

NOTES AND COMMENTS

Seed germination of four Jordanian *Stipa* spp: differences in temperature regimes and seed provenances

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Abstract

Stipa steppes are considered to be an important model ecosystem in arid land ecology owing to their wide geographical distribution and their strong association with human activities. This paper presents data on the seed germination of four Jordanian *Stipa* species (*Stipa capensis*, *Stipa parviflora*, *Stipa arabica* and *Stipa lagascae*) that are widespread throughout various phytogeographical regions in Jordan. We studied variation in seed germination under laboratory conditions among populations and under various temperature regimes. There was significant variation in seed germination with temperature in all four species and seed provenance was significant for three out of the four species. The temperature levels that were most suitable for germination varied from low (8/4°C and 20/10°C) for *S. lagascae* to high (20/10°C and 32/20°C) for *S. capensis*; *S. arabica* and *S. parviflora* germinated equally well at all three temperature regimes. Variations among populations were species specific, but populations with the highest seed germination were always of arid and Saharan Mediterranean origin. Thus, seed germination was negatively correlated with annual precipitation. Such flexibility among temperatures and populations could be interpreted as an efficient survival strategy for species growing under unpredictable environmental conditions. Where artificial reseeded is necessary, differences among species and also among different seed provenances should be taken into account.

Keywords: phytogeography, restoration, *Stipa arabica*, *Stipa capensis*, *Stipa lagascae*, *Stipa parviflora*.

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Introduction

The use of local seed provenances is often recommended for restoration and habitat creation projects because they are thought to be better adapted to local habitat conditions. Thus, germination studies are important for gaining information on the effects of seed storage, pretreatments, the optimal conditions for seed germination and the influence of seed provenance, all of which are crucial for conservation and restoration programs (Ronnenberg *et al.* 2008). *Stipa* species are especially relevant to restoration studies in arid environments because they dominate large parts of the Eurasian zonal vegetation (Lavrenko & Karamysheva 1993). Owing to their strong and long-term

association with human activities, *Stipa* steppes are considered to be highly important model systems in arid land ecology (Cortina *et al.* 2007).

In semiarid and arid regions of the world, the low and irregular distribution of rainfall strongly affects the survival of plants. Their reproductive success decisively depends on whether their seeds germinate in the right place and at the right time (Guterman 1994). In areas with long, dry and hot summers, seed germination should be mainly triggered by interactions in temperature and precipitation to prevent germination occurring shortly after seed maturation following late rainfall in the rainy season before the summer. Such a strategy is very common in many plants in the Negev and Judean Deserts, such as *Stipa capensis* (Poaceae; Guterman 2002).

No reports are available on the germination of *Stipa* species and their populations from Jordan. As species

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with wide distribution ranges often show differences in germination characteristics depending on seed provenance (e.g. Keller & Kollmann 1999; Fenner & Thompson 2005; Ronnenberg *et al.* 2008), we expected a similar pattern for Jordanian *Stipa* species with distribution ranges covering divergent climatic zones. In this context, we investigated germination in four *Stipa* species differing in life form and distributed throughout different phyto-geographical regions in Jordan (*Stipa capensis*, *Stipa parviflora*, *Stipa arabica* and *Stipa lagascae*). Specifically, we addressed the following questions: do the provenances of the four test species differ in germination percentage with respect to their bioclimatic region and what are the optimum temperatures for germination for each species?

Materials and methods

Study region and study species

Biogeographically, Jordan consists of four major phyto-geographical regions (Zohary 1973; Al-Eisawi 1985). These include the Mediterranean (subhumid and semi-arid Mediterranean), Irano-Turanian (arid Mediterranean), Saharo-Arabian and Sudanian (Tropical penetration). In the most recent bioclimatic analysis by Al-Eisawi (1985), nine subdivisions were considered, which fall under four main bioclimatic regions representing a gradient of decreasing precipitation and increasing temperature: (i) subhumid Mediterranean bioclimate;

(ii) semiarid Mediterranean bioclimate; (iii) arid Mediterranean bioclimate; and (iv) Saharan Mediterranean bioclimate.

Four *Stipa* species differing in taxonomical life form, ecology and geographical distribution were studied. The tufted annual *Stipa capensis* Thunp. and the perennial tussockgrass *Stipa parviflora* Desf. (Poaceae; both *Stipa* Section *Stipella* Tzvelev in the sense of Freitag 1985) are widespread in mainly ruderal vegetation of the Irano-Turanian, Saharo-Arabian and Mediterranean regions. The perennial tussock grasses *Stipa arabica* Trin. & Rupr. and *Stipa lagascae* R. & Sch. (both Section *Barbatae* Junge emend. Freitag; Freitag 1985) are semi-desert species of the Irano-Turanian region, extending to the Mediterranean and Saharo-Arabian region (Zohary 1962; Scholz 1991). Flowering and seed dispersal of all study species usually takes place between March and June (Zohary 1962). However, populations located in the Saharo-Arabian and Irano-Turanian phyto-geographic regions start their reproductive cycle earlier in the year than the Mediterranean populations (H. Hamasha, unpubl. data, 2006).

Seed collection and germination tests

Seeds were collected from populations in three out of the four bioclimatic regions, covering most of the natural distribution of *Stipa* species (for ecogeography and climatic profiles of the sampling locations see Table 1; data based

Table 1 Geographical and meteorological descriptions of the seed sources of the *Stipa capensis*, *Stipa parviflora*, *Stipa arabica* and *Stipa lagascae* populations

Population	Bioclimate region†	Geographical		Al	Rn	Ta	Climatic		
		Lt	Ln				Tj	T max	T min
<i>Stipa capensis</i>									
Qatraneh	3	31°12'	36°01'	806	157	24.1	9.4	31.5	2.6
Beren	2	32°07'	35°59'	715	307	24.5	8.9	32.0	3.4
Baun	2	32°23'	35°42'	431	431	26.0	11.1	32.4	6.1
<i>Stipa parviflora</i>									
Azraq	4	31°46'	36°33'	648	90	25.5	8.9	34.4	2.1
Tafila	2	30°32'	35°37'	1237	263	21.2	6.7	28.8	1.5
Humrit Al-Sahin	2	32°05'	35°39'	352	280	26.5	11.7	33.8	6.3
<i>Stipa arabica</i>									
Azraq	4	31°44'	36°32'	648	90	25.5	8.9	34.4	2.1
Tafila	2	30°32'	35°37'	1237	263	21.2	6.7	28.8	1.5
Karak	2	31°06'	35°36'	900	320	22.4	7.4	29.8	1.8
<i>Stipa lagascae</i>									
Tafila	2	30°32'	35°37'	1237	263	21.2	6.7	28.8	1.5
Subayhi-Balqa	2	32°08'	35°40'	492	379	25.5	10.8	32.5	5.7
Jerash	2	32°17'	35°53'	672	427	24.5	9.3	31.1	4.1

† Bioclimatic regions according to Al-Eisawi (1985): 1, subhumid; 2, semiarid; 3, arid; 4 Saharan Mediterranean bioclimate. The climatic profile for each of the sampling sites is based on the data of Hijmans *et al.* (2005). Lt, latitude; Ln, longitude; Al, altitude (m); Rn, mean annual rainfall (mm); Ta, mean hottest month (August) temperature (°C); Tj, mean coldest month (January) temperature (°C); T max, maximum temperature of the warmest month; T min, minimum temperature of the coldest month.

on Zohary 1973, Al-Eisawi 1985 and Hijmans *et al.* 2005). Three of the four species were collected in two bioclimatic regions; only populations of *S. lagascae* were all of semi-arid Mediterranean origin.

With the aim of verifying the existence of differences in germination among populations and at various temperature regimes, we compared the seed germination of 12 populations sampled in May 2007 (three populations per species). Owing to logistical problems, we were not able to conduct initial dormancy tests. Consequently, after sampling, seeds were dry stored in paper bags under ambient laboratory conditions (approximately 20°C). In October 2007, 100 seeds of one population of each species were subjected to a triphenyl tetrazolium chloride test to assess initial seed viability. A total of 450 seeds were selected from each population and were incubated on filter paper moistened with deionised water in 18 Petri dishes with 25 seeds in each dish. As Visser (2001) reported that germination of *S. lagascae* occurs under a wide range of temperatures, three different temperature regimes, representing the environmental conditions of spring, summer and autumn in Jordan, were applied (six repetitions, 32/20°C, 20/10°C and 8/4°C, each at 12 h warm white light/12 h darkness). The dishes were checked three times per week and any germinated seeds were removed. All tests were terminated after 61 days. The data were arcsine square-root transformed and analyzed using a two-way ANOVA. In addition, we compared the germination of two ages of seeds collected from Baun (*S. capensis*) and Azraq (*S. parviflora*) populations in May 2006 and 2007 (17 and 5 months old at the time of sowing) under the same three temperature regimes. The influence of the time of storage on germination was analyzed using a one-way ANOVA. Subsequent post-hoc Tukey's tests revealed potential differences between temperatures and provenances, as well as between years. All statistics were analyzed using SPSS 12.0 (SPSS 2003).

Results

Initial seed viability was >95% in all cases. Temperature had a significant effect on total germination; differences in germination percentages between the various temperatures were highly significant for all species (Table 2; P values ≤ 0.02 in all cases; Fig. 1), but the effects were more pronounced in *S. capensis* and *S. lagascae* (population \times temperature interaction $P < 0.001$). A high percentage of all four species germinated at all three alternating temperatures (8/4°C, 20/10°C and 32/20°C), with the exception of *S. capensis* (Beren population) and *S. lagascae*, which showed lower germination at 8/4°C and 32/20°C, respectively (Fig. 1a,d).

The onset of germination also differed; seeds of *S. capensis*, *S. parviflora*, *S. arabica* and *S. lagascae* populations

Table 2 ANOVA tables showing the effects of the different temperatures (8/4°C, 20/10°C and 32/20°C) and seed populations of *Stipa capensis*, *Stipa parviflora*, *Stipa arabica* and *Stipa lagascae* on seed germination

Source	d.f.	Germination		
		MS	<i>F</i>	<i>P</i>
<i>Stipa capensis</i>				
Populations	2	0.927	92.288	< 0.001
Temperature	2	0.213	21.201	< 0.001
Population \times temperature	4	0.122	12.198	< 0.001
Error	45	0.010		
Sum	54			
<i>Stipa parviflora</i>				
Populations	2	0.041	1.833	0.172
Temperature	2	0.121	5.436	0.008
Population \times temperature	4	0.041	1.851	0.136
Error	45	0.022		
Sum	54			
<i>Stipa arabica</i>				
Populations	2	1.250	63.334	< 0.001
Temperature	2	0.084	4.276	0.020
Population \times temperature	4	0.008	0.392	0.813
Error	45	0.020		
Sum	54			
<i>Stipa lagascae</i>				
Populations	2	0.838	28.340	< 0.001
Temperature	2	4.173	141.12	< 0.001
Population \times temperature	4	0.261	8.833	< 0.001
Error	45	0.030		
Sum	54			

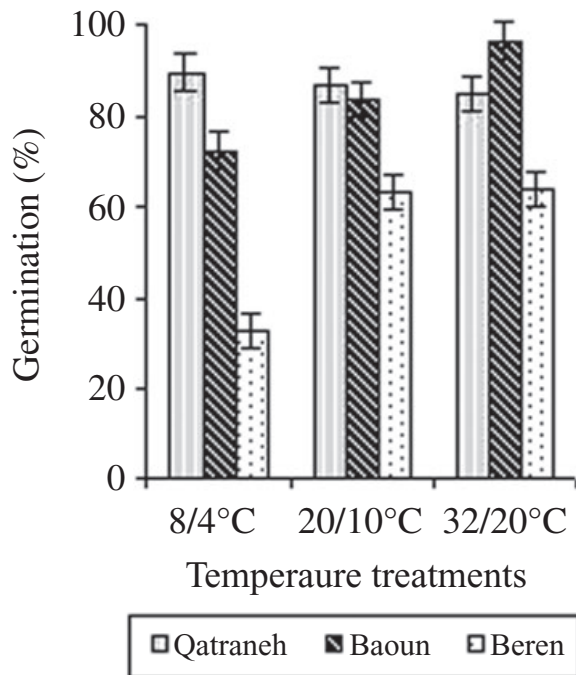
started to germinate after 2, 2, 6 and 20 days of incubation at 32/20°C, respectively; and after 5, 5, 8 and 10 days of incubation at 20/10°C, respectively. At 8/4°C, germination was considerably delayed in all study species (> 12 days in all cases).

The response of seed germination differed significantly among provenances of *S. capensis*, *S. arabica* and *S. lagascae* (Table 2; $P < 0.001$ in all cases; Fig. 1a,c,d) and was insignificant among provenances of *S. parviflora* (Table 2; $P = 0.172$; Fig. 1b). In *S. capensis*, *S. arabica* and *S. lagascae*, seed germination of one or two of the three populations was significantly lower than that of the other populations (Fig. 1a,c,d).

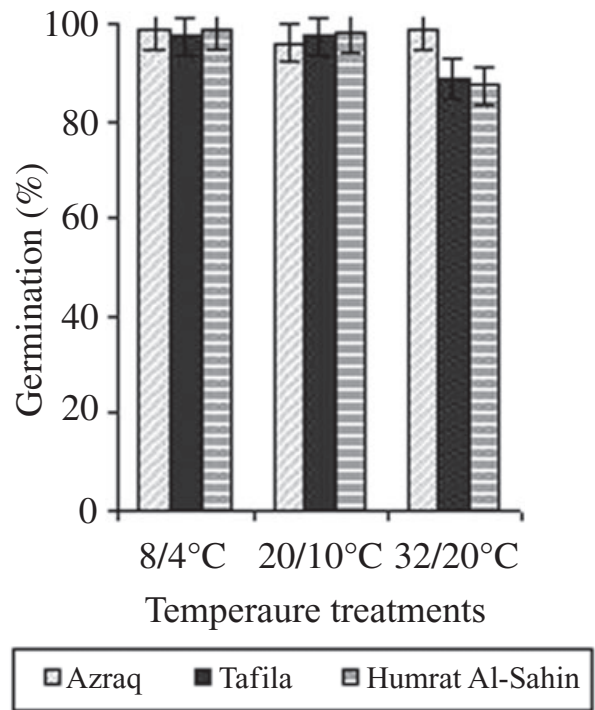
The populations from regions with a high maximum temperature in the hottest month (34.4°C and 31.5°C, respectively) and the lowest mean annual rainfall (90 mm and 157 mm, respectively), namely Azraq and Qatraneh (Table 1), showed higher germination percentages than those from the semiarid Mediterranean regions of Jordan (Table 2; Fig. 1a–c); but these differences were not significant.

The ANOVA examining germination between the two levels of seed age produced insignificant differences in

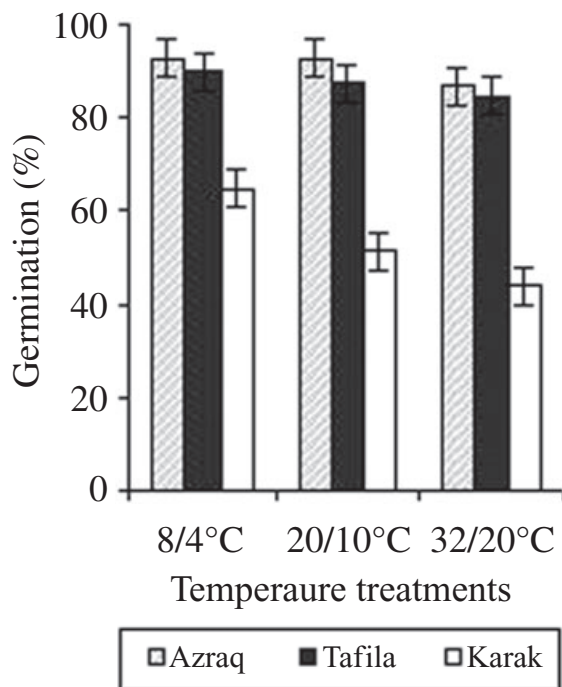
(a) *Stipa capensis*



(b) *Stipa parviflora*



(c) *Stipa arabica*



(d) *Stipa lagascae*

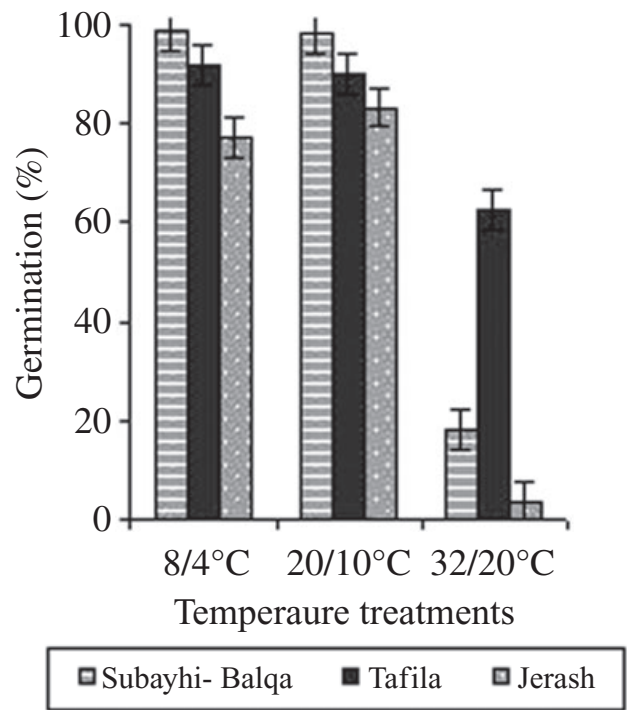


Fig. 1 Germination of various seed populations of (a) *Stipa capensis*, (b) *Stipa parviflora*, (c) *Stipa arabica* and (d) *Stipa lagascae* under the different temperature treatments (significant differences in seed germination among populations and/or temperatures for all four species were revealed by post-hoc Tukey's tests). Populations are arranged in order of decreasing aridity based on precipitation and bioclimatic region.

both *S. capensis* and *S. parviflora* under the three different temperature regimes, with the exception of the Baun population at 8/4°C ($P = 0.009$; germination of the 17 and 5 month old *S. capensis* seeds was 89.7 and 72.7%, respectively).

Discussion

Our results showed that germination of Jordanian *Stipa* seeds can occur over a wide range of temperature regimes. We were not able to assess dormancy, but Visser (2001) reports that fresh seeds of *S. lagascae* have conditional dormancy that is broken progressively in the first year after harvest. After that, germination occurred under a wide range of temperature regimes (Visser 2001), as was also the case for our study species. Our data therefore strongly support the assumption that germination of the four study species is mainly controlled by ambient climatic factors. However, as seeds achieved high germination percentages within only a few days under a wide range of temperature regimes, we assume that water availability, rather than temperature, is the more important factor driving germination in Jordanian *Stipa* species in the field. This is in line with the long imbibition time we assessed for the semidesert species *S. arabica* and *S. lagascae*. However, both ruderals *S. capensis* and *S. parviflora* germinate without any delay under suitable environmental conditions. Zohary (1962) and Feinbrun-Dothan (1986) reported that *S. capensis* usually germinates after the first rains in October–November. Also in agreement with our data, Ronnenberg *et al.* (2007) found that seeds of five mountain steppe species of Central Asia (*Allium polyrhizum*, *Agropyron cristatum*, *Arenaria meyeri*, *Artemisia frigida* and *Artemisia santolinifolia*) germinated best under warm to high temperatures (20/10°C and 32/20°C), whereas temperatures of 8/4°C deferred germination, but did not inhibit it. In addition, they concluded that, in the field, germination of the five species is controlled by ambient temperature and water availability.

The fact that the provenance significantly influenced germination indicates that genetic and environmental factors have a great influence on seed germination traits. The relative importance of both factors is difficult to separate given that the time to the onset of germination and the germination percentages of each population varied. In this regard, Gasque and García-Fayos (2003) found that there was significant variation in seed germination among individuals, populations and years in *Stipa tenacissima*, which also seems to be subjected to both genetic and maternal influences.

The trend for higher germination percentages in arid and Saharan regions than those from semiarid Mediterranean regions is in line with Ronnenberg *et al.* (2008), who reported that the seed viability of *Stipa krylovii* along a

bioclimatic gradient was highest in the driest habitat and decreased with increasing water availability. They also found that seed viability was negatively correlated with annual precipitation over 5 years at the driest study site (K. Ronnenberg, pers. comm., 2005). Shem-Tov *et al.* (1999, 2002) also showed a negative correlation between seed germination of *Plantago coronopus* and *Carrichtera annua* and the average annual precipitation at the site of collection. A possible explanation for higher seed viability under drier site conditions can be drawn from differences in the flowering mode between moister and drier years; the ratio between cleistogamous and chasmogamous flowers of *Stipa* inflorescences was found to be the result of soil moisture conditions (Brown 1952; Ponomarev 1961). Also in accordance, the percentage germination of cleistogamous florets of *Stipa leucotricha* was higher compared with chasmogamous florets (Call & Spoons 1989). This may also hold true for Jordanian *Stipa* as well and corresponds to the limited effectiveness of chasmogamous pollination under dry conditions.

Chen *et al.* (2004) found that in *Hordeum spontaneum*, inhibition of germination and seedling growth by glumellae was higher in mesic ecotypes than in xeric ecotypes. As seeds of our study species were collected from different bioclimatic regions, with wide variations in climate, it is reasonable to assume that variations between populations are an expression of different ecotypes characterized by different germination strategies. The fact that the macroclimate is more mesic in the semiarid Mediterranean regions suggests that *Stipa* populations undergo a change in their germination strategy, which parallels the macroclimatic gradient. It remains to be tested whether dormancy is indeed more common in the semiarid Mediterranean regions of Jordan than in the drier arid Mediterranean and Saharan Mediterranean regions. Moreover, whether differences in germination characteristics originate from maternal or genetic sources is another unsolved question. In this respect, many authors have found that in many species from dry and hot regions, the rate of loss of dormancy increases with the temperature to which the seeds are exposed during summer (Baskin & Baskin 2001; Gutterman 2002; Boeken *et al.* 2004). In the case of Poaceae, the warmer the storage environment and the more time spent in it, the greater the loss of dormancy (Murdoch & Ellis 1992).

Seed age did not influence the germination percentages of two *Stipa* species. Gasque and García-Fayos (2003) also found that seeds of *S. tenacissima* were still able to germinate 28 months after harvesting when stored under laboratory conditions.

In conclusion, germination was variable among different temperature and bioclimatic regions (populations). This flexibility could be interpreted as an efficient survival strategy for species growing under unpredictable

environments, such as the Mediterranean climate (Gutterman 1994). The present study demonstrates that temperature is an important factor for germination. We did not experimentally assess the importance of other potential triggers, but field observations suggest that the amount of rain and the length of the period of increased soil humidity are also decisive. We also assume that, as dispersal occurs in late spring, a lack of water delays seedling recruitment until autumn, when temperatures drop and the winter rains begin. Field studies are necessary prior to any restoration program that focuses on steppe conservation. The influence of seed provenance and the consequent use of local seeds are aspects that have not received much attention in current restoration schemes, but should be considered in the future.

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