowflakes with almost similar visual characteristics. They were then dried at room temperature (24°C) for 72 hours. After the seeds were discharged from the capsules, intact seeds were selected for the experiment. Before applying the treatments, the seeds were disinfected with 5% sodium hypochlorite for 2 minutes. Then, they were washed with distilled water, dried in the room, and applied with the treatments.

The experiment was conducted as a completely randomized design with seven treatments and three replications. The experimental treatments included temperature (4, 24, and 27°C), gibberellic acid (50 and 100 mg L^{-1} GA), and potassium nitrate (0.1 and 0.2% KNO_3). After the treatments were applied, 15 seeds from each treatment were cultured on Whatman filter paper in Petri dishes containing 5 mL distilled water. Then, the Petri dishes were placed in germinators at constant temperatures of 24°C and 27°C. The temperature of 4°C was applied with a refrigerator. The Petri dishes were daily monitored to record the number of germinated seeds and replace the water evaporated from their surface. Finally, the seedling length was measured with a ruler. The germination rate (Maguire, 1962), germination percentage (Scott *et al*., 1984), and seedling length vigor (Abdul-Baki and Anderson, 1973) were estimated by the following equations:

$$\text{Germination Rate: } \left(\frac{a}{1}\right) + \left(\frac{b-a}{2}\right) + \left(\frac{c-b}{3}\right) + \cdots + \left(\frac{n-n-1}{N}\right)$$

in which a, b, c, …, n represent the number of germinated seeds 1, 2, 3, …, n days after seed imbibition, respectively.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds per day}}{\text{Total number of seeds}} \times 100$$

$$\text{Seedling length vigor} = \frac{\text{Germination (\%)}}{\text{Mean of seedling height}} \times 100$$

Experiment 2: Bulb forcing

Dormant snowflake bulbs were collected from the natural habitat of the snowflake in the south of the Anzali Lagoon (Long. 49°38'48'' E., Lat. 37°18'16'' N.) at the end of summer in 2018. Healthy bulbs of similar size were rinsed and disinfected with 2 g L^{-1} benomyl for 15 minutes. For each treatment, 10 bulbs were placed in transparent plastic containers with some pores on their walls.

A randomized complete block design was employed for this experiment whose experimental treatments included (i) 4°C for 8 weeks, (ii) 24°C for 8 weeks, (iii) 27°C for 8 weeks, (iv) 4°C for 4 weeks + 27°C for 4 weeks, and (v) 27°C for 4 weeks + 4°C for 4 weeks. The temperature of 4°C was applied with a refrigerator and the temperatures of 24°C and 27°C with a germinator. The forcing operation was performed in darkness. After it was terminated, the bulbs were planted in a greenhouse with standard conditions in size-7 pots containing vermicompost and garden soil at equal proportions. Table 1 presents the physical and chemical characteristics of the substrate.

Table 1. The physical and chemical characteristics of the substrate used to plant forced snowflake bulbs.

Properties	Substrate (vermicompost + garden soil)
Total N (%)	0.45
Available P (mg kg^{-1})	40.5
Available K (mg kg^{-1})	195
Organic carbon (%)	4.48
Organic matter	7.73
pH	7.26
EC (dS m^{-1})	0.8
Iron	6.87
Sodium	82.5
Lime	6.25
Clay (%)	22
Silt (%)	41
Sand (%)	37

In this experiment, the target parameters were measured on the plants until the withering of the flowers. Leaf and flower numbers were counted during the experiment and their averages were reported. Flowering stem diameter and flower diameter were measured with a caliper, and the height of the flowering stems was determined with a ruler. The number of days to bud emergence was determined by counting the number of days from bulb planting until the emergence of the first visible bud. To measure shoot and bulb fresh and dry weights, the shoots of the plants were separated from their bulb at the end of the experiment. After the shoots and roots were cleaned from the soil remnants, their fresh weight was determined with a digital 0.001-g scale. Then, they were oven-dried at 105°C for 72 hours to find out their dry weight. Chlorophyll content was de-

termined by the procedure described in Mazumdar and Majumder (2003). So, samples were taken at the flowering phase and the leaves were extracted with 80% acetone. Then, the pigment content was read at 660 and 642 nm with a Shimadzu UV-120-02 spectrophotometer (Japan). In the end, leaf chlorophyll content was determined in mg/g fresh weight (FW) by the following equation:

Data analysis

Data collected from the seed priming and bulb forcing experiments were analyzed in the SPSS statistical software and their means were compared with the LSD test.

RESULTS

Experiment 1: Seed priming

Based on the results of the analysis of variance (ANOVA), the effect of temperature, GA, and KNO_3 was significant on the germination percentage and rate at the $P < 0.01$ level and on the seedling length and length vigor at the $P < 0.05$ level (Table 2). The seeds treated with 4 °C and 27 °C did not germinate, so they were removed from the treatments. The comparison of the means showed that the thermal treatment performed poorer than the hormonal and chemical treatments in stimulating snowflake seed germination (Table 3).

Table 2. The analysis of variance for the effect of temperature, gibberellic acid, and potassium nitrate on snowflake seed germination.

S.o.V	df	Germination percentage	Germination rate	Seedling length	Seedling length vigor
Replication	2	0.385^{ns}	146^{*}	9.321^{ns}	58955^{ns}
Treatments	4	1634^{**}	312^{**}	3.92^{*}	66200^{*}
Error	8	19.60	44.94	3.43	40523.94
CV (%)		12.26	54.42	54.48	125.59

*, **, ^{ns} show significance at the $P < 0.05$ and $P < 0.01$ and non-significance at the $P < 0.05$ level based on the LSD test, respectively.

Table 3. The comparison of the means for the effect of temperature, gibberellic acid, and potassium nitrate on snowflake seed germination.

Treatments	Germination percentage	Germination rate	Seedling length (mm)	Seedling lengthvigor
24 ºC	12.44 ^{d}	5.70 ^{b}	2.39 ^{c}	34 ^{c}
50 mg L^{-1} Gibberellic acid	34.22 ^{c}	10.95 ^{ab}	4.01 ^{ab}	178 ^{ab}
100 mg L^{-1} Gibberellic acid	71.55 ^{a}	20.53 ^{a}	5.09 ^{a}	401 ^{a}
0.1 % Potassium nitrate	43.55 ^{b}	16.48 ^{ab}	3.04 ^{b}	144 ^{ab}
0.2 % Potassium nitrate	18.66 ^{d}	7.90 ^{b}	2.48 ^{c}	42 ^{c}

Means with similar letter (s) in each column show the lack of a significant difference at the $P < 0.05$ level based on the LSD test.

Temperature affects germination and plant establishment by adjusting seed dormancy so that a linear relationship has been reported between temperature and germination rate of many plant species (Copeland and Mc Donald, 1995; Ramin, 1997). Often, the germination rate increases with the temperature up to a certain point varying among plant species, and lower or higher tem-

peratures reduce germination rate and percentage (Kumar *et al*., 2011; Copeland and Mc Donald, 1995). In the present research, germination stopped at the lowest (4 °C) and highest temperatures (27 °C). Çiçek *et al*. (2007) revealed that the lower temperature (4 °C) had no impact on accelerating snowflake germination, which is consistent with our findings. According to them, the highest germination (73 %) was obtained from the seeds treated with 24 °C for six weeks. In our study, the highest germination among the thermal treatments was 12.44 % related to the seeds exposed to 24 °C for eight weeks. Based on a comparison of our results and those of Çiçek *et al*. (2007), it seems that exposure duration is also influential on the germination of snowflake seeds in addition to the temperature.

As the results in table 3 show, the highest germination percentage (71.55%), germination rate (20.53), seedling length (5.09 cm), and seedling length vigor (401) were obtained from the seeds treated with 100 mg L^{-1} GA. Germination initiation is accompanied by the synthesis and release of GA in seeds. GA stimulates the activity of hydrolytic enzymes, e.g., α-amylase, which plays a role in the hydrolysis of starch to sugar, thereby increasing the material available to the embryo for its growth and germination initiation (Millaku *et al*., 2010). As well, gibberellin accelerates the exit of rootlets and facilitates seedling establishment by affecting the endosperm cells around the root tip (Ogawa *et al*., 2008). Zarafshar *et al*. (2012) stated that the application of 100 mg L^{-1} GA increased the germination rate and percentage of *Celtis australis*, which corroborates our findings.

In this work, the treatments of 0.1% KNO_3 and 50 mg L^{-1} GA were related to the second and third highest germination rate and percentage of the snowflake, respectively. These two treatments were the best treatments after 100 mg L^{-1} GA in increasing seedling length and seedling length vigor (Table 3). KNO_3 is one of the compounds used to break seed dormancy. The mechanism by which KNO_3 facilitates germination is related to its effect on making a balance in hormone ratios and reducing germinating seed growth inhibitors (Mansouri and Omidi, 2018). We observed that KNO_3 at the rate of 0.2% reduced germination-related traits. Khosh-Khui (2013) reported that the application of high rates of KNO_3 (>0.1-0.2%) should be avoided for seed priming. A positive effect of low levels of KNO_3 has been reported on the germination of *Hippophae salicifolia* (Gupta *et al*., 2011) and *Sorbus pohuashanensis* (Lei *et al*., 2013). According to these studies, the application of KNO_3 at high rates (over 0.2-0.3%) had an adverse impact on the germination rate and percentage, which is similar to our findings.

Experiment 2: Bulb forcing

As the results of ANOVA for the forcing data showed, the effects of the experimental treatments were significant on the number of days to bud emergence, the number of days from bulb planting to flowering termination, the number of leaves, the number and diameter of flowers, the diameter and height of the flowering stem, and shoot fresh weight, and bulb dry weight at the $P < 0.01$ level and on bulb fresh weight at the $P < 0.05$ level. But, they did not influence shoot dry weight and total chlorophyll significantly (Table 4).

The bulbs treated with 4°C for eight weeks did not flower and only exhibited vegetative growth. Although, these plants failed to produce flowers and start their reproductive phase, they produced the heaviest bulbs so that they had the highest bulb fresh weight (28.11 g) and dry weight (8.98 g). The four treatments that resulted in flower initiation did not differ significantly in flowering time, the number of days from bulb planting to flowering termination, flower number and diameter, and stem diameter and height. This implies that low temperatures alone are not beneficial for the flowering or even vegetative growth of snowflakes (Table 5).

The shortest time to flower initiation, an important factor assessed for forcing, was related to the bulbs treated with 27 °C for 4 weeks + 4 °C for 4 weeks (78.3 days) and those treated with 24°C for 8 weeks (94.0 days). However, these treatments had the lowest number of days from bulb planting to flowering termination. The bulbs treated with 27 °C for 8 weeks and those treated with

4°C for 4 weeks + 27 °C for 4 weeks, whose flower emergence was delayed, maintained their flowers longer (149.5 and 143.1 days, respectively). Also, the treatments of 27°C for 8 weeks and 4°C for 4 weeks + 27 °C for 4 weeks outperformed the other treatments in increasing leaf number, flower number, flower diameter, flowering stem length, and total chlorophyll content, although they did not differ significantly (Table 5).

The highest flowering stem diameter (4.66 mm) and height (54.1 cm) were obtained from the treatment of 4°C for 4 weeks + 27°C for 4 weeks and the highest shoot fresh weight (16.83 g) was obtained from the treatment of 24°C for 8 weeks. The bulbs treated with 24°C for 8 weeks had the weakest flowering stem (with a diameter of 2.93 mm and a height of 37.4 cm), the smaller flowers (with a diameter of 14.40 mm), and the fewest number of flowers (2.33 flowers). So, this treatment and the treatment of 4°C for 8 weeks are not recommended for snowflakes (Table 5).

Researchers argue that the storage of dormant bulbs at low temperatures prolongs their dormancy and prevents their physiological maturity, which will result in the production of weak and low-quality flowers (Glover, 2007; Ladan Moghaddam *et al*., 2017). Akbari and Tehranifar (2009) reported that tuberose seedlings treated with 4°C for 8 weeks exhibited very low germination percentage and vegetative growth. They reported that the tuberose bulbs treated with 4°C for 8 weeks produced weaker seedlings and few of them proceeded to the reproductive phase. In our study, the bulbs treated with 4°C did not produce a flowering stem and had lower shoot fresh weight and leaf number than the other treatments. Paz *et al*. (2003) reported that low temperatures retarded flowering and resulted in slow and irregular growth. Some researchers suggest that lower temperatures of bulb storage inhibit vegetative and reproductive growth by reducing enzymatic activities, the inhibition of the hydrolysis of sugars, and their translocation to other parts (de Hertogh and Le Nard, 1993; Nagar, 1995).

Dole (2003) suggests that the duration of the storage of geophytes' reproductive parts at a low temperature plays a significant role in their subsequent growth and flowering. Sandler Ziv *et al*. (2011) reported that the treatment of snowflake bulbs at 9ºC for 2 weeks accelerated their inflorescence emergence. However, the inflorescence length of these bulbs was shorter than those of the bulbs stored at 4ºC and 23ºC. They found that the bulbs stored at 9ºC for 2 weeks recorded the longest leaves and those stored at 23ºC for 12 weeks recorded the shortest leaves. The longest time to flower emergence (63 days) was related to the bulbs stored at room temperature for 12 weeks whereas the bulbs stored at 9ºC for 2 weeks showed the shortest time (39 days).

Based on the results of Dole (2003) and Sandler Ziv *et al*. (2011) and their comparison with the present work, it can be said that the duration of cold treatment plays a decisive role in the flowering of snowflakes. Gilbertson-Ferris (1985) reports that to achieve high-quality *Freesia*, their corms should be stored at 30ºC for 15-16 weeks before receiving cold treatment (13-15ºC). We found that the storage of the snowflake bulbs at 4ºC for 4 weeks + 27ºC for 4 weeks reduced the time required for bud emergence, which in turn advanced their flower initiation. The bulbs stored at 27ºC for 8 weeks produced flowers later, but they kept their flowers for a longer time than the other treatments. However, there was statistically no significant difference between the treatments of 27ºC for 4 weeks + 4ºC for 4 weeks and 4ºC for 4 weeks and 27ºC for 4 weeks. In Sandler Ziv *et al*.'s (2011) study, the longest time to snowflake inflorescence emergence was obtained from the bulbs stored at room temperature for 12 weeks.

de Hertogh and Le Nard (1993) report that the species originated from the Irano-Turanian regions need a period of high temperature (17-25ºC) followed by a cold period (4-9 ºC) for the growth of their flowering stem and blooming. Similarly, we observed that the treatment of 27 ºC for 4 weeks + 4 ºC for 4 weeks was effective in early-flowering. It has also been reported that flower induction and initiation are delayed at high temperatures (25-30 ºC), but they increase the flowering potential of the plants (Khandan Mirkohi *et al*., 2017). Ladan Moghaddam (2017) stated that the storage of tuberose bulbs at a high temperature (20-25 ºC) for a long time retarded flowering. In the present research, the treatment of the bulbs at 27ºC for 8 weeks was related to the

Table 4. The analysis of variance for the effect of forcing treatments on the vegetative and reproductive traits of the snowflake.

S.o.V	df	Time of flowering	Display life	Leaf no.	Flower no.	Flower diameter	Flowering stemdiameter	Flowering stemheight	Shoot fresh weight	Shoot dry weight	Bulb fresh weight	Bulb dry weight	Total chloro-phyll
Replication	2	1016 ^{ns}	1410 ^{ns}	0.056^{ns}	0.350^{ns}	34.03 ^{ns}	1.380 ^{ns}	198 ^{ns}	28.04**	0.211*	42.1^{ns}	2.88**	0.082 ^{ns}
Treatments	4	6747**	11352**	1.058**	3.79**	253**	11.54**	1567**	11.5**	0.046^{ns}	35.62*	5.00**	1.256 ^{ns}
Error	8	929.99	1460.7	0.0699	1.266	31.94	1.424	225.86	1.2618	0.0504	11.819	0.371	1.589
CV (%)	-	37.99	36.72	4.97	56.27	36.32	36.31	38.42	8.11	12.44	14.19	7.994	14.01

*, **, ^{ns} show significance at the $P < 0.05$ and $P < 0.01$ and non-significance at the $P < 0.05$ level based on the LSD test, respectively.

Table 5. The comparison of the means for the effect of forcing treatments on the vegetative and reproductive traits of the snowflake.

Treatments	Time of flowering (day)	Display life (day)	Leaf no.	Flower no.	Flower diameter (mm)	Flowering stemdiameter (mm)	Flowering stemheight (cm)	Shoot freshweight (g)	Shoot dryweight (g)	Bulb freshweight (g)	Bulb dryweight (g)	Total chlorophyll (mg g^{-1} F.W)
T1	94.0 ^{a}	130.0 ^{a}	5.44 ^{b}	2.33 ^{a}	14.40 ^{a}	2.93 ^{a}	37.4 ^{a}	16.83 ^{a}	1.87 ^{a}	21.22 ^{bc}	6.88 ^{bc}	8.76 ^{a}
T2	0. 00 ^{b}	0.00 ^{b}	4.38 ^{c}	0.00 ^{b}	0.00^{b}	0.00 ^{b}	0.00 ^{b}	11.48 ^{c}	1.60 ^{a}	28.11 ^{a}	8.98 ^{a}	9.30 ^{a}
T3	117.5 ^{a}	149.5 ^{a}	6.00 ^{a}	2.66 ^{a}	21.70 ^{a}	4.33 ^{a}	52.2 ^{a}	12.88 ^{bc}	1.77 ^{a}	20.10 ^{c}	5.77 ^{c}	9.58 ^{a}
T4	111.5 ^{a}	143.1 ^{a}	5.55 ^{ab}	2.50 ^{a}	21.26 ^{a}	4.66 ^{a}	54.1 ^{a}	14.01 ^{b}	1.88 ^{a}	26.63 ^{ab}	7.95 ^{ab}	9.35 ^{a}
T5	78.3 ^{a}	97.6 ^{a}	5.22 ^{b}	2.50 ^{a}	20.43 ^{a}	4.50 ^{a}	51.7 ^{a}	13.97 ^{b}	1.89 ^{a}	24.98 ^{abc}	8.50 ^{a}	7.97 ^{a}

Means with similar letter(s) in each column show the lack of a significant difference at the $P < 0.05$ level based on the LSD test. T1 = 24 ºC for 8 weeks; T2 = 4 ºC for 8 weeks; T3 = 27 ºC for 8 weeks; T4 = 4 ºC for 4 weeks + 27 ºC for 4 weeks; T5 = 27 ºC for 4 weeks + 4 ºC for 4 weeks.

highest number of flowers, the thickest flowering stem, and the highest number of days from bulb planting to flowering termination, but this treatment exhibited the longest time to bud emergence, so it is not a proper treatment for bulb forcing.

CONCLUSION

The temperatures of 4ºC and 27ºC are not recommended for the priming of snowflake seeds. The treatment with 24ºC turned out to be weaker than the chemical treatments (KNO_3 and GA). The maximum germination of the snowflake seeds was obtained from their priming with 100 mg L^{-1} GA, and those primed by 0.1% KNO_3 were in the next rank. So, these treatments are recommended for snowflake seed priming and seed-bearing seedling production.

Based on the goal of snowflake forcing, if early flowering is the target, the bulbs are recommended to be treated with 27ºC for 4 weeks + 4ºC for 4 weeks, but if late flowering and the production of flowers with higher quality (higher flower number and diameter, more leaves, longer flower longevity) are the targets, the treatments of 27ºC for 8 weeks or 4ºC for 4 weeks + 27ºC for 4 weeks are suitable. The longer treatment of snowflake bulbs with 4ºC is not recommended since they do not come into flowering.

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