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INVESTIGATION OF SEED GERMINATION CHARACTERISTICS AND RECOVERY OF GERRMINATION IN THE HALOXYLON APHYLLUM UNDER DIFFERENT TEMPERATURE AND SALINITY CONDITIONS

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ABSTRACT

Haloxylon aphyllum is one of the most important species in sandy and saline areas in deserts, and some ecophysiology characters for viability in unfavorable condition have evaluated in this species. Seed germination is the critical stages in life cycle for species survival. In this study investigated salinity and temperature stresses on percentage and velocity of germination using six salinity treatments (0, 100, 200, 300, 400 and 500 mM/l NaCl) under four different temperature regimes (20:10, 30:10, 25:15 and 35:25 ^0C). The results showed that seeds could germinate at very high salt concentration (500mM). However, highest germination percentage was obtained in distilled water and thermoperiod 20:10^0C. Increased on salinity and temperature caused decreased of percentage and velocity of germination. Seed germinated rapidly and no significant change in germination was noticed after 10d. Recovery of seed germination investigated when seeds were transferred to distilled water. The results indicated that recovery percentage was high in seeds were transferred from very saline treatment with lower temperature.

Keywords: *Haloxylon aphyllum, Salinity and temperature stress, Seed germination, Recovery of seed germination*

Introduction

Deserts are regions with irregular precipitation regime and high evaporation potential. In these regions, high level of evaporation causes salts accumulation at soil surface and soil salinization (Danin, 1996). In deserts, various ecophysiological characters and changes are seen in plants including life cycle (annual, perennial), vegetation form (brush, shrub), response to aridity (tolerance and avoidance from aridity), flowering period and kind of seeds distribution and germination (Fahn & Cutler, 1992). One of the plants adapted to desert condition is saxaul (*Haloxylon aphyllum*). This species belongs to Chenopodiaceae family covering majority of desert areas in Asia in which water depth is very low (Danin, 1996). Geographical distribution of this species in Iran covers southern (Kotal, Dalaki and Gawkhane), eastern and central and southern east (Landagh, Anarak, Yazd, Dashte kavir, Bastam, Shahrud and Balochestan) areas (Qhahreman, 1996).

At the economy point of view, saxaul is an important species used for stabilization of sandy lands, range lands, fuel etc. Some characters have been developed in it to be tolerable to the aridity, salinity, Nitrogen shortage and severe wind. In this case, this plant has been classified as Xero-Halophyte (Gutterman, 1993). Some of these characters include decrease of leaf area, having succulent stems to be used for photosynthesis, having succulent roots and cover seeds. These characters ensure its life in severe conditions. Flowering period of this species is between September and October seeds become matured at the end of winter (Qhahreman, 1996). Immatured seeds content 80-86% humidity which will be decreased to 10% two months after seed maturing. The matured seeds are scattered in summer and germinate at the end of summer and early of fall (Qhahreman, 1996).

Germination is the most critical stage in life cycle of this plant covering maximum of tolerance (seed dormancy) and seedling stages (pujol et al, 2000). During seeds distribution in summer till their growth in fall, some unfavorable conditions like aridity and heat are common in deserts. The mature seeds come over dormancy using their hard seed coat. At the beginning of fall, rain can decrease salt contents in the soil and humidity will be sufficient for seed germination.

Consequently, seeds germinate rapidly. This rapid germination is an adaptation found in saxaul under this condition (Rubio-Casal et al, 2000). If rain is low and scattered, seedlings will not grow because soil humidity will be evaporated from sub-surface layer solar heat and salts will be transferred to surface by capillarity force. In this case, seedlings are died due to the salinity and hight temperature and seed germination is stopped. Thus, after a rainy period, only few seeds can germinate at unfavorable conditions and few of them will be survived (Pojol et al, 2000).

Kaul et al (1990) showed that saxaul seeds germinate in light and dark periods. But, increasing the salinity causes germination to be decreased and finally in 102 mol/lit NaCl to be stopped. They observed that maximum germination occurred in salt-free treatments. Similar results for *Atriplex griffithii* (Khan and Rizvi, 1994) and *Nitraria schoberia* (Moshtaghian and Esmaeli, 1997) were observed. The salt tolerance ranges are various in plants. For example, maximum tolerance for *Atriplex patula* and *Tamarix pentanda* are 0/17 and 0/85 mol/lit NaCl, respectively (Ungar, 1996). Khan and Ungar (1996) carried out researches on *Haloxylon recurvum* in Pakistan.

They transferred its seeds from salty treatments to salt-free ones and concluded that some seeds grown in more saline treatments (0.4 to 0.8 mmol/lit) had 40% germination percentage other seeds grown in lower salinity media, had nearby 20% germination percentage. They stated that before germination, the high osmotic pressure and high salinity ranges of medium are necessary for seed germination. This condition caused seeds to be grown uniformaly and the velocity of germination to be increased. The longevity of each seed or germination viability depends on seed humidity content and temperature of environment. The saxaul seeds have a short life time about 10 months (Farant et al 1997, Zheng et al 1998). High salinity couldn't damage seeds (Rubio-Casal et al, 2003). In this study, some eco physiological aspects of seed germination and seed recovery including the effects of salinity and temperature on *Haloxylon aphyllum* were studied.

Materials and Methods

Saxaul seeds were collected from Naeen, Isfahan province. Seeds were stored in plastic pockets at room temperature (14-30°c) and the humidity mean as 26% percentage. They were sterilized by phygon fungicide.

Seed germination under temperature and salinity treatments: seeds were germinated in petri dishes with 90*50mm size having appropriate solution. Duration of experiment, petri dishes were covered in order to decrease evaporation. 25 seeds were used for every treatment with four replicates. In order to find temperature effect on germination, four thermo period (A: 10-20°, B: 10-30°, C: 15-30° and D: 25-35°) were provided by germinator (Khan & Ungar 1984 and Ungar & Khan, 2001).

High temperatures (20, 30, 25, 35°) in 12 hours lightening and low temperatures in 12 hours darkness of florescent lamps were applied.

Seeds germination was investigated under 0, 100, 200, 300, 400 and 500 mmol NaCl under discussed thermo period treatments. In this experiment, seeds germination was assessed by determination of germination percentage and germination velocity in a 20 days period. For this purpose, germinated seeds were counted daily and transferred. Germinated seeds mean those which are emerging from Petri dishes. For determination of germination rate in different treatment corrected Timpsons index of germination was used.

$$(VG) = \frac{\sum G}{t} \qquad (1)$$

Velocity of germination (VG) is expressed as percentage, G as germination per day and t as total time of germination. The more the rate of VG is, the more the velocity of germination (Pujol et al, 2000). In this research, the maximum level of this index was 35 (100/20)

Recovery of seed germination

After 20 days, seeds that couldn't germinate in different treatments were transferred to distilled water in order to determine seed germination under temperature and salinity stress. For this purpose, the equation of germination recovery (RG) was used (Pujol & et al and Vicente, 2004).

$$(RG)=[(a-b)/(c-b)]*100 \qquad (2)$$

RG= percentage of seeds recovery

a= total number of germinated seeds in distilled water

b= total number of germinated seeds in saline treatment

c= total number of germinated seeds

The aim of this research is to study whether environmental factors (temperature and salinity strees) or seed damaging are the reasons causing seeds not to germinate.

Statistical analyzing

For statistical analyzing, SPSS software (ver. 10) was used. For variance analyzing, ANOVA was used and the normality test was done by kolomogrov-smirnov and levene test. The rate of germination percentage was converted to Arcsine before analyzing and the results presented in standard error form.

Results

The results of daily mean germination is showed in figure 1. The results show that in treatments having some salt concentration (for example 1-0mmol/lit), germination is decreased by increasing of temperature. In all temperature treatments, the highest germination percentage occurs in distilled water. Germination percentage is decreased by increasing of salinity. The lowest germination percentage occurred in 25-35$^{°c}$ thermo period in salt-free treatment.

The total mean germination percentage based on standard error was calculated and results are showed in figure 2. Results analyzing using ANOVA showed that seed germination in

Haloxylon aphyllum is affected by salinity ($p=0.0001$ and $f=107.2$), thermo period ($p=0.0001$ and $f=19.57$) and interaction of these two factors ($f=3.416$ and $p=0.000$).

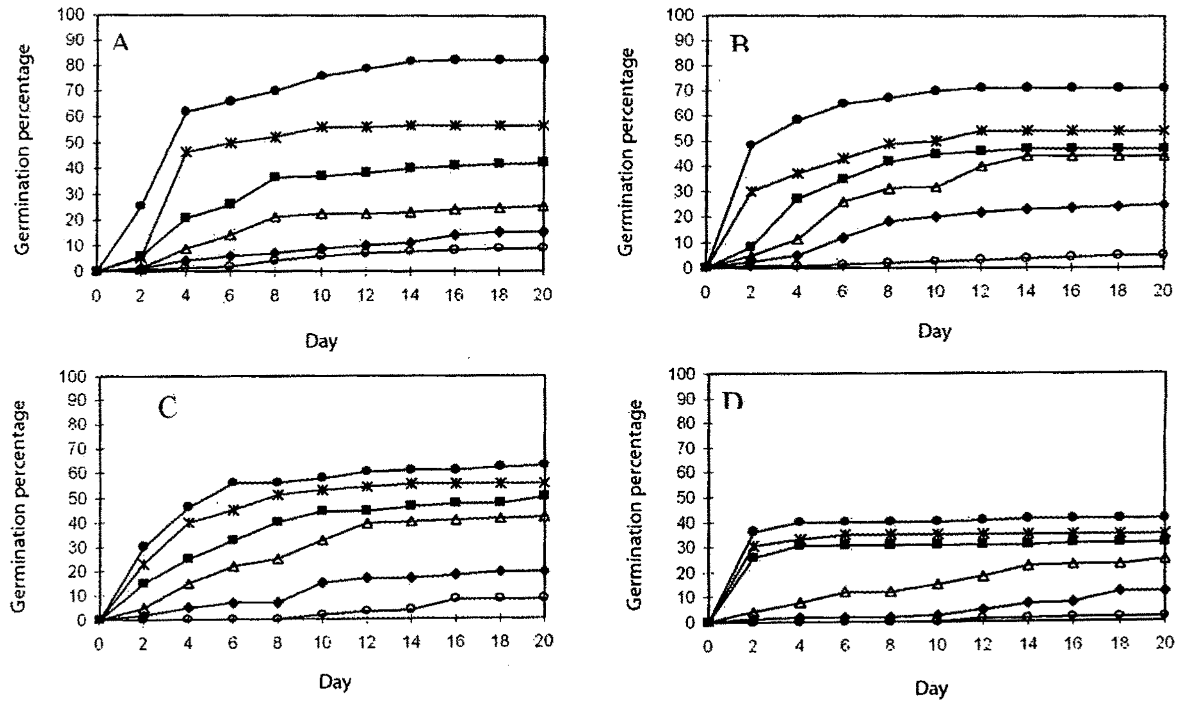

Figure 1. Diagram of daily mean germination percentage in treatments (0, 100, 200, 300, 400 and 500 mmol/lit and thermo period)

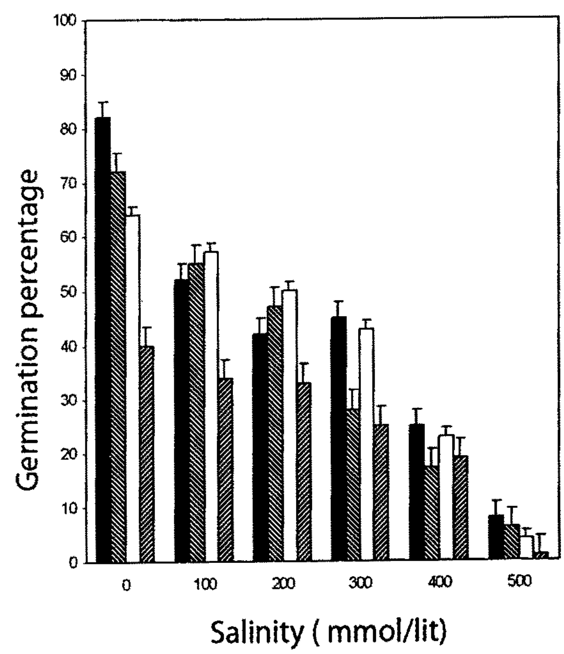

Figure 2: Diagram of final germination and standard errors

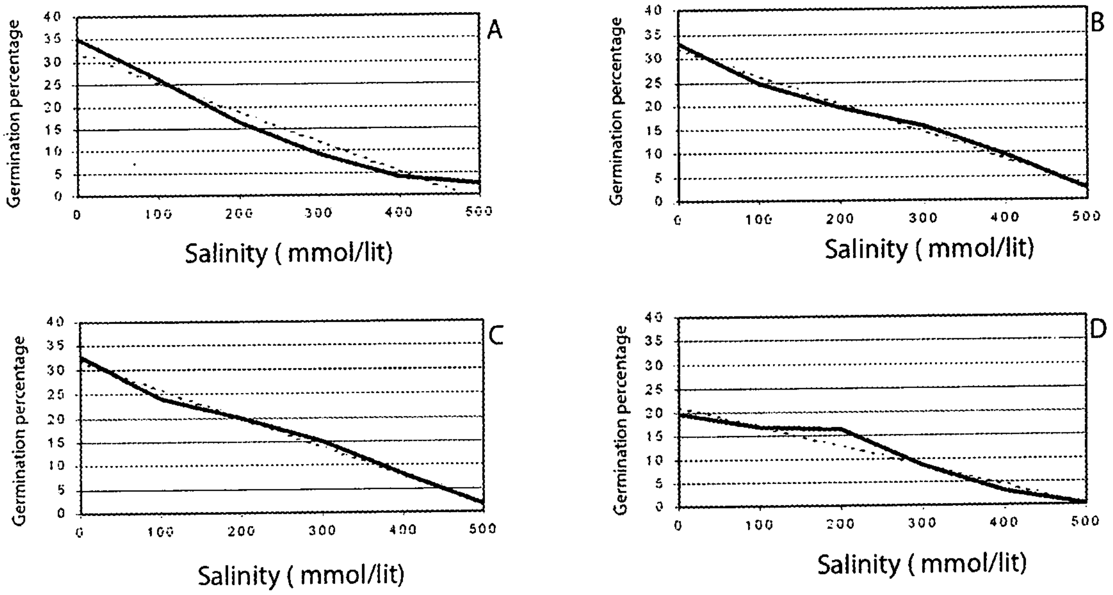

Figure 3: Diagram of germination velocity in different NaCl concentrations and thermo period

With using of corrected Timpson index, germination velocity (VG) was calculated in different treatments and its results were showed in figure 3. The germination velocity was decreased by increasing of salinity. The germination velocity was similar in low and medium temperature but decreased in high temperature. In order to understand results, the best slope line for data was drawn using Excel for A to D thermo periods software and these slope were -0.07, 0.05, -0.06 and -0.4, respectively. Negative denotation demonstrates the inverse relationship between salinity and germination velocity. The slope of line shows the decline of germinate velocity in low temperatures is faster than in high temperatures. Thus, germination velocity in low temperatures is affected by salinity level.

After 20 days, other seeds that couldn't germinate under saline treatments were transferred to dishes having distilled water and recovery of seeds germination (RG) were investigated in a 20 day period (equation 2) and showed in table 1.

Table 1: Recovering of germination percentage after transferring to distilled water

Temperature (°c)				
35:25	25:15	30:10	20:10	mm/L
1.5±8	0	0	12.5±56	0
0	3.2±14	0.7±2	0.5±21	100
0.7±3	1±6	2.1±6	2.5±5	200
3.5±17	7.8±34	2.6±24	1.3±14	300
1.2±6	1.2±40	2.4±21	2.5±50	400
1.8±9	2.7±44	3.2±35	2.5±37	500

These results showed that germination percentage under 25-35$^{\circ c}$ thermo periods (high temperature) was very low in all treatments but enhanced by increasing of salinity.

Discussion

In this study, the effects of salinity and temperature on *Haloxylon aphyllum* were investigated. According to the results, germination velocity of first days was high and after ten days, reached to the maximum level in all treatment.

By increasing of salinity, germination velocity was decreased. Seeds grow in low temperature and distilled water germinated better than other treatments. Increasing in salinity and temperature caused decreasing in germination percentage and delaying in germination. Thus, under 500 mmol/lit NaCl and 25-35° thermo periods, germination was stopped. These results are as the same those of other research done on Halophyte species. In many studies, Halophyte species had shown the same behaviors under saline environment including delaying in germination, decreasing of germination percentage and velocity and finally seed dormancy due to high osmotic pressure and shortage of available water (Ungar and Khan 1984, Pujol et al 2000 and Rubio-casal et al 2003). Naturally, when seeds are growing under inappropriate conditions like high salinity and temperature which are out of tolerance capacity of seeds, germination is stopped and seeds become dormant. With commencement of raining and decreasing of salinity and osmotic pressure, germination will be occurred rapidly (Vicente, 2004). In this study, in seeds transferred from saline treatment to distilled water, recovery of germination was investigated. Germination level in seeds transferred from very saline treatment (500 and 400 mmol/lit NaCl) with low or medium thermo period was 40 to 50%, but lower in other concentrations and thermo periods. Thus, excessive salinity doesn't damage seeds or cause ionic toxicity for seeds but causes high osmotic pressure leading to lack of ability of seeds to germinate. The germination ability will be maintained and seeds will germinate under favorite conditions. Germination velocity of seeds transferred to distilled water was more rapid than those of primary treatments.

After 5 days, germination was reached to its maximum level. Similar results (e.g. Pujol et al, 2000) were found for four Halophyte species. The recovery of seeds grown under 500 mmol/lit NaCl and 25-35° thermo period was very low. This shows that seeds grown under high salinity and temperature simultaneously may lose their germination ability for ever.

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