



# Eco-physiological studies on desert plants: germination of *Halothamnus iraqensis* Botsch. seeds under different conditions

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**Abstract:** With the aim to investigate if the halophyte *Halothamnus iraqensis* Botsch. can be suitable for re-vegetation and remediation of salt-affected lands, this study evaluated (1) the effects of photoperiod, thermoperiod, storage period and wings' presence on its seed germination, and (2) the ability of its seeds to have successful germination recovery after salt stress. Germination tests in different photoperiods (12 h light/12 h darkness and total darkness) and thermoperiods (15°C/20°C and 20°C/25°C) were conducted for seeds collected in 2012, 2013, 2014, 2015 and 2016. The seeds collected in 2016 were sown under different salinity levels (0, 100, 200, 400 and 600 mM NaCl) to assess the salinity tolerance during the germination. Wings' presence highly inhibited seed germination of this species in both photoperiods and thermoperiods under all salinity level treatments. In addition, the germination recovery occurred well when seeds were deprived of their wings. The photoperiod of 12 h light/12 h darkness and the thermoperiod of 15°C/20°C were the best conditions for seed germination. Germination percentages of *H. iraqensis* seeds decreased with the increasing storage duration, especially after three years of the collection. In addition, *H. iraqensis* seeds were able to germinate under different salinity levels, and their germination percentages decreased with increasing salinity levels. *H. iraqensis* seeds have the ability to recover their germination after alleviating the salt stress, irrespective of photoperiod, highlighting the halophilous character of this species.

**Keywords:** wings' presence; storage period; photoperiod; thermoperiod; salt stress; *Halothamnus iraqensis* Botsch.

**Citation:** Arvind BHATT, Narayana R BHAT, Valentina MURRU, Andrea SANTO. 2019. Eco-physiological studies on desert plants: germination of *Halothamnus iraqensis* Botsch. seeds under different conditions. Journal of Arid Land, 11(1): 75–85. <https://doi.org/10.1007/s40333-019-0121-7>

## 1 Introduction

Desert species are well adapted to cope with high temperatures, irregular water availability and high salinity, hence they are able to successfully colonize and survive under harsh environmental conditions (Khan and Weber, 2006; Rewald et al., 2011). Substrate salinity is one of the factors that mainly affects species germination and growth in arid and semi-arid regions (Paul, 2012; Flowers and Colmer, 2015; Song and Wang, 2015; Bhatt and Santo, 2017a). Halophytes have the ability to adapt to the dry saline soil conditions through morphological, anatomical and physiological adaptations (Flowers and Colmer, 2008; Hasanuzzaman et al., 2014; Song and Wang, 2015). Therefore, planting halophytic plants could be a potential way to rehabilitate the salt-affected lands

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Received 2018-03-15; revised 2018-05-02; accepted 2018-06-21

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in arid and semi-arid regions. Furthermore, halophytes might be used as fodder and fuel or for medicinal purposes (Hasanuzzaman et al., 2014; Gairola et al., 2015; Song et al., 2016; Xu et al., 2016).

Seed germination is one of the most important phases in the life cycle of halophytes and germination responses to different environmental factors play an important role in determining their distribution in saline environments (Song et al., 2005; Li et al., 2010). In particular, under natural conditions, germination of halophytic species is controlled by environmental factors such as light (Huang et al., 2003; Bhatt et al., 2016a), temperature (El-Keblawy et al., 2013; Bhatt et al., 2016b) and soil salinity (Khan et al., 2002; El-Keblawy and Bhatt, 2015; Bhatt and Santo, 2016). The requirement to adapt to each of these environmental factors varies among species, and therefore each halophyte responds differently during its seed germination phase (Khan and Ungar, 1997; Noe and Zedler, 2000).

The *ex-situ* storage of germplasm is a necessary step for the long-term conservation of rare and endangered species, and the duration of successful storage without the loss of seed viability varies among species. Thus, knowing the seed longevity of a species is essential for its effective management and long-term conservation (Li et al., 2008; Xu et al., 2016). Seed banks are widely used for the conservation of threatened and economically important species, due to the possibility to conserve large amounts of genetic diversity (seeds) within a small volume, and consequently with relatively low management costs (Martin et al., 2001). However, seed storage may often negatively influence seed viability in relation to conditions (e.g., temperature and humidity) and time of storage (Shaban, 2013; Mahmood et al., 2016). It is a fundamental part of maintaining a seed bank by continuous monitoring seed viability through germination testing at regular intervals, which allows evaluating the genetic and physiological erosion during the storage period (Ruiz et al., 1999). Some studies have been already conducted to assess the seed viability after *ex-situ* storage of some desert halophytes, such as *Salsola imbricata* (Zaman et al., 2010; El-Keblawy, 2014), *Zygophyllum qatarense* (Zaman, 2013) and *Haloxylon salicornicum* (El-Keblawy, 2014). However, the effects of storage on seed viability are unknown for most of the desert halophytes.

The genus *Halothamnus* Jaubert & Spach belongs to the Amaranthaceae family and comprises 21 species distributed in arid and semi-arid habitats of Saudi Arabia, United Arab Emirates (UAE), Yemen, Kuwait, Turkey, Armenia, China, Pakistan and Afghanistan (Kothe-Heinrich, 1993; Musaddiq et al., 2015). Several species of this genus are used as fodder and for medicinal purposes (Kinzikaeva, 1968; Musaddiq et al., 2015). Previous studies confirmed that all the species of *Halothamnus* have a unique  $C_4$  photosynthesis mechanism and this gives them more resistance to drought and high salinity as compared to  $C_3$  plants (Zalensky 1981; Yin and Wang, 1997).

*Halothamnus iraqensis* Botsch. is a perennial branched shrub that grows up to 50 cm in height. It is widely spotted in Saudi Arabia, UAE, Kuwait, Oman and Iraq (Kothe-Heinrich, 1993). The seeds of *H. iraqensis* are enclosed by a persistent wing which is retained in the mature fruit with the pericarp (Mandaville, 2011). The wings' presence helps this species in its seed dispersal, which occurs through anemochory (Kothe-Heinrich, 1993). Most species of the *Halothamnus* (including *H. iraqensis*) which are frequently grazed by camels, sheep and goats have excellent sand-fixation ability and can tolerate extreme temperatures, high drought and high salinity. Therefore, *H. iraqensis* has the potential to be used for rehabilitating the degraded arid and salt-affected lands (Kinzikaeva, 1968). However, until now, there is no information available on the effects of long-term seed storage and role of wing on its seed germination and salinity tolerance. It could be in help for restoration or rehabilitation of salt-stressed habitats to understand the long-term seed storage behavior of *H. iraqensis* (Fenner and Thompson, 2005; Liu et al., 2011).

The aims of this study were to investigate the effects of photoperiod, thermoperiod, storage period and wings' presence on the germination of *H. iraqensis* seeds and the ability of the seeds to have successful germination recovery after alleviating the salt stress.

## 2 Materials and methods

### 2.1 Study area

Kuwait is characterized by a desert climate with a long, dry and hot summer and a cool winter. The day time temperature usually exceeds 50°C in summer and below 4°C in winter (Annual Statistical Report, 2006). Precipitation is scarce, with less than 114 mm annually and mostly occurring in October to March in the next year (Omar et al., 2007). Soil in Kuwait is mostly sandy with high infiltration rate and is calcareous in nature (Omar et al., 2007).

### 2.2 Seeds collection

Seeds of *H. iraqensis* were collected from a natural vegetation population in Julaia, Kuwait (28°53'29"N, 48°14'17"E), in the period of their natural dispersal (December) in 2012–2016. The seeds were collected from 50 randomly selected plants and mixed together to represent the genetic diversity of the population. They were then stored in brown paper bags at room temperature (20°C±2°C) in Kuwait Institute for Scientific Research (KISR) Seed Bank until the experiment started in December 2016. The seeds collected in 2016 were used for the experiments within a week after the collection. When the seeds were used for the experiments after the storage, the seed lot collected during each year was divided into two sub-lots, in which one lot of seeds was deprived of wings (de-winged seeds; wings were carefully removed by hand) and the other remained intact (winged seeds or intact seeds). The seed mass of the lot for each year was determined by weighing three replicates of 50 seeds each.

### 2.3 Effects of photoperiod, thermoperiod, storage period and wings' presence on seed germination

The seeds of *H. iraqensis* collected in 2012–2016 were incubated in two growth chambers to investigate the photoperiod and thermoperiod requirements during the germination. The chambers were set at night/day (12 h/12 h) temperature regimes of 15°C/20°C and 20°C/25°C in either continuous darkness (dark treatment) and 12 h light/12 h darkness (light treatment). In the dark treatment, the petri-dishes were wrapped in two layers of aluminum foils to prevent any exposure to light. The germination tests were conducted in 9-cm petri dishes containing three disks of Whatman No. 1 filter paper moistened with 10 mL of distilled water. Four replicates of 25 seeds each were used for each treatment. The seeds were considered to be germinated with the emergence of the radicle ( $\geq 2$  mm). Germinated seeds were counted and removed daily for a 30 d period. However, seeds incubated in the dark were checked only at the end of the test. At the end of the germination test, the ungerminated seeds were dissected to evaluate the embryo status and viabilities (living and therefore white; turgid and brown and therefore dead) under a binocular microscope. The permeability of winged and de-winged seeds, was evaluated by measuring the seed weight after an imbibition test in distilled water for 24 h.

### 2.4 Effects of salinity on seed germination

Germination tests under different salinity levels (0, 100, 200, 400 and 600 mM NaCl) were conducted to assess the salinity tolerance for seeds of *H. iraqensis* collected in 2016. Four replicates of 25 seeds each were used per treatment for both winged and de-winged seeds. Seeds were sown in 9-cm diameter petri dishes on three layers of Whatman No. 1 filter paper, moistened with 10 mL of the saline solution for each salinity level. The petri dishes were sealed with parafilm to minimize evaporation and external contamination. The petri dishes were incubated at 15°C/20°C in either dark or light treatments. For the dark treatment, the dishes were wrapped in aluminum foils as described above. Germinated seeds were counted and removed every day for the light treatment and were counted at the end of the test for the dark treatment.

### 2.5 Germination recovery

The seeds failed the salinity tolerance test were placed in petri dishes with distilled water to germinate again under the same conditions of the experiments mentioned above to test the effects of photoperiod and thermoperiod. The germinated seeds were counted in the same way as the other

experiments. At the end of the germination test, the ungerminated seeds were dissected to evaluate the embryo status and viabilities under a binocular microscope.

## 2.6 Data analysis

The germination percentage was calculated as the mean percentage of the four replicates (standard deviation, SD), and the recovery percentage (RP) was calculated by the following equation:

$$RP = [(a - b) / (c - b)] \times 100\%, \quad (1)$$

where  $a$  is the total number of seeds germinated gonging through the salinity tolerance test plus those that germinated again in the germination recovery stage,  $b$  is the total number of seeds germinated gonging through the salinity tolerance test and  $c$  is the total number of seeds used in the salinity tolerance test (Khan et al., 2000; Bhatt and Santo, 2016).

The Shapiro-Wilk test was conducted to evaluate the normality before the subsequent analyses. Arcsine-transformed germination percentages were analyzed by ANOVA and consequent Fisher's least significant differences (LSD) post-hoc test. Data were graphed using Sigmaplot 11.0 (Systat Software Inc., London, UK) and the statistical analyses were carried out using Statistica 7.0 for Windows (Software Statsoft Release 7).

## 3 Results

### 3.1 Effects of photoperiod, thermoperiod, storage period and wings' presence on seed germination

The mean seed mass values of winged and de-winged seeds of *H. iraqensis* were 9.7 and 4.3 mg, respectively. *H. iraqensis* seeds germinated differently ( $P < 0.001$ ) under different treatments including photoperiod, thermoperiod, storage period and wings' presence (Table 1). The treatments of the above factors and their interactions showed highly significant ( $P < 0.001$ ) effects on the germination percentages of *H. iraqensis* seeds, with exceptions of the interactions among photoperiod, thermoperiod and wings' presence, and among photoperiod, thermoperiod, storage period and wings' presence, which only showed significant effect ( $P < 0.05$ ). Furthermore, the interaction between photoperiod and thermoperiod showed no-significant effect ( $P > 0.05$ ) on the germination percentages of *H. iraqensis* seeds.

**Table 1** Effects of photoperiod, thermoperiod, storage period, wings' presence and their interactions on the germination percentage of *H. iraqensis* seeds

Treatment	SS	df	MS	F	P
Photoperiod (P)	7182.42	1	7182.42	424.157	***
Thermoperiod (T)	435.61	1	435.61	25.724	***
Storage period (Y)	24,529.43	4	6132.45	362.147	***
Wings'presence (W)	147,379.64	1	147,379.65	8703.520	***
P×T	25.60	1	25.64	1.512	ns
P×Y	826.67	4	206.73	12.204	***
T×Y	657.46	4	164.42	9.706	***
P×W	5017.68	1	5017.67	296.315	***
T×W	739.61	1	739.63	43.677	***
Y×W	17,457.43	4	4364.42	257.737	***
P×T×Y	443.43	4	110.90	6.546	***
P×T×W	102.40	1	102.44	6.047	*
P×Y×W	1199.42	4	299.85	17.708	***
T×Y×W	553.40	4	138.40	8.170	***
P×T×Y×W	166.60	4	41.70	2.460	*
Error	2032.04	120	16.96		

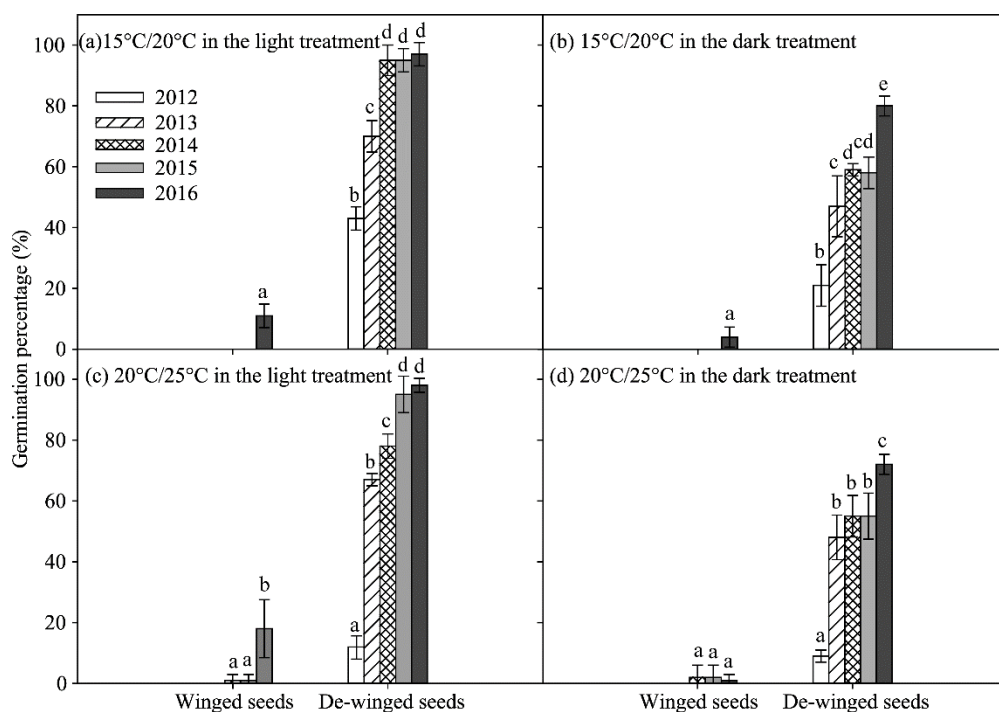
Note: ns, no significant correlation at  $P > 0.05$  level; \*, significant correlation at  $P < 0.05$  level; \*\*\*, highly significant correlation at  $P < 0.001$  level. SS, sum of squares; df, degrees of freedom; MS, mean square; F, fisher variable.

### 3.1.1 Temperature at 15°C/20°C

In the light treatment, only the winged seeds collected in 2016 showed germination ability of germination percentage (12%), while winged seeds collected in other years were unable to germinate (Fig. 1a). The de-winged seeds collected in all years were able to germinate with the highest germination percentage of 93% observed for the seeds collected in 2014, 2015 and 2016, while the lowest germination percentage of 40% was appeared in the de-winged seeds collected in 2012. The same pattern was observed in the dark treatment but with significantly lower germination percentages compared with the light treatment (Fig. 1b). In particular, the de-winged seeds of 2016 showed an absolutely high germination percentage of 80% in the dark treatment.

### 3.1.2 Temperature at 20°C/25°C

In the light treatment, the germination percentage was only 2% for the winged seeds collected in 2014 and 2015, and was 20% for the winged seeds collected in 2016 (Fig. 1c). The de-winged seeds of all years germinated and the seeds collected in 2015 and 2016 had the highest germination percentage (96%). With respect to the light treatment, the winged seeds in the dark treatment showed lower germination percentages than those in the light treatment and had no significant difference among the winged seeds collected in 2014, 2015 and 2016 (Fig. 1d). Moreover, the de-winged seeds also showed lower germination percentages in the dark treatment. Only the de-winged seeds collected in 2016 reached the highest germination percentage of 70%.



**Fig. 1** Germination percentages of winged and de-winged seeds of *H. iraqensis* collected in 2012, 2013, 2014, 2015 and 2016 under different photoperiod and thermoperiod treatments

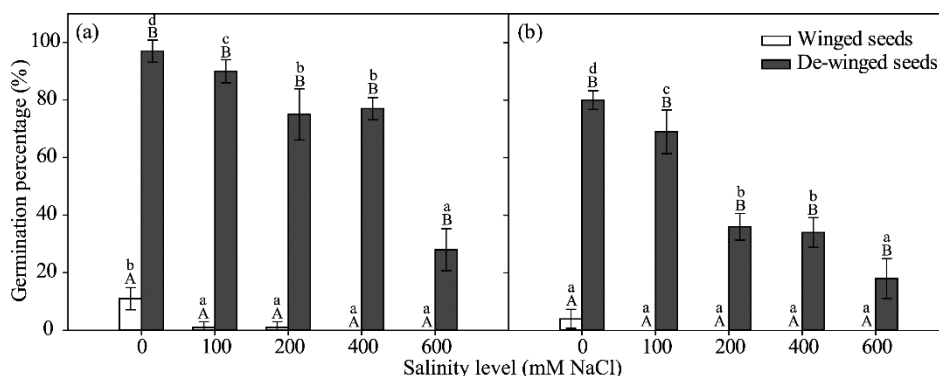
### 3.2 Effects of salinity on seed germination at 15°C/20°C

The photoperiod, wings' presence and salinity and their interactions significantly ( $P < 0.001$ ) influenced the germination percentages of *H. iraqensis* seeds (Table 2). In the light or dark treatment with different salinity levels (0, 100, 200, 400 and 600 mM NaCl), no more than 12% of winged seeds germinated and the highest germination percentage occurred in total absence of NaCl (Fig. 2). In the light treatment, the germination percentages of de-winged seeds decreased with salinity increasing, ranging from 97% under 0 mM NaCl to 28% under 600 mM NaCl. The same pattern of de-winged seeds was observed in the dark treatment, with the germination percentages ranged from 80% under 0 mM NaCl to 20% under 600 mM NaCl.

**Table 2** Effects of photoperiod, wings' presence, salinity and their interactions on the germinations percentage of *H. iraqensis* seeds

Treatment	SS	df	MS	F	P
Photoperiod (P)	3864.20	1	3864.20	206.274	***
Wings' presence (W)	68,913.80	1	68,913.80	3678.673	***
Salinity (S)	12,316.80	4	3079.20	164.370	***
P×W	2928.20	1	2928.20	156.310	***
P×S	744.80	4	186.20	9.940	***
W×S	8831.20	4	2207.80	117.854	***
P×W×S	964.80	4	241.20	12.875	***
Error	1124.00	60	18.73		

Note: \*\*\*, highly significant correlation at  $P < 0.001$  level.



**Fig. 2** Germination percentages of winged and de-winged seeds of *H. iraqensis* at 15°C/20°C in the light treatment (a) and in the dark treatment (b) under different salinity levels (0, 100, 200, 400 and 600 mM NaCl). Bars mean standard deviations. Different capital letters indicate the significant differences among winged and de-winged seeds under the same salinity level at  $P < 0.001$  level. Different lowercase letters indicate the significant differences among the salinity levels for winged or de-winged seeds at  $P < 0.05$  level.

### 3.3 Germination recovery after salt exposure

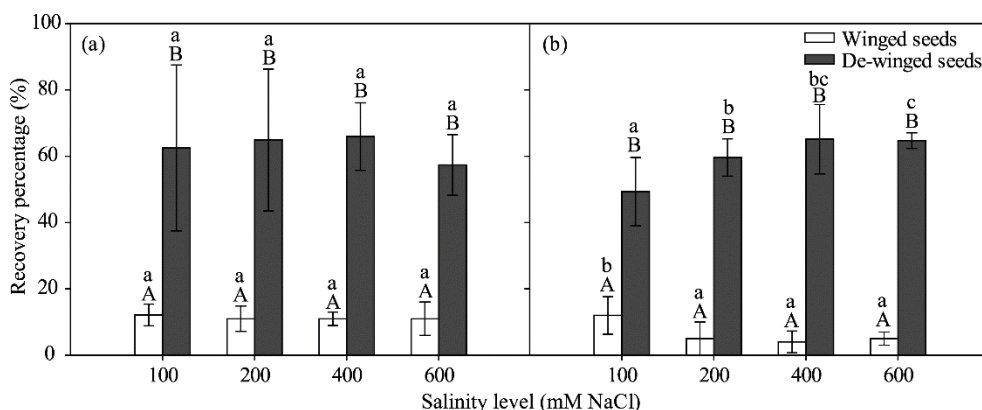
The unique factor which highly significantly influenced ( $P < 0.001$ ) the germination recovery percentages of *H. iraqensis* seeds was the wings' presence. The photoperiod, salinity and the interactions of photoperiod, wings' presence and salinity had no significant influence on the germination recovery of *H. iraqensis* seeds (Table 3). No more than 15% of winged seeds had the ability to recover the germination after the salinity tolerance test (Fig. 3). When seeds were deprived of their wings, the germination recovery percentage increased considerably by 60% in the light treatment and at least 50% in the dark treatment. At the end of the germination recovery test, the ungerminated seeds were dissected to evaluate the embryo status and viabilities under a binocular microscope, and no more than 5% of seeds were dead.

**Table 3** Effects of photoperiod, wings' presence, salinity and their interactions on the germination recovery percentages of *H. iraqensis* seeds after salt exposure and washed with distilled water

Treatment	SS	df	MS	F	P
Photoperiod (P)	240.95	1	240.95	2.339	ns
Wings' presence (W)	43,777.19	1	43,777.19	425.015	***
Salinity (S)	57.00	3	19.00	0.185	ns
P×W	12.98	1	12.98	0.126	ns
P×S	125.88	3	41.96	0.407	ns
W×S	433.81	3	144.60	1.404	ns
P×W×S	373.04	3	124.35	1.207	ns
Error	4944.07	48	103.00		

Note: \*\*\*, highly significant correlation at  $P < 0.001$  level; ns, non-significant correlation at  $P > 0.05$  level.





**Fig. 3** Germination recovery percentages of winged and de-winged seeds of *H. iraqensis* at 15°C/20°C in the light treatment (a) and in the dark treatment (b) after the exposure to different salinity levels (100, 200, 400 and 600 mM NaCl). Bars mean standard deviations. Different capital letters indicate the significant differences among winged and de-winged seeds under the same salinity level at  $P < 0.001$  level. Different lowercase letters indicate the significant differences among the salinity levels for winged or de-winged seeds at  $P < 0.05$  level.

## 4 Discussion

### 4.1 Effects of photoperiod on the germination of *H. iraqensis* seeds

Our results showed that the light treatment significantly enhanced the germination percentages of *H. iraqensis* de-winged seeds, indicating that these seeds are light-dependent (positively photoblastic, however, they are also able to germinate in the total darkness). Therefore, they might have a higher ability of germination if they remain at or near the soil surface when other environmental conditions (e.g., moisture availability, optimal temperature, etc.) are favourable. The light-mediated germination of *H. iraqensis* seeds might be an adaptation strategy to avoid their germination too deep under the soil, where conditions could be insufficient for successful seedling emergence due to the low energy reserves (Milberg et al., 2000).

### 4.2 Effects of thermoperiod on the germination of *H. iraqensis* seeds

As shown in Figure 1, *H. iraqensis* seeds collected in 2012–2014 showed significantly higher germination percentages at 15°C/20°C than at 20°C/25°C; however, the thermoperiod had no obvious impacts on the seeds collected in 2015 and 2016. Generally, seeds go through various physiological changes during storage, such as the loss of dormancy, temperature and light sensitivity (Bewley and Black, 1982; Probert, 2000). Therefore, we believe that the germination temperature would be different for seeds with different storage durations. Based on our results, combined with the local meteorological data (not shown), we speculate that *H. iraqensis* seeds could germinate well in January to April with the temperature of 13°C–25°C if the seeds were kept for no more than two years. Furthermore, if the seeds were kept for more than two years, the better germination season is January to March with the temperature of 13°C–19°C. Based on the seed storage duration, different adaptation strategies can help the species to survive in the harsh environmental conditions.

### 4.3 Effects of wings' presence on the germination of *H. iraqensis* seeds

The results obtained in our study demonstrated that the presence of wings, independent of all the other factors, highly inhibited the seed germination of *H. iraqensis* and imposed seed dormancy, indicating that the wing of this species probably contains inhibitors. In *Aellenia autrani* (Post) Zoh., the presence of persistent wing has been found responsible for the leading seed dormancy (Negbi and Tamari, 1963); and in *Halothamnus subaphyllus* (C.A. Meyer) Botsch., the wing was reported to contain germination inhibitors, e.g., phenolic components, abscisic acid and nicotinic acid (Ionesova, 1970). The presence of wing was also reported to cause the physiological dormancy in several species of Amaranthaceae (Baskin and Baskin, 2014; Bhatt and Santo, 2017b).

Specifically, the presence of wing can inhibit the germination of a species, being a mechanical barrier for seed radicle growth. Moreover, the wing presence can reduce the correct water absorption because the wing is a tissue that can accumulate chloride ions or high concentrations of abscisic acid (Takeno and Yamaguchi, 1991; El-Keblawy et al., 2013; Xing et al., 2013; Bhatt et al., 2016a, b). In this study, both winged and de-winged seeds of *H. iraqensis* were able to absorb water, which means that wing will not stopped the correct water absorption, however it does inhibit the seed germination. In our case, some winged seeds (<19%; Figs. 1 and 2) collected in 2014, 2015 and 2016 were able to germinate, indicating that the wing might not be fully rigid at that time to create a mechanical barrier for radicle emergence. However, the wing might become more rigid with storage duration, which might explain why the seeds with longer storage duration did not germinate. Further investigations are needed for a better understanding of the germination inhibition mechanism due to the wings' presence.

#### 4.4 Effects of storage period on the germination of *H. iraqensis* seeds

Germination percentages of *H. iraqensis* seeds decreased with the increasing storage duration, especially after three years of the collection. This might be caused by the aging of the seeds or the storage conditions. Storage duration can alter the endogenous hormone level and seed-coat phenolics, which ultimately affect the seed germination. With the increasing storage duration, phenolics and abscisic acid concentrations significantly increased (Li et al., 2008; Xu et al., 2016). Thus, decreasing level of gibberellic acid and increasing phenolics combined with the increasing storage duration of *H. iraqensis* seeds could be responsible for the poor germination. In this study, *H. iraqensis* seeds were able to maintain the germination percentages up to 95% and 70% after the collection for three and four years, respectively, under the photoperiod and thermoperiod of 12 h/12 h of light at 15°C/20°C or 20°C/25°C, indicating that the seeds are tolerant to desiccation and can be successfully stored at room temperature up to 3–4 years without excessive loss of their viability. In the natural desert habitat, the longevity of seeds may be attributed to the presence of persistent wing by providing physical and chemical barriers to the unfavorable and unpredictable desert environmental conditions. In this study, seeds were stored with the presence of wing at room temperature and wings were only removed at the time of the test and therefore our results can be predictive about the possibility of seed longevity under natural conditions. However, further studies are needed to determine how seeds without wings can maintain their viability if they are stored in similar conditions of the winged seeds in our study.

#### 4.5 Effects of salinity on the germination of *H. iraqensis* seeds

*H. iraqensis* seeds, when deprived of the wings, have better ability to germinate under all the tested salinity levels. On the contrary, when wings were maintained, only less than 2% of seeds germinated under all the tested salinity levels (Fig. 2). This pattern might be an indication that wing is likely a source of accumulation of salts which inhibit the germination in this species as reported in other species of Amaranthaceae (Baskin and Baskin, 2014). Germination percentages of *H. iraqensis* seeds decreased with increasing salinity level, but a very high germination percentage (77%) was recorded for de-winged seeds under 400 mM NaCl, reflecting the reasonably high salinity tolerance of seeds of this species. These findings indicate that *H. iraqensis* has the potential to be used in salt-affected lands by sowing its seeds directly instead of planting adult individuals, which can greatly reduce the economic costs of restoration ecology activities. When the *H. iraqensis* seeds are sown in saline substrate, germination percentages are higher in the light treatment than in the dark treatment, and increasing salinity negatively affected germination percentages in the dark treatment as compared to the light treatment. This pattern might indicate that seeds remain near the soil surface could be able to germinate even with few rainfall events that could reduce the surface salinity. However, if they are buried too deep in the soil, the unavailability of light, together with the higher salinity level, could reduce the possibility of their germination. Our results are in accordance with previous studies (El-Keblawy and Bhatt, 2015; Bhatt et al., 2016a, b; El-Keblawy et al., 2016) which reported that light and salinity have positive interactive effects on seed germination compared with the total darkness in presence of salinity and, therefore,



some of the halophyte seeds germinate better in presence of light when salts are also present in the substrate.

#### 4.6 Germination recovery

Our results indicated that ungerminated seeds under different salinity levels of *H. iraqensis* were able to recover their germination ability after alleviating the salt stress by transferring them to distilled water. However, the winged seeds have very low germination recovery percentages as compared to the de-winged seeds, indicating that the presence of wing has negative effects on the germination recovery after the salinity tolerance test (Fig. 3). The winged seeds showed very poor germination under the highest tested salinity level (600 mM NaCl), but their germination percentages increased by transferring them to distilled water. Similar results were obtained for *Salsola affinis* (Wei et al., 2008). Studies on other species (Cordazzo, 1994; Keiffer and Ungar, 1997) showed that the salinity might weaken the effects of wing on seed germination and stimulate the germination of winged seeds, but this was not the case for *H. iraqensis*, for which winged seeds had very low germination percentages under all the tested salinity levels (with respect to de-winged seeds). The germination recovery ability of *H. iraqensis* seeds was influenced exclusively by wings' presence, while the photoperiod and salinity level had no effect on the germination recovery percentages.

Our findings showed that *H. iraqensis* seeds can maintain viable during high salt exposure and they might be able to germinate after being washed with distilled water. Similar results were obtained from other desert halophytic species, when the ungerminated seeds from saline solutions were transferred to distilled water (El-Keblawy et al., 2013; Bhatt et al., 2016a, b). It is a well-known fact that the germination recovery ability of seeds is a species-specific property both in halophyte and salt tolerant species (Ungar, 1995; Song et al., 2005). From a natural viewpoint, in the field, this ability of *H. iraqensis* seeds to maintain viable under high salinity level might be an evolved physiological adaptation which helps the seeds to survive under the saline desert habitat conditions. From a practical viewpoint, this property indicates that *H. iraqensis* seeds can be sown in salt-stressed habitats or the salinized land because they can help the recovery of the habitats or land.

## 5 Conclusions

This study allowed the understanding that wings' presence highly inhibits the germination of *H. iraqensis* seeds. In addition, the storage duration affects the germination ability of seeds. Germination percentages of *H. iraqensis* seeds decreased with the increasing storage duration, especially after three years of the collection, indicating that the storage duration should not exceed 2–3 years. Photoperiod and thermoperiod are important factors influencing the germination of *H. iraqensis* seeds. The photoperiod of 12 h light/12 h darkness and the thermoperiod of 15°C/20°C are the best conditions for seed germination. Moreover, the results of our study indicate that seeds of *H. iraqensis* are well adapted to saline habitats, which can tolerate high salinity levels in the soil and are able to recover well after alleviating the salt stress.

## Acknowledgements

This study was funded by Kuwait Institute for Scientific Research. The authors are very grateful to Dr. John A MALTON, Dr. Samuel C ALLEN and Prof. Yougasphree NAIDOO for the linguistic revision of the manuscript.

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