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The Effect of Osmotic Conditions on Germination of (*Haloxylon aphyllum* L.) Seeds and Recovery Under Water Stress

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Abstract: *Haloxylon aphyllum* L. is a an important perennial plant for conservation in Asian, especially Iran dune desert. The effects of water potential on germination and recovery responses after transfer to distilled water and osmotic potential were studied in *Haloxylon aphyllum* L. seeds collected from Fars (neirize) dune desert located in the elevations of 1632 m.a.s. in center of Iran in Asian desert. Treatments included 2 pretreatment levels (0, -1.2 MPa) and 6 levels of polyethylene glycol PEG (6000) potentials (0, -0.3, -0.6, -0.9, -1.2, -1.5 MPa), using a completely randomized block design. Result showed that osmotic potential had a significant effects on germination percentage, germination rate, seedling length and seedling dry weight. Total of them decreased with increased osmotic potential. Recovery of seed increased of seedling characteristics after being transferred to distilled water and osmotics potential. As prime witht Poly Etilen Glycol (PEG) concentration increased threshold to osmotic potential.

Key words: Germination, *Haloxylon aphyllum* L., osmotic conditions, polyethylene glycol, water stress, recovery

INTRODUCTION

Haloxylon aphyllum L. is a leaf succulent perennial plant belong to the family chnopodiaceae which 1200 species same (Ali and Qaiser, 2001). *Haloxylon aphyllum* L. is a perennial shrub that is widely distributed in Turkey, Syria, Iraq, Iran, Afghanistan, Kashmir, India and Central Asia same (Jafari, 1966). It is mainly distributed in the Northeast and Southwest and center dune desert of Iran. In Iran, this plant is mainly distributed in Dune deserts of Fars (neirize) provenance, center of Iran in Asian desert. Iran is a country in the mid-latitude belt of arid and semi-arid regions of the Earth. Approximately 60% of Iran is classified, as arid and semi-arid much of the country has a desert climate with an average annual precipitation of <300 mm (Mansoory, 1992). This specie have a main role in restoration of these areas (Huang *et al*., 2003). In Asian desert, the average annual rainfall is between 200 and 30 mm or even less (Fu, 1989). Haloxylon species regenerated by seed and establishment of the species in new site depend on seed dispersal, germination and establishment of seedlings. Seed germination in the plant life cycle is the critical stage for survival, especially under arid and unpredictable environmental conditions like those of the Mediterranean ecosystems (Gimenez-Benavides *et al*., 2005). Seed and uniformity of germination are limiting in forest trees (Fenner, 1992). Seed germination plays an important role on the regeneration of plant species, especially under unpredictable environmental conditions like those of the Mediterranean ecosystems (Gimenez-Benavides *et al*., 2005). Poor germination and seedling establishment are regarded, as the major causes of low densities in Mediterranean forests (Close and Wilson, 2002). Also, seed performance depends on vigor (Copeland and McDonald, 1995). Seed quality is also critical for the early vigor of a new plant. Early vigor is a combination of the ability of the seed to uniformity germinate and emerge after planting and the ability of the young plant to grow and develop after emergence (Hou and Wang, 2002). Several factors in the environment determine germination occurs (Bewley and Black, 1994). Seeds especially with low vigor, sown in cold spring are often exposed to numerous environmental hazards during germination. Temperature and moisture levels appear to be critical, at germination and emergence stage (Bodsworth and Bewley, 1981). Poor establishment my result and as a consequence, a low competitive ability allow weed to over come sown grasses. Water constant in soil is an important environmental factor for controlling of seed germination (Kramer and Kozlowiski, 1976). Seed germination in field conditions occurs after Winter rains after that the soil dries out quality. In dry forests, species germination and early establishment must occur during

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the first of wet season when water is more available too (Khurana and Singh, 2001). Seed priming is a treatment that partially hydrates seed, so that germination processes begins but radical emergence doesn't occur (Welbaum *et al*., 1998). Osmopriming to improve germination early Spring, at low temperatures especially with PEG solutions, may be of great practical importance particularly in Mediterranean environments priming of wheat seed in osmoticum or water may improve germination and emergence (Ashraf and Abu-Shakra, 1978) and promote vigorous root growth (Carceller and Soriano, 1972) under low soil water potential compared with checks. Primed seed with PEG6000 exhibited increased in germination rate of the 3 herbage grass at low temperatures (Mauromicale and Cavallaro, 1996). Priming of seed in osmotic solution has been used to improve the rate and uniformity of germination of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Al-Karaki, 1998). There is little information available about seed germination responses *Haloxylon aphyllum* L. to water stress and there is not data on the recovery rate of seed from osmotically induced water stress. *Haloxylon aphyllum* L. is one of the dominant species of Iran dune deserts present but little data is available on germination responses to water stress are poorly understood. Researcher need anyway to impove germination rate on increases duration of germination. The aim of this research were to investigate, the effects of water stress on germination and seedling vigor and response of recovery under water stress conditions for the improve of water stress threshold by increase germination phase tolerate to water stress limited. Prime is one of these methods.

MATERIALS AND METHODS

Mature seed lots of *Haloxylon aphyllum* L. were harvested Fars (neirize) dune desert Fars dune desert (29°35' 54°00' E) located in the elevations of 1632 m.a.s. in center of Iran in Asian desert. Iran is a country in arid and semi-arid regions of the Earth. Approximately 60% of Iran is classified, as arid and semi-arid, much of the country has a desert climate with an average annual precipitation of <300 mm (Mansoory, 1992). After the decreasing of humidity, the seeds were stored within bags in a refrigerator (5°C) until the beginning of each experiment. This study was carried out at the Department of Dessert Region Management, College of Agriculture, University of Shiraz, Iran. Seeds were treated using the 0.2% fungicide Benomil before the germination test. The treatments were 6 levels of polyethylene glycol PEG (6000) potentials including control, -0.3, -0.6, -0.9, -1.2, -1.5 MPa. *H. aphylum* seeds were primed in critical PEG potential (-1.2 MPa) where seeds imbibed but did not germinate for 3 day, at 25°C then seeds were washed with distilled water and transfer to 6 levels of polyethylene glycol PEG (6000) potentials. The recovery percentage was determined by the following formula (Kaya *et al*., 2006):

$$\text{Percent recovery} = a-b/c-b\times100$$

Where:

a = The total number of seeds germinated after being transferred to distilled water
b = The total number of seeds germinated at different osmotic potentials
c = The total number of seeds

In all experiments, 4 replications of 50 seeds of 2 provenances were sown, at -0.3, -0.6, -0.9, -1.2, -1.5 MPa. On Whatman No. 1, filter paper in Petri dishes (50 mm diameter) in light condition (Ghaedi *et al*., 2009) at 25°C (Taghvaei and Ghaedi, 2010). The filter paper was moistened with about 5 mL of treatment, so that about half of the seeds were immersed in the solution. During the experiment, lost solution water was replaced when necessary. The seeds were considered to have germination when the emerging radical were over 5 mm long (Young *et al*., 1981). The numbers of germinated seeds were recorded on daily basis. After 9 days, the final number of germinated seeds was calculated, as well as the percentage of germination. Mean time to full germination was calculated according to the equation of Roberts and Ellis (1981). The germination rate was calculated by inverse of mean time to full germination (Tobe *et al*., 2000; Flores and Briones, 2001).

$$GR = 1/MTG$$

Where:

GR = Germination Rate
MTG = $\sum (ni.ti)/\sum n$
MTG = Mean time to full germination
n = No. of seeds newly germinating at time t
t = No. of day from sowing

Shoot Length (SL cm) was measured on the length from the top to the shoot basis and Root Length (RL cm) is the length from top of the root. Shoot Dry Weight (SDW g) and Root Dry Weight (RDW g) were measured after drying for 24 h in an oven, at 70°C (ISTA, 2002).

Threshold: Some methods have been developed previously to describe species relative productivity in response to stress but among those methods the regression germination percentage method is the most specifically salient to stress (Taghvaei, 2008;

Covell *et al*., 1986) regression analysis is generally recognized as the best statistical tool for the investigation of relationships among relative germination and water stress. The germination collected at control to -0.9 MPa osmotic potentials were used to construct 2 linear regressions to describe the decreases and threshold of germination in stress condition.

Data analyses: Data were checked for normality and then were analyzed using Mstac statistical software. Treatment means were separated by Duncan test, if the F-value of the treatments was significant at the 0.05 or 0.01 probability levels.

RESULTS

Germination Percentage (GP): Germination percentage of *H. aphyllum* seed was significantly ($p<0.01$), affected by osmotic potential (Table1). The germination percentage of *H. aphyllum* decreased with increase osmotic potential. There was also a noticeable decrease in germination percentage at -0.6 MPa (Table 2). As germination percentage, reached from 92% in control to 56.6% in -0.6 MPa. No seed germinated observed in -0.9 MPa (Table 2). Prime with PEG for 3 day improved germination percentage. Over all final germination percentage was coMParable in both non-prime and prime seed, germination percentage in primed seed was significantly greater than non-primed seed (Fig. 1). A germination continued in -0.9 MPa in primed seed but no germination observed in -0.9 MPa (Table2).

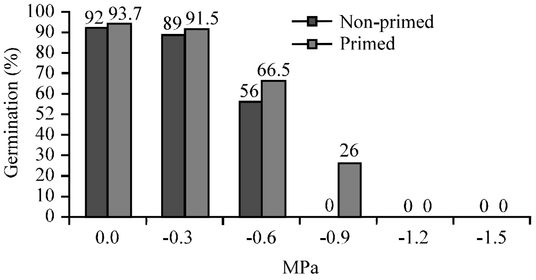

Fig. 1: *Haloxylon aphyllum* Germination Percentage (GP) response to osmo potential

Germination rate: Osmotic potential significantly ($p<0.01$) affected germination rate (Table 1). The average of germination rate in control was 0.51 seed h^{-1} but it decrease by 0.19 seed h^{-1} in -0.6 MPa (Table 2). Prime significantly improved the germination rate (Fig. 2). The average germination rate in control for non-primed seed was 0.51 seed h^{-1} while in seed primed was 0.81 seed h^{-1}. The germination rate at -0.3 and -0.6 MPa in seed primed was significantly greater than non seed primed (Table 2).

Mean Time to full Germination (MTG): Mean time to full germination significantly ($p<0.01$) increased by osmotic potential (Table 1). Osmotic potential treatments of -0.3 to -0.6 MPa increased mean time to full germination by 51.87 and 124.49 h, respectively (Table 2). Prime with PEG significantly decreased of mean time to full germination (Fig. 3). The mean time to full germination was shorter in primed seed (29.98, 30.29 and 45.73 h), than non seed primed (51.87, 58.9 and 124.49 h) in control -0.3 and -0.6 MPa, respectively (Table 2).

Seedling Length (SL): The seedling length was significantly affected by water stress treatment (Table1). The average of seedling length in control was 3.8 cm but at -0.6 MPa was 0.856 cm. Prime increased seedling length. There were significant difference ($p<0.01$) between non seed primed and seed primed in all level of osmotic potential studied. As there was no significant between control and -0.3 MPa in primed seeds (Fig. 4).

Table 1: Analysis of variance for seed and seedling parameters

Sources	Degree of freedom	G (%)	MGT	GR	SL	SDW
Replication	3	11.570	13.26	0.005	0.045	0.00^{**}
Drought (A)	5	16367.120^{**}	8146.18^{**}	0.662^{**}	24.850^{**}	0.00^{**}
Pretreatment (B)	15	180.188^{**}	1481.97^{**}	0.649^{**}	23.270^{**}	0.00^{**}
$A^{*}B$	5	40.008	4271.89^{**}	0.068^{**}	3.036^{**}	0.00^{**}
Error	35	1823.240	1736.90	0.005	0.050	0.00^{**}

*,**Significant at $p<0.05$ and <0.01, respectively

Table 2: Variation in seed and seedling parameters mean among osmotic potential level

Osmatic potential (MPa)	G (%)		MTG (h)		GR (seed h^{-1})		SL (cm)		SDW (g)	
	np	p	np	p	np	p	np	p	np	p
0	92.00	93.70	51.80	29.98	0.510	0.810	3.800	47.78	0.003	0.00350
-0.3	89.00	91.50	58.90	30.29	0.410	0.790	1.940	4.61	0.002	0.00300
-0.6	56.00	66.50	124.50	45.73	0.190	0.530	0.856	3.53	0.001	0.00200
-0.9	0.00	29.00	0.00	62.60	0.000	0.380	0.000	1.96	0.000	0.00100
-1.2	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.000	0.00000
-1.5	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.00	0.000	0.00000
	39.58^{b}	43.45^{a}	39.22^{a}	28.10^{b}	0.186^{b}	0.418^{a}	1.090^{b}	2.48^{a}	0.001^{b}	0.00158^{a}

Means with the same letter on each column are not significantly different ($p<0.05$); P = Primed seed; NP = Non-primed seed; G (%) = Germination percentage; GR = Germination Rate (per day); MTG = Mean of Time Germination; SL = Seedling Length (cm); SDW = Seedling Dry Weight (g); Germination Rate (GR)

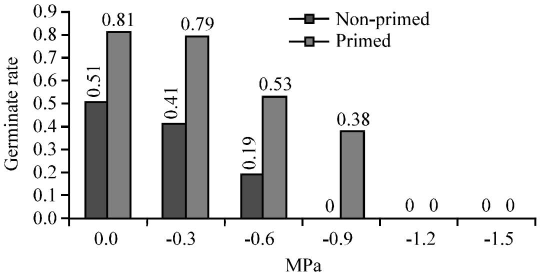

Fig. 2: *Haloxylon aphyllum* germination rate response to osmo potential

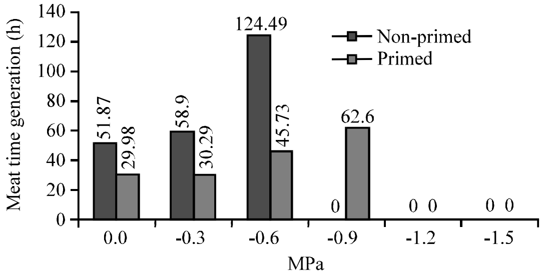

Fig. 3: *Haloxylon aphyllum* Mean Time to full Germination (MTG) response to osmo potential

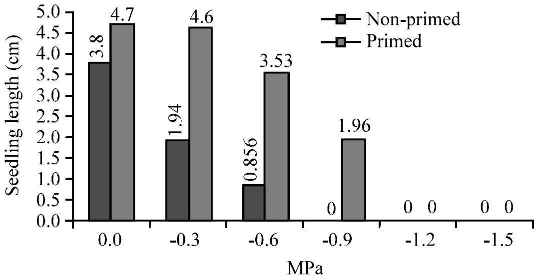

Fig. 4: *Haloxylon aphyllum* Seedling Length (SL) response to osmo potential

Seedling dry Weight (SW): Water stress treatment significantly ($p<0.01$) decreased seedling dry weight (Table 1). Seedling dry weight decreased, as the severity of osmotic potential increased (Table 2). The average of seedling dry weight in control was 0.003 g but reached to 0.0002 g at -0.6 MPa. Priming for 3 days significantly ($p<0.01$) increased seedling dry weight compared to non-prime seed (Fig. 5). There were no significant difference between the seedling dry weight primed seed and non-primed seed in control level (Table 2).

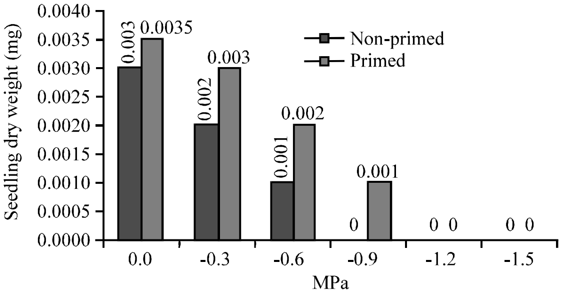

Fig. 5: *Haloxylon aphyllum* Seedling Dry Weight (SDW) response to osmo potential

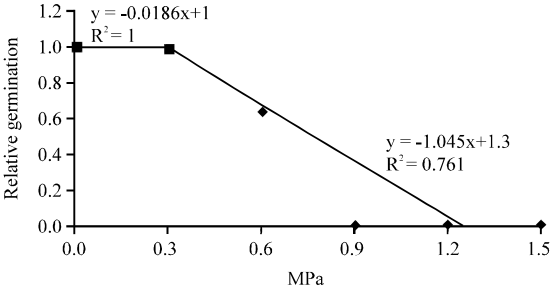

Fig. 6: *Haloxylon aphyllum* relative germination response to osmo potential before primed

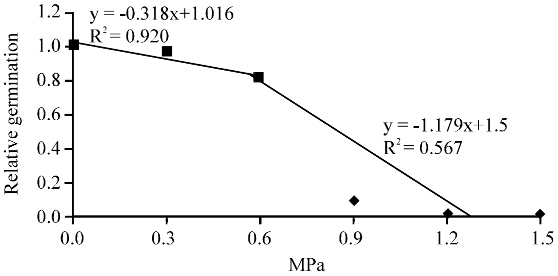

Fig. 7: *Haloxylon aphyllum* relative germination response to osmo potential after primed

Threshold: Prime increased threshold of germination to osmotic potential of *H. aphylullm* seeds (Fig. 6 and 7). The highest of germination were obtained, at control in non-primed and prim seed, however them was decreased slowly with increased of osmotic potential (Fig. 7). In the non-primed seed germination suddenly decreased at -0.3 (Fig. 6). But in primed seed, this suddenly slope showed in -0.06 MPa (Fig. 7).

The intersection of tow linear between 0 to -0.03 and -0.3 to 09 Mpa and between 0 to -0.06 and -0.6 to 1.2 MPa showed threshold germination to osmotic potential for non-primed (Fig. 6) and primed (Fig. 7), respectively.

DISCUSSION

The initial germination of *H. aphyllum* sensitive to osmotic potential. Osmotic potential decreased of germination traits especially. Osmotic potential of PEG solution reduced germination percentage, germination rate, seedling length and seedling dry weight. Similar finding were reported about other halophtic species (Khan and Rizvi, 1994; Tobe *et al*., 2000). Germination of *H. aphyllum* decreased with an increased osmotic potential and inhibited at the low of osmotic potentia l (-0.9 MPa). Low osmotic potential down water uptake by seed, there by inhibiting germination (Dodd and Donovan, 1999; Katembe *et al*., 1998). The 2nd phase water uptake involves the movement of water across the cells membranes of seed depend on the osmotic potential of the surrounding solution (Bewley and Black, 1994). Osmotic priming of *H. aphyllum* seeds increased of germination percentage, germination rate, seedling length and seedling dry weight. The seed of *H. aphyllum* remained viable after 3 days in -1.2 Mpa. The prime was beneficial effect on seed vigour. When seeds of *H. aphyllum* were transferred to all treatment of osmotic potential, germination was faster under control and osmotic potential conditions. But, the recovery of germination percentages decreased significantly with an increase of osmotic potential (Table 1, Fig. 1). Similar finding was reported about Atriplex species (Katembe *et al*., 1998). There are reports that priming permits early DNA replication (Bray *et al*., 1989), increased RNA and protein synthesis (Ibrahim *et al*., 1983), greater ATP availability (Mazor *et al*., 1984), faster embryo growth (Dahal and Bradford, 1990), repair of deteriorated seed parts (Giri and Schillinger, 2003) compared with cotrol. The seeds of halophyte remain viable after long period of exposure to salinity osmotic (Keiffer and Ungar, 1995) and primed neutrality germinated rapidly after lather rain in arid land. Primed increased osmotic potential tolerance in germination phase.

CONCLUSION

In arid environment rapid germination and early seedling vigor tend to maximize use of available soil water after Winter, resulting in increased dry matter accumulation. In this area, temperature raise rapidly and evaporate soil water, resulting osmotic potential and limited optimum duration for germination and decreased germination percentage and plant per land. In this study, demonstrated that germination of *H. aphyllum*, as a plant in these area sensitive to osmotic potential. Seed priming with PEG improved tolerance to osmotic potential by increasing the percentage and rate of germination. *H. aphyllum* seeds in top soil in salty dune desert after late Winter expose with osmotic potentials above of their tolerance limits and primed, so increased during of germinated after rainy periods.

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