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Effect of Stratification on Germination of *Leucojum Aestivum* L. Seeds, a Valuable Ornamental and Medicinal Plant

[1]Emrah Çiçek, [2]Mustafa Aslan and [3]Fahrettin Tilki

[1]Düzce University, Faculty of Forestry, 81620-Duzce, Turkey.
[2]Western Black Sea Forest Research Institute, 14100-Bolu, Turkey.
[3]Artvin Coruh University, Faculty of Forestry, 08000-Artvin, Turkey.

Abstract: *Leucojum aestivum* L. is a valuable medicinal and ornamental plant. Despite its enormous industrial potential and the declining status of its natural population, little or no attention has been give to conservation and propagation of this species by seed. *Leucojum aestivum* seeds with capsule were collected from its natural habitat in Akyazı, Turkey. Initial seed viability using the tetrazolium method and 1000 seed weight were determined. Field germination of non-treated seeds in nursery was also determined. Seeds were cold-stratified at 4 ºC for 0, 3, 6 and 7 weeks and warm-stratified at 22 ºC for 3, 6 and 7 weeks in sand for laboratory germination. The results of this study revealed that viability of seeds was 98%. Unstratified seeds gave 0% germination in laboratory, and cold stratification didn't break seed dormancy. Improved germination responses (73% germination) were obtained when dormant seeds of *Leucojum aestivum* were pre-treated for 6 weeks of warm stratification. 82% emergence without pretreatment was occurred after 6 months of sowing in the nursery.

Key words: Alkaloid, *Leucojum aestivum,* Germination percentage, Seed treatment

INTRODUCTION

Total number of vascular plant species in Turkey is about 9000 of which about 3000 is endemic to Turkey[1]. More than 500 geophytes are found in Turkey and some of them are exported to abroad for a long time[2]. But due to massive collection for industries most plants with bulb are not allowed to harvest and export. Only 20 species including *Leucojum aestivum* L. (snowflake) (*Amaryllidaceae*) are allowed to collect for industries. But most of the exported bulb of *Leucojum aestivum* are being collected from its natural habitats without any permission, and current practices of harvesting are unsustainable[3,4].

Leucojum aestivum is a valuable medicinal and ornamental plant native to South Europe, Turkey, the Balkans, Caucasia and Northern Iran[2,5]. It grows from see level to 1000 m altitude in Turkey and common on *Fraxinus angustifolia* dominated bottomland forests. But these bottomland forests were not properly used and the ecosystems were degraded, declined or destroyed due to urbanization, agricultural practices or poplar cultivation[6]. Thus, the natural ecosystem of *Leucojum aestivum* were reduced in size and destroyed. Also due to overgrazing in its natural habitats the regeneration of the species by seed is heavily constrained[3,4]. Over-exploitation has lead either to decline or loss of its natural populations.

This economically important species has been used for ornamental purpose in Europe but now bulbs and leaves of *Leucojum aestivum* is being used as a medicinal purpose. Extracts from *Leucojum aestivum* exhibited significant antiviral activities[7]. Alkaloids including galanthamne and other chemicals (lections, chelidonic acid etc) used in pharmacy[8,9,10]. Galanthamne alkaloid obtained from *Leucojum aestivum* is also used to treat Alzheimer[11].

Although there are some studies on alkaloids found in *Leucojum aestivum*[7,11,12,13], vegetation propagation[14,15,16], bulb yield and some snowflake characters[17,18,19], influence of chemical composition of soils on the galanthamne content[20], and site characteristics[4], there is currently no information on production by seed, seed and seed germination of *Leucojum aestivum*.

Despite its enormous industrial potential and the declining status of its natural population, little or no attention has been give to conservation and propagation of this species by seed. Seeds that fail to germinate under favorable environmental conditions are considered to be dormant and various methods are used to break dormancy in several species[21,22]. For sustainable use, propagation and conservation purposes and due to a growing demand for bulb and leaves of this species by the pharmaceutical industries, seed dormancy and germination behavior of this species

Corresponding Author: Dr. Fahrettin Tilki, Artvin Coruh University, Faculty of Forestry, 08000-Artvin, Turkey
E-mail: fahrettintilki@yahoo.com

should be studied. To our knowledge, such information is not available for snowflake. Thus, the aim of the study was determine whether seeds of *Leucojum aestivum* possess dormancy, and if so how can it be broken.

MATERIALS AND METHODS

Leucojum aestivum seeds with capsule were collected from its natural habitat in Akyazı, Turkey (lat. 40º 48' N, long.30º 33' E, alt. 25 m a.s.l.). The fruits harvested were transported to the handling place and kept in laboratory for 5 days. And then seeds were extracted manually from the capsule. The seeds were kept in room condition (20 ºC) for a few days and then the moisture content of the seeds and 1000 seed weight was determined based on 8 replications of 100 seeds[23]. Seeds were sterilized with 10% ethyl alcohol to prevent fungal infection and then stored at 4 ºC in refrigerator until use.

Initial seed viability was determined on seeds using the tetrazolium method[23]. The seeds were soaked in distilled water for 24 h, bisected longitudinally, and then soaked in 1% solution of 2,3,5-triphenyl tetrazolium chloride. Seeds with red-stained embryos were considered viable.

To test field germination of non-treated seeds, a randomized block design with six replications was employed at Düzce nursery (lat. 40°50' N, long. 31°10' E, alt. 140 m a.s.l.). Each experimental unit (plot) was 1.2 m^2 (1x1.2 m) and contained five rows. 250 seeds were sown in each experimental unit (50 seeds in each row, 50x5=250). Seeds were covered with sieved nursery soil (0.5-1.0 cm thick) and then all plots were covered with peat (3 cm thick). No watering and irrigation were applied in the plots. The nursery soil was sandy clay loam with a soil pH of 7.2-7.5 in the rooting zone. The climate is mild with humid summers. Annual precipitation averages approximately 840 mm and the temperature averages 13ºC. The growing season averages 210-220 days.

In mid-November most of the seeds sown in the nursery were germinated. But epicotyl was not developed. Epicotyl was seen in early February 2007, and then germination percentage (emergence) was recorded in late February until no further germination/emergence occurred. The criterion for the germination in the field was the growing the young shoot above the soil surface.

Seeds were cold-stratified at 4 ºC for 0, 3, 6 and 7 weeks and warm-stratified at 22 ºC for 3, 6 and 7 weeks in sand. For each experiment, there were four replicates with 100 seeds each per treatment arranged in a completely randomized design. The seeds were germinated in Petri dishes (15 cm in diameter) containing two layers of filter paper. Germination tests were performed in a germination cabinet at constant 20ºC in darkness. Germination in laboratory was monitored in each day for 28 days. Data was subjected to analysis of variance (ANOVA), and percent data were arcsine transformed before performing ANOVA. When significant differences were found, Duncan' New Multiple Range Test was performed for comparison of the means.

RESULTS AND DISCUSSIONS

Moisture content of the seeds was 33% and 1000 seed weight was 94 g. The results of this study revealed that viability of seeds was 98%. 82% emergence without pretreatment was occurred after 6 months of sowing in the field/nursery and the germination was hypogeal.

Table 1: Effects of stratification on germination percentage

Stratification	Germination (%)
Control	0a
3 weeks of cold stratification	0a
6 weeks of cold stratification	0a
7 weeks of cold stratification	0a
3 weeks of warm stratification	5b
6 weeks of warm stratification	73c
7 weeks of warm stratification	pregermination

Means followed by the same letter are not significantly different at $p<0.05$.

No emerged in laboratory occurred without pretreatment indicating that the seeds were dormant. Cold stratification was not affecting in breaking seed dormancy and gave no germination (0%). Improved germination responses were obtained when dormant seeds of *Leucojum aestivum* were pre-treated for 6 weeks of warm stratification, and 6 weeks of warm stratification increased germination to 73%. Increasing the duration of stratification to 7 weeks caused the seeds to germinate during stratification period.

The reason why field germination was higher than laboratory germination was that seeds were germinated at 20 ºC in laboratory but seeds were sown in nursery bed in July and sown seeds were stratified in nature from late July to February, and then germinated until late February. Thus, seed germination in laboratory should be performed at higher temperatures (e.g. 25 ºC or 30/20 ºC).

Since seeds sown in nursery in July were germinated in mid-November, it might be stated that they didn't need cold stratification. Laboratory germination study supported this idea and cold stratification didn't increase germination.

The present study points out that seeds of this species should be sown from mid-summer to early autumn without stratification or in early spring after 6 weeks of warm stratification. Taking into account the differences observed in the results obtained by nursery

and laboratory studies, further experiments should be conducted to study the role played by various stratification pretreatments and germination temperatures in the response to these treatments.

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