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EFFECT OF WATER SUPPLY AND SOWING DEPTH ON SEEDLING EMERGENCE IN TWO HALOXYLON SPECIES IN THE JUNGAR BASIN

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Abstract

Haloxylon ammodendron and *H. persicum* are important part of the desert ecosystems, particularly in shifting sand dune areas, and these plants are common in the Jungar Basin of the northwestern China. To obtain basic information for the conservation and reintroduction of these two species, we studied the impact of water supply and sowing depth on the seedling emergence. Our results showed that proportion and rate of emergence increased with quantity of irrigation rate and sowing depth initially and then it declined, leading to the seedling mortality. Seeds, which were sown for 0.5 cm or 1.0 cm depth and having 20 mm of water supply, resulted in the highest emergence percentage and rate, whereas surface sowing of 0 cm depth and 10 mm of water supply led to 100% of seedling mortality within 30 days. Based on these results and taking into account the pattern of precipitation in the Jungar Basin, sowing of *H. ammodendron* and *H. persicum* for 0.5–1.0 cm depth before the snow cover begins melting in early spring is a critical point for the artificial restoration of these plants.

Key words: Emergence proportion; Emergence rate; Seedling; *Haloxylon ammodendron; Haloxylon persicum*.

Introduction

The key point for the successful plant cover regeneration is a seed germination and seedling emergence, which are influenced by many factors, including temperature, soil moisture, and the depth of sowing and the a depth of burying are the most important among them (Gutterman, 1993; Zheng *et al.,* 2003). *Haloxylon ammodendron* requires low temperatures for germination (Zhang & Zou, 1995) and the burying depth is a very important factor controlling the distribution and composition of vegetation in the desert ecosystems (Vander, 1974; Vleashouwers, 1997).

Germination is directly related to the burying depth (Gutterman., 1993; Maun., 1998; Huang, 1998; Zhang, 2001): shallow depth, and maintaining the micro-environment moist, favour germination and subsequent growth of seedlings, whereas seeds lying on the soil surface tend to dry out (Maun., 1994; Huang, 1998; Ren, 2002). In the case, when seeds are buried deeper, both germination and seedling emergence are likely to be adversely affected, and in general, seed germination and seedling emergence decrease with sowing depth. Under natural conditions, the sowing depth is not regulated, which makes the natural regeneration of *H. ammodendron* depending of the vagarious nature. Therefore, we want to study the main factors, affecting on the natural regeneration of *H. ammodendron*.*H. ammodendron* and *H. persicum*, belonging to the family Chenopodiaceae, which are common part of the desert ecosystem in the Jungar Basin in the northwestern China. *H. ammodendron* is the most widely distributed species in the Asian deserts (Wu, 1995; Huang, 2003; Lv, 2012;Xu, 2014) and, together with *H. persicum*, plays an important role in the maintaining the structure and function of the arid ecosystem. However, land reclamation, cultivation, overgrazing, indiscriminate cutting, and digging have destroyed the stands of *H. ammodendron* over the last five decades and led to the formation of shifting sand dunes in the Jungar Basin. For stopping degradation and habitat restoration, it is important to study the factors for improvement of the *H. ammodendron* and *H. persicum* regeneration.

Reproduction with seeds is the only way for developing of the *H. ammodendron* population; germination, therefore, has direct impact on their regeneration (Huang, 2003). Most studies of *H. ammodendron* seed germination were confined only by laboratory experiments and have been focused on temperature, light, salt concentration, length of storage and fruit wing role (Khan, 1996; Tobe, 2000; Wang, 2004; Tobe, 2005; Wei, 2006). Our study is focused on the effects of the available water amounts at the time of sowing and sowing depth on the seed germination and seedling emergence of *H. ammodendron* and *H. persicum* due to understand how a single precipitation event can affect these two parameters. The point is that the extent of snowfalls govern the thickness of the snow cover layer and thus the burying depth of seeds, and precipitation amounts govern the water availability during the time of seed germination in early spring. We also checked the possible impact of weather factors on the process of natural regeneration. Thus our study has important theoretical and practical significance for restoration of natural arid environment and controlling the desertification in the Jungar Basin.

Materials and Methods

Study area: The study area is situated near the desertification control station (44°11.401′ N, 89°33.357′ E) in the Qitai County (southeastern Jungar Basin. Xinjiang Province, China). The local climate is characterized by harsh conditions as during summer, as during winter. The mean annual rainfall amount is 176 mm and mean annual evaporation rate is 2141 mm. The soils are mainly aeolian sands and vegetative cover of the sand dunes comprises mostly from *H. ammodendron*, *H. persicum*, *Tamarix ramosissima*, and *Calligonum leucocladum* (Wu, 2005).

Experimental design: Winter snowfalls accounts 15%–25% of the annual precipitation amounts in the

Junger Basin. Since the snowcover melts within the short time in spring, the soil moisture remains high throughout this season. March is the most crucial time for the seed germination and seedling growth for *H. ammodendron* and *H. persicum* under natural conditions of the Gurbantunggut Desert. Winter rainfalls in Qitai are common during over 4 months, with average amount is about 40 mm (average for 55 years, 1951–2005). The snow melt was simulated by irrigation during sowing time. The simulation involved five levels of irrigation (10 mm, 20 mm, 40 mm, 60 mm, and 80 mm, respectively).

Seed collection and storage: Mature fruits of *H. ammodendron* and *H. persicum* were collected randomly in the Turpan Eremophytes Botanic Garden, China during November of 2007. The fruits were dried out in the open air and their wings were removed. The seeds were stored at –5°C in the icebox until the germination experiment, which was conducted in March of 2008. The seeds' viability was assessed before the experiments: germination percentage under natural conditions was 91.1±4.1% in *H. ammodendron* and 93±2.7% in *H. persicum*; these are the highest values recorded in this study.

Experimental set-up: The experiment began in mid-March of 2008, when the snowcover had melted on the ground. Experimental sands was collected near the sand dunes of the desertification control station. Plastic cylinders, each of which was 11 cm in diameter and 30 cm in length, were filled with sands and buried in a flat leeward site of the sand dunes. The treatments, which were the same for both species, consisted of different sowing depths from 0 to 5 cm and different water amounts supply through irrigation (10–80 mm) during sowing. Twenty seeds were sown per each depth (0 cm, 0.5 cm, 1.0 cm, 2.0 cm, 3.0 cm, 4.0 cm, and 5.0 cm) and plastic cylinders were watered until the depth of the standing water was 10 mm, 20 mm, 40 mm, 60 mm, or 80 mm. The entire experiment was replicated five times. During rainy days, water-proof plastic was used to block natural rainfall, so other factors except rainfall remained as close as possible to those found in natural conditions. The seeds and emerging seedlings were observed and the data were recorded daily.

Measure of indicators: Two indicators were used to quantify germination of seeds and emergence of seedlings: emergence percentage (the number of seedlings from the total number of seeds sown) and the rate of emergence (Rozema, 1975), which was measured the emerge speed of seedlings and calculated the emergence rate by formula:

$$\text{Emergence rate} = \sum \frac{100G_i}{nt_i}$$

where n was the number of seeds per each treatment and G_i was the time (t_i) or number of days taken by the seedlings for emerging (ti = 0,1,2,3 ..., ∞), where the faster the seedlings emerging was, the greater was the value.

Results

Effects of the sowing depth and water supply amounts on the emergence rate: The emergence rate increased with water supply amounts (Fig. 1), which, however, then decline after sufficient maximum. The emergence rate in both species was maximum, when they received 20 mm of water supply, and was significantly different (p<0.01) from cases, when they received 10 mm and 80 mm of water . The emergence rate was high when plants had 40 mm, 60 mm, and 80 mm of irrigated water but the three treatments from them did not differ significantly (p<0.01) among themselves, whereas the differences between treatments of 40 mm or 60 mm and 10 mm or 80 mm were significant (p<0.01). In surface-sown seeds (0 cm depth), the emergence rate was low and increased with depth, reaching its maximum value at 0.5 cm and 1.0 cm, which was significantly different (p<0.01) from that for other depths and declined thereafter until 5.0 cm, when the emergence rate was equal to 0. The best combination of the sowing depth and water supply amounts was 0.5–1 cm of depth and 20 mm of water, which led to the emergence rate of 32±10.3% in *H. ammodendron* and 37.0±7.1% in *H. persicum* (difference between 0.5 cm and 1.0 cm was insignificant). The *H. ammodendron* differed from *H. persicum* by some minor details, when the first species had emergence rate equal to 0, with 20 mm of water supply and sowing depth of 4.0 cm.

Effects of the sowing depth and water supply amounts on the emergence rate: In both species, the emergence rate increased with increase of water supply in the surface-sown seeds (Fig. 2), being highest value of 80 mm water, moreover, the emergence rate in *H. ammodendron was* higher than in *H. persicum*. However, when seeds were buried into sands, the emergence rate increased, with water supply up to 60 mm, while for 80 mm, the rate decreased again, and the emergence rate in *H. persicum* was higher than that in *H. ammodendron*. Among five levels of water supply, the rate was the highest for water supply of 20 mm and lowest for 10 mm. For same amounts of water supply, the emergence rate decreased with depth, being significantly higher (p<0.01) for 0 cm, 0.5 cm, and 1.0 cm than for 3.0 cm and 4.0 cm. The differences in the emergence rate for depth of 0 cm, 0.5–2.0 cm, and 3–4 cm were significant and the rate was relatively high for water supply of 20 mm, 40 mm, and 60 mm, being the highest for 20 mm and lowest for 10 mm and 80 mm.

Interaction effect of species, water amounts, and sowing depth on the seeding emergence and emergence rate: A three-way ANOVA showed that the interaction between species had no effect on the emergence rate (Table 1). The interaction between species and sowing depth was significant for emergence proportion, but not for the emergence rate. Finally, both parameters were influenced by each of three interactions, namely (a) species and water amounts, (b) the water amounts and sowing depth and (c) species, water amounts and sowing depth.

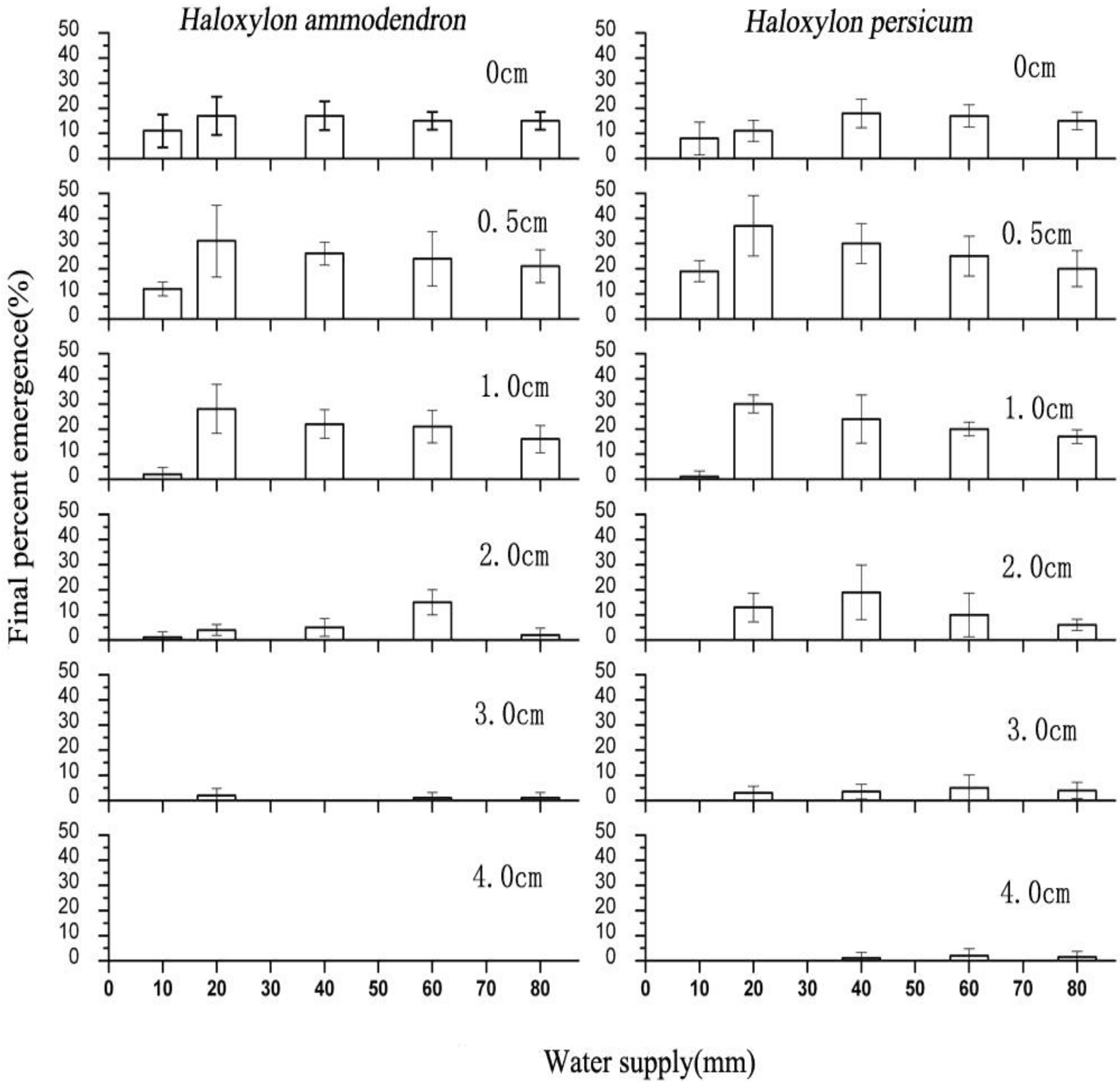

Fig. 1. Final percent emergence of seedlings of different sawing depth and water supply.

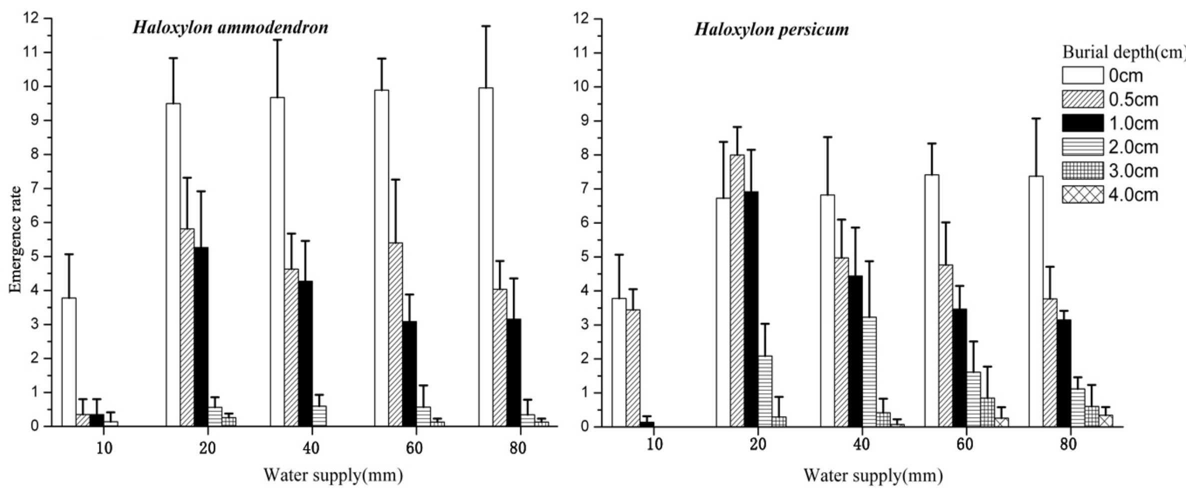

Fig. 2. Emergence rate of seedlings of r different sawing depth and water supply.

Table 1. Germination (%) and rate of seedling emergence as affected by water supply and depth of sowing in *Haloxylon ammodendron* and *H. persicum*.

Source	Df	Emergence		No-emergence	
		F-value	p-value	F-value	p-value
species(S)	1	0.479	0.489	1.509	.220
water(W)	4	30.637	0.000	39.572	.000
depth(D)	6	179.360	0.000	254.114	.000
S*W	4	2.605	0.036	1.228	.299
S*D	6	5.347	0.000	6.110	.000
W*D	24	6.886	0.000	7.181	.000
S*W*D	24	2.011	0.004	1.820	.012
Error	280				
Total	350				

A three-way ANOVA with respect to the data on percentage emergence and the rate of emergence showed that neither was affected by the interaction between species

Table 2. Survival (%) of seedlings as affected by water supply and depth of sowing in *H. ammodendron* and *H. Persicum*.

Species	Depth (cm)	Water supply (mm)				
		10	20	40	60	80
H. ammodendron	0	100Aa	59.1±8.9Ba	42.1±24.7Ba	38±20.1Ba	38.2±8.5Ba
	0.5	38.9±20.1Ab	20±10.1Bb	17.3±9.2Bb	13±6.6Cb	11.1±9.0Cb
	1.0	20±10.1Ab	13±6.6Bc	10.6±6.8Bb	8.0±6.8Bb	2.7±1.1Cc
	2.0		0		0	0
	3.0		0		0	0
	4.0					
H. persicum	0	100 Aa	49.0±8.9Ba	36.3±8.3Ba	20±10.0Ca	16.6±15.5Ca
	0.5	100 Aa	20±10.1Bb	0	8.0±4.0Bb	0
	1.0	21±10.1 Ab	13±6.6Bc	0	0	0
	2.0		0	0	0	0
	3.0		0	0	0	0
	4.0					

Survival (%) of seedlings are presented by the means ± standard deviations of five replicates
Comparisons were performed different sawing depth and water supply (*p*< 0.01; Fisher's LSD test)

Table 3. Seedling mortality as affected by water supply and depth of sowing in *H. ammodendron* and *H. persicum*.

Species	Depth (cm)	Water supply (mm)				
		10	20	40	60	80
H. ammodendron	0	3.28±0.82	2.45±1.41Aa	1.6±1.12Ba	0.80±0.25Ba	0.83±0.18Ba
	0.5	Aa	0.33±0.10Bb	0.2±0.04Bb	0.13±0.05	0.13±0.05
	1.0	0.80±0.25	0.13±0.08	0.08±0.04	Bb	Bb
	2.0	Ab	Bb	Bb	0.06±0.04	0.04±0.02
	3.0	0.55±0.12	0	0	Bb	Bb
	4.0	Ab	0		0	0
H. persicum	0	3.27±0.81	1.62±1.12	1.33±0.68	0.55±0.12	0.44±0.29
	0.5	Aa	Ba	Ba	Ba	Ba
	1.0	2.94±0.85	0.33±0.30Bb	0	0.18±0.11Bb	0
	2.0	Aa	0.25±0.13	0	0	0
	3.0	0.55±0.25	Ab	0	0	0
	4.0	Ab	0	0	0	0

Seedling mortality are presented by the means ± standard deviations of five replicates
Comparisons were performed different sawing depth and water supply (*p*< 0.01; Fisher's LSD test)

Effects of the water amounts and sowing depth on the seedling mortality: The mortality proportion in both species was higher at 10 mm of water supply than that at 20 mm (Table 2). Surface sowing and 10 mm of irrigation led to 100% of mortality, whereas the mortality rate dropped significantly as the water amounts increased more than 20 mm. At 10 mm, mortality was the highest for all depths. In both species, mortality rate dropped with increasing depth. Although mortality rate in *H. ammodendron* was lower than in *H. persicum* overall, 0% mortality was recorded only for *H. ammodendron* seedlings and *H. persicum* seedlings under some conditions.

The seedling mortality in *H. ammodendron* and *H. persicum* decreased with increasing of water supply and depth (Table 3) Seedling mortality in *H. ammodendron* fell when the water supply was ≥20mm and the depth was ≥0.5mm. The seedling mortality in *H. persicum* was higher at 0.5 cm depth and 10 mm of water supply, but fell markedly when the water supply was ≥20 mm as well as when the depth was ≥0.5 mm. Mortality rate in *H.ammodendron* and *H. persicum* seedlings decreased markedly as water supply increased up to 20 mm or higher and also when the seeds were buried deeper than 0.5 cm or more. The sowing depth increases mortality rate consequently the number seedlings is decreased.

Discussion

Soil moisture is the key factor in ecosystem restoration in the Jungar Basin, and the level of soil moisture directly influences on the seeds' germination and seedlings' emergence (Zheng, 2006), and our experiments with *H. ammodendron* and *H. persicum* seeds support this statement. At the same sowing depth, both percentage and rate of emergence for both these species increased with increasing of water supply and then decreased when the supply was too excessive. At 20 mm of irrigation, emergence percentage was the highest. If water supply is inadequate, seeds cannot absorb enough water and therefore the emergence percentage would be low; even when seeds germinate, the seedlings may fail for emerging. In dry soil, the water potential is not very different from that of desiccated seeds. For such low osmotic potentials, water does not enter into the seeds and thus cannot induce the seeds germinating. Seeds of desert shrubs vary in their ability to germinate when they are under the moisture stress. When the water supply would be excessive, the sand will be saturated and both water and air would be lacking; such near-anaerobic conditions are detrimental for germination and establishment (Baskin, 1998). This example explains why the emergence percentage was higher even in the case of the surface-sown seeds if the water supply was adequate. However, roots of the growing seedlings need water in larger quantity, especially in upper layers. If the top layer does not hold adequate moisture, the seedlings would die. In *H. ammodendron*, once the root system will grow beyond the upper layer, the seedlings will dry if roots will encounter into a dry layer (Yang & Zou, 1995). Only in the case, when the precipitation is abundant enough to infiltrate into the dry layer, then the seedlings can escape drought stress and survive (Li, 2004).

Among all stages in a plant, the seed has the highest resistance to extreme stresses from environment, whereas seedlings of many plant species represent the most sensative stage (Gutterman, 1993; Ren 2002). Of the great total number of seeds produced by any plant, only a small part of them develops into seedlings. For various reasons, losses because of seed dispersal and seedling emergence are most substantial. Many studies of plant germination have focused on the effects of specific environmental factors on seed germination and seedling establishment (Maun, 1994; Zhang, 2001; Irfan *et al*., 2014). The sawing depth of seed is most important factor for the seed germination and seedling emergence (Ren *et al*., 2002): if seeds are buried too deep, they either fail to germinate because of excess moisture, low temperature, poor gas exchange, and higher CO_2 levels in their micro-environment (Gutterman, 1993; Keeley & Fotheringham, 1997) or germinate but fail to emerge because the seed reserves are exhausted in the struggle. In the present experiment, seeds of *H. ammodendron* and *H. persicum* were germinated when were buried for 1.0–4.0 cm depth. But when they were buried deeper (5 cm), most germinated seeds were failed to emerge as seedlings. A proper depth has a positive impact on the seed emergence, seedling establishment, and subsequent growth. If seeds were buried too deep, the plant growth would be negatively affected and it may even die, thereby the plant stands depend on the sowing depth (Maun, 1994). Seedlings, which are developing from seeds that are buried too deep, fail to emerge for a number of reasons: (a) the food reserves in the endosperm or cotyledons may be exhausted before the seedling manages to break through the soil (Li *et al.*, 2004; Zhu, 2006); (b) soil in the deeper layers is more compact or dense, because of the weight of the upper layers , This not only reduces aeration but also makes it difficult for roots to spread in the rhizosphere; (c) poor roots growth means in turn the poor growth of the above-ground parts, which leads to developing of weak plants in general (Seiwa, 2002). Our study showed that the optimal depth was beneficial to the establishment of seedlings for both species, the emergence being significantly higher when the seeds were buried for depth of 0.5 cm or 1.0 cm in sands than when they were buried for depth of 3 cm or 4 cm or not buried at all; and when they were buried deeper than 5 cm, seedlings of both the species failed to emerge. A small proportion of seeds turned to be dormant, especially when buried too deep, probably because of factors mentioned above, namely high soil moisture, low temperatures, poor aeration, and higher concentrations of CO_2 (Gutterman, 1993; Huang, 1998; Yu & Wang, 1998). In general, dormancy is ecologically advantageous phenomenon because seeds can survive in the dormant state during long time (Gutterman, 1993; Huang, 2000), and they are 'ready' to germinate as soon as the upper layers of sand would be removed away by wind or water stream, enabling them to germinate when the depth is more suitable (Maun., 1996; Huang, 1998).

According to Bond*.*, (1999) and Tobe*.*, (2007), larger seeds, having more reserves than smaller ones, produce taller seedlings despite of thicker layer they have to traverse. Bond *et al*. (1999) reported that the depth for maximum seedling emergence is proportional to the cube of seed weight. In *H. ammodendron* and *H. persicum* (mean seed weight of 3.12 mg and 4.94 mg, respectively), although many of the deeply buried seeds could germinate, seedling elongation was adversely affected by sowing depth (Tobe*.,* 2007) Tobe obtained the similar results for two deciduous shrubs that grow on desert sand dunes in China. He demonstrated that widely differing seed weights had no marked effect on the threshold depth from which the seedlings were able to emerge. Larger seeds tend to have a bigger endosperm, which supplies the energy required to emerge from greater depths (Seiwa*.,* 2002). One thousand seeds of *H. ammodendron* weighed 3.12±0.29 g on average and those of *H. persicum* weighed 4.94±0.38 g. Therefore a few seedlings of *H. persicum* could emerge from seeds would be buried for depth of 4.0 cm and heavier seeds are an adaptation to the sand environment. However, the difference in seed weight between *H. ammodendron* and *H. persicum* was not affected on the threshold depth for germination for these species because the inhibition of seedling elongation can also be attributed and the exhaustion of seed reserves while penetrating a harder layer of sands having the higher moisture content (Hornbaker*.,* 1997).

Germination percentage decreased with increasing moisture stress. It appears that the shifting dunes constitute a soil habitat with an inherent risk for seeds to be buried so deep that their seedlings would fail to emerge. A modest depth 0.5–1.0 cm is recommended for sufficient growth of Haloxylon, while more shallow depth would lead to the drying seeds before they can start to germinate and deeper sowing would lead to failing of seedling emerge, because of exhaustion of seed reserves. Under natural conditions, seed germination process is complicated and influenced by many natural factors such as salinity, drought, light, and temperature. It is possible to control these conditions, and the present study recommends that seeds of *H. ammodendron* and *H. persicum* would be buried for depth of 0.5–1.0 cm and irrigated by water amount supply of 20 mm.

Conclusions

For optimal depth of sowing, emergence percentage and rate of emergence in *H. ammodendron* and *H. persicum* increased with the amounts of irrigation initially and declined thereafter. Both species exhibited the highest percentage of emergence when it was supplied 20 mm of water.

Emergence percentage was higher for sowing depths of 0.5cm and 1.0 cm than for 0 cm, 3.0 cm, and 4.0 cm. Not a single seed germinated when it was buried for depth of 5 cm. The depth and seedling morality were negatively correlated in both species: no one seedling can survive when the seeds were not buried at all (surface sowing) and were supplied only with 10 mm of water.

There is the extreme climatic conditions in the Jungar Basin, where sufficient precipitation is seldom. Early spring represents the best conditions with respect to soil moisture and temperature: the melting snow supplies an adequate moisture for seedling emergence in *H. ammodendron* and *H. persicum* and warm weather provokes the faster growth. Thus, considering the natural conditions in the Junger Basin and the results of this study, seeds of *H. ammodendron* and *H. persicum* need to be buried artificially for depth of 0.5–1.0 cm in early spring for assistance of natural restoration of these species.

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