

# Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan

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## Abstract

*Salvia spinosa* L. and *Salvia syriaca* L. are perennial medicinal herbs that occur in the Mediterranean, Irano-Turanian and Saharo-Arabian phytogeographic regions of Jordan. With respect to the seed germination requirements, prevailing environmental conditions in each phytogeographic region may promote local adaptation and consequently affect the distribution range of the species. Using seeds of both species collected from populations across the three regions, we tested responses to variations in temperature and salinity under laboratory conditions. Both species showed significant differences in cumulative germination percentages and germination rates (modified Timson Index) with temperature, while origin only significantly affected *S. spinosa* seeds. Both species germinated best under the highest temperature regime (32/20°C). The low temperature regime (8/4°C) completely inhibited germination in *S. syriaca*, whereas it led to 80% to 95% germination in *S. spinosa*, with significant variation being recorded between the phytogeographic regions. For both species, salt solutions of 0, 25 and 50 mM NaCl yielded the highest germination percentages and rates, which sharply and significantly declined at higher concentrations (100 and 200 mM NaCl). Our results provide evidence of local adaptation of the study species to salinity and temperature in the respective maternal environments, particularly in the Irano-Turanian and Saharo-Arabian regions. Such differentiation should be accounted for in future conservation planning.

**Keywords:** germination percentage, germination rate, phytogeographic region, *Salvia spinosa*, *Salvia syriaca*.

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## Introduction

Medicinal plants are coming under increasing pressure from habitat destruction, bio-prospecting and climatic change (Roberson 2008). In addition, overharvesting has placed many wild medicinal species at risk of extinction as a result of reduced effective population sizes, soil seed bank depletion and loss of genetic diversity (Cruse-Sanders *et al.* 2005). Thus, overharvested popula-

tions may be driven to extinction more quickly than those exposed to population fragmentation and habitat destruction alone (Vance 2002).

Detailed information on germination behavior is important to the understanding of species in terms of their establishment, tolerance of abiotic factors, and dynamics across environmental gradients. Furthermore, it can provide clues to a species' survival strategy with respect to seed dormancy, pretreatments, optimal conditions for seed germination and the influence of seed provenance (Baskin & Baskin 1998; Ronnenberg *et al.* 2008; Hamasha

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& Hensen 2009). The study of seed germination behavior has provided the data required for developing and implementing effective strategies and protocols for the *ex situ* and *in situ* conservation of, for example, *Arabis kennedyae* (Andreou *et al.* 2011) and *Argyranthemum* species (Francisco-Ortega *et al.* 1994).

For habitat restoration projects, the use of native species and local seed provenances is often recommended because they are expected to be better adapted to the local climate and soil conditions (Bischoff *et al.* 2006). Seed provenance has been shown to significantly affect germination, indicating that genetic and environmental factors have a strong influence on seed germination traits (Bischoff *et al.* 2006). Inter-population variability in the response of germination behavior to environmental conditions has been widely reported for many species in accordance with differences among their habitats of origin (e.g. Hamasha & Hensen 2009), and can be attributed to both maternal influences and genetically based local adaptation.

A number of abiotic factors, such as temperature, salinity, light and soil moisture, can have significant individual and cumulative species-specific effects on germination, thereby affecting the distribution of plants in natural habitats (Huang *et al.* 2003; El-Keblawy & Al-Rawai 2005; Gorai *et al.* 2011). In addition, environmental factors can influence the onset, percentage and rate of germination for different species (Cendán *et al.* 2013). For example, when moisture conditions are favorable, salinity and temperature represent major factors affecting seed germination and initial seedling establishment in arid and saline regions (Huang *et al.* 2003; El-Keblawy & Al-Rawai 2005; Al-Khateeb 2006; Maraghni *et al.* 2010). Knowledge of the effects of temperature and salinity on germination may therefore be useful in evaluating germination characteristics or establishment potential for species in arid regions (Jordan & Haferkamp 1989; Maraghni *et al.* 2010). In addition, such knowledge will complement the limited data available on the influence of soil salinity as a driver in plant ecology and phytogeography (Bui 2013).

Species with wide distribution ranges can show either high phenotypic plasticity (Banta *et al.* 2012) or intraspecific differentiation in germination behavior under varying environmental conditions (Keller & Kollmann 1999; Fenner & Thompson 2005; Ronnenberg *et al.* 2008). However, variation in phenotypes may be determined not only by the genotype and the environment of the individuals but also by maternal effects (Roach & Wulff 1987), and Gutterman (2000) contends that phenotypic variation is caused by the local conditions under which seeds mature. Maternal environmental conditions can have a strong impact on plant fitness (Lacey & Herr 2000), which in turn can affect the quality of the offspring (Mousseau & Fox 1998) and influence seed germination (Rossiter 1996, 1998). Maternal effects are considered to be adaptive

when they enhance offspring fitness in an environment similar to that experienced by the parental generation (Rossiter 1996, 1998; Donohue & Schmitt 1998).

In addition, differences in genotype and phenotype can interact with environmental factors such that fitness can vary among populations as a function of both local adaptation and the local environment (Banta *et al.* 2012). In this respect, species exhibiting greater intraspecific variation can have a greater chance of coping with climate change, as it may buffer species against associated adverse effects (Thuiller *et al.* 2004). Genetically determined local adaptation can be evaluated by examining the germination traits of plants with different provenances under a variety of environmental conditions (Luzuriaga *et al.* 2006; Sales *et al.* 2013). However, as plants with broad geographic distributions are generally associated with high colonization and persistence, plants with limited distribution are expected to have less success in plant recruitment than more widespread species (Astegiano *et al.* 2013).

In Jordan, medicinal plants have attracted increasing scientific (Hudaib *et al.* 2008; Al-Quran 2011; Nawash *et al.* 2013) and commercial attention (World Bank 2003). For example, recent overharvesting has pushed *Salvia fruticosa* L. to extinction from the wild in Jordan (Al-Eisawi 1996). Therefore, there is increasing pressure on wild plant populations, from which most medicinal plants are harvested, and there is an urgent need to conserve medicinal plants in their habitats for future generations (Al-Quran 2011).

For the present study, we investigated local adaptation in the germination behavior of two *Salvia* species from Jordan. *Salvia spinosa* L. and *Salvia syriaca* L. (Lamiaceae) are two native medicinal plant species (Oran & Al-Eisawi 1998; Ulubelen 2003) that occur across the Mediterranean, Irano-Turanian and Saharo-Arabian phytogeographic regions of Jordan (Zohary & Feinbrun 1966; Zohary 1973; Danin 1992). We assessed the diversity between seed provenances of the study species in order to test for intraspecific variation in seed germination and local adaptation. For instance, several studies have demonstrated that seeds that originate from populations subjected to high-salinity germinate more readily and earlier than those from less saline environments (Ungar 1991; Van Zandt & Mopper 2004; Yao *et al.* 2010). Therefore, this approach may serve as a model for testing differences in germination between individuals from different provenances, and our results may provide valuable knowledge to support conservation and restoration programs for such species elsewhere. In addition, to test whether germination behavior reflects local adaptation to the prevailing environmental conditions of the three seed-source regions (Mediterranean, Irano-Turanian and Saharo-Arabian), we tested for intraspecific variation in percentages and rates of seed germination between our seed provenances.

The objectives of this study were: (i) to verify inter-population variability in germination responses (percentage and rate) to temperature and salt stress, and with respect to phytogeographic region; and (ii) link any such variation, as well as the levels of temperature and salinity that can trigger or inhibit germination in populations of both species, with their distribution range and local adaptation.

## Materials and methods

### *Study area and study species*

Jordan is situated between longitudes 35°40' and 39°E and between latitudes 29°30' and 34°N, and it is unique in its natural diversity. Biogeographically, Jordan consists of four major phytogeographic regions, namely the Mediterranean, Irano-Turanian, Saharo-Arabian and Sudanian regions, and its environmental heterogeneity is very remarkable (Zohary 1973; Al-Eisawi 1996). The climate is characterized by moist cold winters and hot dry summers, resulting in extreme variability in rainfall across

the year. Rainfall decreases from west to east and from north to south (Al-Eisawi 1996). Generally, the content of soluble salts in the soils increases with decreasing rainfall. Salinity ranges from 9 to 600 mM within Jordanian soils, with the highest levels occurring in the Jordan Valley (Al Qudah 2001), where environmental conditions of the Sudanian region prevail.

According to geographic information and climatic data from 31 meteorologic stations as presented by Al-Eisawi (1996), the Mediterranean, Irano-Turanian and Saharo-Arabian regions of Jordan are characterized by elevational ranges of 700–1700 m a.s.l., 400–700 m a.s.l. and 600–700 m a.s.l., respectively. Annual rainfall is 300–600 mm, 150–250 mm and 50–100 mm, respectively. Mean temperature in summer is 20°C, 25°C and >30°C, respectively (Table 1), and soil salinity is equivalent to 9 mM, 24 mM and 150–200 mM NaCl, respectively (Al Qudah 2001).

*Salvia spinosa* and *S. syriaca* are perennials that can grow to 30–60 cm and 30–80 cm tall (Zohary & Feinbrun 1966), respectively, with the flowering season extending from April to June for *S. spinosa* and until July for *S. syriaca*. The

**Table 1** Geographic, meteorologic and site descriptions of the seed sources of the *Salvia spinosa* and *Salvia syriaca* populations

Population	Phytogeographic region	Geography		Climate					No. ind.
		Lt	Ln	Al	Rn	Tm	Ta	Tj	
<i>Salvia spinosa</i>									
Gafgafa	Med	32°.37'	35°.92'	782	412	17.2	24.4	9.1	18
Abo Bana	Med	30°.87'	35°.67'	1148	220	14	20.7	6.5	17
Fjaej	Med	30°.57'	35°.63'	1263	300	12.3	19.4	4.4	19
Kings Road	Med	30°.08'	35°.43'	1616	270	14.1	21.6	5.8	67
Sarrot	Ira	32°.17'	35°.95'	534	250	15	27	4	23
Humret Sahen	Ira	32°.10'	35°.66'	650	280	19.6	26.5	11.7	19
JUST	Ira	32°.48'	35°.98'	588	250	15	27	4	173
Al Jezzah	Ira	31°.68'	35°.96'	715	157	16.7	23.6	8.4	53
Safawi	Sah	31°.99'	36°.85'	615	75	18.7	27	9.2	31
Al-Azaraq	Sah	31°.87'	36°.74'	560	91	17.7	25.1	8.7	15
Wadi Rum	Sah	29°.60'	35°.38'	960	59	25.1	39.2	9.3	12
Borqu Castle	Sah	32°.60'	38°.01'	545	82	18	26	7	13
<i>Salvia syriaca</i>									
Ras Yousif	Med	32°.72'	35°.87'	820	473	15.2	22.4	7.1	80
Airport Highway	Med	31°.87'	35°.88'	921	275	12.3	23.6	8.4	35
Madaba	Med	31°.74'	35°.84'	781	320	18.3	24.9	10.5	>2000
Abo Bana	Med	30°.87'	35°.67'	1148	220	14	20.7	6.5	19
Dieban	Ira	31°.59'	35°.79'	636	301	18.3	24.9	10.5	>300
Karak	Ira	31°.20'	35°.73'	747	179	17.4	24.1	9.5	250
Rehab	Ira	32°.35'	36°.02'	796	220	15	24	4	277
Sarrot	Ira	32°.17'	35°.95'	534	250	15	27	4	63
Mafrq 2	Sah	32°.37'	36°.21'	526	155	16.9	24.8	7.4	51
Mafrq 1	Sah	32°.33'	36°.19'	722	160	15	24	4	42

Phytogeographic regions based on Al-Eisawi (1996): Med, Mediterranean; Ira, Irano-Turanian; Sah, Saharo-Arabian. The climate data for the sampling sites were obtained from Hamasha and Hensen (2009), and the Jordanian Meteorological Department (unpublished data). Lt, latitude; Ln, longitude; Al, altitude (m); Rn, mean annual rainfall (mm); Tm, temperature mean (°C); Ta, mean hottest month (August) temperature (°C); Tj, mean coldest month (January) temperature (°C). No. ind., number of individuals per sampled population.

majority of *Salvia* species are pollinated by bees (Claßen-Bockhoff *et al.* 2004), whereas flowers of both species receive visits from honeybees and bumblebees (field observation). Seed production per mature plant ranges from 1500 to 10 000 seeds for *S. spinosa*, and from 100 to 2000 for *S. syriaca*. Nutlets of both study species contain a pectinaceous mucilage layer that imbibes a large amount of water when moistened (Oran 1997). Populations of *S. spinosa* inhabit the Irano-Turanian region and extend to the Mediterranean and Saharo-Arabian regions. Despite having the same Chorotype as *S. spinosa*, *S. syriaca* is more common in the Mediterranean region but much rarer in the Saharo-Arabian region (Zohary & Feinbrun 1966; Danin 1992).

#### *Seed collection and germination tests*

Sampling of both *Salvia* species aimed to cover most of their natural distribution within Jordanian borders. Seeds were collected from populations of three out of the four phytogeographic regions, covering the whole extent of their occurrence. Sampling from microhabitats that seemed to be extremely saline or that may have been affected by agricultural activities was avoided. In the period between May–June and late June–July 2013, mature seeds were collected from 12 and 10 natural populations of *S. spinosa* and *S. syriaca*, respectively. Four populations per region were sampled, except for *S. syriaca* in the Saharo-Arabian region, where we only found two populations with mature seeds (Table 1). Seeds were collected from three to 10 individuals per population depending on the population size (Table 1). Seeds of both species were placed in paper bags and stored at room temperature (20°C). We germinated a small proportion (20 seeds per population) of the collected seeds directly after collection (August 2013) at the Jordan University of Science and Technology seed laboratory to assess seed dormancy. After 3 months of storage, 50 seeds per population were subjected to triphenyl tetrazolium chloride to assess initial seed viability. In *S. spinosa*, the average mass per seed lot (5 × 25 seeds) was calculated for the four populations from each region. Populations of the Irano-Turanian region produced larger and heavier seeds than those of the Mediterranean and Saharo-Arabian regions (0.164 g, 0.146 g and 0.142 g, respectively). Owing to the large percentage of empty seeds in *S. syriaca*, we were not able to calculate the average mass of seed lots. In order to eliminate the effect of the empty seeds from our results, seeds were soaked in distilled water for 2 minutes before each experiment, with any floating seeds subsequently being discarded.

Experiments were carried out with five replicates of 25 full intact seeds per population. Seeds were germinated on moistened filter paper (Whatman No. 1) in 70-mm-

diameter Petri dishes. Germination was carried out at 12 h warm white light /12 h darkness (Light Thermostat, Typ 1301, RUMED, Halle (Saale), Germany) and checked every 2 days, with seedlings being removed. To ensure randomness among treatments and populations, the horizontal and vertical positions for all Petri dishes inside the germination chambers were shifted every 2 days.

#### *Effects of temperature and phytogeographic regions on germination*

To examine variation in germination among populations from different phytogeographic regions in response to the various temperature regimes, we simulated the mean temperature regime prevailing in all regions during the main germination period (8/4°C and 20/10°C, November and February–March, respectively) and during summer (32/20°C) (after Hamasha & Hensen 2009). Germination of seeds from all populations was tested with these three simulated temperature regimes on filter paper moistened with 10 mL of distilled water. The tests were terminated after 38 days.

#### *Effects of salinity and phytogeographic region on germination*

Due to the limited availability of *S. syriaca* seeds, and based on the results from the former experiment, we examined the influence of salinity and provenance on seed germination in both species by incubating seeds only under the optimum temperature regime (32/20°C). Temperature can interact with salinity to affect seed germination (Luan *et al.* 2014) and the evaluation of tolerance of salinity without temperature interaction can be carried out more effectively at the most suitable germination temperatures than at lower or higher temperatures.

We germinated seeds on one layer of filter paper moistened with 10 mL of either distilled water (control) or different NaCl solutions (25, 50, 100 and 200 mM). Petri dishes with lids were tightly sealed using Parafilm to prevent evaporation. On a weekly basis, seeds were transferred to new Petri dishes with new filter paper, lids and fresh solutions to maintain salt concentrations close to the target levels throughout the germination period. All tests were terminated after 22 days.

#### *Statistical analysis*

All statistical analyses were performed in R-3.0.2 (R Core Team 2013). We used the cumulative proportion of germinated seeds per dish by the end of the experiment (i.e. germination probability or percentage) and the modified Timson Index (TI) as response variables. TI

describes the germination velocity and is calculated as follows:  $TI = \Sigma G/t$ , where  $G$  is the percentage of seed germination at 1-day intervals, and  $t$  is the overall germination period (Pérez-Fernández *et al.* 2006). In this equation, the TI value increases with germination velocity as well as with the number of germinated seeds. We used linear and generalized linear mixed effects models to analyze TI and germination probability, respectively (R-package lme4, Bates *et al.* 2013). While we assumed a normal error distribution for TI based on model analytic plots (Crawley 2013), germination percentage (probability) was analyzed as a binomial variable using the *logit link* function.

In an explorative analysis we tested the effect of seed mass on the probability of *S. spinosa* germination and TI by means of logistic and linear regression, respectively, with both analyses including a random effect of population. As the effects were not significant, we excluded seed mass from further analysis. All models included the main effects (phytogeographic region and temperature or salinity treatment) and two-way interactions of the latter two with phytogeographic region on germination probability and TI, respectively. These models also included a random effect of population. The significance of these main effects and interactions was assessed using Wald  $\chi^2$  tests, and pairwise comparisons were performed with Tukey's post-hoc tests ( $P < 0.05$ ).

## Results

Neither species showed dormancy at room temperature (25°C in Jordan), with 85% of *Salvia spinosa* seeds and 50% of *Salvia syriaca* seeds germinating. Initial seed viability values for *S. spinosa* and *S. syriaca* were 90% and 60%, respectively. The low percentage of viable seeds for *S. syriaca* was due to the presence of seed predators. Seed mass in *S. spinosa* had no significant effect on germination percentages ( $P = 0.156$ ).

### Effects of temperature and phytogeographic regions on germination

The analysis of probability of germination and the TI showed a significant effect of temperature, phytogeographic region and their interactions on both metrics of seed germination of *S. spinosa* ( $P$  values  $\leq 0.02$  in all cases; see Table 2). Phytogeographic region showed no significant effect on the probability of germination ( $P = 1$ ) and TI ( $P = 0.191$ ) of *S. syriaca* seeds, whereas temperature and its interaction with phytogeographic region did ( $P$  values  $\leq 0.04$  in all cases; see Table 2). In both species, the highest TI was reached under the 32/20°C regime, followed by the 20/10°C regime (Fig. 1). Variation in the day germination commenced and the rate of germination with respect to seed affinity (phytogeographic region) was only significant under the low temperature regime (8/4°C) in *S. spinosa* (Fig. 1). Saharo-Arabian populations of the former species germinated 8 days earlier than Mediterranean populations and achieved the highest germination percentages (95%) under this low temperature regime. In contrast, seeds of all affinities in *S. syriaca* failed to germinate under the low temperature regime (8/4°C). Higher TI values for both species were achieved under the higher temperature regime (32/20°C) than the lower one (20/10°C) (Fig. 1).

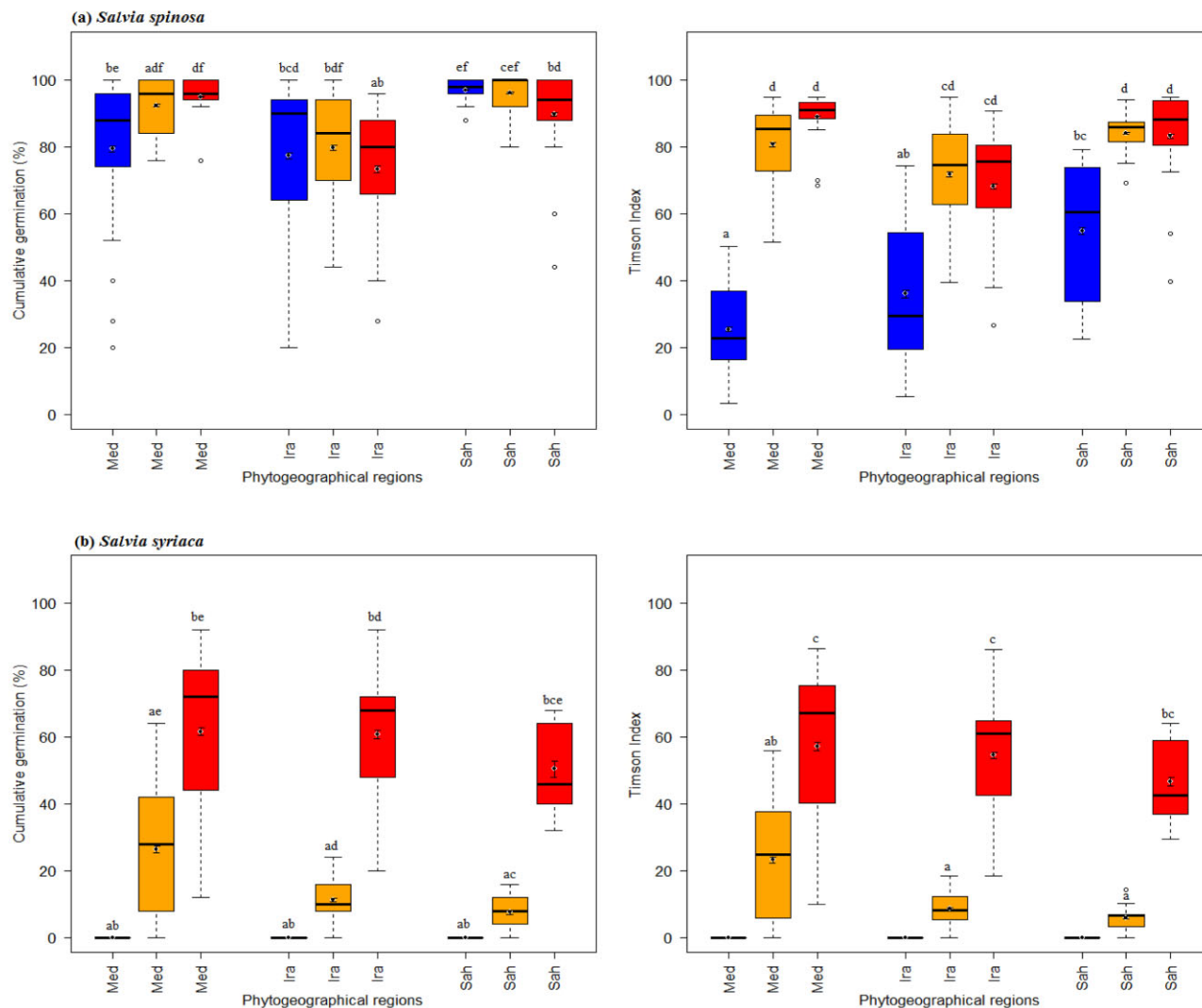
### Effects of salinity and phytogeographic region on germination

In both study species, the highest percentages and rates of germination were recorded in 0, 25 and 50 mM NaCl. Seed affinities had slight but insignificant effects on final germination in the saline treatments (Fig. 2). NaCl concentrations > 50 mM increasingly hampered seed germination in both *Salvia* species, but some seeds still germinated at 200 mM. Both probability of germination and TI decreased significantly ( $P < 0.001$  in all cases; see Table 2).

**Table 2** Results of the mixed model analyses for the effects of temperature regime (8/4°C, 20/10°C and 32/20°C), salinity concentration (0, 25, 50, 100 and 200 mM of NaCl) and phytogeographic region on seed germination for *Salvia spinosa* and *Salvia syriaca* populations

Source	<i>Salvia spinosa</i>						<i>Salvia syriaca</i>					
	Cum-germ (%)†			TI‡			Cum-germ (%)			TI		
	d.f.§	Chisq¶	P	d.f.	Chisq	P	d.f.	Chisq	P	d.f.	Chisq	P
Treatment (temperature)	2	68.83	<0.001	2	346.79	<0.001	2	572.14	<0.001	1	105.43	<0.001
Phytogeographic region	2	12.07	0.002	2	14.76	<0.001	2	0	1	2	3.31	0.191
Treatment × phytogeographic region	4	78.74	<0.001	4	57.27	<0.001	4	26.62	<0.001	2	6.39	0.041
Treatment (salinity)	4	606.87	<0.001	4	569.40	<0.001	4	313.52	<0.001	4	171.88	<0.001
Phytogeographical region	2	7.78	0.02	2	3.53	0.171	2	14.30	<0.001	2	1.47	0.479
Treatment × phytogeographic region	8	151.05	<0.001	8	27.45	<0.001	8	42.35	<0.001	8	5.49	0.704

† Cum-germ (%), cumulative germination percentages; ‡ TI, Timson Index; § d.f., degree of freedom; ¶ Chisq, Chi square test (Wald  $\chi^2$ ).

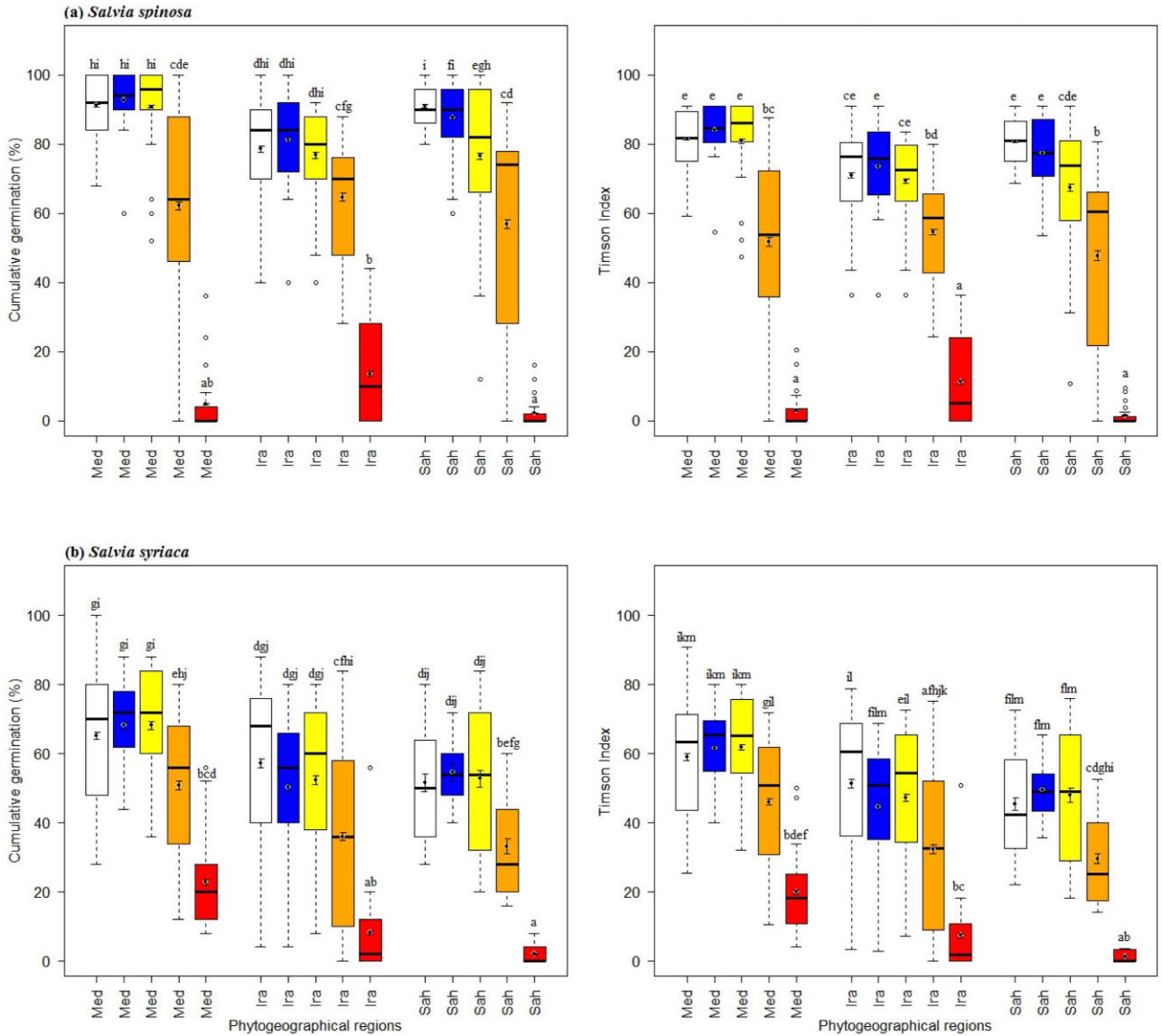


**Fig. 1** Cumulative germination percentages (analyzed as germination probability up to day 38) and modified Timson Index (TI) for seeds of (a) *Salvia spinosa* and (b) *Salvia syriaca* from different phytogeographic regions and under different day/night temperature regimes. Factor level combinations with the same letter are not significantly different at the 0.05 significance level (post-hoc Tukey's test). Black dots (•) represent arithmetic means while error bars represent standard errors. Boxplots show the following parameters of the original data distributions: boxes, interquartile range; horizontal line, median; whiskers, either maximum/minimum value or 1.5 times the interquartile range of the data (whichever is smaller); points, outliers (with respect to whiskers' range). ■, 8/4°C; ■, 20/10°C; ■, 32/20°C.

with increasing NaCl concentration, and pairwise comparisons revealed the threshold of 50 mM under which seeds of both species germinated well. Phytogeographic region had a significant effect on probability of germination ( $P$  values  $\leq 0.02$  in both species; see Table 2) but a non-significant effect on TI ( $P$  values  $\geq 0.171$  in both species; see Table 2). The interaction of salinity with phytogeographic region was highly significant in terms of probability of germination and the TI for *S. spinosa* seeds ( $P < 0.001$  in both cases; see Table 2), and for the probability of germination of *S. syriaca* seeds ( $P \leq 0.001$ ) but not TI (Table 2;  $P = 0.704$ ). Post-hoc tests revealed that *S. spinosa*

seeds from the Irano-Turanian region showed a significantly higher probability of germination than seeds from the Saharo-Arabian region under extreme salinity (200 mM; Fig. 2), whereas in *S. syriaca*, seeds from the Mediterranean region showed a significantly higher probability of germination than seeds from the Saharo-Arabian region under extreme salinity (200 mM).

In both species, and for all affinities, seeds started to germinate after 1–3 days of incubation. At each level of salinity (0, 25, 50, 100 and 200 mM), the differences in the germination rates of the two species among the three phytogeographic regions were not significant. In both species,



**Fig. 2** Cumulative germination percentages (analyzed as germination probability up to day 22) and modified Timson Index (TI) for seeds of (a) *Salvia spinosa* and (b) *Salvia syriaca* from different phytogeographical regions (Med, Mediterranean; Ira, Irano-Turanian; Sah, Saharo-Arabian) and at different NaCl concentrations (0, 25, 50, 100 and 200 mM). Factor level combinations with the same letter are not significantly different at the 0.05 significance level (post-hoc Tukey’s test). Black dots (•) in boxplots represent arithmetic means while error bars represent standard errors. Boxplots show the following parameters of the original data distributions: boxes, interquartile range; horizontal line, median; whiskers, either maximum/minimum value or 1.5 times the interquartile range of the data (whichever is smaller); points, outliers (with respect to whiskers’ range). □, 0 mM; ■, 25 mM; ■, 50 mM; ■, 100 mM; ■, 200 mM.

and among populations of the same seed affinity, TI values showed a sharp and mostly significant decline with increasing salinity above 50 mM (Fig. 2). In terms of the Timson Index, and among the phytogeographic regions for *S. spinosa*, the rate of decline with increasing salinity differed significantly (i.e. the rate of decline was higher in both the Mediterranean and Saharo-Arabian populations than in the Irano-Turanian population) (Fig. 2).

**Discussion**

Our results show that seeds of both species were not dormant after ripening. Germination in *S. spinosa* seeds can be triggered under a wide range of temperature regimes, from 8/4°C to 32/20°C, which indicates that they are able to germinate at any time from spring to winter in habitats where moisture is available. The inability of *S.*

*syriaca* seeds to germinate at 8/4°C indicates a limit around these temperatures for the species. With regard to salinity, both species achieved the highest percentage and rate of germination at 0, 25 and 50 mM NaCl, but they largely failed to germinate at 200 mM NaCl.

Both *Salvia* species achieved optimum germination rates at 32/20°C. In agreement, Côme (1993) found that seeds of *S. officinalis* germinated within a range of 10–25°C, while those of *S. sclarea* showed a slightly broader range of optimal temperatures (10–30°C). Gorai *et al.* (2011) reported that seeds of medicinal Tunisian sage (*S. aegyptiaca*) germinated under a wide range of temperatures and achieved the highest germination percentage (77%) at 30°C. They also concluded that variation in the optimal temperature and germination rates between species might constitute an adaptive strategy to harsh environmental conditions.

Our results support the assumption that adaptive processes generated variation in germination between and among species populations. In a similar study on four Jordanian *Stipa* species, there was significant variation in seed germination with temperature for all four species, and seed provenance had a significant effect for three of the species (Hamasha & Hensen 2009). At the species level, the ability of Saharo-Arabian populations of *S. spinosa* to germinate and achieve a high final germination percentage under an 8/4°C temperature regime more rapidly than Mediterranean and Irano-Turanian populations suggests that the former populations are better adapted to germinating under cold conditions. Among species, the ability to germinate at low temperatures may partially explain the deeper distribution of *S. spinosa* populations in the Saharo-Arabian region. Such adaptation may be interpreted as seeds of populations in arid and semi-arid areas (Irano-Turanian and Saharo-Arabian regions) being able to respond to moisture availability in late autumn and early winter to enable germination when temperatures are relatively low, making them less likely to suffer from desiccation. Our assumption is in line with Hamasha and Hensen (2009), who reported that variability in soil moisture imposes fluctuating selection pressures on different bioclimatic regions, thereby influencing phenologic patterns such as flowering time and timing of seed germination. Moreover, steep climatic gradients might shape the genetic structure of plant populations (Hamasha *et al.* 2013), which is supported by the differences in germination ability between the two *Salvia* species under winter temperatures, as *S. syriaca* populations have limited distribution in the Saharo-Arabian region.

In the present experiment, the seeds of both *Salvia* species showed tolerance of lower salinity concentrations (25 and 50 NaCl mM), with a rapid decline in germination percentages with increasing salinity above 50 mM. At the

lower concentrations, germination percentages were similar to those of the control (Fig. 2). In agreement, Gorai *et al.* (2011) found that seed germination of *Salvia aegyptiaca* at 30°C tolerated moderate salinity levels and was only completely inhibited at 300 mM NaCl. In another study, moderate salinity levels of 50 and 100 mM NaCl had no significant effect on the germination of mucilaginous seeds of *Diploaxis harra* at the most suitable temperature regime, and germination was completely inhibited at 200 mM (Tlig *et al.* 2008). Therefore, it is possible that for *Salvia* species, mucilage can serve as a kind of “filter” and/or “sorbent” to help mitigate the effects of low salt concentrations (25 and 50 mM), as proposed by Yang *et al.* (2011). The same experiment demonstrated that intact achenes of *Artemisia sphaerocephala* had higher germination percentages with increasing osmotic potential and NaCl concentration than those with mucilage removed. Elsewhere, mucilage water uptake in *Henophyton deserti* seeds was unaffected by low and moderate salt concentrations but inhibited under high polyethylene glycol (PEG)-6000 concentrations (Gorai *et al.* 2014).

In our study, at salt concentrations of 100 and 200 mM, phytogeographic region started to have a significant effect on germination percentages (Fig. 4). This significant variation may have resulted from adaptive differentiation or maternal effects on seed characteristics and germination behavior or both. Maternal plants can influence seeds by contributing organelles, endosperm, seed coats and other structures (Roach & Wulff 1987; Baskin & Baskin 1998). Van Zandt and Mopper (2004) found that seeds produced by maternal plants growing under high salinity germinated earlier and in greater numbers than seeds from low-salinity plants, and that such plants can produce larger, more salt-tolerant seeds (Yao *et al.* 2010). Therefore, factors that cause maternal effects can potentially affect seed size (Van Zandt & Mopper 2004). Variation in seed mass or size has an adaptive function with respect to germination in many plant species and especially in halophytes (Khan & Weber 2008). Several authors found that tolerance of salinity is proportional to seed mass in species such as *Salicornia europaea* (Ungar 1987), *Atriplex triangularis* (Khan & Ungar 1984) and *Chenopodium album* (Yao *et al.* 2010). Moreover, according to Lu *et al.* (2010), the mucilage layer produced by the larger *Diptychocarpus strictus* seeds increased seed width by 50%, while in smaller seeds it only increased by 5%. Therefore, we expect that the thicker mucilage layer produced by larger seeds can absorb and filter more water, as may be the case for the largest seeds found in our study, which were of Irano-Turanian origin. We therefore propose that for our study species, populations with larger seed masses may be able to tolerate higher salinity. Moreover, we assume that such tolerance is further enhanced by the higher production of mucilage in larger seeds than in smaller seeds.



In summary, we assume that the ability of Irano-Turanian and Saharo-Arabian populations to produce larger seeds than Mediterranean populations can be interpreted as an advantageous strategy for both *Salvia* species with respect to their responsiveness and adaptation to salinity in different phytogeographic regions. According to Al Qudah (2001), salinity in Jordanian soils is about 9 mM in the xeric moisture regime (Mediterranean), 24 mM in the transitional xeric-aridic region (Irano-Turanian), 150 mM in xeric-aridic soils (Saharo-Arabian), and it ranges from 200 mM in some locations in the Saharo-Arabian region to reach a maximum of 600 mM in the Sudanian region. In addition, the low germination percentage recorded under 200 mM saline conditions demonstrates that both study species have a low tolerance of salinity at the germination stage. Flowers *et al.* (2010) use a threshold of 200 mM NaCl to separate halophytes from glycophytes, thus both of our study species are glycophytes (Danin 1992). This limit of tolerance partly explains why these species do not exist in the Sudanian region of Jordan, which is dominated by halophytes as salt concentrations can reach up to 600 mM NaCl (Al Qudah 2001). However, our study could be complemented by further investigation of adaptation to small-scale differences in soil salinity at the local level. One way to achieve this would be by involving populations from different microhabitats of varying salinity in each region and to include analyses of soil samples.

Successful seedling establishment is correlated with seed germination traits, which evolve in response to selection regimes characteristic of particular habitats, especially climatic regimes. Species regulate the onset and level of germination in response to environmental conditions. Therefore, we conclude that information on seed germination responses to temperature and salinity for each phytogeographic region should be taken into account before restoration projects commence, as this will facilitate the successful establishment of seeds in the given region. Seed collection, either for restoration projects or seed banks, should focus on selecting seeds from the respective provenances, as restoration with local seeds is recommended not only for germination but also for seedling survival and plant maturation.

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