

Research Note

Germination response of *Salsola schweinfurthii* (Chenopodiaceae) to salinity and winged perianth removal

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Abstract

Salsola schweinfurthii is a perennial branched halophytic shrub that inhabits arid environments in and around the Arabian Peninsula. Its tolerance to extreme drought renders it suitable for urban arid landscaping. Germinability of intact and de-winged seeds (winged perianth removed) was determined under two photoperiods (0 and 12 hours light per day), three thermoperiods (daily low/high of 15/25, 20/30 and 25/35°C) and five salinity levels (0, 100, 200, 400 and 600 mM NaCl). Germination was maximised (93%) by de-winging and incubation in 12 hours light at 25/35°C. Intact and de-winged seeds both exhibited positive photoblastism. Germination of intact seeds was entirely prevented by the lowest level of salinity, and only slightly less prevented in de-winged seeds. Ability to germinate returned after saline solution was replaced with distilled water. The ability for *S. schweinfurthii* seeds to remain viable through a temporary period of salinity indicates an adaptation to unpredictable soil surface conditions in arid environments. Artificial propagation rates might be increased by removing perianths and sowing before or after mid-winter.

Experimental and discussion

Population expansion in Arabian Gulf states has produced an increasing demand for green urban landscapes, while groundwater supplies have declined in both quality and quantity (Al-Rashed and Sherif, 2000; Yagoub, 2004). Use of endemic xerophytes in green outdoor spaces could reduce maintenance costs (Vickers, 2006). Propagation information is available for commercialised exotic species but lacking for endemic species (Shahin and Salem, 2014), which are therefore underutilised.

Salsola schweinfurthii Solms-Laub. (Chenopodiaceae) is a perennial branched halophytic shrub growing to 0.8 m height and found in Saudi Arabia, Oman, Egypt,

Jordan, United Arab Emirates and Qatar (Miller *et al.*, 1996; Norton *et al.*, 2009). It is of interest for urban landscaping in arid regions due to its visual appearance and tolerance of limited, degraded groundwater. The present study was conducted to investigate the germination ecology, including the influence of wing perianths, light, temperature and salinity on germination, and the ability of the seeds to remain viable through temporary periods of saline stress.

Salinity of desert soils can be highly spatiotemporally variable, due to micro-topographic variations and unpredictable precipitation (Ungar, 1995). Temporal control of germination in arid ecosystems is more important than spatial in most seasons (Venable *et al.*, 2008), but spatial opportunities for recolonisation regularly arise. The botanical significance of salinity varies with phenology, and germination is particularly affected due to the increased difficulty of seed water uptake (Yan *et al.*, 2013). Consequently, ability to germinate is a key mechanism for desert halophytes to have spatiotemporal control over plant establishment (Gutterman, 2002).

Germination in natural conditions is normally limited by water stress, salinity or adverse temperature (Neo and Zedler, 2000; Al-Khateeb, 2002). Light, temperature and salinity affect germination in different ways among halophytic species (e.g. Khan *et al.*, 2000a, b; Mehrun *et al.*, 2007; Zaman *et al.*, 2010; Pahani *et al.*, 2012; El-Keblawy *et al.*, 2013; El-Keblawy and Bhatt, 2015). Among *Salsola* spp., germination is affected by the presence of winged perianths. In *Salsola affinis* C.A. Mey. ex Schrenk the perianths inhibited germination by restricting water absorption, but also protected the seed from salinity damage (Wei *et al.*, 2007; Xing *et al.*, 2013). In *S. komorovii*, dimorphic wing types contained different levels of abscisic acid, which inhibited germination at differential rates (Takeno and Yamaguchi, 1991).

Wings are a common evolutionary adaptation to facilitate seed/achene dispersal by wind (Chaloner and Sheerin, 1981; Howe and Smallwood, 1982; Jurado *et al.*, 1991). Dimorphism of achenes has been reported to aid seed dispersal adaptation to uncertain environmental conditions, favouring absence of wings when conditions are favourable and presence when not (Brändel, 2007). *S. schweinfurthii* seeds mature in November / December and have wing perianths to facilitate wind dispersal (Ridley, 1930).

Mature seeds of *S. schweinfurthii* were collected from 50 naturally occurring plants in Al Wakra, Qatar (25.1769N, 51.6148E) in December 2014. Plants were visually selected to represent the genetic diversity of the population. The coastal location is hyper-arid, with a sporadic annual precipitation of 152 mm occurring mostly between November and March (International EMECs Center, 2003), and evapotranspiration of between 2 and 10 mm day⁻¹, in December and June, respectively (Abu Sukar *et al.*, 2007). Midsummer is hot and humid, with daytime temperatures peaking above 50°C in August and humidity approaching 100% in May.

Visually abnormal seeds were removed. The rest were cleaned and stored in paper bags at room temperature (20 ± 2°C) until use in April 2015. Wings were then removed from half the seeds using an improvised rubber thresher, developed to minimise seed coat scarification during wing removal (El-Keblawy *et al.*, 2013). The mass (mean and standard deviation, SD) was determined for intact and de-winged seeds by weighing three replicates of 50 seeds each.

Germination of intact and de-winged seeds was studied for 10 days after seed hydration, under three thermoperiods and two photoperiods using three LMS (UK) incubators. For each treatment, four replicates of 25 seeds were placed in 90 mm-diameter tight-fitting Petri-dishes containing one disk of Whatman No. 1 filter paper and 10 ml distilled water. Thermoperiods were night/day temperatures of 15/25, 20/30 and 25/35°C, chosen to simulate 12-hour temperature averages for mid-winter (January) and surrounding months (December and February, November and March, respectively). Photoperiods were 0 and 12 hours light daily, to simulate buried and exposed seeds, respectively. Night/day temperatures lasted for 12 hours and corresponded with night/day photoperiod. The 0 hours photoperiod was simulated by wrapping Petri-dishes in two layers of aluminum foil (Parafilm, Sigma-Aldrich, UK). Germination was defined as the presence of a radicle greater than 2 mm length. Germinated seedlings were counted and removed daily from the 12 hours photoperiod treatments, and at the end of the 10 days for the 0 hours photoperiod treatments. Germination was considered completed after three consecutive days (days 10-12 after hydration) of no seeds germinating in the 12 hours photoperiod treatments.

Intact and de-winged seeds were treated with 0, 100, 200, 400 and 600 mM NaCl solutions. Four replicates of 25 seeds each were used for each treatment. Germination was conducted in 90 mm-diameter tight-fitting Petri-dishes containing one disk of Whatman No. 1 filter paper and 10 ml saline solution, and sealed with Parafilm to minimise evaporation. Treatments were incubated at one thermoperiod (20/30°C) and two photoperiods (0 or 12 hours light).

Seeds that failed to germinate in saline treatments were rinsed and placed in distilled water for a further 10 days. Treatments were incubated at the same thermoperiod (20/30°C) and photoperiods as during the first 10 days. Recovery percentage (RP) was calculated per replicate to represent the number of seeds germinating in distilled water as a percent of those not germinating under saline conditions (Yang *et al.*, 2010):

$$RP = \frac{(a - b)}{(c - b)} \times 100$$

where: a = total number of seeds germinated (saline + distilled treatments)

b = number of seeds germinated in the saline treatment

c = total number of seeds tested (25)

Saline and distilled treatment germinations were calculated as (b/c) × 100 and (a/c) × 100, respectively.

Germination percentages of replicates were normalised by arcsine square-root transformation, and assessed by ANOVA and Fisher's Least Significant Differences (LSD) *post hoc* test, using IBM SPSS Statistics 21. Shapiro-Wilk test for normality was used. Note that tables 1 and 2 display untransformed percentages. Seeds that appeared to not contain viable embryos at the end of the experiment were excluded from analysis. Mean germination time (MGT, mean days ± SE) was calculated according to Ellis and Roberts (1981), where D is the number of days from hydration to germination and N is the number of seeds germinated on day D.

$$MGT = \frac{\sum DN}{\sum N}$$

Germination of *S. schweinfurthii* was significantly affected by photoperiod, salinity, by thermoperiod to a lesser extent ($P < 0.001$ for all three factors), and by their interactions. Germination percentage was greatest for de-winged seeds exposed to light (table 1) and not salinity (table 2). Thermoperiod had only a marginally significant effect ($P = 0.044$) with no clear trend, although its interaction with wing removal was significant ($P = 0.007$). Germination of intact seeds was completely prevented by salinity and was greatly reduced in seeds with removed perianth. Removal of perianth wings reduced seed mass by 40.1%, from 1.41 to 0.85 mg. The interaction between wing removal and 12-hour light photoperiod was highly additive ($P < 0.001$). This interaction was present in two experiments, but was absent in seeds recovering from salt exposure.

Table 1. Germination and mean germination time of *Salsola schweinfurthii* seeds with perianth wings intact and removed, under six photoperiod / thermoperiod regimes. Mean germination time could only be assessed in treatments subjected to light. Values with the same superscript letter within a column are not significantly different based on Fisher's Least Significant Difference at $P = 0.05$ significance.

Photoperiod (hours of light per day)	Thermoperiod (°C for 12 hours night/day)	Germination (%)		Mean germination time (days)	
		Wings intact	Wings removed	Wings intact	Wings removed
0	15 / 25	33 ^{ab}	34 ^a	-	-
	20 / 30	40 ^b	44 ^a	-	-
	25 / 35	20 ^a	38 ^a	-	-
12	15 / 25	44 ^b	82 ^b	2.82 ^b	4.35 ^b
	20 / 30	43 ^b	88 ^b	1.52 ^a	2.82 ^a
	25 / 35	35 ^{ab}	93 ^b	1.52 ^a	2.40 ^a

Table 2. Germination of *Salsola schweinfurthii* seeds with intact and removed winged perianths, in five salinity and two photoperiod treatments, after 10 days in saline, followed by 10 days in distilled water. Thermoperiod was 20/30°C alternating on a 12-hour cycle. Values with the same superscript letter within a column are not significantly different based on Fisher's Least Significant Difference at $P = 0.05$ significance.

Photoperiod (hours of light per day)	Salinity (mM NaCl)	Germination (%) of seeds with winged perianth intact			Germination (%) of seeds with winged perianth removed		
		10 days saline	10 days distilled	Recovery percentage	10 days saline	10 days distilled	Recovery percentage
0	0	40 ^b	-	-	44 ^b	-	-
	100	0 ^a	57 ^a	57	1 ^a	77 ^a	76.8
	200	0 ^a	58 ^a	58	0 ^a	78 ^a	78
	400	0 ^a	49 ^a	49	0 ^a	68 ^a	68
	600	0 ^a	53 ^a	53	0 ^a	64 ^a	64
12	0	43 ^b	-	-	88 ^b	-	-
	100	0 ^a	59 ^a	59	22 ^{ab}	78 ^a	71.8
	200	0 ^a	53 ^a	53	5 ^a	78 ^a	76.8
	400	0 ^a	65 ^a	65	5 ^a	76 ^a	74.7
	600	0 ^a	53 ^a	53	5 ^a	72 ^a	70.5

The greater tolerance to thermoperiod is consistent with that of other species within the Chenopodiaceae family, such as *S. imbricata* (Zaman *et al.*, 2010) and *Suaeda aralocaspica* (Wang *et al.*, 2008), and may be an adaptation of the species to germinate under conditions of optimal moisture and salinity, with less regard to temperature. Seeds are dispersed in November / December (Mandaville, 1990) when average, minimum and maximum temperatures are 24, 12 and 42°C, respectively (Islam *et al.*, 2009). Seeds may germinate from this time until April if there is rainfall, or they may remain dormant for another season. The period of December to April has a greater chance of rainfall (Boer, 1997). Mean germination time (MGT) was significantly longer at the coldest thermoperiod and after wing removal ($P < 0.001$), but these factors acted independently (table 1).

Salsola schweinfurthii seeds exhibited positive photoblastism, which has previously been linked to soil seed bank longevity (Benítez-Rodríguez *et al.*, 2004). Seeds buried too far from light are inhibited from germination if their seed reserves are insufficient to produce a successful emergent seedling (Kozłowski, 2000). It is possible that the perianth decays over time, making the seed increasingly susceptible to germination at depth.

Response to saline conditions was temporal. Of seeds that were inhibited from germinating in saline conditions, most germinated when saline conditions were removed (table 2). Recovery percentage (RP) was significantly greater in de-winged seeds ($P < 0.001$) but not affected by initial salinity level or photoperiod. Under natural conditions, seeds are more likely to germinate when salt has been removed by rainfall, dew or irrigation. Similar results have been observed in other species, (Gul and Weber, 1999; Huang *et al.*, 2003; Zia and Khan, 2004; Qu *et al.*, 2008; El-Keblawy and Bhatt, 2015). Halophyte germination percentages are normally greatest in non-saline conditions (Baskin and Baskin, 1998) and for *S. vermiculata*, MGT was increased (Guma *et al.*, 2010). Perianth wings increased the suppression of germination under saline conditions and, as with photoblastism, it is possible that this suppression is weakened over time as the wing decomposes. Perianth wing removal increases germination percentages in many Chenopodiaceae species (Baskin and Baskin, 1998), possibly by providing a mechanical barrier to radicle emergence (Wei *et al.*, 2008), or by removing tissue that has accumulated chloride ions (Beadle, 1952).

In conclusion, *S. schweinfurthii* seeds are most likely to germinate if they are at the soil surface in moist, non-saline conditions, in temperatures representative of winter and parts of autumn and spring. The winged perianth plays a significant role in regulating germination, and seeds can remain viable during a period of exposure to high salinity. Artificial propagation rates might be optimised by removing perianths and sowing before or after mid-winter.

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