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Factors Affecting Germination Performance of Four Endemic *Sideritis* Species in Turkey

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ABSTRACT

The genus *Sideritis* is an indigenous plant to temperate and subtropical regions of Turkey, especially for Mediterranean and Aegean region, and is extensively used as herbal tea and spice. The seeds of *Sideritis* species have a difficulty in germination, which limits their cultivation. This study was conducted to evaluate the germination performance of 4 endemic *Sideritis* species (*Sideritis condensata*, *S. libanotica ssp. linearis*, *S. leptoclada* and *S. tmolea*) harvested freshly or stored for 1 and 2 years in terms of germination percentage and mean germination time and to determine the suitability of seed treatments (Hydration and gibberellic acid (GA_3) application, and their combination with pre-chilling) for overcoming germination difficulty in the species. Germination percentages of these species ranged from 28.5% to 77.0%. The lowest germination rate and the highest mean germination time (11.9 day) were determined in *S. libanotica ssp. linearis* in fresh seeds. Improved germination and shortened mean germination time were achieved using 200 mg L^{-1} GA_3 application. Seed storage did not influence the germination of the *Sideritis* species. It was found that pre-chilling did not prerequisite for germination of the species. It was concluded that GA_3 treatment should effectively be used as a method for improving germination of endemic *Sideritis* species regardless of seed age.

Keywords: Dormancy; Hydration; Gibberellic acid; Pre-chilling; Storage; Germination

Türkiye'nin Dört Endemik *Sideritis* Türünde Çimlenme Performansını Etkileyen Faktörler

ESER BİLGİSİ

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ÖZET

Sideritis cinsi özellikle Akdeniz ve Ege Bölgesi gibi Türkiye'nin ılıman ve subtropik bölgelerinde bulunan ve yaygın olarak çay ve baharat olarak kullanılan bitkilerdir. *Sideritis* türlerinin tohumlarında çimlenme problemleri görülmekte ve

bu durum tarımını sınırlandırmaktadır. Bu çalışma, taze, 1 ve 2 yıl depolanmış 4 endemik *Sideritis* türündeki (*Sideritis condensata*, *S. libanotica ssp. linearis*, *S. leptoclada* and *S. tmolea*) çimlenme problemlerini gidermek amacıyla tohum uygulamalarının (hidrasyon, gibberellik asit (GA_3) ve ön üşütme kombinasyonları) etkinliğini belirlemek amacıyla çimlenme yüzdesi ve ortalama çimlenme süreleri değerlendirilerek yürütülmüştür. Türlerin çimlenme yüzdesi % 28.5-% 77.0 arasında değişim göstermiştir. En düşük çimlenme oranı ve en yüksek ortalama çimlenme süresi (11.9 gün) *S. libanotica ssp. linearis* türünün taze tohumlarında belirlenmiştir. Çimlenme yüzdesinde artış ve ortalama çimlenme süresinde kısalma 200 mg L^{-1} GA_3 uygulamasından elde edilmiştir. Depolama süresi *Sideritis* türlerinin çimlenmesini etkilememiştir. Ön üşütme ise incelenen türlerin tohumlarının çimlenmesi için gerekli bulunmamıştır. Sonuç olarak, tohum yaşına bağlı olmaksızın, GA_3 uygulamasının endemik *Sideritis* türlerinin çimlenmesini artırmada etkili bir şekilde kullanılabilecek bir yöntem olduğu belirlenmiştir.

Anahtar Kelimeler: Dormansi; Hidrasyon; Gibberelik asit; Ön üşütme; Depolama; Çimlenme

1. Introduction

There are approximately 12,000 plant species on the flora of Turkey, about 3,750 of them are endemic (Baydar 2013). The number of plants used for medicinal purposes are 1,000-2,000 (Arslan et al 2002). Turkey is a gene center for Lamiaceae family, which comprises a lot of aromatic plants. The family includes 546 species, 45 genus and totally 731 taxa with the endemic plant ratio of 44.2% (Ertan et al 2000; Arslan et al 2002; Gümüşçü 2014).

The genus *Sideritis*, a member of the Lamiaceae family, has more than 150 species which are distributed in temperate and tropical regions of the Northern Hemisphere (Tomas-Barberan et al 1988). 46 species and 53 taxa of genus *Sideritis* have existed and 40 of *Sideritis* taxa are endemic in Turkey (Davis 1982). The genus *Sideritis* is naturally found in the western Anatolia. The species are annual or perennial, herbaceous or little bushy plants in the habitat. *Sideritis* species are often utilized in folk medicine in Turkey and Europe for their anti-inflammatory, anti-rheumatic, digestive and antimicrobial properties (Kılıç 2006). They have been traditionally consumed as herbal tea, known in Turkey as mountain tea, especially in Mediterranean and Aegean regions (Uçar & Turgut 2009). Furthermore, essential oils obtained from *Sideritis* species have been used for tonic, carminative, antispasmodic, diuretic.

They have a great export potential for medicinal, aromatic and ornamental purposes while their cultivation has been limited due to low germination performance and slow seedling growth. Some reports on germination of *Sideritis* species showed that the germination performance was varied with temperature, light, seed treatment and species (Evstatieva & Popova 1998; Esterelles et al 2010; Kadis et al 2010; Yankova-Tsvetkova et al 2013). They reported controversial results about germination behavior of different *Sideritis* species and have not developed a standard method. The objective of the present study was to determine the appropriate combination of seed treatments i.e. hydration, gibberellic acid (GA_3) and pre-chilling to improve germination performance of some endemic *Sideritis* species. Also, it was determined if their seeds required for after-ripening by using different seeds stored for 1 and 2 years.

2. Material and Methods

This study was carried out at the Department of Field Crops, Faculty of Agriculture, Eskişehir Osmangazi University, Turkey. The seeds of 4 *Sideritis* species (*S. leptoclada*, *S. condensata*, *S. libanotica ssp. linearis* and *S. tmolea*) were collected from natural pastures in Dalaman-Muğla, Akseki-Antalya, Bozkır-Konya and Tire-İzmir respectively, between 2009 and 2010. The seeds were planted in seedbed under uncontrolled greenhouse and the seedlings were transplanted to the experimental gardens of the division of Medicinal and Aromatic Plants in Çumra Vocational High School of Selçuk University in order to produce sufficient seed material under the

same conditions. Plant density was arranged as 70×50 cm and each plot was consisted of five rows with 25-30 plants. The seeds from each species were collected from 15 plants in 2011 (2 years storage), 2012 (1 year storage) and 2013 (used as newly harvested seeds) years and stored in paper bags in a seed storage room (temperature: 15-25 °C; humidity: 40%-60%) until the start of experiment.

Seeds from each species harvested at different years (fresh, 1 and 2 years storage) were used as control (T_c). Hydration was performed by soaking seeds into distilled water for 6 h (T_1); 4 days pre-chilling between moistened filter papers at 4±1 °C after hydration (T_2); soaking in 200 mg L^{-1} gibberellic acid (GA_3) solution for 6 h (T_3) as described by Gümüşçü (2014); 4 days pre-chilling between moistened filter papers at 4±1 °C after T_3 treatment (T_4), and 4 days pre-chilling at 4±1 °C followed by germination between filter papers moistened with 200 mg L^{-1} GA_3 solution (T_5). The treated seeds with GA_3 were thoroughly rinsed with distilled water three times. After all of the treatments, the seeds were surface-dried and dried back to their original moisture content at room temperature (about 22 °C, 45% relative humidity) determined by changes in seed weight.

Four replicates of 50 seeds from each treatment were germinated in 3 moistened, rolled filter papers using 8 mL of distilled water. Before the germination test, seeds from each species were treated with Thiram (80%) against fungal contamination. Each rolled paper was then put into a sealed plastic bag to prevent evaporation. Seeds were germinated at 20±1 °C (Estrelles et al 2010) in the dark for 14 days. A seed was considered germinated when the emerged radicle was visible. Germinated seeds were counted daily for 14 days. The germination percentage and the mean germination time (MGT) of the seeds from each species were determined at the end of the germination test (ISTA 2003). MGT= $\Sigma(Dn)/\Sigma n$, where, n is the number of seeds which germinate on day D and D is the number of days counted from the beginning of germination test.

The experimental design was two factors arranged in a completely randomized design with 4 replications. Data given in percentages were subjected to arcsin transformation before statistical analysis. Analysis of variance was performed for all investigated parameters using SPSS 16. Significant differences among mean values were compared by Duncan's Multiple Range test ($P \leq 0.05$).

3. Results and Discussion

Germination percentage and mean germination time of *Sideritis* species in relation to seed storage were shown in Figure 1. Among the *Sideritis* species, a significant difference was found ($P \leq 0.05$) for germination percentage and mean germination time. One year storage of the species gave the lowest germination percentage except for *S. libanotica ssp. linearis*. Minimum germination was determined in fresh harvest seeds of *S. libanotica ssp. linearis*. Similarly, mean germination time varied with storage time and the species. Seeds stored one year showed higher the time to germination than that of the other years. In general, seeds harvested freshly and stored two years needed longer the time to germination.

Seed treatments and seed storage significantly influenced germination percentage and mean germination time of *S. condensata* (Table 1). Control seeds showed that the germination percentage ranged from 41.5% to 47.0%. Seed storage did not affect germination percentage of control seeds. However, germination percentage was clearly enhanced by GA_3 treatment (T_3), and it reached to maximum values with 67.5% in fresh seeds, 82.0% in one year storage and 88.0% in seeds stored for two years. Older seeds gave better response to GA_3 treatment compared to fresh seeds. Higher mean germination time was obtained from control seeds with low germination percentage. Seed treatments shortened the mean germination time and T_4 method was the most effective to decrease germination time.

Germination percentage was apparently affected by seed storage and better performance was observed in the seeds of *S. libanotica ssp. linearis* stored for 1 year (Table 2). Seed treatments enhanced germination percentage and accelerated the mean germination time. GA_3 after pre-chilling (T_5) showed the highest germination in both fresh and 1 year stored seeds while older seeds was promptly influenced by T_4. Considering seed treatments GA_3 remarkably reduced the mean germination time.

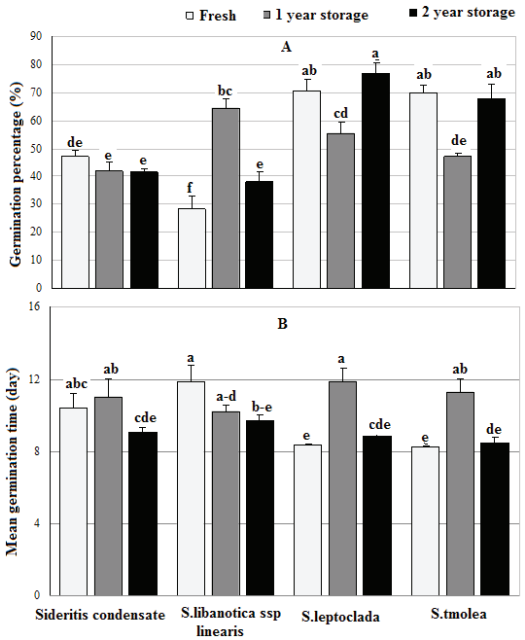

Figure 1- Changes in germination percentage (%, A) and mean germination time (day, B) of *Sideritis* species after 1 and 2 years storage

Şekil 1- Sideritis türlerinin 1 ve 2 yıl depolamadan sonraki çimlenme yüzdesi (%, A) ve ortalama çimlenme süresindeki (gün, B) değişim

Table 1- Effects of seed treatments on germination percentage (%) and mean germination time (day) of the seeds of *Sideritis condensata* harvested freshly or after storage for 1 and 2 years

Çizelge 1- Taze hasat ile 1 ve 2 yıl depolanan Sideritis condensata tohumlarının çimlenme yüzdesi (%) ve ortalama çimlenme süresi (gün) üzerine tohum uygulamalarını etkileri

Treatment	*Fresh*	*1 year storage*	*2 year storage*	*Mean*
		Germination percentage (%)		
Control (T_c)	47.0^{fg}	42.0^{g}	41.5^{g*}	43.5
Hydration (T_1)	56.0^{d-g}	56.0^{d-g}	60.0^{def}	57.3
Hydration + pre-chilling (T_2)	62.0^{de}	68.0^{cd}	51.0^{efg}	60.3
200 mg L^{-1} GA_3 (T_3)	67.5^{cd}	82.0^{ab}	88.0^{a}	79.2
200 mg L^{-1} GA_3 + pre-chilling (T_4)	62.0^{de}	68.0^{cd}	86.0^{ab}	72.0
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	49.0^{efg}	76.5^{bc}	75.5^{bc}	67.0
Mean	57.3	65.4	67.0	
		Mean germination time (day)		
Control (T_c)	10.42^{a}	10.98^{a}	9.07^{b}	10.16
Hydration (T_1)	5.50^{def}	6.21^{d}	7.65^{c}	6.45
Hydration + pre-chilling (T_2)	4.90^{d-h}	5.65^{def}	5.75^{de}	5.43
200 mg L^{-1} GA_3 (T_3)	5.50^{def}	5.44^{def}	5.97^{de}	5.64
200 mg L^{-1} GA_3 + pre-chilling (T_4)	3.70^{h}	3.91^{gh}	5.25^{d-g}	4.29
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	4.55^{e-h}	4.57^{e-h}	4.19^{fgh}	4.44
Mean	5.76	6.13	6.31	

*, values show the real germination percentages but variance analysis was performed using arcsin transformed values. Means followed by the same letter(s) are not significantly different at P≤0.05

Table 2- Effects of seed treatments on germination percentage (%) and mean germination time (day) of the seeds of *S. libanotica ssp. linearis* harvested freshly or after storage for 1 and 2 years

Çizelge 2- Taze hasat ile 1 ve 2 yıl depolanan S. libanotica ssp. linearis tohumlarının çimlenme yüzdesi (%) ve ortalama çimlenme süresi (gün) üzerine tohum uygulamalarını etkileri

Treatment	*Fresh*	*1 year storage*	*2 year storage*	*Mean*
		Germination percentage (%)		
Control (T_c)	28.5^{g}	64.5^{c}	38.0^{fg}*	43.7
Hydration (T_1)	45.0^{def}	69.0^{bc}	30.0^{fg}	48.0
Hydration + pre-chilling (T_2)	42.0^{efg}	65.0^{c}	34.0^{fg}	47.0
200 mg L^{-1} GA_3 (T_3)	65.0^{c}	82.0^{ab}	57.0^{cde}	68.0
200 mg L^{-1} GA_3 + pre-chilling (T_4)	63.0^{c}	68.0^{bc}	59.0^{cd}	63.3
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	80.5^{ab}	83.5^{a}	55.0^{cde}	73.0
Mean	54.0	72.0	45.5	
		Mean germination time (day)		
Control (T_c)	11.86^{a}	10.20^{b}	9.72^{bc}	10.59
Hydration (T_1)	7.22^{de}	7.19^{de}	8.44^{cd}	7.62
Hydration + pre-chilling (T_2)	5.32^{fg}	6.25^{ef}	7.92^{d}	6.50
200 mg L^{-1} GA_3 (T_3)	5.13^{fgh}	5.41^{fg}	5.25^{fgh}	5.26
200 mg L^{-1} GA_3 + pre-chilling (T_4)	3.72^{gh}	3.85^{gh}	3.85^{gh}	3.81
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	3.76^{gh}	4.68^{gh}	4.06^{gh}	4.17
Mean	6.17	6.26	6.54	

*, values show the real germination percentages but variance analysis was performed using arcsin transformed values. Means followed by the same letter(s) are not significantly different at $P \leq 0.05$

Germination characteristics of *S. leptoclada* in relation to seed treatments and storage are shown in Table 3. Germination percentage of control seeds ranged from 55.0% to 77.0% and the minimum value was detected in 1 year stored seeds. It was improved by seed treatments and the application of 200 mg L^{-1} GA_3 + pre-chilling was found as the predominant method in fresh seeds of *S. leptoclada*. One year stored seeds surprisingly gave lower germination percentage than fresh and two years storage. No seed treatments manage to improve the germination percentage of seeds stored for 1 and 2 years. Moreover, they were disabled for decreasing the mean germination time of seeds stored for 2 years. Among the seed treatments, the shortest mean germination time (3.36 day) was recorded in 200 mg L^{-1} GA_3 followed by pre-chilling.

Control seeds of *S. tmolea* showed that one year storage gave the lowest germination percentage with 47.0%. The application of 200 mg L^{-1} GA_3 significantly enhanced the germination percentage of *S. tmolea* seeds. The higher germination percentage was determined in fresh seeds compared to seed storages. Seed treatments led to decrease the mean time to germination but, the pre-chilling followed by 200 mg L^{-1} GA_3 was found better than the other methods.

Germination test showed that viability of the seeds of *Sideritis* species stored for one and two years was different. The least average germination percentages were recorded in *S. condensata* with 43.5% and *S. libanotica ssp. linearis* with 43.7%. In addition, a clear significant difference was observed in seeds of *S. libanotica ssp. linearis*, *S. leptoclada* and *S. tmolea* stored for 1 year.

Table 3- Effects of seed treatments on germination percentage (%) and mean germination time (day) of the seeds of *Sideritis leptoclada* harvested freshly or after storage for 1 and 2 years

Çizelge 3- Taze hasat ile 1 ve 2 yıl depolanan Sideritis leptoclada tohumlarının çimlenme yüzdesi (%) ve ortalama çimlenme süresi (gün) üzerine tohum uygulamalarını etkileri

Treatment	*Fresh*	*1 year storage*	*2 year storage*	*Mean*
		Germination percentage (%)		
Control (T_c)	70.5^{b-e}	55.5^{fgh}	77.0^{abc}*	67.7
Hydration (T_1)	75.0^{a-d}	52.0^{ghi}	70.0^{b-e}	65.7
Hydration + pre-chilling (T_2)	61.0^{efg}	41.0^{i}	$64.0d^{-g}$	55.3
200 mg L^{-1} GA_3 (T_3)	81.0^{ab}	48.0^{hi}	73.0^{b-e}	67.3
200 mg L^{-1} GA_3 + pre-chilling (T_4)	84.0^{a}	47.0^{hi}	65.0^{c-f}	65.3
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	53.0^{f-j}	46.0^{hi}	76.5^{abc}	58.5
Mean	70.8	48.3	70.9	
		Mean germination time (day)		
Control (T_c)	8.38^{bc}	11.89^{a}	8.87^{b}	9.71
Hydration (T_1)	4.63^{ghi}	8.19^{bc}	7.98^{bcd}	6.93
Hydration + pre-chilling (T_2)	4.06^{hi}	6.32^{ef}	7.78^{bcd}	6.05
200 mg L^{-1} GA_3 (T_3)	4.76^{gh}	6.79^{def}	6.66^{def}	6.07
200 mg L^{-1} GA_3 + pre-chilling (T_4)	3.36^{i}	8.00^{bcd}	7.83^{bcd}	6.40
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	3.83^{hi}	7.30^{cde}	5.72^{fg}	5.62
Mean	4.84	8.08	7.47	

*, values show the real germination percentages but variance analysis was performed using arcsin transformed values. Means followed by the same letter(s) are not significantly different at $P \leq 0.05$

Any significant increase or decrease trend was not detected as storage time was extended. This shows that the seeds of *Sideritis* species did not require ripening after harvest. Generally, the seeds harvested in 2012 gave lower germination and delayed germination time; demonstrating that the seed quality of *Sideritis* species might be affected adversely by the climatic conditions like rainfall and temperature during flowering along with seed development stage.

In general, all the seed treatments increased germination percentage and shortened the time to seed germination compared to that of control (Table 4). However, it was found that GA_3 considerably improved germination performance in the seeds of *S. condensata* and *S. libanotica ssp. linearis*. The effectiveness of GA_3 on germination or decreasing of seed dormancy has been reported by Gümüşçü (2014) in *Sideritis* species, El-Dengawy (2005) in loquat, Dissanayake et al (2010) in guayule and Nadeem et al (2012) in *Ochradenus arabicus*, İpek et al (2008) in cumin. The seeds of *S. condensata* and *S. libanotica ssp. linearis* do not germinate sufficiently, while the seeds of *S. leptoclada* and *S. tmolea* exhibit very slow germination confirming the existence of dormancy. The reason of dormancy may be due to the presence of some inhibitors, low internal hormone or undeveloped embryos. Khan & Ungar (1997) and Dissanayake et al (2010) reported that the application of gibberellic acid in declining innate and environment-induced dormancy by means of reducing inhibitors and increasing promoter level. GA_3 induces enhanced cell wall plasticity which cause converting the starches to simple sugars. The sugars lead to absorb high amount of water, which

Table 4- Effects of seed treatments on germination percentage (%) and mean germination time (day) of the seeds of *Sideritis tmolea* harvested freshly or after storage for 1 and 2 years

Çizelge 4- Taze hasat ile 1 ve 2 yıl depolanan Sideritis tmolea tohumlarının çimlenme yüzdesi (%) ve ortalama çimlenme süresi (gün) üzerine tohum uygulamalarının etkileri

Treatment	*Fresh*	*1 year storage*	*2 year storage*	*Mean*
		Germination percentage (%)		
Control (T_c)	70.0^{b-e}	47.0^{g}	68.0^{b-e}*	61.7
Hydration (T_1)	73.0^{bcd}	55.0^{efg}	61.0^{c-g}	63.0
Hydration + pre-chilling (T_2)	70.0^{b-e}	54.0^{efg}	57.0^{efg}	60.3
200 mg L^{-1} GA_3 (T_3)	92.8^{a}	51.0^{fg}	78.0^{b}	73.9
200 mg L^{-1} GA_3 + pre-chilling (T_4)	79.0^{b}	58.0^{d-g}	77.0^{b}	71.3
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	78.0^{bc}	66.0^{b-f}	78.0^{b}	74.0
Mean	77.1	55.2	69.8	
		Mean germination time (day)		
Control (T_c)	8.25^{b}	11.25^{a}	8.51^{b}	9.34
Hydration (T_1)	3.36^{g}	7.12^{c}	5.23^{de}	5.24
Hydration + pre-chilling (T_2)	3.39^{g}	6.23^{cd}	4.82^{ef}	4.81
200 mg L^{-1} GA_3 (T_3)	6.06^{cd}	6.32^{cd}	4.59^{ef}	5.66
200 mg L^{-1} GA_3 + pre-chilling (T_4)	4.07^{efg}	4.82^{ef}	3.75^{fg}	4.21
Pre-chilling + 200 mg L^{-1} GA_3 (T_5)	3.18^{g}	4.88^{ef}	3.74^{fg}	3.93
Mean	4.72	6.77	5.10	

*, values show the real germination percentages but variance analysis was performed using arcsin transformed values. Means followed by the same letter(s) are not significantly different at $P \leq 0.05$

result in a decrease in cell water potential, finally end up cell elongation and growth (Arteca 1996; Nadeem et al 2012).

The superiority of pre-chilling on germination of *Sideritis* species was not found in the study. However, the beneficial effects of pre-chilling were reported by Yılmaz & Tonguç (2013) in *Fraxinus ornus* subsp. *cilicica*, Ali et al (2010) in *Descurainia sophia* and *Plantago ovata* and Szewski & Folin (2009) in big bluestem (*Andropogon gerardii* Vitman). Its efficiency on germination of *Sideritis* was apparently promoted by GA_3 treatment rather than hydration. Our results are in agreement with Watkinson & Pill (1998) who observed that pre-chilling with GA_3 gave higher increase in germination of Indiangrass. Furthermore, Keshtkar et al (2009) found that the highest germination was obtained when the seeds of *Ferula assa-foetida* were treated with 250 mg L^{-1} GA_3 and pre-chilling.

4. Conclusions

The beneficial effects of gibberellic acid on germination of the investigated endemic *Sideritis* species were found. All the species germinated higher than 60% when either GA_3 treatment alone or together with pre-chilling were applied. It was concluded that the seeds of *Sideritis* species should be germinated after they were treated with 200 mg L^{-1} GA_3 for 6 h.

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