Sensitivity of Seed Germination to Temperature and Light in *Helichrysum amorginum*

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BulletinUASVM Horticulture 77(1) / 2020

Print ISSN 1843-5254, Electronic ISSN 1843-5394

DOI:10.15835/buasvmcn-hort: 2020.0007

Abstract

Helichrysum amorginum is a perennial shrub, endemic on the Greek island of Amorgos and used for medicinal, cosmetic and ornamental purposes. Information concerning germination behavior and seedling survival is considered essential for the establishment and cultivation of wild species. The objective of this study was to investigate the effect of temperature and light on germination of *Helichrysum amorginum* seeds. A set of experiments was conducted under completely randomized design with four replications of 200 seeds at three different suboptimal germination temperatures (15, 20 and 25°C) in light and dark conditions for 10 days. Seed material was collected from two different fields of the island (Aegiali and Katapola). The germination performance was evaluated by final germination percentage and mean germination time. The effect of temperature on seed germination percentage and mean germination time was significant in all cases. The presence of light enhanced germination, especially at the optimum temperature of 20°C. In most cases the mean germination time increased at the lower temperature of 15°C. Seeds from Aegiali exhibited higher germination ability than the seed lot from Katapola. Knowledge of seed germination behaviour is of major importance in order to establish effective protocols for seed propagation in *Helichrysum amorginum*.

Keywords: germination percentage, germination time, *Helichrysum amorginum*, light, temperature

Introduction

The genus *Helichrysum* Mill. belongs to the *Asteraceae* family, which comprises over 600 species distributed in Africa, Asia, Australia and Europe. It is represented in the Mediterranean area by 25 native species and in Greece by 10 species among which 5 are endemic: *H. amorginum* Boiss. & Orph., *H. doerfleri* Rech.f., *H. heldreichii* Boiss., *H. taenari* Rothm., *H. sibthorpii* Rouy. In addition to its ornamental value, since the flowers retain their form and colour when dried, increasing interest in this genus has been shown recently by

the cosmetic and pharmaceutical industries due to the essential oils produced by the glandular hairs present on the leaves and flower heads of the plant. The volatile fraction of the essential oil showed anti-inflammatory, anti-allergic, antimicrobial and antioxidant properties (Akaberi *et al.*, 2019). *Helichrysum amorginum* Boiss & Orph is a perennial dwarf white tomentose shrub that can reach 30 cm in height. The flower is a capitulum, small in size with white involucre bracts, sometimes flushed pinkish towards the base, and yellow florets. It is endemic (characterized as vulnerable)

on the Greek island of Amorgos where it grows in arid soils, from the cliffs above the sea to the inland hills. The flowers, produced from May to July, are used locally for medicinal, cosmetic and ornamental purposes (Chinou *et al.*, 2004).

Cultivation of threatened or vulnerable endemic species can offer a sustainable alternative to the harvesting of wild populations, yielding economic gain to local communities (Liu *et al.*, 2018). The definition of germination protocols for endemic, rare, threatened or vulnerable species characterized by small populations is an important step for their cultivation and consequently for their commercial exploitation, considering that seed germination is a critical stage for plant establishment (Sawilska, 2007). It is common knowledge that temperature, soil moisture, light and oxygen are the major factors controlling seed germination. Each species is characterized by a temperature range over which germination is favorable, and occasionally responding differently to constant or alternating temperature regimes, reflecting the climatic and ecological conditions to which the species is adapted (Baskin and Baskin, 2014, Picciau *et al.*, 2019). In addition, light is also important for seed germination and light requirements can vary with temperatures. Some seeds germinate similarly in light and darkness, while others do it more readily under light or dark conditions (Afolayan *et al.*, 1997; Doussi and Thanos, 1997; Sawilska, 2007; Casalini *et al.*, 2017). Although some information is available concerning the germination behavior of the *Helichrysum* species which originate in the Mediterranean basin, there is no reference in the literature to *Helichrysum amorginum* germination requirements. Therefore, the objective of this study was to investigate the effect of temperature and light on the germination of *Helichrysum amorginum* seeds.

Materials and Methods

Seed material was collected from two different fields on the Greek island of Amorgos situated in Aegiali and Katapola (North region, altitude 236 m and South region, 30 m above sea level respectively). Seeds were surface sterilized in a 1% sodium hypochlorite solution, rinsed in distilled water and dried before the experiment (4.4% seed moisture). A set of experiments was conducted under a completely randomized design

with four replications. Seeds were germinated at three different constant temperatures 15, 20 and 25 °C in light and dark conditions, maintained in a thermostatically controlled incubator (± 1 °C) for 10 days. For each experiment samples of 800 seeds (four replicates of 200 seeds each) were placed in Petri dishes between two filter papers moistened with 7 mL of sterile distilled water. Petri dishes were hermetically sealed with parafilm to prevent evaporation and then randomized in an incubator. The seeds were kept in the dishes to assess germination. This was scored when the radicle approached approximately 2 mm length, and data were collected daily until no additional germination had occurred for 48 h.

At the end of the tests the final percentage of germination was calculated. Using the daily counts, the mean germination time (MGT) was calculated for each lot using the formula cited by Ellis and Roberts (1980) given below:

$$\text{MGT} = \sum n.D / \sum n$$

where n = number of seeds newly germinated at time D; D = days from the beginning of the germination test, $\sum n$ = final germination.

Data of the final germination percentage (arcsine transformed) and mean germination time were statistically analyzed by a completely randomized two-way analysis of variance (ANOVA) using the StatSoft software (1999). The analysis of variance was conducted considering light conditions and temperatures as fixed factors. When 'F' ratios were significant, means were separated by the Student-Newman-Keuls (SNK) test at ≤ 0.05 .

Results and Discussion

The cumulative seed germination time courses during imbibition of seeds originating from Katapola and Aegiali at different temperatures and light conditions are shown in Figures 1 and 2. The effect of temperature on seed germination percentage and mean germination time was significant in all cases (Tab. 1). In this study, seeds germinated at 20 °C showed a higher rate of final germination percentage and a lower mean germination time compared to the seeds from other treatments. Final germination percentage ranged from 26% to 76% and from 51% to 85% at 15 °C and 20 °C, respectively compared to the higher temperature of 25 °C. Comparable results

Figure 1. Cumulative germination of *Helichrysum amorginum* seeds from Katapola exposed to light and dark conditions at 15, 20 and 25 °C
Vertical bars represent the standard error

were reported by Picciau *et al.* (2019) who found the highest germination percentage of *Helichrysum microphyllum* subsp. *tyrrhenicum* seeds from Sardinia and Corsica at 15 °C and 20 °C.

This is common in many native species grown under a Mediterranean climate, where seed germination occurs at 15 - 20 °C, which ensures that seeds complete germination during the rainy period in autumn, thus allowing seedlings to avoid arid conditions during summer (Thanos *et al.*, 1995; Doussi and Thanos 2002; Luna *et al.*, 2008; Kadis and Georgiou 2010). In addition, other *Helichrysum* species, *H. aureonitens* from South Africa and *H. cassinianum* from Western Australia, native to regions where most of the rain falls in the warmer summer periods, tended to have germination maxima at higher temperatures of 25- 30 °C (Mott 1974; Afolayan *et al.*, 1997). Mean germination time ranged from 4 to 5 days at the optimum temperature of 20°C. Higher or lower temperatures from the optimum caused a significant delay in mean germination time (7 to 9 days and 6 to 7 at Katapola and Aegiali, respectively) regardless of light (Tab. 1).

The effect of light on seed germination percentage was significant at $p < 0.05$ (Tab. 1). The presence of light enhanced germination, especially at the optimum temperature of 20°C.

The presence of darkness reduced germination regardless of temperature. This reduction was

more severe in the seeds from Aegiali (58% and 42% germination at 15 and 25 °C respectively) than in the seeds from Katapola (26% at 15 °C) (Tab. 1). Similar results regarding the favorable effect of light were observed in seed germination of *H. aureonitens* (Afolayan *et al.*, 1997), *H. serotinum* and *H. stoechas* (Luna and Moreno, 2009) and *H. italicum* subsp. *microphyllum* (Willd.) Nyman (Casalini *et al.*, 2017).

Seeds from Aegiali exhibited higher germination ability than the seed lot from Katapola (Figs. 1 and 2, Tab. 1). Several environmental factors (e.g. light, moisture, temperature, orientation and altitude) can cause differences in the germination response among populations of the same species. In this study, the differences in germination behavior between populations may be related to the altitude of the different locations, which reflect the different microclimatic conditions prevailing in the natural habitat where the autochthonous plants of this species are located in the northern rock cliffs of Amorgos island. In this case, the Aegiali area is at a higher altitude, while Katapola is near the coast (236m and 30m above sea level, respectively). Correlations between intraspecific differences in seed germination and altitude have been reported in previous studies (Vera 1997; Chamorro *et al.*, 2013). Conversely, as declared by Picciau *et al.* (2019), differences in seed

Figure 2. Cumulative germination of *Helichrysum amorginum* seeds from Aegiali exposed to light and dark conditions at 15, 20 and 25 °C
Vertical bars represent the standard error

Table 1. Results from an analysis of variance showing the effects of temperature and light on final germination percentage and mean germination time of *Helichrysum amorginum* seeds from Katapola and Aegiali.

Katapola Temperature (°C)	Germination (%)		Mean germination time (days)	
	Light	Dark	Light	Dark
15	35.0 Aa	26.0 Ba	8.22 Aa	7.03 Aab
20	59.0 Ab	51.0 Ab	5.60 Aa	5.08 Aa
25	20.0 Ac	21.0 Aa	7.71 Aa	9.49 Ab
Average	38.0 A	32.7 A	7.18 A	7.20 A
Significance				
L		*		ns
T		***		*
L x T		ns		ns
Aegiali Temperature (°C)	Germination (%)		Mean germination time (days)	
	Light	Dark	Light	Dark
15	76.0 Aab	58.0 Ba	6.88 Aa	6.79 Aa
20	85.0 Aa	78.0 Ab	3.74 Ab	4.08 Ab
25	64.0 Ab	42.0 Ba	5.46 Ac	6.69 Ba
Average	75.0 A	59.9 B	5.36 A	5.86 A
Significance				
L		*		ns
T		**		***
L x T		ns		ns

Note: ns, not significant; significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values belonging to the same characteristic with different lower-case letters within a column indicate significant differences between temperatures mean values and different upper-case letters within a row indicate significant differences between the means of light and dark conditions tested according to Student-Newman-Keuls test at $p < 0.05$.

germination of *Helichrysum microphyllum* subsp. *tyrrhenicum* were not correlated with altitude

Conclusion

The results of the present experimental work have led to the conclusion that low temperatures

and light conditions affected positively seed germination of this endemic species. The overall results of this experiment provided useful information in order to establish effective protocols for seed propagation in *Helichrysum amorginum*.

This is necessary for successful establishment and small-scale cultivation within the species' native habitat, which may offer a sustainable solution for the conservation of the wild population of this endemic species and could contribute positively to local economic development.

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