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The seed characteristics of *Salvia dorystoechas* B.T. Drew

Mustafa YILMAZ[1], Tolga OK[2], Münevver ARSLAN[3], Hakan DEMİRBAĞ[* 4], Hafize KÜÇÇÜK[1], Cansu GÜNAY[1]
Muhammed Emirhan GÜNDOĞDU[1]
ORCID: 0000-0002-8250-1882; 0000-0002-9546-3547; 0000-0003-2645-1486; 0000-0003-2778-6376;
0009-0006-9950-2124; 0009-0004-8519-3215; 0009-0007-7276-4009

[1] Bursa Technical University, Faculty of Forestry, Bursa, Türkiye
[2] Sütçü İmam University, Faculty of Forestry, Kahramanmaraş, Türkiye
[3] The General Directorate of Forestry, Forest Regional Directorate of Giresun, Giresun, Türkiye
[4] The General Directorate of Forestry, Research Institute for Forest Soil and Ecology, Eskişehir, Türkiye

Abstract

Purpose: The aim of this study was to determine the seed characteristics of *Salvia dorysthoechas*, a local endemic shrub growing in southern Türkiye.
Method: Two seed sources were used in the study: (1) from the natural population in Antalya - Feslikan Plateau and (2) from 3-year-old *Salvia dorystoechas* plants of Feslikan origin grown in Eskişehir Forest Nursery. In order to determine dormancy, seeds were subjected to four different duration of prechilling treatments. Prechilling treatments were applied to moist filter paper in petri dishes at 4°C for 2, 4 and 6 weeks. Germination tests were carried out using filter paper placed in 12 cm diameter petri dishes at 20°C with 300 (3×100) seeds.
Findings: 1000-seed weight was determined as 0.586 g. The shape of the thin seed was slightly concave. The average length, width and thickness of the seeds are 4.29 mm, 1.57 mm and 0.67 mm, respectively. There was no physical dormancy due to the impermeable seed coat. The seed has a physiological dormancy at a light depth and 2 weeks of prechilling treatment was sufficient to eliminate dormancy. The germination percentage of the seeds without any pretreatment (control) exceeds the half of the total germination capacity. Germination occurs rapidly and the germination percentage reaches maximum levels within a week after the prechilling treatments.
Conclusion: The seed characteristics of this endemic and narrowly distributed species were studied in detail for the first time. It is expected that this study will contribute to the production of the species from seed and the *ex situ* conservation.

Keywords: Çalba tea, Mediterranean areas, Seed germination, Prechilling, Endemic.

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Salvia dorystoechas B.T. Drew'in tohum özellikleri

Özet

Amaç: Bu çalışmanın amacı Türkiye'nin güneyinde yetişen nadir endemik bir çalı türü olan *Salvia dorystoechas*'ın tohum özelliklerini belirlemektir.
Metod: Çalışmada iki tohum kaynağı kullanılmıştır: (1) Antalya - Feslikan Yaylası'ndaki doğal popülasyondan ve (2) Eskişehir Orman Fidanlığı'nda yetiştirilen Feslikan kökenli 3 yaşındaki *Salvia dorystoechas* bitkilerinden. Dormansiyi belirlemek için tohumlar dört farklı sürede katlama işlemine tabi tutulmuştur. Katlama işlemleri 4°C'de petri kaplarında nemli filtre kâğıdı üzerinde 2, 4 ve 6 hafta boyunca uygulanmıştır. Çimlenme testleri 20°C'de 12 cm çapındaki petri kaplarında filtre kâğıdı üzerinde 300 (3×100) tohumla gerçekleştirilmiştir.

[*] *Corresponding author*: Tel.: +902223240248; Fax.: +902223241802; E-mail: hakandemirbag@ogm.gov.tr
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Bulgular: Türün 1000-tohum ağırlığı 0,586 gr olarak belirlenmiştir. Tohumun şekli ince ve hafif içbükeydir. Tohumların ortalama uzunluğu, genişliği ve kalınlığı sırasıyla 4,29 mm, 1,57 mm ve 0,67 mm'dir. Tohum kabuğundan kaynaklanan fiziksel dormansi yoktur. Tohum, hafif derinlikte dormansiye sahiptir ve dormansiyi gidermek için 2 haftalık katlama işlemi yeterlidir. Herhangi bir ön işlem uygulanmayan tohumların (kontrol) çimlenme yüzdesi, toplam çimlenme kapasitesinin yarısını aşmaktadır. Çimlenme oldukça hızlı olup, katlama işlemlerinden sonra çimlenme yüzdesi bir hafta içinde maksimum seviyelere ulaşmaktadır.
Sonuç: Bu endemik ve dar dağılımlı türün tohum özellikleri ilk kez ayrıntılı olarak incelenmiştir. Çalışmanın türün tohumdan üretilmesine ve *ex situ* korunmasına katkıda bulunması beklenmektedir.

Anahtar Kelimeler: Çalba çayı, Akdeniz bölgesi, Tohum çimlenmesi, Katlama, Endemik.

1. Introduction

Salvia dorystaechas B.T. Drew(Feslikan sage, Devren thyme, Çalba tea) is an endemic medicinal plant with limited natural distribution in the West of Antalya, southern Türkiye (Figure 1).

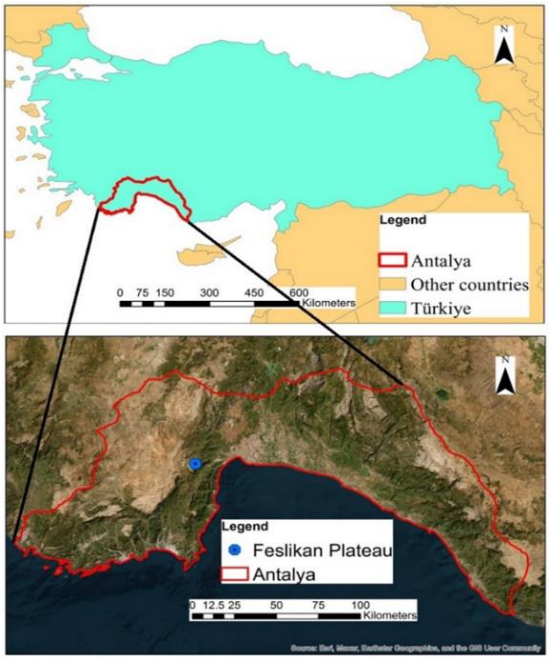

Figure 1. The area seed collected (Feslikan plateau)

The species is 40-100 cm tall, densely pilose, with short soft hairs (pubescent) and many stemless glands. Flowers are in the form of a spike (Figure 2). Fruit is upright, without bending, it remains on the plant until the next season. Flowering is in the 5th-7th months [1; 2].

Figure 2. *Salvia dorystoechas* in natural site, Feslikan Plateau-Antalya

The plant generally prefers stony and rocky areas exposed to the sun. It is also encountered among bushes and maquis vegetation. The main forest tree species around natural distribution sites are *Pinus brutia*, *Cupressus sempervirens*, *Quercus coccifera*, *Juniperus* spp., and *Cedrus libani*. It naturally occurs at altitudes ranging from sea level to 2000 m [3]. Natural populations of the species are under risks due to overgrazing, urbanization, excessive collection by local people [4].

The plant has a rich chemical content [2], strong antioxidative capacity so that it used as a potential antioxidative edible source due to its different classes of phenolic compounds [5; 6].

The genus *Salvia* is one of the most widely distributed the world, occupying many different habitats. There are around 900 species of *Salvia* in all around the world. Türkiye is one of the genetic diversity centers for *Salvia*. There are 100 *Salvia* species in the country, 53 of which are endemic [7].

The seeds of *Salvia* species can exhibit quite different characteristics in terms of size and physiological characteristics due to the great heterogeneity of its natural habitats. Physiological dormancy is common in *Salvia* seeds under different environmental conditions [8]. Physical dormancy is also observed for the seeds of some species [9]. Light can also significantly affect the germination percentage of some *Salvia* species [10]. Removing the nutlet covering tissues, prechilling, and application of GA3 are common treatments that are effective in overcoming the seed dormancy [8; 9; 11; 12]. *Salvia dorystoechas* can also be produced by tissue culture. Cotyledons were determined as the most suitable explants for creating callus cultures of the plant [13].

There is a knowledge gap regarding the physiological properties of *S. dorysthoechas* seeds. In a previous study, seed germination rates of this species were found to be very low. In natural conditions, the generation of new individuals through germination appears to be very difficult. [14]. In another study [2], cold stratification partially increased the germination percentage in some populations. Due to low seed germination of *Salvia dorysthoechas*, a narrowly distributed endemic medicinal plant, the examination of the factors that affect the germination and dormancy of this plant is especially needed.

The main objectives of this study on *S. dorystoechas* seeds were to determine the basic morphological traits of the seeds and to explore the dormancy phenomenon and the pretreatment requirements for germination.

2. Metarial and method

Two seed sources used in the study:

(1) The seeds were collected on 06.08.2021 and 12.09.2021 from a natural population in Antalya - Feslikan Plateau, 1880-1900 m. The seed collection site is in the transition from the upper forest limit to the alpine zone, with very rare *Juniperus foetidissima* and other semi-woody species such as *Origanum minutiflorum* and *Dahpne oleoides* subsp. *oleoides*. The seed collection area has east and southeast aspects where the bedrock is limestone.

(2) Additional seeds were collected on 09.08.2024 from three-year-old *Salvia dorystoechas* plants of Feslikan origin, cultivated at the Eskişehir Forest Nursery (Figure 3).

Figure 3. The seeds and spikes of *Salvia dorystoechas*

2.1. Morphological characteristics

The dimensions of the seed (length, width and thickness) were measured using 30 seeds with an electronic caliper with a sensitivity of 0.01 mm. 1000-seed weight was calculated based on 800 (8×100) seeds according to ISTA rules [15].

2.2. Dormancy level and pretreatment requirements

The preliminary germination tests with the few seeds demonstrated that *S. dorystoechas* seeds have partial physiological dormancy. Therefore, to determine the presence of dormancy, dormancy depth, and pretreatment requirements for germination, the seeds from natural population and the forest nursery were pretreated with four different pretreatment regimes including control. The prechilling treatments were applied at 4°C for 2, 4, and 6 weeks. During prechilling treatments at 4°C, the seeds were placed on moist double-layered filter paper in petri dishes. To maintain adequate moisture levels throughout the pretreatment period, distilled water was sprayed as needed.

2.3. Germination tests

The germination tests were performed on double-layered filter papers that were placed in 12-cm diameter petri dishes with 300 (3 dishes × 100 seeds) seeds at 20°C. 20°C is the recommended germination temperature for *Salvia* seeds (Thanos and Doucci, 1995). The seeds were considered germinated when their radicles protruded approximately 3 mm and displayed geotropism (Figure 4). The petri dishes were examined every two days, and the germinated seeds were counted and removed. The germination tests were terminated after 28 days.

Figure 4. Some germinated seeds of *S. dorythoechas* on filter paper

The germination percentage of seeds (GP) and the mean germination time (MGT) were calculated using the formulas below [16].

$$GP(\%) = \frac{\sum n_i}{N} x100 \quad (1) \qquad MGT = \frac{\sum (t_i . n_i)}{\sum n_i} \quad (2)$$

1. Where GP (%) is the percentage of seeds that germinated, ni is the number of germinated seeds at week i, and N is the total number of incubated seeds per test.
2. Where MGT is the mean germination time, t_i is the number of weeks since the beginning of the test, and n_i is the number of germinated seeds recorded for week t_i.

2.4. Statistical analyses

The data for GP and MGT were evaluated by analysis of variance (ANOVA). The treatment means were tested using Duncan multiple-range test. Arcsine square root (√P) transformations were applied to the GP values to normalize the error distributions prior to the variance analyses.

3. Results

3.1. Morphological characteristics of the seeds

The seeds were slightly concave in shape (Figure 5). The seed was thin, its thickness was less than half of its width. The average 1000-seed weight was 0.586 g.

The average length and width, and thickness of the seeds were 4.29±0.366 mm, 1.57±0.134 mm, and 0.67±0.081 mm, respectively.

Figure 5. The shape and dimensions (mm) of *Salvia dorythoechas* seeds

3.2. Germination features

The results showed a significant effect of seed source and prechilling on the germination percentage (GP) (Table 1). On the other hand, mean germination time (MGT) had a similar appearance in both seed sources and only affected by prechilling treatment. A seed source × prechilling interaction was significant for seed germination (p=0.018) but not effective on MGT.

The seeds in the control treatment (without any pretreatment) germinated at more than half of the maximum germination percentage it could achieve. The average germination percentages of NP seeds and FNG seeds after 2 weeks of prechilling were 89.0% and 56.3%, respectively (Table 2). Two weeks of prechilling eliminated dormancy completely in both seed sources, and the seeds reached the highest germination ratio under these conditions. With longer stratification processes of 4 and 6 weeks, germination accelerated while the germination percentages were the similar (Figure 6).

Table 1. Effects of seed source and prechlling duration on germination percentage (GP) and mean germination time (MGT) of *S. dorystoechas* seeds, results of factorial ANOVA

Source of variation	df	GP			MGT		
		MS	F	P-value	MS	F	P-value
Seed source (SS)	1	2730.66	994.02	0.000	0.753	1.407	0.253
Prechilling (P)	3	640.29	233.08	0.000	113.231	211.663	0.000
SS * P	3	12.33	4.48	0.018	0.198	0.370	0.775
Error	16	2.75			0.535		

Table 2. Germination percentage (GP) and mean germination time (MGT) of *S. dorythoechas* seeds collected from natural population and the forest nursery after the different prechilling durations

Prechilling	**Natural Population**		**Forest Nursery Grown**	
	GP (%)	**MGT (d)**	**GP (%)**	**MGT (d)**
0 week (Control)	62.3 b*	13.31 d	20.7 b	12.66 c
2 week	89.0 a	5.98 c	56.3 a	5.31 b
4 week	88.3 a	4.63 b	60.3 a	4.48 ab
6 week	88.3 a	3.28 a	60.3 a	3.34 a

* The values on the same line column followed by the same letters are not significantly different at $p < 0.05$.

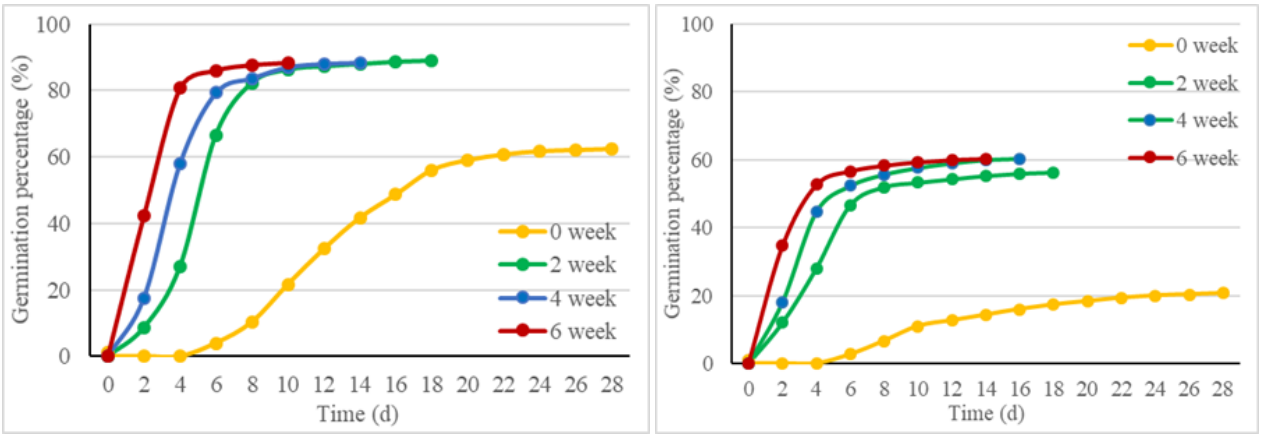

Figure 6. The germination percentages of *S. dorystoechas* seeds collected from the natural population (A, left) and the forest nursery (B, right) after the different prechilling durations

4. Conclusions and discussion

Salvia dorythoechas is an endemic plant with limited distribution in southern Türkiye. Natural populations are under anthropogenic effects that threaten their existence in some places. The seed characteristics of the species has not been sufficiently known and this research has revealed them in detail, which will contribute to its propagation and ex situ conservation.

Seeds of *Salvia* species come in many different sizes and weights. The 1000-seed weight of *S. dorythoechas* is lighter than most of the seed weights of *Salvia* species [17]. The slightly concave seed is thin and small with a length of about three times its width. The1000-seed weight of the species (0.586 g) is quite light compared to the seeds of some natural *Salvia* species in Türkiye such as *S. aramiensis* (8.19 g). Some *Salvia* species contain chemical mucilage in their seeds that prevents germination [18]. Physical dormancy has also been observed in the seeds of *S. mirzayanii* [19] and *Salvia verticillata* [9]. There is no physical dormancy that restricts water and air passage in *S. dorysthoechas* seeds. Current research demonstrated that *S. dorysthoechas* has only a physiological dormancy at a slight depth.

Physiological dormancy is generally eliminated by cold treatment at 4 °C. Two weeks of prechilling (cold treatment) were sufficient to eliminate the dormancy of the *S. dorysthoechas* seeds. Germination accelerated with the extension of the cold treatment. In line with this, about 7 weeks prechilling treatment positively affected the germination percentage in the seeds of *S. smyrnaea* [20]. Similarly, 10-30 d cold stratification was effective in breaking dormancy in the seeds of *S. aramiensis* [21]. On the other hand, seeds of *S. cyanescens* [22], *S. officinalis*, *S. coccinea*, and *S. farinacea* [11] have no dormancy and are capable of achieving maximum germination without any pretreatment.

The seeds obtained from the forest nursery have apparently lower germination ability than those collected from the natural populations (Table 2). This is probably due to the relatively higher proportion of empty seeds in NGS sources, because after 2 weeks, extending the prechilling period did not lead to an increase in the germination percentage. In other words, most of the nursery grown seeds that did not germinate after the prechilling treatment were empty.

The optimum germination temperature of *Salvia* seeds ranges from 10°C to 30°C [23]. For example, *S. fruticosa* and *S. pomifera* ssp. pomifera seeds showed higher germination percentage at lower temperatures of 10 and 15°C [24]. *S. sclarea* seeds showed better germination performance between 15 and 25 °C [25]. Since the natural distribution of *S. dorystoechas* is found in a wide range from sea level to 2000 m, the germination behavior of seeds from populations at different altitudes should be examined at different temperatures.

For *S. dorysthoechas* production in the nursery, it will be sufficient to sow the seeds at the beginning of March without any stratification. Alternatively, seeds may be sown at the beginning of April after stratification for 2 or 4 weeks under Mediterranean climatic conditions at low to mid altitudes.

Salvia seeds can be stored alive for a long time in cold conditions [17]. Similarly, the dry seeds stored in the refrigerator for three years showed very high germination (Table 2). As with other species whose seeds have remained viable for decades, the seeds of this narrowly distributed endemic species should be stored in seed banks for long-term conservation purposes.

It is an ornamental plant was high value with its thanks to strong fragrance and attractive appearance [2]. The species can be grown in suitable places and used as an ornamental plant. The cultivation of this medicinal and aromatic plant by the private sector for commertial purposes should also be considered and carried out for commercial purposes.

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Ethical statement: This study does not require ethical approval.

Author contributions: All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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