Seed germination characteristics of some medicinally important desert plants from the Arabian Peninsula

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Abstract

The arid climate of Saudi Arabia supports many medicinally important species. Germination behaviour is crucial to their establishment in the face of low rainfall and high summer temperatures that produce high evapotranspiration and salt accumulation in the surface soil. We investigated the seed germination biology of three medicinal species from Wadi ad Daygah in central Saudi Arabia: Salvia spinosa (Lamiaceae), Ochradenus arabicus, and Ochradenus baccatus (Resedaceae). We examined the responses of freshly collected seed to constant and alternating temperature, light, and salinity (NaCl). None of the species showed innate dormancy. All achieved high germination percentages over a wide range of diurnally alternating temperatures (5/15, 10/20, 15/25, 20/30 and 25/35 °C). However, the range of temperature for successful germination was narrower at constant temperature, especially for S. spinosa. The rates of germination suggested that all the temperatures examined were suboptimal. Basal temperatures for germination were 5-8 °C. Germination of all the species was promoted by light. All were tolerant of salinity up to 80-100 mM NaCl. Osmotically enforced failure to germinate with increasing salinity was reversible after transfer to fresh water. Understanding these adaptive characteristics will assist the development of effective strategies for the conservation of medicinally important species in arid environments.

Keywords: arid environments; Saudi Arabia; seed germination; temperature response; salinity; desert plants

Introduction

Desert plants tend to be rich sources of secondary metabolites, synthesised in adaptive responses to the multiple environmental stresses of their habitat: extreme temperatures, intense solar radiation, drought, salinity and predation. Many of these compounds are biologically active and consequently of medicinal value (Ksouri et al., 2012). For medicinal plants to be investigated and potentially exploited it is necessary to cultivate them, initially from wild collections and seed banks. A prerequisite for this is clearly a good understanding of their requirements for seed germination, and whether their germination and dormancy responses vary in populations from different localities.

The timing of germination and the environmental cues that regulate it are especially critical for seedling establishment in desert species because they can experience such severe environmental limitations on growth and survival (Mayer and Poljakoff-Mayber, 1989; Gutterman, 1993). In the field, germination from a seed bank in the soil can be regulated by various environmental factors including, temperature and its daily alternation, light, soil moisture content and soil salinity (Baskin and Baskin 2014; Menon *et al.*, 2014; Bhatt and Pérez-García, 2016). Although species of arid areas may have a relatively narrow or wide range of constant temperature for germination, it is also not uncommon for seed dormancy to be substantially relieved by diurnally alternating temperatures and exposure to light. Saline conditions may also enforce dormancy and affect long-term seed viability.

Salvia spinosa (Lamiaceae), Ochradenus arabicus and Ochradenus baccatus (Resedaceae) are perennial desert plants characteristic of the flora of Saudi Arabia (Mandaville, 1990; Migahid, 1996; Al-Turki 1997; Collenette 1998, 1999; Chaudhary, 1999). They are all of known medicinal value and are used in folk medicine in Saudi Arabia (Al-Yousuf *et al.*, 2002; Lu and Foo, 2002; Rahman *et al.*, 2004; Nawash and Al-Horani, 2011; Alqasoumi *et al.* 2012). Salvia spinosa ranges from N. Africa to N.W. Afghanistan but is restricted to the central and northern regions of Saudi Arabia. Ochradenus baccatus is distributed from Libya to Pakistan but is likewise only found in central and northern Saudi Arabia, where it widely distributed in different habitats (Al-Turki, 1997; Chaudhary, 1999). Ochradenus arabicus is endemic to the Arabian Peninsula (Khan *et al.*, 2012).

The germination of *Salvia spinosa* seed from Jordan has been investigated previously by Al-Gharaibeh *et al.* (2017). Seed dormancy and germination of *Ochradenus baccatus* from the Kuwait desert has also been examined (Suleiman *et al.*, 2011; Bhatt and Pérez-García, 2016) but no information for either species is available for Saudi Arabian material. On the other hand, Nadeem

et al. (2012) have studied the effects of plant growth regulators (GA3 and BAP), KNO₃ and thiourea in breaking seed dormancy of *Ochradenus arabicus* from Saudi Arabia. Notwithstanding this varied information on germination biology, it is not clear how their responses to the major environmental cues - temperature, salinity and light - might be of adaptive value to these species. For instance, warmer conditions would signal the end of an adversely cold season and a daily amplitude in temperature would be a precursor to consistently very high summer temperatures. Both temperature variation and light perception are consistent with seeds not being buried with sand beyond their capacity to emerge. The capacity to survive saline conditions could allow dormancy to be enforced by low water potentials until accumulated rainfall becomes sufficient for seedling establishment.

We hypothesised that three co-occurring medicinal species, representing two distantly related plant families, would have evolved similar seed germination characteristics that enable them to exploit episodes of rainfall in an extremely arid environment. Thus, the aim of work described in this paper was to provide comparative information on the germination biology of *Salvia spinosa*, *Ochradenus arabicus* and *O. baccatus* from Wadi ad Dayqah in the desert of central Saudi Arabia. Specifically, we sought to examine the effects on seed germination of: (a) alternating and constant temperatures; (b) sodium chloride concentration; and (c) light and dark. Investigating seed germination will also provide important information for development and implementation effective strategies and protocols for the *ex situ* and *in situ* conservation of these species (*e. g.* Al-Turki *et al.*, 2019, 2020).

2. Materials and methods

2.1. Study site

Wadi ad Dayqah is located in the central region of Saudi Arabia, about 60 km north of Riyadh (Fig. 1). This wadi is c. 22 km long and c. 1 km wide, originating at an altitude of 789 m to the east of Hazoa village (N 21° 58' 15.78, E 058° 51' 35) and running to the west of Salbouk, N 25° 04' 22, E 046° 20' 25.8) at 699 m (near Salbouk village). Its valley penetrates the limestone rocks of the Tweeiq Mountains. Rainfall is regular in pattern (Riyadh region Meteorological station, 2000 – 2007) being predominantly in winter and spring: January (15mm), February (12mm), March (25mm), April (28 mm). The highest temperature recorded was 43.1°C, 42.2°C and 40.3°C for June, July and August respectively and minimum temperatures ranging from 10.2°C for December, 8.7°C, for January and 10.7°C in February.

The pH of the soils in the habitats of these three species is generally alkaline: 8.53 (*Salvia spinosa*), 8.81 (*Ochradenus arabicus*) and 8.90 (*O. baccatus*). Electrical conductivity (EC) measurements were 0.47 mmhos cm⁻¹ (*Salvia spinosa*), 0.71 mmhos cm⁻¹ (*Ochradenus arabicus*) and 0.38 mmhos cm⁻¹ (*O. baccatus*). Corresponding concentrations of sodium and chloride ions were: *Salvia spinosa* (0.89 mM Na⁺100 g⁻¹, 2.07 mM Cl⁻ 100 g⁻¹); *Ochradenus arabicus* (0.99 mM Na⁺ 100 g⁻¹) (2.19 mM Cl⁻ 100 g⁻¹); *O. baccatus* (0.79 mM Na⁺ 100 g⁻¹, 2.05 mM Cl⁻ 100 g⁻¹).

2.2. Seed collection and germination protocol

Mature seeds of *Salvia spinosa*, *Ochradenus arabicus* and *O. baccatus* were collected from 15-40 plants, randomly selected from natural populations in Wadi Dayqah on 10 April 2007. Seeds were air-dried, cleaned and examined immediately. Then seeds were placed in incubators (LEEC, Nottingham, UK, Model PL33) under controlled conditions, in 9 cm diameter Petri dishes on three layers of Whatman No. 1 filter paper that had been moistened with 5 ml of distilled water or different concentrations of NaCl solution. Petri dishes were sealed by plastic film to prevent evaporation. Germination was defined as emergence of a radicle. Effects of constant temperature, fluctuating temperature, light and salinity were investigated in separate experiments for two reasons: a single factorial experiment would have been impracticable; in addition, their combined effects in the field would be likely to be sequential, rather than simultaneous.

2.4. Effects of temperature

In the first experiment, seeds were incubated at four constant temperatures (10, 15, 25 and 35°C), with 12 h darkness and 12 h light each day. In the second experiment, seeds were incubated at five alternating temperatures (5/15, 10/20, 15/25, 20/30 and 25/35°C), with the lower temperature during 12 h darkness and the higher temperature during 12 h light). In every treatment, five replicates of 20 seeds were used. Seed germination was counted daily for 30 days. Final total germination was recorded as well as the time taken to reach 50% of the final germination percentage, across all the replicates (t_{50}).

2.5 Effects of light and dark

Seeds were incubated at 10/20°C (12 h:12 h each day), in either continuous fluorescent light or in continuous darkness. Germination in the light was monitored each day, at which time germinated seeds were counted and removed from the Petri dishes, for 30 days. After this no additional seeds germinated. For continuous darkness, the dishes were wrapped in aluminum foil to prevent any exposure to light and seed germination was counted only after 30 days at the end of experiment.

2.6 Effects of salinity

Seeds were incubated at seven concentrations of NaCl (0, 20, 40, 60, 80, 100 and 150 mM) in a regime of 12 h at 10°C in the dark and 12h at 20°C in the light each day. Five replicate Petri dishes were used for every treatment, with 20 seeds per dish, as previously. The number of seeds germinated was counted daily for 30 days and germinating seeds were removed from the Petri dishes. Final total germination was recorded as well as the time taken to reach 50% of the final germination percentage, across all the replicates (t_{50}).

Seeds remaining ungerminated at the end of experiment were rinsed twice in distilled water and then transferred to dishes moistened with distilled water; then they were incubated for 15 days under the same conditions and germination recorded daily, to assess whether salinity had inhibited germination. After this, ungerminated seeds were tested for viability with a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) (Mackay, 1972; Moore, 1985). Seeds were placed in TTC solution in Petri dishes covered with aluminum foil to exclude light, before being incubated for 24 h at 10/20°C. Red staining indicated living tissue.

2.7 Statistical analysis

Germination percentages were arcsine transformed before statistical analysis, in order to meet expectations of normality and homogeneity of variance, and subjected to a one-way analysis of variance (ANOVA), with post hoc tests to indicate significance differences between treatments (Sokal and Rohlf, 1981). The speed of germination was assessed as the reciprocal of the time to 50% of final germination (1/t50). Linear and two-parameter hyperbolic regressions were fitted and drawn using Sigmaplot 11 (Systat Software Inc.).

3.1 Response to temperature

Salvia spinosa seeds were able to germinate over only a narrow range of constant temperature, with close to 100% final germination at 15 °C and 25 °C but no germination at all at the higher and lower temperatures (Fig. 2). In contrast, almost all seeds (95-100%) germinated across the whole range of alternating temperatures from 5/15 °C to 25/35 °C (Fig. 2). Examination of the rate of germination $(1/t_{50})$ indicates that temperature treatments in both constant and alternating regimes (taking the mean temperature for alternating regimes) were in the suboptimal range (Fig. 3). The two estimates of the base temperature for germination (derived from the linear regressions) were very similar, at 8.4 °C for constant temperature and 8.0 °C for the mean of alternating temperature.

The germination of *Ochradenus arabicus* at constant temperature also was greater than 95% at both 15 °C and 25 °C, although low final germination occurred below this apparent optimum and moderate germination above it (Fig. 2). Again, good germination was evident over a wider range of temperatures under the alternating regime; highest final germination was greatest in the 20/30 °C and 25/35 °C treatments but was only slightly (although significantly) lower at 15/25 °C and 10/20 °C. Only the 5/15 °C treatment was clearly very suboptimal. On the other hand, the rates of germination (1/*t*₅₀) showed that temperature treatments in both constant and alternating regimes were in the suboptimal range (Fig. 3). The two estimates of the base temperature for germination were 5.9 °C for constant temperature and 9.2 °C for the mean of alternating temperature.

Overall final germination *Ochradenus baccatus* was rather lower than the other two species. At constant temperature there was a clear optimum at 15 °C, with significantly lower values at 10 °C and 25 °C, and significantly by far the lowest at 35 °C (Fig. 2). The optimum was essentially the same (10/20 °C) under an alternating regime, with 83% germination. However, in contrast, germination was only slightly (although significantly) lower at 5/15 °C and was maintained at 55% up to 25/35 °C. As with the other two species, the rates of germination (1/ t_{50}) showed that temperature treatments in both constant and alternating regimes were in the suboptimal range (Fig. 3). In this case, the two estimates of the base temperature for germination were distinctly different at 2.5 °C for constant temperature and 7.7 °C for the mean of alternating temperature.

3.2 Response to light

The germination of all three species was significantly promoted by light. The effect was particularly strong in the two species of *Ochradenus*, where the exclusion of light reduced final germination from 75% to 6% and 14%, respectively, for *O. arabicus and O. baccatus*; although the reduction in the dark was smaller in *Salvia spinosa*, it was nevertheless highly significant (Fig. 4).

3.3 Response to salinity

The final germination percentage of seeds of all three species was inhibited progressively by increasing NaCl concentration (Fig. 5). In *Salvia spinosa* the reduction in germination (from 100% in distilled water) was relatively small up to a concentration of 80 mM NaCl. However, germination was only 13% at 100 mM, a highly significant reduction, and there was none at all at 150 mM NaCl. Examination of the rate of germination ($1/t_{50}$) showed it was greatest in distilled water and declined hyperbolically with increasing NaCl concentration (Fig. 6). Subsequent transfer of ungerminated seeds to distilled water resulted in most of them germinating, including 90% of the original number that had remained ungerminated after previously having been subjected to 150 mM NaCl (Table 1). Few were dead or remained dormant.

Germination of *Ochradenus arabicus* showed the greatest tolerance to salinity, with relatively small reductions up 100 mM NaCl and it was the only species able to germinate at 150 mM NaCl, albeit achieving only 30% final germination, in comparison with 78-96% at lower NaCl concentrations (Fig. 5). The rate of germination $(1/t_{50})$ again was greatest in distilled water and declined hyperbolically with increasing NaCl concentration (Fig. 6). Subsequent transfer of ungerminated seeds to distilled water resulted in most of them germinating, including all of those remaining ungerminated after previous subjection to 150 mM NaCl (Table 1). Few were dead or remained dormant.

Final germination percentage in *Ochradenus baccatus* was 60% in distilled water and declined more or less linearly to 30% at 100 mM NaCl, with no germination at 150 mM NaCl. As with the other two species, the rate of germination (1/*t*₅₀) was greatest in distilled water and declined hyperbolically with increasing NaCl concentration (Fig. 6). Subsequent transfer of ungerminated seeds to distilled water resulted in most of them germinating, including 60% of the original number that had remained ungerminated after previously having been subjected to 150 mM NaCl (Table 1). However, rather larger numbers than for the other species were found to be dead and remaining dormant.

4 Discussion

Although perennial species inhabiting the arid wadis of the Saudi-Arabian desert are necessarily adapted to survive the rigours of extreme drought, high temperatures and the consequent evaporative accumulation of salts in the rooting zone, windows of opportunity for the establishment of vulnerable seedlings are nevertheless limited. Seed germination characteristics would be expected to have evolved to exploit episodes of rainfall. Consequently, it is not surprising that *Salvia spinosa*, *Ochradenus arabicus* and *O. baccatus* from Wadi ad Dayqah showed broadly very similar germination responses to the environmental factors tested experimentally, despite coming from phylogenetically unrelated plant families: *Salvia* is classified in the order Lamiales of the Asterid clade, whereas *Ochradenus* is in the order Brassicales of the Rosid clade (The Angiosperm Phylogeny Group *et al.*, 2016).

Freshly collected seed of all three species could germinate rapidly after imbibition, indicating an absence of innate dormancy. This is consistent with results for populations of S. spinosa from three different phytogeographic regions (Mediterranean, Irano-Turanian and Saharo-Arabian) of Jordan (Al-Gharaibeh et al., 2017). However, in a recent comparison of 15 species of Salvia from Iran, Abdollahi et al. (2021) used a standardized treatment for breaking seed dormancy, although it is not clear whether this was strictly necessary for the two accessions of S. spinosa. On the other hand, there appears to be evidence of infraspecific variation in respect of dormancy in *Ochradenus*. Bhatt and Pérez-García (2016) reported that freshly matured seeds of O. baccatus from Qatar were not able to germinate, even when they were treated with gibberellic acid (GA3), and required long-term storage to break dormancy. Similarly, Gairolam and El-Keblawy (2020) found non-deep dormancy in populations from Qatar and the UAE that could be broken with GA3 but they had previously stored the seeds for 25 months. Suleiman et al. (2012) also obtained good germination, without any pre-treatments, in stored material from a Kuwait Seed Bank. Unlike our findings, there have been two previous reports of deep dormancy in the Arabian endemic O. arabicus: Nadeem et al. (2012) found little germination in freshly collected seeds from Saudi Arabia, although it was improved by moderate amounts of GA3 and storage for up to 12 months; similar conclusions can be drawn from the work of Gairolam and El-Keblawy (2020) with a population from the UAE, albeit with substantially lower overall germination rates. The ability to respond rapidly to rainfall after dormancy enforced by predictable annual drought could be regarded as adaptive in a desert environment, although delayed, asynchronous germination might also be of advantage to these desert perennials (Nadeem et al., 2012).

All three species could germinate well over a remarkably wide range of temperatures. This agrees with previous findings for S. spinosa (Al-Gharaibeh et al., 2017) but the temperature responses of the Ochradenus species seem not to have been investigated previously. O. arabicus appeared more sensitive to inhibition of germination at the lower extremes of temperature and O. baccatus to those at the highest temperatures. Furthermore, all species had a narrower range of temperature for high final germination at constant temperature than under diurnally alternating temperatures. This was particularly marked in S. spinosa, which showed no germination below 15 °C or above 25 °C at constant temperature but 95-100% germination from 5/15 °C to 25/35 °C. Similarly, Al-Gharaibeh et al. (2017) reported 95% germinability of S. spinosa under a diurnal alternation as low as 4/8 °C. Steinbauer and Grigsby (1957) found that out of 85 species selected from 15 families, more than 80% showed higher germination at alternating temperatures compared to constant temperatures. Inhibition of seed germination at constant temperature has been regarded as a depth-sensing mechanism, as temperature amplitude is reduced with increasing soil depth (Baskin and Baskin, 2014) and it would be advantageous for seeds buried beyond their capacity to emerge to remain dormant. This idea is also consistent with promotion of germination by light found for all three species, as light flux also declines rapidly with soil depth. A considerable portion of the seed in these species must be light sensitive and this is a well-known phenomenon in many species (Baskin and Baskin, 2014). There is previous evidence for there being little or no germination in the absence of light for O. arabicus, although in this study light had little effect on the germination of O. baccatus (Gairolam and El-Keblawy, 2020). Such light stimulation has been reported for Salvia mellifera in fire prone environments (Keeley, 1986) but there was no light-promoting effect in S. verbenaca, an invader of arid rangelands (Javaid et al., 2018).

The generally high germinability of seeds of these species over such a wide range of alternating temperatures might suggest that temperature per se is not critical for the timing of germination. However, the rate of germination $(1/t_{50})$ suggests otherwise, as it increased linearly over the whole range of temperatures investigated, under both constant and alternating regimes. Thus, the optimal average temperature was no lower than 30 °C for any of the species, allowing rapid germination with rising spring temperatures. Possibly of more significance is the basal temperature, below which no germination occurs. Taking the average of estimates from constant and alternating temperature regimes, this was clearly c. 8 °C for *Salvia spinosa* and *Ochradenus arabicus* and c. 5 °C for *O*.

baccatus. This could affect timing by protecting seeds from germinating in the coldest part of the wet season (i.e. winter) and allowing germination in the more favourable warmer part (i.e. spring).

Although none of the three species investigated is a halophyte, the germination of all of them showed appreciable tolerance of salinity. There was only a gentle decline in total germination salinity below an NaCl concentration of 80 mM. Salvia spinosa was the least tolerant, and a similar response for this species was recorded by Al-Gharaibeh et al. (2017). Ochradenus baccatus was marginally more tolerant in our experiment, although it showed little germination at 100 mM NaCl in the experiment of Bhatt and Pérez-García (2016). O. arabicus was the only species that showed any germination at 150 mM. The rapid decline in the rates of germination with increasing salinity reinforces its adverse initial impact on all three species. However, as in halophytes, the effect of salinity was a dormancy imposed by osmotic restriction of water uptake (Woodell, 1985); although there was some mortality, the majority of seeds remained viable could germinate after they were transferred to fresh water. In contrast many glycophytes suffer irreversible toxic effects from high ion concentrations, particularly chloride concentrations (e.g. Munns et al., 1995; Munns, 2002). We could speculate on the significance of these findings for species in the Arabian desert: in the field, dormancy imposed by the evaporative accumulation of salts would tend to postpone germination until episodes of rain in winter or spring leached them from the surface soils and allowed germination at time more favourable for seedling establishment.

5 Conclusion

This study provides a comprehensive comparison of the factors that regulate the seed germination behavior of three sympatric plants of medicinal importance in flora of Saudi Arabia (*Salvia spinosa*, *Ochradenus baccatus* and *O. arabicus*). The findings help to explain the reproductive adaptations that confer fitness on these species in an arid environment with erratic, seasonal rainfall and very high summer temperatures. These species had no innate (physiological) seed dormancy and could therefore be able to exploit favorable conditions rapidly. They had distinctive germination responses to temperature, light and salinity. Germination was promoted by diurnally alternating temperatures, over a wide range of temperatures for germination were determined as 5-8 °C, which would inhibit germination in the coldest parts of winter when prospects for survival would not be optimal. Although all three species are salt-tolerant for glycophytes, moderate salinity progressively enforced dormancy (an osmotic effect), which was removed by return to non-saline conditions; this would

promote germination under favorable conditions for establishment after episodes of winter rainfall. The combined effects of temperature, light and salinity on seed germination provide a framework that can explain seedling establishment and, ultimately, the occurrence of these species in a severely arid environment. The findings provide information that will assist in the development and implementation of effective strategies for the *ex situ* and *in situ* conservation of such medicinally important species.

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Table 1. Fate of seeds that did not germinate in the salinity test. Proportions (%) of original number germinating on transfer to distilled water, remaining dormant, and dead (as judged by the tetrazolium test) in *Salvia spinosa, Ochradenus arabicus* and *Ochradenus baccatus* at 10/20 °C (12h light/12h dark)

Species	NaCI (mM)	Germinating	Dormant	Dead
Salvia spinosa	0	-	-	-
	20	-	-	3
	40	-	3	3
	60	4	-	4
	80	8	-	3
	100	67	-	2
	150	95	-	5
Ochradenus arabicus	0	-	-	4
	20	-	-	6
	40	-	4	4
	60	7	-	3
	80	10	-	2
	100	12	5	5
	150	70	-	-
Ochradenus baccatus	0	-	20	20
	20	22	11	10
	40	25	20	6
	60	20	15	17
	80	35	12	10
	100	40	10	20
	150	60	20	20



Fig. 1. Location of Wadi ad Dayqah in the central region of Saudi Arabia. The area from which seed was collected is shown in red.



Fig. 2. Response of final seed germination to constant temperature (left-hand panels) and alternating temperature (right-hand panels) for (A) *Salvia spinosa*, (B) *Ochradenus arabicus* and (C) *O. baccatus*. Vertical bars represent \pm SE. Columns that do not share a letter are significantly different (P<0.05).



Fig. 3. Relationship between the rate of seed germination (1/time to 50% germination, $1/t_{50}$) to constant temperature (•) and alternating temperature (•) for *Salvia spinosa, Ochradenus arabicus* and *O. baccatus*.



Fig. 4. Response of final seed germination to light for *Salvia spinosa*, *Ochradenus arabicus* and *O. baccatus*. Vertical bars represent \pm SE. Columns that do not share a letter are significantly different (P<0.05).



Fig. 5. Response of final seed germination to salinity (NaCl concentration) for *Salvia spinosa*, *Ochradenus arabicus* and *O. baccatus*. Vertical bars represent \pm SE. Columns that do not share a letter are significantly different (P<0.05).



Fig. 6. Relationship between the rate of seed germination (1/ time to 50% germination, $1/t_{50}$) and salinity (NaCl concentration): (a) *Salvia spinosa*, y = -0.2793+(1.2789*32.3019)/(32.3019+x), $r^2 = 0.99$, P = 0.0005; (b) *Ochradenus arabicus*, y = -0.1546+(1.1599*23.0656)/(23.0656+x), $r^2 = 0.99$, P < 0.0001; (c) *Ochradenus baccatus*, y = -0.0118+(1.0117*5.4687)/(5.4687+x), $r^2 = 0.99$, P < 0.0001;