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Priming Effects on Germination Parameters of Vulnerable Salvia aramiensis Rech. f. Harvested from Different Locations

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ABSTRACT: Salvia aramiensis Rech. f. *is a perennial sub-shrub from the Lamiaceae family that is locally endemic in the Hatay province of Türkiye. The study aimed to evaluate the location and priming effects on germination parameters of* ***S. aramiensis*** *seeds. Six districts of Hatay (Antakya, Arsuz, Belen, Dörtyol, Samandağ, and Yayladağı) were surveyed and seeds were collected separately during summer 2020. Seed moisture (%) and thousand seed weight (TSW-g) were determined. A total of 33 priming applications were applied and control groups (no treatment) were included, however, only ten of them showed germination. Germination rate (%), mean germination time (MGT), germination index (GI), and T50 were determined. Locations and priming were found to be statistically significant ($p<0.05$) on germination parameters. The seed moistures ranged between 5.76 and 7.87% and the TSW seed varied between 5.77 and 10.05g. The highest germination rate was observed in Belen district seed samples as 65.50% with 30 d cold stratification/2000 ppm GA_3 treatment combination. MGT ranged between 2.87 and 7.55 days and the highest result was obtained from Antakya district. GI results ranged between 0.42 and 11.30 and T50 results ranged between 1.82 and 6.94 days. In the present study, Belen location showed importance according to the studied parameters.*

Keywords: *Germination, sage, dormancy, stratification, hormone, seed priming.*

Farklı Lokasyonlardan Toplanan Salvia aramiensis Rech. f. Tohumları Üzerine Önişlem Uygulamalarının Etkisi

ÖZ: Salvia aramiensis Rech. f. *Türkiye'nin Hatay ilinde yerel endemik olan Lamiaceae familyasına ait çok yıllık bir yarı çalıdır. Çalışmada* ***S. aramiensis*** *tohumlarının çimlenme parametreleri üzerine lokasyon ve priming etkilerinin değerlendirilmesi amaçlanmıştır. Hatay'ın altı ilçesinde (Antakya, Arsuz, Belen, Dörtyol, Samandağ ve Yayladağı) araştırma yapılmış ve 2020 yazında ayrı ayrı tohumlar toplanmıştır. Tohum nemi (%) ve bin tane ağırlığı (TSW-g) belirlenmiştir. Çalışma kapsamında 33 adet önişlem tohumlara uygulanmış ancak sadece 10 önişlem uygulamasında çimlenme tespit edilmiştir. Çalışmada çimlenme oranı (%), ortalama çimlenme süresi (MGT), çimlenme indeksi (GI) ve T50 belirlenmiştir. Çimlenme parametreleri üzerine lokasyon ve priminglerin istatistiksel olarak önemli ($p<0,05$) olduğu belirlenmiştir. Tohum nemleri %5,76-7,87 aralığında, TSW ise 5,77-10,05 g arasında değişmiştir. En yüksek çimlenme oranı %65,50 ile Belen ilçesi tohum örneklerinde 30d soğuk katlama/2000ppm GA_3 uygulaması kombinasyonunda gözlenmiştir. MGT 2,87-7,71 gün arasında değişmekte olup en yüksek sonuç Antakya ilçesinden elde edilmiştir. GI sonuçları 0,42-11,30, T50 sonuçları ise 1,82-6,94 gün arasında değişmiştir. Bu çalışmada Belen lokasyonu incelenen parametrelere göre önem göstermiştir.*

Anahtar kelimeler: *Çimlenme, adaçayı, dormansi, katlama, hormon, önişlem.*

INTRODUCTION

The Lamiaceae family contains the genus *Salvia* with approximately 1000 species, many of which have medicinal and economical value. In the Flora of Turkey, *Salvia* is presented with 99 species including 58 endemic species (Guner *et al*., 2012). *Salvia aramiensis* Rech. f. is one of the 24 *Salvia* species from Hatay province of Türkiye (Bahadirli and Ayanoglu, 2021). *S. aramiensis*, also known as "Hatay sage", is a local endemic sub-shrub perennial, that grows between 250 and1000 m of Amanos Mountains, and has been used as an herbal product and incense (Turkish= pohur) in the Eastern Mediterranean region of Türkiye. According to the Flora of Turkey (Davis, 1982), the species exists in Türkiye, Lebanon and Syria yet neither Lebanon nor Syria published any scientific paper about it. Celep *et al.* (2010) suggested the IUCN threat category for the national level of the *S. aramiensis* should be changed to VU (vulnerable) due to the threats of construction, land clearing and fire. That study was more than a decade ago and since then more natural damage has occurred. Also, field trips showed that unconscious and careless collecting of populations prevented flowering during the summer season (Figure 1). This situation also makes it necessary to start cultivation.

Figure 1. A flowerless plant showing the effects of over harvested from Yayladağı district (a), and un-harvested plant at the same time from Belen district (b).

Şekil 1. Aşırı hasat edilen çiçeklenme aşamasına geçememiş Yayladağı lokasyonundan bir bitki (a) ve aynı zamanda fotoğrafı çekilmiş hasat edilmemiş Belen lokasyonundan bir bitki (b).

Studies showed that there is a great variation in essential oil content and components of *S. aramiensis* (Demirci *et al.*, 2002; Karaman *et al.*, 2007; Ayanoglu *et al.*, 2012; Bahadirli and Ayanoglu, 2019). *S. aramiensis* has importance as an herbal tea regarding its high 1,8-cineole and low camphor and thujone contents (Bahadirli, 2020). The biological activities of the species have not yet been investigated thoroughly, yet anti-Alzheimer and antioxidant activity were studied (Ertas *et al.*, 2017; Alim *et al.*, 2018; Bahadirli, 2022). Infused tea from *S. aramiensis* leaves has been used for cough, cold, flu, and stomach pain among local people (Guzel and Guzelsemme, 2018). Cultivation of *S. aramiensis* at different climates was also studied and Mediterranean climate were recommended (Yenikalayci, 2022).

Many medicinal plants are traded by collecting them from the natural flora. In order to meet the increasing demands for these plants and to protect the natural flora, intensively collected plants must be cultivated. At the same time, the germination rate of many medicinal and aromatic plants from nature is a problem. The seed coat, unripe seeds, and dormancy are the main factors affecting seed germination success even in viable seeds (Ellis *et al.*, 1985a). Priming applications such as removing seed coats, hormonal priming, stratification, hydro priming, and chemical priming, are some useful methods depending on the species to obtain a higher germination rate, time, and quality. In one study, Thanos (1993) stated that 49% of *Salvia fruticosa* seeds were empty. Many studies focus on different *Salvia* species' germination behaviour. Effects of storage temperatures on *S. officinalis* (Kretschmer, 1989), effects of different light regimes (Liu *et al.* 2006), and hydro priming (Dastanpoor *et al.*, 2013) were investigated in previous studies. Research of germination applications such as light, chemicals, and priming on *S. officinalis*, *S. fruticosa*, *S. pomifera*, and *S. tomentosa* showed different germination behaviours even in the same genus and same Clade (Ozcan *et al.*, 2014). Local environmental conditions that affect seeds until maturation could have a strong impact on germination behaviour (Lacey and Herr, 2000).

Preliminary studies with 10-day cold stratification and hydro priming for 24 hours on *S. aramiensis* seeds gave low germination rates, and these results revealed the necessity of searching for solutions to germination problems. Different locations and priming applications for instance hormones, hydro priming, chemical priming, stratification, mechanical abrasion, hormones, and stratification combinations were investigated to determine their effects on *Salvia aramiensis* seed germination.

MATERIALS AND METHODS

Seed source, study area, and seed collection

Salvia aramiensis is a sub-shrub perennial plant that grows at altitudes between 250 and 1000 m (Davis, 1982). Plant height is up to 1 meter, petals are lilac to pink and leaves are thin, grey-green. Pollination is done by insects and type is cross-pollination (Claßen-Bockhoff *et al.*, 2004). *Salvia* species including *S. aramiensis* have a superior ovary that is deeply 4 lobed and the fruit is a schizocarp of four nutlets (1-3) (Ryding, 2001; Zona, 2017). The species is Eastern Mediterranean Element and grows naturally in rocky places under *Pinus brutia* (Davis, 1982).

The fieldwork was carried out in the Amanos Mountains, which are approximately 180 kilometres long, and mostly located in the province of Hatay. The Amanos Mountains have a unique plant diversity with the vegetation belonging to the Black Sea climate zone as well as the classical Mediterranean climate. Seed materials were collected from different populations in Antakya, Arsuz, Belen, Dörtyol, Samandağ and Yayladağı districts of Hatay province, Türkiye (Figure 2)

The climate is mostly characterized as the rainy Mediterranean. Annual rainfall, mean temperature, mean of the hottest month, and mean of the coldest month of the districts between 2019 July- 2020 July are given in Table 1. Initially, from May to July of 2020, locations were checked for seed maturation, and in the middle of July, seed collections started. For each population, seeds were collected from 25-50 individuals depending on the seed quantity. The collected seeds were placed in paper bags and carried to the laboratory, then air-dried for three days at room temperature (24 °C) before being packed for further investigations.

Figure 2. Distribution map of *S. aramiensis* seed collection localities.
Şekil 2. *S. aramiensis* tohumlarının toplandığı lokasyonların dağılımını gösteren bir harita.

Table 1. Locality, geographic and meteorological descriptions of *S. aramiensis* populations.
Çizelge 1. *S. aramiensis* populasyonlarının lokalitesi, coğrafi ve meteorolojik tanımlamaları.

Population	Area	Geography			Climate			
		Latitude	Longitude	Altitude	Rn	Tm	Tmax	Tmin
Antakya	Fırnız	36°26'27"	36°16'56"	1333	706.4	18.4	43.5	-4.1
Arsuz	Gözcüler	36°21'59"	35°57'28"	483	384.1	20.0	44.0	-2.8
Belen	Kömürçukuru	36°26'07"	36°08'28"	996	556.0	16.3	41.2	-7.8
Dörtyol	Örencik	36°54'41"	36°16'6"	545	690.8	19.2	45.4	-3.2
Samandağ	Yaylıca	36°20'40"	36°8'54"	976	507.0	19.3	43.6	-1.2
Yayladağı	Dağdüzü	36°04'30"	36°05'11"	351	602.0	16.8	43.5	-7.4

*Meteorological data were obtained from Hatay Meteorology Directorate. Latitude (N), Longitude (E), Altitude (m), Rn; total annual rainfall (mm), Tm; annual mean temperature (°C), Tmax; mean of the hottest month (September) temperature (°C), Tmin; mean of the coldest month (February) temperature (°C)

Seed physiology

Seed moisture: The moisture content was determined with the method from the International Seed Testing Association (ISTA, 1985). Five grams of seeds from locations were weighed separately with three replications. The samples were held for 17±1 hours at 103±2 °C in the oven, and at the end of the duration, the seeds were left in a desiccator until constant weight was weighed. The moisture content (%) was calculated according to the formula given below.

Seed moisture content (%) = $\left[\frac{M2-M3}{M2-M1}\right] \times 100$

M1= Petri dishes weight

M2= Petri dishes weight + seed weighed

M3= Petri dishes weight + seed weighed after oven-dried

Thousand seed weight: The weight of hundred seeds with ten replications was used to calculate thousand seed weight (TSW).

Germination experiments

The experiment was conducted at the Field Crops Biotechnology Laboratory of Hatay Mustafa Kemal University in 2021. Experiment was applied according to ISTA (1985) descriptions. *S. aramiensis* seeds were sanitized with 5% NaClO (Sodium hypochlorite) for 15 minutes and washed thoroughly with sterilized water. Different treatments of hydro priming, chemical priming, stratification, pre-chill, stratification+GA_3 combination, mechanical priming and dry seeds (control) were used (Table 2). In hydropriming, seeds were soaked for the indicated time periods with designated water at the appropriate temperature. In chemical priming, seeds were soaked in prepared solutions for the appropriate period. For cold priming, seeds were wrapped in a coarse filter paper, moistened with 3 ml of water, and placed in a resealable plastic bags. No further watering was found necessary during cold stratification period. In pre-chilling, seeds were placed in a plastic petri dish, no wrapping or watering were applied. Cold stratification and GA_3 are the most advised and used methods for many ornamental and medicinal plants seeds (ISTA, 1985). Therefore they were chosen for combination. Cold stratification and GA_3 were combined with the stratification process applied first followed by GA_3 application. Abrasion of seeds was done with sandpaper. For the control, seeds were directly placed in petri dishes. At end of the treatment time, all of the seeds were dried on a filter paper and then placed in 11 cm petri dishes on top of Whatman No.1 paper. A total of 34 treatments with four replicates of 50 seeds (50x4=200) in each treatment were used. Seeds were incubated in a germination chamber with 24 h dark regime at 25±0.5 °C for 28 days. Two ml distilled water was applied in the beginning. Afterwards, watering was done when necessary. The petri dishes were wrapped with plastic bags to minimize water losses during the experiment (Caliskan *et al.*, 2012).

Table 2. Seed treatment methods and germination status.
Çizelge 2. Tohumlara uygulanan önişlemler ve çimlenme durumları.

Treat. No	Priming Method		Treatment		Germination Status
1	Hydro priming	Water (10 mL)	25 °C°	3 days	NG
2			25 °C°	7 days	NG
3			65 °C	10 minutes	NG
4			65 °C	30 minutes	NG
5			65 °C	1 hour	NG
6	Chemical priming	GA_3	500 ppm	24 hours	G
7			1000 ppm	24 hours	G
8			2000 ppm	24 hours	G
9			3000 ppm	24 hours	G
10		Ethylene	250 ppm	24 hours	NG
11			750 ppm	24 hours	NG
12		Salicylic acid	500 ppm	24 hours	NG
13			1000 ppm	24 hours	NG
14		Seaweed	2%	24 hours	NG
15			4%	24 hours	NG
16		KNO_3	0.20%	24 hours	NG
17			1%	24 hours	NG
18		$MgSO_4$	0.50%	24 hours	NG
19			1%	24 hours	NG
20	Stratification	Cold stratification	4 °C	10 days+24 hour	NG
21			4 °C	20 days+24 hour	NG
22			4 °C	30 days+24 hour	NG
23			4 °C	45 days+24 hour	NG
24	Pre-chill	Short time pre-chill	- 5°C	10 minutes	NG
25			- 5 °C	20 minutes	NG
26			- 5 °C	30 minutes	NG
27	Combination	Stratification+GA_3	1000	10 days+24 hour	G
28			2000	10 days+24 hour	G
29			3000	10 days+24 hour	G
30			1000	30 days+24 hour	G
31			2000	30 days+24 hour	G
32			3000	30 days+24 hour	G
33	Mechanical	Abrasion			NG
34	Control	Without treatment			NG

NG=Not germinated G=Germinated.

Evaluation of germination and statistical analysis

Counting of germinated seeds was done every other day and germinated seeds were removed from petri dishes. Only ten treatments resulted in germination and the control were included for further statistical analysis (Table 2).

Germination rate (%) is an estimate of the germinability of the population of seeds. The equation to calculate germination percentage is:

$$\mathrm{G}\ (\%) = \frac{\sum_{i=1}^{k} ni}{N} \times 100$$

Where n_i is the number of seeds germinated in the i^{th} time, N is the total number of seeds used.

Mean germination time is a measure of the rate and time spread of the germination. It indicates time spent germinating. The following formula was used to calculate the mean germination time (Ellis and Roberts 1980).

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{n=1}^{k} n_i}$$

Where $n_i t_i$ is the product of seeds germinated at interval i^{th} with the corresponding time interval and n_i is the number of seeds germinated in i^{th} time.

The germination index is an estimate of the days it takes a certain germination percentage to occur. The germination index can be calculated by using the following expression from Association of Official Seed Analysts (AOSA and SCST, 1993).

$$GI = \sum_{i=1}^{k} (n_i / t_i)$$

Where n_i is the number of seeds germinated in the i^{th} time and t_i is the time taken for seeds to germinate at i^{th} count.

The time to reach 50% germination (T_{50}) indicates how much time was taken for half of the seeds to germinate (Coolbear *et al.*, 1984).

$$T50 = \frac{ti + \left[\left(\sum_{i=1}^{k} ni - ni\right)x(ti - tj)\right]}{ni - nj}$$

To determine the value of n_i and n_j, the cumulative number of seeds germinated for the conditions given below should be reviewed.

$$n_i < \sum_{i=1}^{k} ni < nj$$

Where n_i is the nearest cumulative number of seeds germinated (Cni) < $\frac{\sum_{i=1}^{k} ni}{2}$, n_j is the nearest cumulative number of seeds germinated (Cnj)> $\frac{\sum_{i=1}^{k} ni}{2}$, t_i is the time interval corresponding to n_i and t_j is the time interval corresponding to n_j.

The experiment was conducted based on repetitive random plots with four replicates. All measured variables were subjected to factorial analysis of variance (ANOVA) to test for the significant effect between the factors using with MSTAT-C. Factor 1 was the location and factor 2 was priming applications. The pattern of difference between means was analysed using Fishers' protected least significant difference test (LSD) at $p<0.05$ significance level (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

In the present study, germination parameters were evaluated for vulnerable threatened categorized *Salvia aramiensis*. Different collecting sides and priming were used to determine their effect on germination parameters.

Seed physiology

Effects of locations on seed moisture content and thousand seed weight were found to be statistically ($p<0.05$) significant (Table 3). Seed moisture contents ranged between 5.76 and 7.87%, higher seed moisture content was found in samples from Arsuz while the lowest result was from Dörtyol samples. Arsuz followed by Samandağ locations had the highest seed moisture. Thousand Seed Weight (TSW) results ranged between 5.77 and 10.05 g, and the highest TSW were from samples of Belen while the lowest TSW were obtained from Dörtyol samples.

Table 3. Seed moisture (%) and thousand seed weight (g) values of *S. aramiensis* seeds from six districts of Hatay.
Çizelge 3. Hatay'ın altı farklı lokasyonundan toplanan *S. aramiensis* tohumlarına ait tohum nemi (%) ve bin tohum ağırlığı (g) değerleri.

No	Locations	Seed Moisture (%)	TSW
1	Antakya	7.56±0.02^{c}	8.47±0.14^{b}
2	Arsuz	7.87±0.02^{a}	8.13±0.18^{b}
3	Belen	7.29±0.01^{e}	10.05±0.35^{a}
4	Dörtyol	5.76±0.02^{f}	5.77±0.23^{c}
5	Samandağ	7.856±0.01^{b}	8.45±0.23^{b}
6	Yayladağı	7.39±0.03^{d}	8.24±0.18^{b}
7	Mean	7.29	8.19

*Significance level $p<0.05$

Thousand seed weight for *S. officinalis* were found 8.03 g considering that *S. officinalis* and *S. aramiensis* are in the same clade our results are compatible except the location Dörtyol (Liu *et al.*, 2006). Study showed that *S. hispanica* TSW ranges between 1.32-1.30 g comparing to *S. aramiensis* considerable low (Ixtaina *et al.*, 2008). However, the mean moistures were similar to our results as in *S. hispanica*.

Seed germination

The present study evaluated germination parameters of *S. aramiensis* seeds collected from different districts of Hatay, Türkiye. A total of 34 (including control) priming were applied however only GA_3 (Gibberellic acid) and cold stratification combinations with GA_3 resulted in germination. Statistical analysis was applied to ten priming treatments and the control groups.

Germination rate (%), mean germination time (day), germination index (day), and T50 (day) were determined to show the effects of locations and treatments on *S. aramiensis* seeds. All the parameters were found to be statistically important ($p<0.05$) on germination parameters.

Germination rate (%) shows the total germinated seeds in the experimental period and also demonstrate the seeds' vigour. Germination rate ranged from 0 to 65.5%, but in all control samples, no germination was observed (Table 4). The highest germination rate was observed in Belen district seed samples at 65.5% with 30d/2000ppm GA_3 treatment combination. When the locations are compared, Belen district had the highest germination rate followed by Samandağ and Yayladağı locations. The lowest germination rates were observed generally in 500 ppm GA_3/24h treatments. Treatment effects were found to statistically significant, and 2000/3000 ppm GA_3 and its combination with 30-day stratification were found to have the highest germination effect. Since there was no germination observed from stratification treatment alone, it was shown that GA_3 is more effective on germination.

Table 4. Effects of treatments and locations on *S. aramiensis* seed germination rate (%).
Çizelge 4. Önişlemler ve lokasyonlara göre *S. aramiensis* tohumlarının çimlenme oranları (%).

Treat. No	Germination Rate^{+}						Treat. Mean^{+++}
	Antakya	Arsuz	Belen	Dörtyol	Samandağ	Yayladağı	
1	9.0±1.0$^{[/}$	5.5±0.5$^{]}$	11.0±0.6$^{Z[}$	6.0±0.8$^{\]}$	19.5±1.0^{UV}	17.0±1.0^{V-X}	11.33^{e}
2	24.0±0.8ST	11.0±1.3$^{Z[}$	32.0±1.9^{MN}	16.0±1.4^{W-Y}	39.0±1.7^{H-K}	35.0±1.9^{LM}	26.17^{d}
3	38.5±1.0^{I-L}	20.0±0.8^{UV}	60.5±0.8^{B}	12.5±0.5^{YZ}	46.0±1.2^{EF}	35.5±0.6^{KL}	35.5^{a}
4	42.0±1.4^{GH}	16.0±0.8^{W-Y}	48.5±1.0^{E}	13.0±1.0^{YZ}	49.0±1.2^{DE}	43.5±1.0^{FG}	35.33^{a}
5	28.0±0.9^{O-Q}	24.0±0.9ST	37.5±0.5^{I-L}	13.5±0.6^{X-Z}	26.5±0.5^{P-S}	22.0±0.6^{TU}	25.25^{d}
6	25.0±0.5^{Q-T}	13.5±1.0^{X-Z}	52.0±0.6^{CD}	19.0±0.6^{U-W}	27.5±1.0^{P-R}	53.5±1.4^{C}	31.75^{b}
7	38.0±0.9^{I-L}	14.0±0.8^{X-Z}	27.0±1.0^{P-S}	15.0±1.4^{XY}	37.0±0.5^{J-L}	40.5±1.2^{G-I}	28.58^{c}
8	31.5±0.5^{N}	13.0±0.7^{YZ}	59.5±1.0^{B}	14.5±0.3^{X-Z}	39.5±0.9^{H-J}	31.0±1.3^{NO}	31.50^{b}
9	36.0±1.0^{J-L}	17.0±0.4^{V-X}	65.5±1.0^{A}	20.0±1.0^{UV}	47.5±1.8^{E}	31.0±1.3^{NO}	36.17^{a}
10	36.5±1.5^{J-L}	24.5±1.3^{R-T}	59.5±0.5^{B}	18.5±1.3^{VW}	47.0±1.7^{E}	29.0±1.3^{N-P}	35.83^{a}
Loc. Mean	28.05^{d++}	14.41^{e}	41.18^{a}	13.45^{f}	34.41^{b}	30.7^{c}	-

1: 500 ppm GA_3/24h, 2: 1000 ppm GA_3/24h, 3: 2000 ppm GA_3/24h, 4: 3000 ppm GA_3/24h. 5: 10d/1000 ppm GA_3, 6: 10d/2000ppm, 7: 30d/1000ppm, 8: 30d/1000ppm, 9: 30d/2000ppm, 10: 30d/3000ppm, No germination observed in control and not placed in table. Significant level for all categories is $p<0.05$. $^{+}$ Means are result of locations and treatments interactions, LSD=3.032; $^{++}$Locations means statistical results, LSD= 0.9143, $^{+++}$Treatment means statistical results, LSD=1.2

Mean germination time (day) (MGT) showed elapsed time to germination and results are given in Table 5. MGT results ranged from 0 to 7.55 days. After control, the lowest result was obtained from Dörtyol location with 2.87 days and the highest result was obtained from seeds of Antakya district treated with 1000 ppm GA_3/24h as 7.55. MGT should be lower with high germination rates. Belen district also stands out with its fast germination time. Dörtyol district seeds showed the lowest mean germination time, however, germination rates were also comparatively low. A comparison of treatments showed that stratification could shorten the germination time.

Table 5. Effects of treatments and locations on *S. aramiensis* seed MGT (day)
Çizelge 5. Önişlem ve lokasyonlara göre *S. aramiensis* tohumlarının ortalama çimlenme süreleri (gün)

Treat. No	Mean Germination Time						
	Antakya	Arsuz	Belen	Dörtyol	Samandağ	Yayladağı	Treat. Mean
1	7.55 ± 0.5^{A}	$7.17\pm0.3^{A-C}$	7.40 ± 0.3^{AB}	$7.06\pm0.5^{A-C}$	$6.71\pm0.1^{A-D}$	$6.68\pm0.4^{A-D}$	7.01^{a}
2	$6.71\pm0.5^{A-D}$	$7.35\pm0.4^{A-C}$	$7.33\pm0.4^{A-C}$	$5.84\pm0.5^{D-H}$	$6.39\pm0.5^{B-E}$	$5.36\pm0.5^{E-J}$	6.50^{b}
3	$5.38\pm0.3^{E-J}$	$6.26\pm0.3^{C-F}$	$5.35\pm0.4^{E-J}$	$4.57\pm0.4^{I-R}$	$5.55\pm0.5^{E-I}$	$5.14\pm0.4^{F-L}$	5.37^{c}
4	$5.96\pm0.5^{D-G}$	$5.92\pm0.5^{D-G}$	$5.19\pm0.3^{F-L}$	$4.57\pm0.5^{I-R}$	$5.29\pm0.5^{E-K}$	$4.84\pm0.5^{G-N}$	5.30^{c}
5	$5.11\pm0.2^{F-M}$	$4.60\pm0.5^{I-Q}$	$5.54\pm0.5^{E-I}$	$3.32\pm0.4^{J-T}$	$5.11\pm0.4^{F-M}$	$4.55\pm0.5^{I-R}$	4.87^{d}
6	$4.37\pm0.3^{J-S}$	$4.03\pm0.3^{L-V}$	$3.86\pm0.5^{N-W}$	3.0 ± 0.3^{VW}	$3.76\pm0.4^{N-W}$	$3.44\pm0.4^{Q-W}$	3.74^{e}
7	$3.55\pm0.5^{P-W}$	$4.74\pm0.2^{H-O}$	$3.96\pm0.5^{M-W}$	$3.78\pm0.4^{N-W}$	$4.23\pm0.2^{J-U}$	$3.63\pm0.4^{O-W}$	3.98^{e}
8	$4.68\pm0.6^{H-P}$	$4.11\pm0.3^{L-V}$	$4.14\pm0.5^{K-V}$	$3.55\pm0.5^{P-W}$	$3.22\pm0.4^{S-W}$	$3.73\pm0.5^{N-W}$	3.90^{e}
9	$3.79\pm0.2^{N-W}$	$3.51\pm0.4^{P-W}$	$3.53\pm0.4^{P-W}$	2.84 ± 0.1^{W}	$3.11\pm0.6^{U-W}$	$3.18\pm0.3^{T-W}$	3.32^{f}
10	$3.15\pm0.3^{T-W}$	2.96 ± 0.4^{VW}	$3.39\pm0.5^{R-W}$	$3.36\pm0.2^{S-W}$	$3.28\pm0.3^{S-W}$	3.02 ± 0.3^{VW}	3.19^{e}
Loc. Mean	4.57^{a}	4.60^{a}	4.52^{ab}	3.90^{d}	4.24^{bc}	3.96^{cd}	-

1: 500 ppm GA_3/24h, 2: 1000 ppm GA_3/24h, 3: 2000 ppm GA_3/24h, 4: 3000 ppm GA_3/24h. 5: 10d/1000 ppm GA_3, 6: 10d/2000ppm, 7: 30d/1000ppm, 8: 30d/1000ppm, 9: 30d/2000ppm, 10: 30d/3000ppm, No germination observed in control and not placed in table. Significant level for all categories is $p<0.05$. Standard Errors were given near mean results as ±; $^{+}$ Means are result of locations and treatments interactions, LSD=0.9515; $^{++}$Locations means statistical results, LSD= 0.2869; $^{+++}$Treatment means statistical results, LSD=0.3884

Germination index shows the effectiveness of treatments on germination count and time. GI results ranged between 0.42 and 11.30 (Table 6). The highest GI was obtained from Belen location with 30d stratification/2000ppm GA_3 treatment, while the lowest GI result was determined from Arsuz location with 500 ppm GA_3/24h treatment. Between the locations, Belen location showed higher performance, while stratification treatments increased GI. GI is a sensitive index predicting seed quality and decrease in the GI results shows that a seed lot has a low germination ability and also a longer germination time.

T50 shows the time required for half of the seeds to germinate and T50 results are given in Table 7. Low T50 numbers show that germination has occurred faster than the higher T50 results. In the present study, T50 ranged between 1.82 and 6.94 days, but control was not calculated due to the zero germination. The lowest T50 was obtained from Arsuz location with 30d stratification/3000ppm GA_3 treatment, while the highest T50 was in Antakya location with 500 ppm GA_3. Comparison of the locations yielded two groups that were statistically similar; one was Antakya, Arsuz and Belen locations the other group was Dörtyol, Samandağ and Yayladağı.

Seed weight could play an important role to show germination behaviour. Abdollahi *et al.* (2012), in their study with 40 *Salvia* species from Iran, found that lighter seeds had superior germination rate comparing to heavier seeds. In contrast to this, in the present study Belen location had a higher germination rate with highest seed weight. Although the Belen location seeds had higher seed moisture, seed weight was also high, therefore, we could assume their endosperm was heavier.

There are several studies on *Salvia* species that examined germination. The effect of priming KNO_3, GA_3, PEG, and their combinations on *S. officinalis* seed germination rate and mean germination time were studied (Gorai *et al.*, 2011; Sonmez *et al.*, 2019). In that study, germination rates were found to be generally higher than our results, especially in the control where germination rate was 34% while in the present study no germination was observed in the control group. Furthermore, MGT (day) was found to be longer than that of the present study, and KNO_3 was extended to the MGT. At the same time GA_3 was found to shorten the MGT. Similar to our results, GA_3 was found to promote germination rate. Different studies have revealed the diversity of seed germination times of *Salvia* species. *S. hispanica* seed germination lasted five days (de Paive *et al.*, 2016).

Table 6. Effects of treatments and locations on *S. aramiensis* seed GI.
Çizelge 6. Önişlem ve lokasyonlara göre *S. aramiensis* tohumlarının çimlenme indeksi değerleri.

Treat.No	Germination Index (GI)						
	Antakya	Arsuz	Belen	Dörtyol	Samandağ	Yayladağı	Treat. Mean
1	0.68±0.1$^{\text{\]}}$	0.42±0.1$^{\text{]}}$	0.88± 0.1$^{[-]}$	0.48±0.1$^{\text{]}}$	1.76±0.1$^{X-[}$	1.41±0.1$^{Z-\}$	0.94^{g}
2	2.09±0.4^{V-Z}	0.85±0.1$^{\text{\]}}$	2.34±0.4^{U-Z}	1.51±0.3$^{Y-\}$	3.34±0.4^{P-S}	3.61±0.4^{O-R}	2.29^{f}
3	4.17±0.2^{L-P}	1.76±0.3$^{X-[}$	6.19±0.4^{F-H}	1.49±0.4$^{Y-\}$	4.76±0.4^{J-N}	4.01±0.5^{N-Q}	3.73^{d}
4	4.20±0.2^{L-P}	1.56±0.2$^{Y-\}$	5.02±0.3^{I-M}	1.58±0.1$^{Y-\}$	5.31±0.2^{H-J}	5.04±0.2^{I-L}	3.78^{d}
5	3.26±0.5^{P-T}	3.11±0.5^{Q-U}	4.05±0.4^{N-P}	1.86±0.4^{X-Z}	3.08±0.4^{R-U}	2.80±0.3^{R-W}	3.03^{e}
6	3.55±0.2^{O-R}	1.83±0.2^{X-Z}	8.33±0.6^{CD}	3.66±0.5^{O-R}	4.36±0.4^{K-O}	9.27±0.6^{B}	5.17^{b}
7	6.47±0.4^{BC}	1.91±0.4^{W-Z}	4.06±0.4^{N-P}	2.41±0.4^{T-Y}	5.55±0.4^{H-J}	6.86±0.3^{EF}	4.54^{c}
8	4.13±0.5^{M-P}	1.93±0.5^{W-Z}	8.84±0.4^{BC}	2.62±0.4^{S-X}	7.61±0.4^{DE}	5.11±0.6^{I-K}	5.04^{b}
9	5.75±0.4^{G-I}	2.95±0.4^{R-V}	11.30±0.3^{A}	4.09±0.6^{N-P}	9.15±0.6^{BC}	5.77±0.5^{G-I}	6.5^{a}
10	6.90±0.4^{EF}	4.99±0.4^{I-M}	10.69±0.5^{A}	3.43±0.3^{O-S}	9.25±0.4^{B}	5.53±0.3^{H-J}	6.8^{a}
Loc. Mean	3.75^{d}	1.94^{e}	5.61^{a}	2.1e	4.92^{b}	4.49^{c}	-

1: 500 ppm GA_3/24h, 2: 1000 ppm GA_3/24h, 3: 2000 ppm GA_3/24h, 4: 3000 ppm GA_3/24h. 5: 10d/1000 ppm GA_3, 6: 10d/2000ppm, 7: 30d/1000ppm, 8: 30d/1000ppm, 9: 30d/2000ppm, 10: 30d/3000ppm, No germination observed in control and not placed in table. Significant level for all categories is $p<0.05$. Standard Errors were given near mean results as ±; $^{+}$ Means are result of locations and treatments interactions, LSD=0.7781; $^{++}$Locations means statistical results, LSD= 0.2346; $^{+++}$Treatment means statistical results, LSD=0.3177

Table 7. Effects of treatments and locations on *S. aramiensis* seed T50.
Çizelge 7. Önişlem ve lokasyonlara göre *S. aramiensis* tohumlarının T50 değerleri.

Treat.No	T50						
	Antakya	Arsuz	Belen	Dörtyol	Samandağ	Yayladağı	Treat. Mean
1	6.94±0.2A	6.31±0.1^{A-E}	6.69±0.2^{AB}	6.27±0.5^{A-F}	5.64±0.2^{C-I}	5.85±0.2^{B-H}	6.28^{a}
2	5.97±0.2^{A-G}	6.47±0.2^{A-C}	6.39±0.4^{A-D}	5.01±0.2^{G-L}	5.44±0.3^{D-J}	4.78±0.4^{I-M}	5.68^{b}
3	4.52±0.2^{J-O}	5.29±0.2^{F-K}	4.53±0.2^{J-O}	3.93±0.3^{M-R}	4.69±0.1^{I-N}	4.12±0.3^{L-Q}	4.51^{c}
4	4.91±0.3^{H-M}	5.36±0.2^{E-J}	4.5±0.3^{J-O}	3.92±0.2^{M-R}	4.42±0.4^{J-O}	4.27±0.5^{K-P}	4.56^{c}
5	4.22±0.2^{L-P}	3.63±0.1^{O-T}	4.51±0.2^{J-O}	3.93±0.1^{M-R}	3.88±0.3^{M-R}	3.61±0.2^{O-T}	3.96^{d}
6	3.90±0.2^{M-R}	3.67±0.2^{N-S}	2.81±0.2^{S-Y}	2.15±0.2^{W-Y}	3.35±0.2^{P-U}	2.48±0.1^{U-Y}	3.06^{e}
7	2.93±0.2^{R-X}	4.04±0.3^{L-Q}	3.34±0.2^{P-U}	2.74±0.5^{S-Y}	3.51±0.1^{O-U}	2.52±0.2^{U-Y}	3.18^{e}
8	4.0±0.2^{L-Q}	3.15±0.2^{Q-W}	3.28±0.2^{P-V}	2.60±0.4^{T-Y}	2.03±0.4^{XY}	2.67±0.2^{S-Y}	2.95^{e}
9	2.77±0.2^{S-Y}	2.78±0.2^{S-Y}	2.5±0.3^{U-Y}	1.93±0.4^{XY}	2.05±0.5^{XY}	2.21±0.3^{W-Y}	2.37^{f}
10	2.15±0.2^{W-Y}	1.82±0.2^{Y}	2.49±0.1^{U-Y}	2.27±0.4^{V-Y}	1.97±0.2^{XY}	2.16±0.1^{W-Y}	2.14^{f}
Loc. Mean	3.85^{a}	3.87^{a}	3.73^{a}	3.16^{b}	3.36^{b}	3.15^{b}	-

1: 500 ppm GA_3/24h, 2: 1000 ppm GA_3/24h, 3: 2000 ppm GA_3/24h, 4: 3000 ppm GA_3/24h. 5: 10d/1000 ppm GA_3, 6: 10d/2000ppm, 7: 30d/1000ppm, 8: 30d/1000ppm, 9: 30d/2000ppm, 10: 30d/3000ppm, No germination observed in control and not placed in table. Significant level for all categories is $p<0.05$. Standard Errors were given near mean results as ±; $^{+}$ Means are result of locations and treatments interactions, LSD=0.8522; $^{++}$Locations means statistical results, LSD= 0.2569; $^{+++}$Treatment means statistical results, LSD=0.3479

Several studies have examined on pre-treatment effects on germination of *Salvia* species, however, to the best of our knowledge, the present study is the first for *Salvia aramiensis*. *S. aramiensis* is a local endemic for Türkiye yet it is very important to study this species both for natural resources conservation and, also to determine the pre-treatment method for cultivation. *S. aramiensis* has been used by local herbalists around Hatay province yet the species could be more popular regarding its good quality essential oil (Demirci *et al.*, 2002; Bahadirli, 2020). The present study showed not only the importance of priming but also collecting localities. Dastanpoor *et al.* (2013) found hydro-priming could induce seed germination of *S. officinalis* to 85.5%, while in the present study hydro-priming did not affect seed germination. Hormone applications were a major preference for germination treatments, regarding their high impact and GA_3 is one of the most used treatment. *S. officinalis*, *S. fruticosa*, *S. tomentosa* and *S. verticillata* seed germination were increased with GA3 application; furthermore, *S. officinalis* had the highest germination rate at 95.1% (Ozcan *et al.*, 2014; Tursun, 2019). Ethylene was found to be effective in increasing seed germination of *S. pomifera* and *S. tomentosa* while in the present study ethylene treatment of 250 ppm and 750 ppm did not affect germination (Ozcan *et al.*, 2014).

In general, seed dormancy occurs in *Salvia* species, however, in some samples from species such as *S. officinalis*, *S. coccinea* and *S. farinacea*, no dormancy occurs (Ellis *et al.*, 1985b). Localities where seeds were collected was also found to have important effects on germination, for instance Al-Gharaibeh *et al.* (2017) found that *S. syriaca* and *S. spinosa* species collecting location, temperature and salinity have a great impact on seed tolerance to salinity as well as germination. Ellis *et al.* (1985b) found that cold stratification and hormones are effective for germination of dormant seeds. Experiments with salinity, temperature and PEG are also crucial that they could show the response of species to stress factors (Javaid *et al.*, 2018; Sonmez *et al.*, 2019). On the other hand, for species with low germination ability like *S. aramiensis*, dormancy breaking treatments should be first defined and stress factors applied later.

Thanos (1993) found the germination time of *S. fruticosa* seeds was very low compared to those of other Lamiaceae species, and regarding this result, the present experiments were planned to be conducted for 30-days. Furthermore, during the experiment, it was observed that *S. aramiensis* seeds started and completed germination very quickly. Also, abnormal seedlings were observed from late germinated seeds. GI in *S. officinalis* was 45.56%, while in our result it was observed as too low this could be due to species diversification, genetic and environmental factor effects (Liu *et al.*, 2006).

CONCLUSIONS

S*alvia aramiensis* is an important tradable commodity for Türkiye. Nevertheless, species are only collected from nature widely, hence their cultivation should be started very soon. As the amount of collection from nature increases, it is very important to preserve the plants in their natural flora. In the present study, for the first time, seed and germination parameters of this species using seeds from six different locations were presented. The results showed that seed collecting locations and priming methods have a high impact on germination. Also, GA_3 and stratification combinations could positively affect germination parameters. According to the studied locations, Belen district was significant. Pre-treatment studies should be continued for further investigations on breeding and cultivation.

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