

[Research]

## Seed germination response of *Haloxylon persicum* (Chenopodiaceae) to different hydrothermal conditions and sand burial depths

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### ABSTRACT

Daily counts of germinated seeds of white saxaul (*Haloxylon persicum*) incubated at 35 different hydrothermal environments (10-25°C and 0 to -3 MPa) were carried out under controlled laboratory conditions. The seeds were then buried in sand at 4 different depths in well drained pots constantly moisturized for three weeks. In all these hydrothermal conditions, the courses of germination were completed in less than 12 days. Both temperature and water deficit conditions significantly affected the seed germination. Water potential values of -1.5 MPa reduced the germination percentage to less than one fourth of the potential viability in all ambient temperature regimes and seed germination almost completely ceased at water deficit of -3 MPa. As water potential decreased from 0 to -1.5, base temperature values slightly increased from 5.4 to 6.5 °C. Six-cm depth of burial was enough to reduce germination by 50% and on an average 85% of germinated seeds at 8-cm depth failed to reach the soil surface. No seedling appeared on top of the sand bed in pots labeled "12-cm burial depth".

**Keywords:** Seed germination, hydrothermal time analysis, seed burial, *Haloxylon persicum*.

### INTRODUCTION

Stretching for more than a thousand kilometers in length, the arid deserts of the central Iran plateau are covered mostly by gravel and shifting sands (Zohary, 1974). Since 1950s several national projects of sand dune fixation have been underway in the country and their achievements in combating desertification are widely recognized (Koocheki, 1996). As part of the green belt development programs, white saxaul (*Haloxylon persicum* Bunge ex Boiss. and Buhse), a dominant shrub-like C<sub>4</sub> tree (Pyankov et al., 1999), was successfully planted over scattered areas around Iran's central desert to protect villages, roads and railways, as well as for fuel and fodder production (Nemati, 1986; Akhiani et al., 1997).

Despite being less expensive and more effective, direct seeding of this species, unlike planting of seedling, has not been promising. A number of reasons have been proposed to explain the unsuccessful plant establishment after aerial seeding. Improper seeding time and burial in sand

are considered to be the main constraints (Matin et al., 1994).

To determine the proper time and location for aerial seed sowing, knowing the threshold levels of temperature and seed water content as well as the interaction between these independent sources of variation to retain adequate germination is essential. The first aim of this research is therefore to study germination behavior of the white saxaul seeds at different water potential and temperature conditions. To quantify the response of germination rate to the gradient of temperature change and water availability over time, the results will be formulated under a population-based hydrothermal time empirical model, which was proposed by Gummerson (1986) and then better described by Bradford (1995).

In the second step, the experiment aims to assess the potential of fully imbibed white saxaul seeds to overcome sand burial stress. Sand deposition within Iran's central desert region sometimes reaches up to several meters; consequently, not only a good knowledge of speed and direction of the

dominant winds is needed, also careful assessments of seed ability to tolerate the adverse effects of sand accretion and to germinate and grow up through the sand, are the main factors that have to be included in afforestation projects in the region.

The result of this study determines threshold depths for germination of buried seeds and emergence of seedlings. The danger of excavation and continuation of growth of the exhausted seedlings of this species are not studied here and requires further investigation.

## MATERIALS AND METHODS

### *Seed sources and collection*

During December 2006, fruit clusters of white saxaul were collected by hand from a 30 year-old plantation in Häresābād near Sabzevār (36° 06' N, 57° 36' E). After debris were cleaned up, the fruits (winged seeds) were air-dried at room conditions for a week and then stored in dark and dry conditions at 4°C for three months. At the time of the experiment, wings of the fruit were removed and healthy seeds with medium and large grade sizes were selected. Primary germination tests with the seedlot showed viability of more than 95%.

### *Hydrothermal time experiment*

The population-based hydrothermal time model (Gummerson, 1986; Bradford, 1995) was used to explain germination behavior of the white saxaul seeds. Tests were carried out in 9 cm Parafilm-sealed glass Petri dishes containing two layers of Whatman no. 1 filter paper at five different ambient temperatures (10, 15, 18, 20 and 25°C) using an unlit Environmental Simulator (Weiss Umwelttechnik GmbH, Germany). Seven osmotic solutions of 0, -0.5, -1, -1.5, -2, -2.5 and -3 MPa were prepared by deionized water and Polyethylene Glycol (PEG) 8000 (Sigma Chemicals, P-2139) according to Michel (1983). Their water potential values were then confirmed using a Vapor Pressure Osmometer (Model 5520, Wescor, Inc., USA) and corrected for the effect of temperature. To avoid possible exclusion of PEG molecules, the paper substrates were first subjected to low water potential solutions for half an hour before transferring into the Petri dishes.

Four replications of 50 seeds were considered for each temperature × water

potential combination. Osmotic solutions of petri dishes were refreshed and germinated seeds were discarded everyday for 5 days after starting the experiment. The petri dishes were left intact for the rest of the counting days. Seeds with the radical emerged to 2 mm were counted as germinated seeds. Number of counting days was based on the last observed germinated seed. Differences between the 35 hydro × thermal conditions were tested by a Two-Way Analysis of Variance upon final germination percentage (arc-sine transformed) at confidence level of 95%.

Cumulative percentages of germination at each temperature regime were probit-transformed and the reciprocal time to median germination ( $1/t_{50}$ ) was calculated for each water potential treatment. The  $1/t_{50}$  values were then used to determine optimum temperature treatments for seed germination. The different fractions of germination rates were also regressed against associated optimal as well as sub-optimal temperatures; and base temperature ( $T_b$ ), which is the temperature below which germination does not occur, was assessed by interpolating the corresponding value to zero median germination time (Holt and Orcutt, 1996; Steinmaus et al., 2000).

Germination rates ( $1/t_g$ ) to obtain 20, 40, 50, 60, 80 percent germination (g) were determined for each temperature regime and linearly regressed against corresponding water potential where they were applicable (Finney, 1971). The reverse of the slope of each regression line was considered as hydrotime constant value ( $\theta_H$ ) and the point where regression line intercepts x-axis represented the base water potential ( $\Psi_{b(g)}$ ), which is the water potential above which seed percentile "g" will germinate (Bradford, 1990).

Along with changing the  $\Psi_b$  values, which is essential in a hydrothermal model, a modified base water potential value  $\{\Psi_{b(g)} = \Psi_{b(g)T_0} + k_T(T-T_0)\}$  was elucidated at supra-optimal temperatures to improve the homogeneity in normal distribution of final germination. Where  $\Psi_{b(g)T_0}$  is the base water potential to achieve g% germination at optimum temperature ( $T_0$ ) and  $k_T$  is the slope of linear regression line between supra-temperatures and the corresponding values of the non-modified  $\Psi_b$  as dependent variable

(Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003). Using the repeated probit regression method as described in Dahal and Bradford (1990), the possible hydrothermal time constant ( $\theta_{HT}$ ) was calculated. The Pearson Chi Square Goodness of Fit test ( $\alpha = 0.05$ ) was used to determine whether the interpolated values indicated a satisfactory goodness of fit for the regression lines (Minitab Inc., 2000).

#### Seed burial experiment

Well washed and oven-sterilized sea sand was poured into 16×19 cm black polyethylene pots up to a base mark and moistened. The drainage outlets at the bottom of pots were covered with ceramic particles to prevent sand loss. Samples of 100 fully imbibed seeds were placed on the sand surface and appropriate amount of sand was uniformly added to bury them at 0, 1, 4, 8 or 12 cm depths. The pots were loosely covered with black plastic bags and kept moisturized by frequent water spraying under lab incubation conditions at 22-25°C. After three weeks, all the plant materials were dug up and washed under running water over a small-mesh sieve.

Total number of germinated seeds and emerged seedlings were counted separately. The germinated seeds were considered as those with at least two millimeter long radicle. The rest of the seeds were considered to be non-germinated. These seeds were surface sterilized and subjected to a germination test under previous experiment conditions to check their viability. Four replications were allocated to each treatment. Arcsin square root transformed data from all burial depths were subjected to one-way ANOVA followed by Tukey's test at 5% probability level.

## RESULTS

### Hydrothermal time experiment

None of the germination tests showed seed dormancy, and final germination percentages were higher than 60% for all temperature regimes in pure water (water potential=0 MPa) (Figure 1). An overall significant statistical difference was observed in each, as well as interaction between the two sources of variation (Table 1).

**Table 1.** Two-Way Analysis of Variance of arcsine transformed percentage of the total seed germination at different water potential (WP) and temperature (T) regimes

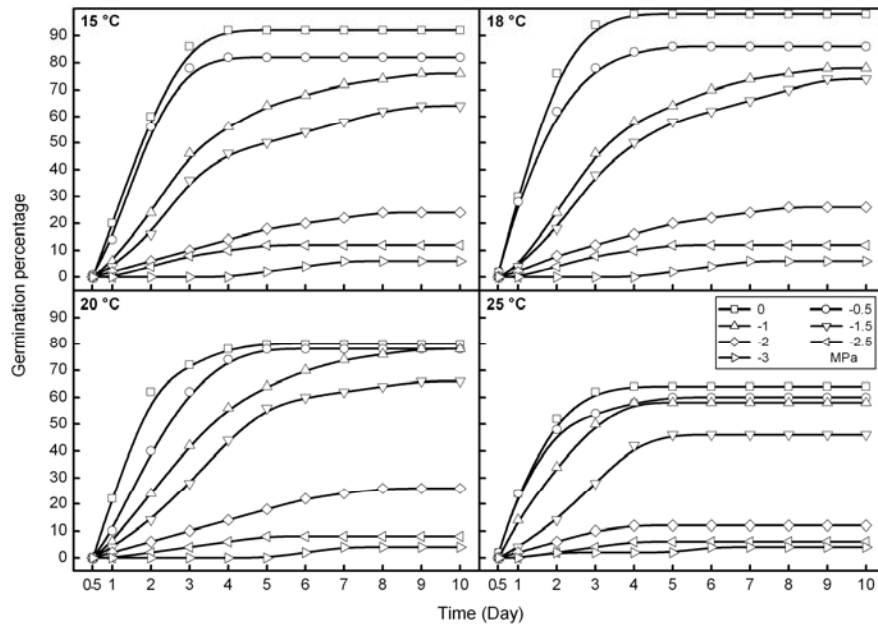
Source of variation	Degree of freedom	Sum of Square	Mean Square	F statistic	p
T	4	2199.4	549.8	83.2	0.00
WP	6	59851	9975.2	1510.5	0.00
T×WP	24	1528.1	63.7	9.6	0.00
Error	106	680.2	6.4		

The same table shows that in contrast with temperature, water deficit has a major inhibitory effect on the seed germination, yielding a higher mean sum of square value (Table 1). Under all temperature regimes, the total seed germination percentage decreased as water potential became more

negative, but this decrease was more radical at water potential values less than -1.5 MPa (Figure 2). The drastic effect of low water potential on seed germination prevented the calculation of reciprocal time to median germination ( $1/t_{50}$ ) at of -2, -2.5 and -3 MPa (Table 3).

**Table 2.** Mean values for base ( $T_b$ ) and optimum ( $T_o$ ) temperatures (°C ± standard deviation), to achieve 50% germination at different water potential regimes

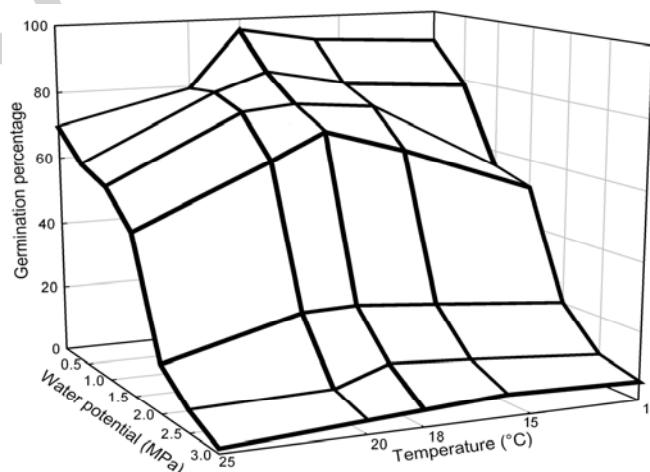
	Water Potential (MPa)			
	0	-0.5	-1	-1.5
$T_b$	5.4 ± 0.0	5.6 ± 0.1	6.4 ± 0.2	6.5 ± 0.1
$T_o$	17.3 ± 0.2	17.4 ± 0.4	17.7 ± 0.0	17.6 ± 0.2



**Fig 1.** Effect of temperature and water potential on cumulative germination percentage of white saxaul seeds.

Figure 2 also shows a steady progress in germination of seeds exposed to 0 till -2.5 MPa water deficit conditions. Therefore 18 °C was regarded as optimum temperature treatment for all water potential regimes, except -3 MPa, which germination rate was almost constant across all thermal environments. Temperatures below 18°C were considered as sub-optimal temperature treatments. This result was better clarified by plotting different times to acquire the 16th, 50th and 84th germination percentiles in pure water, which are reported to represent the median different germination

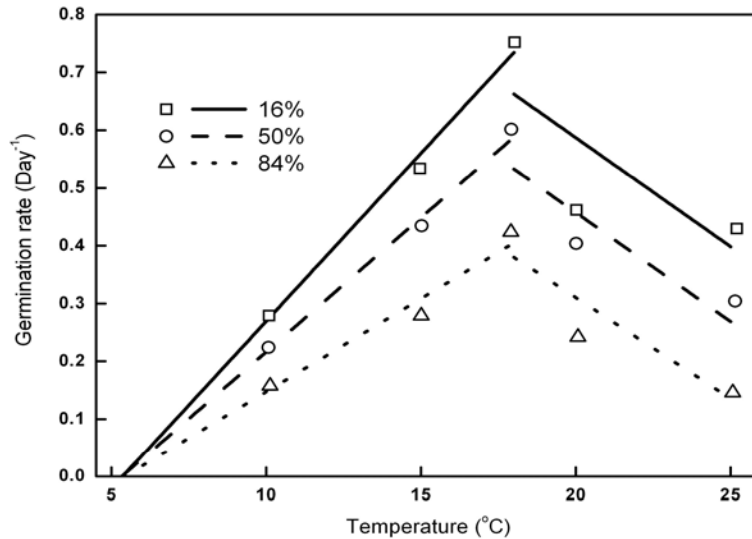
rates (1/tg). Using extrapolating method (Alvarado and Bradford, 2002), the base temperature value of 5.4 °C was calculated for all three germination percentiles in pure water (Figure 3). The same method was used to calculate the base and optimum temperatures to obtain 50% germination at 0 till -1.5 MPa water deficit conditions. Approximately similar optimum temperature values were obtained for all water potential regimes, but the base temperatures gradually increased as water potential decreased (Table 2).



**Fig 2.** An unfitted germination percentage surface for white saxaul seeds incubated under simultaneous effects of temperature and water.

The hydrotime constant value ( $\theta_{HT}$ ) decreased until the optimum temperature treatment and then increased as temperature further rose to 20 °C (Table 3). There were also different results for base water potential to achieve 50% germination  $\Psi_{b50}$  (MPa) at different thermal environments. The standard deviation ( $\sigma_{\Psi b(50)}$ ) values were also constant over the supra-temperature range of 18-20° C (F test,

$P < 0.05$ ) (Table 3). Since  $\sigma_{\Psi b}$  was clearly different between three optimum and suboptimal treatments, a general hydrothermal time model, which describes seed germination behavior across all temperature  $\times$  water potential regimes, could not be developed (Kebreab and Murdoch, 2000). A constant value of 88.25 (MPa. $^{\circ}$ C.Day) might account for  $\theta_{HT}$  for supra-temperature range of 18 to 20° C.

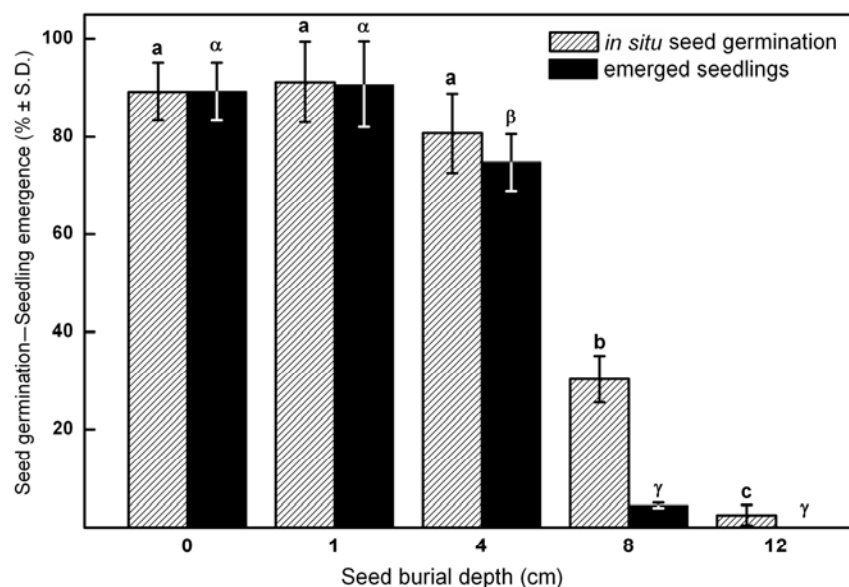


**Fig 3.** Different times to acquire the 16th, 50th and 84th germination percentiles are plotted versus different temperatures in pure water conditions. Considering the optimum temperature treatment of 18°C as the turning point, extrapolating shows almost the same base and optimum temperature for the three fractions.

#### Seed burial experiment

Analysis of variance for both the total germination and the number of emerged seedlings, showed highly significant differences among burial depth treatments ( $P = 0.00$ ) (ANOVA tables are not presented). Only 0.5 and 7 percent of epicotyls of germinated seeds did not reach the soil surface at 1- and 4-cm burial

depths, respectively. From statistical point of view, no difference was found between the three shallow burial depths at confidence probability of 0.95, though seed germination and seedling emergence at 1-cm burial depth were slightly higher than corresponding values at two other treatments (Figure 4).



**Fig 4.** The effect of burial depth on seed germination and seedling emergence. The vertical bars represent  $\pm$ SD. The bars are tagged with the same to indicate statistically significant differences at  $P < 0.05$ . Values with different Roman alphabets for seed germination and different Greek alphabets for seedling emergence indicate significant differences ( $P < 0.05$ ) [one-way ANOVA followed by Tukey's test].

Considering the sharp decline in both seed germination and seedling emergence, the same figure shows that 8 cm is a milestone among the 4-cm burial depth classes. In average, 85% of the germinated seeds at this depth failed to reach the soil surface, and no statistically difference was observed between the numbers of emerged seedlings at 8- and 12-cm burial depths. Disregarding the few seeds that germinated at 12-cm burial depth, germination was almost seized at this depth and no seedling appeared on the sandy soil surface. In almost every unemerged germinated seeds

in the two deep burial treatments, radicle emergence was observed without plumule growth. Laboratory germination tests showed that none of the ungerminated seeds from 0- till 4-cm burial depths germinated; but  $10.1 \pm 2.6$  and  $9.2 \pm 2.8$  percent (mean  $\pm$  standard deviation) of ungerminated seeds, dug up from respectively 8- and 12-cm burial depths were germinated on paper in Petri dishes. Unlike many under-sand (*in situ*) germinated seeds, cotyledons and plumule were normal and intact in these seeds.

**Table 3.** The components of the hydrothermal time relationships, grouped based on different temperature treatments (T)

T (°C)	$\theta_H \pm \sigma$ (MPa.Day)	$\Psi_{b50}$ (MPa)	$\sigma_{\psi b}$ (MPa)	$k_T$	$r^2$
10	$17.37 \pm 7.13$	-3.22	0.28	---	0.95
15	$7.49 \pm 4.39$	-2.57	0.16	---	0.94
18	$4.34 \pm 2.71$	-2.45	0.38	---	0.90
20	$9.77 \pm 6.81$	-2.75	0.34	0.15	0.89

$\theta_H$  is hydrotime constant;  $\Psi_{b50}$  is base water potential to achieve 50% germination with its standard deviation of

$\sigma_{\psi b}$ . The homogeneity of the supra-optimal temperatures was improved according to Alvarado and Bradford (2002) for 20 °C;  $k_T$

is the slope of linear regression line between supra-temperatures and the corresponding values of the non-modified  $\Psi_b$ . The standard deviation for  $\theta_H$  at 25 °C was higher than its correspondent average value and was not presented

### Discussion

Following large losses due to seed dispersal, seed germination and subsequent seedling growth are the most crucial stages in the life cycle of a plant (Gutterman, 1993). During these periods, low soil moisture levels and unfavorable thermal conditions are the main limiting factors. Desert plants have evolved different strategies to adapt to increase the chance of a successful regeneration (Wang et al., 1997). Among them, white saxaul uses a highly environment-dependent strategy of mass

production of short-lived and fast germinating seeds (Amani and Parvizi, 1996). In natural regeneration or direct seeding afforestation projects, the success of using this strategy requires careful selection of appropriate site/time combinations. The winged seeds (fruits) of saxaul species are not recalcitrant and tolerate desiccation, but their germination capacity virtually vanishes within less than ten months, forming no soil seed bank (Jafari et al., 2003). Hence, seeding time is limited to a short period of time at the start of the growing season. Early seeding guarantees receiving enough precipitation, but increases frost risk. Late seeding, on the other hand, may not provide sufficient time for seedlings to lignify before the onset of winter.

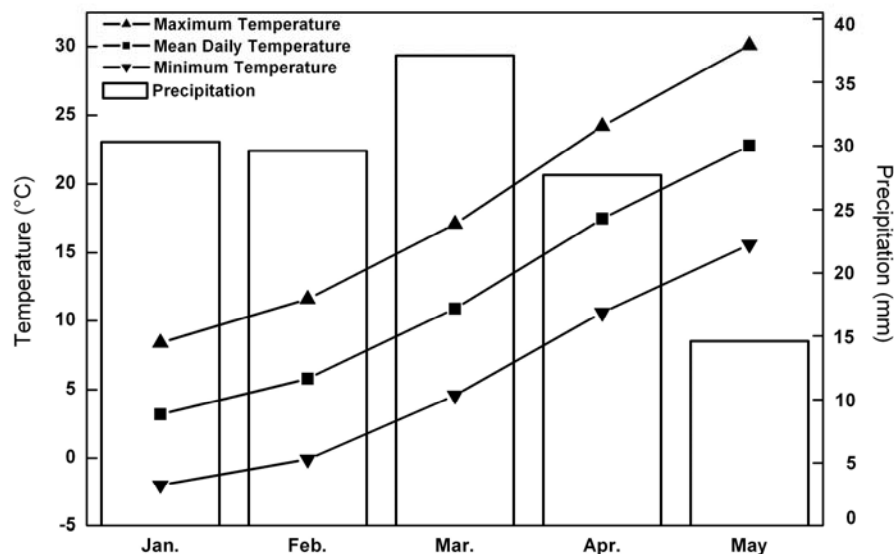


Fig 5. The mean monthly temperatures (Scatter diagram) and precipitation (bar diagram) values of first five months over 50- year period for Sabzevār climatology station, Iran

The results of this study led to better understanding of threshold temperatures and water potentials in seed germination of white saxaul. Base temperature ( $t_b$ ) changed merely by 1°C over all water potential conditions. It would, therefore, be suitable to consider mean daily temperature as an indicator of start time for afforestation projects in the region. Weather records for fifty years at Sabzevār climatology station (Unknown, 2006) indicates mid February as the earliest time that the  $t_b$  for seed germination (5.4-6.5°C) is provided (Figure 5).

Seeds of white saxaul showed a relatively high germination percentage under water deficit conditions. However the germination rate was greatly affected by water deficit and a rapid and not steady decline was observed in germination rate as water potential decreased below -1 Mpa. Similar results were previously reported when seeds incubated in saline conditions (Tobe et al., 2000). This range of water deficit levels are frequently reported in saline and sodic soils, which are not considered as the ecological niche of *Haloxylon persicum* (Akhani and Ghorbanli, 1993).

At the end of winter, the earliest base temperatures are usually provided coinciding with the highest level of precipitations in the region (Figure 5). Therefore soil water potential should not be the main obstacle at that time. But past experiences shows that in practice, a few consecutive weeks of mild temperature, accompanied by an above-average monthly precipitation with no major sand-shifting wind have to be considered as the favorite conditions (Song et al., 2005).

The results showed that raising temperature changed the base water potential values, which was previously shown to be the cause of rejection of a hydrothermal time model (Kebreab and Murdoch, 1999). The model failure can also be characterized by different standard deviation values for median base water potential at different thermal environments (Wang et al., 2005). As water became less accessible, the cardinal temperatures surged slightly. Increase in base temperature was previously considered as the reason for failure of the model as well (Fyfield and Gregory, 1989). The hydrothermal results showed that a successful prediction of seeding time base on monthly mean temperature can be taken into account only in wet years. A 3-factor regression might be calculated to describe the significant interaction between seed moisture content and ambient temperature over time, but the coefficients could not be extrapolated to other seedlots, thus the data is not presented.

Increase in hydrotime constant values along the suboptimal temperature gradient can be speculated as the indicator of seed germination complexity. The complexity becomes more apparent when seeds are buried in sandy soils, because ambient temperature and moisture content change at different burial depths (Maun, 1998). The results confirmed that, apart from moderating the stress impact of environmental conditions, burial in sand has major effects on seed germination physiology (Gutterman, 1993; Baskin and Baskin, 2001).

Although not statistically significant, shallow buried seeds performed better than the seeds were grown on the soil surface. The pots that were used in this

study were well-covered and water vapor saturated, but water content balance might be disrupted in unburied seeds. On the other hand, seed burial more than a few centimeters was a recurrent event in seed germination of white saxaul. About 6 cm depth of burial was enough to reduce germination by 50%.

Since the sands were well-drained, the main inorganic obstacle to seed germination under burial conditions, assumed to be either the lack of enough storage in seeds to overcome soil pressure, or physical overburden on apical meristems, which consequently retards the upward growth (Maun, 2004). Zheng and coworkers (2005) summarized the results of other researches on deeply buried small seeds with the same symptoms. The hindering effect of the upper sand layer may be simplified as the difference between the number of *in situ* germinated seeds and emerged seedlings. The large number of seeds with seized hypocotyl growth in the two deep burial treatments possibly shows the significant effect of soil pressure on the plumule. Likewise the small number of unemerged germinated seeds in the shallow burial treatments may indicate the effect of extreme low storage in the seeds.

Dry seeds of *Haloxylon persicum* which are stored for a year under cold and dark conditions lose their viability to a great extent up to 90% (personal observations). Obviously this storage conditions never happens in nature, but the treatment used for buried seeds in this study (soaking for time period more than a week) is also not common under shifting sand conditions (Kardavani, 1999). Nevertheless the results were consistent with what was expected, and showed that burial of imbibed seeds for less than a month has deleterious effect on seed survival and increase the rate of biotic decay (Finch-Savage, 1995). Only a small portion of the non-germinated seeds could resume their germination after digging up, which cannot form a reliable seed bank for a minimum seed life span of one year.

#### ACKNOWLEDGMENT

The author thanks Department of Agronomy and Plant Breeding, ShahreKord University for permission to let him use the



lab space and environmental simulator machine. The technical assistance of Mr. Gharekhani and Mr. Abedini are acknowledged. Special thanks are also extended to Department of Plant Biology, Isfahan University to authorize the researcher to access their osmometer. The helpful comments of Dr. Saadollah Houshmand are greatly appreciated.

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(Received: Nov.14-2010, Accepted: Apr.1-2011)

## پاسخ جوانه زنی بذر *Haloxylon persicum* (Chenopodiaceae) به شرایط متفاوت دمایی- رطوبتی و عمق‌های مختلف دفن در ماسه

ع. سلطانی

### چکیده

آزمایش جوانه‌زنی در شرایط آزمایشگاهی برای بذرهای تاغ سفید (*Haloxylon persicum*) قرار گرفته تحت ۳۵ نوع شرایط متفاوت دمایی - رطوبتی (۲۵-۱۰ درجه سانتیگراد و صفر تا ۳- مگاپاسگال) انجام شد. سپس بذرها در چهار عمق مختلف ماسه دریایی برای سه هفته در گلدانهای کاملاً زهکش شده کشت شدند. دوره جوانه‌زنی در هیچ یک از شرایط دمایی-رطوبتی از ۱۲ روز فراتر نرفت. پتانسیل آبی ۱/۵- درصد جوانه‌زنی را به کمتر از یک چهارم جوانه‌زنی در شرایط بهینه کاهش داد و جوانه‌زنی کاملاً در پتانسیل آب ۳- متوقف شد. همانطور که پتانسیل آب از صفر به ۱/۵- کاهش یافت، مقادیر دمای حداقل از ۵/۴ به ۵/۶ درجه سانتیگراد افزایش یافت. عمق دفن ۶ سانتی‌متری برای کاهش ۵۰ درصدی ظهور گیاهچه‌ها در سطح برگ کافی بود و ۰.۸۵٪ و ۰.۱۰٪ بذرهای جوانه‌زده به ترتیب در عمق ۸ و ۱۲ سانتی‌متری، گیاهچه به سطح خاک نرسید.