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Effect of drought stress on the germination parameters of *Cupressus* seeds

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Abstract: To study the effect of drought stress on germination and some physiological characteristics of *Cupressus arizonica* and *C. sempervirens* seeds including germination percentage, mean germination time (MGT), germination speed, germination energy, vigor index and recovery percentage, a factorial experiment in completely Randomized Design (CRD) was arranged with five treatments of osmotic potential and 4 replications. The water potential of the germination substrates (0, -2, -4, -6 and -8 bars) was conducted using PEG-6000 solutions. The seeds were kept for 37 days in germinator and 8-16 hours (radiation-darkness) and at 20 ± 0.5°C. The results indicated that drought stress had significant effect on seed physiological characteristics in both species. Decreasing water absorption potential from 0 to -8 bars, significantly reduced germination percentage, speed, energy, and vigor index and increased mean time of germination in both species. The highest recovery percentage was observed in -8 bars. Germination percentage and germination speed, vigor index and recovery percentage of *C. sempervirens* was better than *C. arizonica* that probably is more resistant species to drought stress.

Keywords: *Cupressus* seeds, Polyethylene glycol 6000, Recovery percentage, Vigor Index.

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Introduction

Arizona Cypress (*Cupressus arizonica* ***var arizonica*** Greene) and Medite Cypress (*Cupressus sempervirens* var. horizantalis (Mill.) Gord) two species belonging to the *Cupressaceae* family are very important forest tree species for multiple purposes in forestry because of their ability to grow in adverse environments such as calcareous, clayish, dry and poor soils (Gallis et al., 2006) and have important roles in the landscape, local economy, plantations and city green areas (Bagnili and Vendramin, 2009). Different factors are affecting recruitment in forest species and the establishment of new forest areas. One of the main processes is germination, because we can infer some information on the strategy of the species to cope with drought (avoidance, tolerance), and also, knowledge has important implications in the management of the seed during nursery (Kozlowski and Pallardy, 2002). Suitable conditions for seed germination and seedling growth are the most important factors that affect the natural regeneration of forests (Vickers and Palmer, 2000). Decrease in water potential in germination medium needed for germination process to start because of drought prevent water absorption (Almansouri et al., 2001). Moisture conditions immediately prior to and during germination play a dominant role in regulating germination (Karssen, 1982). According to Qkcu et al. (2005) seeds supplied with insufficient moisture will show unsynchronized seedling emergence which is an important problem in nurseries. Drought is one of the most important factors that limit trees germination in Iran. Therefore successful tree culture depends on the ability of seeds to germinate under low soil moisture conditions. One technique for studying the effect of water stress on germination is to simulate stress conditions using artificial solutions to provide variable water potentials (Falusi et al., 1983). PEG is an osmotically active but physiologically inert substance, which is unable to penetrate the seed coat because of its high molecular mass. Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita et al., 1979). Water is withdrawn from the cell and the cell wall because PEG does not enter the apoplast, however, some researchers have reported toxic effects caused by PEG (Fan and Blake, 1977), even though PEG is a nonionic, long chain, non-penetrating and inert polymer. A large number of studies have been carried out on the effects of drought stress on the germination of forest tree species. Effects of drought stresses induced by polyethylene glycol (PEG) on germination of Mongolian pine (*Pinus sylvestris* var. *mongolica* L.) indicated that the seeds from both provenances did not germinate when PEG concentration was more than 25%. The germination capacity and germination rate of natural seeds were significantly higher than those of plantation seeds for all treatment levels. The mean growth rates of radical and hypocotyl from natural seeds were significantly higher than those from plantation seeds at all treatment levels below 20% PEG treatment ($P<0.05$) (Zhu et al., 2006). Effects of drought stress using PEG-6000 with potentials 0, -2, -4, -6 and -8 bars by Boydak et al. (2003) were examined on germination in six provenances of Brutia pine (*Pinus brutia* Mill.) seeds from different bioclimatic zones in Turkey. Their results showed that a decrease in water potential produced a marked reduction in germination percentage, germination speed and germination value. Changes in germination of Stone pine (*Pinus pinea* L.) seeds under various abiotic stresses osmotic potentials of -0.30MPa (10% polyethylene glycol [PEG]), -0.58 MPa (18% PEG), -0.80 MPa (21% PEG), -1.05MPa (24% PEG) showed that PEG caused the most detrimental effects on *Pinus* seeds. On the other hand, with increasing the osmotic potential, the germination was completely inhibited (Muscolo et al., 2007). Several reports indicate that seeds show appropriate response in germination stage, will had better growth and stronger root system in seedling stage (Berkat and Briske, 1982; Gupta et al., 1993). Also, the ability seeds to maintain viability after an extended period of exposure to drought stress have been investigated by Maraghni et al. (Maraghni et al., 2010). Most seeds show an important recovery of germination when stress conditions are alleviated, indicates that they may be more drought tolerant than actively growing plants (Ungar, 1996). The purpose of this study was to evaluate the influence of drought stress on germination indices, vigor and recovery percentage of *C. arizonica* and *C. sempervirens* induced by PEG 6000 and selection the better resistant species for seedling production.

Materials and Methods

Seeds of *C. arizonica* and *C. sempervirens* species were collected in 2008 with equally in size and weight were supplied from the Caspian forests seed center in Mazandaran, Amol. The characteristics of seeds are shown in Table 1. The seeds were extracted, cleaned and stored for 15 days in a dark and cool place at 4 ºC until used. Before the germination tests, damaged and insect infected seeds were discarded, and the empty ones were eliminated using the floating method in distilled water. The design was set up as a factorial experiment, using a completely randomized design (CRD) with four replications for each treatment of water potentials and with two species and 100 seeds for each replication, 5 water potential levels and a total of 2000 seeds for each one of species and this research was carried out at the laboratory of Tarbiat Modares University, Faculty of Natural Resources, Noor, Iran. The water potential of the germination substrates (0, -2, -4, -6 and -8 bars) was determined using PEG-6000 solution, prepared as described by Michel and Kaufman (Michel and Kaufmann, 1973). Osmometer device (wescor-5520 USA) was used for the accurate measurement of osmotic potential solution. PEG 6000 was dissolved in water and placed in a shaker bed (25 ºC) for 16 h. The seeds were surface sterilized in 0.58% sodium hypochlorite solution for one minute. After applying sterilization, the seeds were washed several times with distilled water. Germination tests were performed in 9-cm-diameter glass petri dishes on two layers of filter paper saturated with 7 ml of water solutions. PEG-6000 solutions were renewed every 48 h under sterile conditions to ensure relatively constant $\Psi\pi$ in the treatments. Experiments were carried out in a temperature controlled growth chamber at 20 ± 0.5 ºC and 8-16 hours (radiation-darkness). Germination counts were performed daily for 37 d and germination was considered to have occurred if the radicle protruded 2 mm from the seed coat. Seeds with abnormal radicals were excluded from the germination counts (Edwards and Wang, 1995). All seeds from the previous germination tests which did not germinate after 37 days at different PEG-6000 solutions, were placed in new petri dishes with filter paper moistened with deionized water and incubated under the same conditions for additional 20 days to study the recovery of germination. Percentage germination was recorded for 20 days at 2-day intervals. Germination equations are showed in Table 2.

Table 1. The characteristics of seeds.

Species	Seed provenance	Viability (%)	Purity (%)	Moisture (%)	Number (per Kg)	1000 seed weight (per g)
Cupressus arizonica	Gorgan	26	87	13.5	128700	4.9
C. sempervirens	Gorgan	33	97	13.1	145306	4.6

Table 2: Germination indices calculation equations

[1]	Germination percentage (28)	$G = \frac{n}{N} * 100$
[2]	Mean germination time (22)	$MGT = \sum(\frac{ni\ ti}{ni})$
[3]	Germination speed (22)	$GS = \sum(\frac{ni}{ti})$
[4]	Germination energy (28)	$GE = \frac{PV}{N} * 100$
[5]	Vigor index (18)	$SVI = [SL\,(cm) * G]$
[6]	Recovery percentage (24)	$RP = \frac{(a-b)}{(c-b)} * 100$
ni = the number of germinated seeds on day ti PV= maximum of cumulative percentage germination SL= seedling length a = the total number of seeds germinated after being transferred to distilled water b = the total number of seeds germinated in PEG solution		N= number of seeds initiated n = the total number of germinated seeds during the germination test ti = the number of days during the germination period (between 0 and 37 days) c = the total number of seeds

Data analysis

Data were statistically analyzed using SPSS software program (Ver.17 for Windows) and Excel. Distribution was tested for normality by Kolmogorov - Smirnov, and homogeneity of variances tested by Levene test. Two - Way - ANOVA was used to determination the interaction between treatments of water potentials and species on germination indices, vigor index and recovery percentage. Wherever the treatment effect was significant, Fisher's protected LSD test (p = 0.05) was carried out to compare the means. Germination indices, vigor index and recovery percentage between two species in the same water potential level treatment was analyzed by t-test. Also, cumulative germination percentage chart in 37-day period (to be sure all seed germination) was constructed.

Results

Analysis of variance showed highly significant differences among both species and water stress treatments. The interaction between treatments was significant in percentage and speed of germination, vigor index and recovery percentage (Table 3). Seeds in control media (zero osmotic potential), had the highest percent, speed, energy of germination and vigor index but decreased significantly by decreasing water potential (more negative). The mean germination time (MGT) increased with the increase in PEG concentration. The highest recovery percentage was observed in -8 (Table 4). Result interaction between water stress *species showed that the highest percentage and speed of germination and vigor index was observed in *C. sempervirens* seeds and control media (0 bar) (Table 4). T-test analyzes between *C. arizonica* and *C. sempervirens* species showed that percentage and speed of germination, vigor index and recovery percentage were greater in *C. sempervirens* seeds than *C. arizonica* (Table 5). Cumulative percentage germination decreased significantly by decreasing water potential (Figure 1. and Figure 2).

Table 3. The Results analysis of variance species and water stress treatments

Source of variation	GP	MGT	GS	GE	VI	RP
Species	26.19^{**}	1.66^{ns}	7.05^{*}	0.66^{ns}	8.1^{**}	5.33^{*}
Water stress	35.35^{**}	11.53^{**}	15.71^{**}	21.94^{**}	38.46^{**}	14.22^{**}
Species* water stress	2.92^{*}	0.08^{ns}	7.03^{*}	1.82^{ns}	9.2^{*}	6.86^{*}
Error	8.43	10.61	0.61	3.78	5.26	11.2

** Signifiant differences (P < 0.01) * Signifiant differences (P < 0.05) (^{ns}): Non signifiant differences (P > 0.05)
GP: germination percentage , MGT: mean germination time , GS: germination speed
GE: germination energy, VI: vigor index, RP: recovery percentage

Table 4: Effects of water potentials on germination traits, vigor index and recovery percentage.

	T	GP	MGT	GS	GE	VI	RP
C. arizonica	(T1) 0	18.75 ± 0.4a B	10.02 ±0.2b	2.46 ± 0.01a B	12 ±0.2a	10.89 ± 0. 6a B	4.45 ± 0.04c D
	(T2) -2	14.5 ± 0.3b BC	11.31 ±0.1b	1.39 ± 0.00b BCD	9 ±0.1b	7.03 ± 0.03b CD	8.42 ± 0.18bc CD
	(T3) -4	10.5 ± 0.23c CDE	11.97 ±0.03b	1.32 ± 0.03b BCD	5.75 ±0. 1c	3.13 ± 0.02c E	11.83 ± 0.9ab BC
	(T4) -6	9.25 ± 0.12c DE	14.77 ±0.05ab	1.1 ± 0.00bc CD	4.25 ±0.03c	2.58 ± 0.01c E	10.27 ± 0.7ab BC
	(T5) -8	7 ± 0.11c E	19.56 ± 0.34a	.42 ± 0.00c D	4 ±0.01c	1.45 ± 0.01c E	15.45 ± 0.19a AB
C. sempervirens	(T1) 0	27.75 ± 0.7a A	10.73 ± 0.65c	4.19 ± 0.06a A	13.25 ±.37a	17.21± 0.82a A	4.69 ± 0.07c D
	(T2) -2	18.5 ± 0.31b B	12.13 ± 0.79c	2.51 ± 0.02b B	6.75 ± 0.47b	8.69 ± 0.66b BC	12.53 ± 0.05b BC
	(T3) -4	18 ± 0.9b B	13.6 ± 0.02bc	1.92 ± 0.02bc BC	5.25 ± 0.25b	4.09 ± 0.27c DE	11.92 ± 0.1b BC
	(T4) -6	11.75 ± 0.6c CD	17.08 ± 0.34ab	0.93 ± 0.01bc CD	6.5 ± 0.75b	3.38 ± 0.51c E	15.2 ± 0.31ab AB
	(T5) -8	7.5 ± 0.28c DE	20.74 ± 0.25a	0.41 ± 0.03c D	5.75 ± 0.25b	2.03 ± 0.68c E	18.35 ± 0.08a A

Significant differences among water potentials are presented by different letters in column each species. T: Water potentials (bars), GP: germination percentage , MGT: mean germination time , GS: germination speed GE: germination energy, VI: vigor index, RP: recovery percentage - Significant differences in interaction between water stress *species presented by the capital different capital letters in column using Duncan test for 10 combined treatments (interaction effects).

Table 5: Effect of species on germination traits, vigor index and recovery percentage

Parameters	*C. arizonica*	*C. sempervirens*
Germination percentage	12b	16.7a
Mean germination time	13.53a	14.85a
Germination speed	1.34b	2a
Germination energy	7a	7.5a
Vigor index	5.01b	7.08a
Recovery percentage	10.08b	12.53a

Significant differences between species are presented by different letters in rows by T-test.

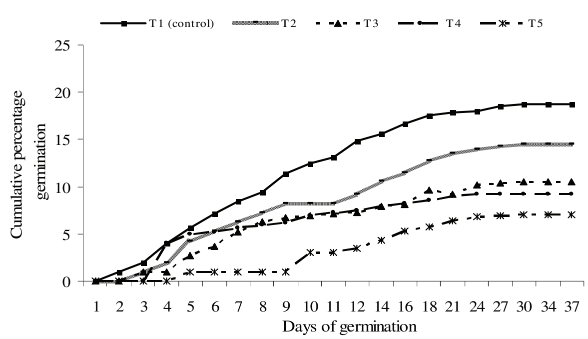

Figure 1: Cumulative percentage germination process under water potentials in *C. arizonica.* (T1): 0 bar (control), (T2): -2 bar, (T3): -4 bar, (T4): -6 bar, (T5): -8 bar of water potentials.

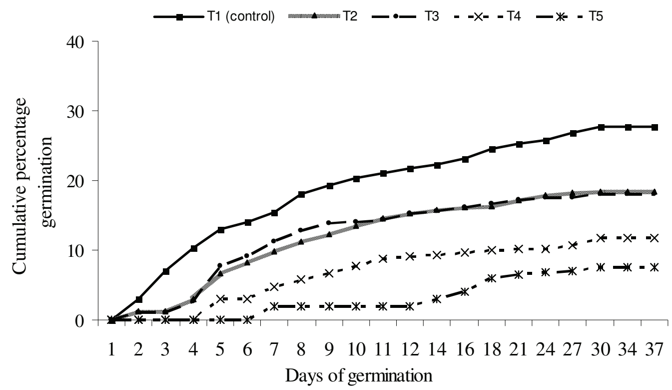

Figure 2: Cumulative percentage germination process under water potentials in *C. sempervirens*. (T1): 0 bar (control), (T2): -2 bar, (T3): -4 bar, (T4): -6 bar, (T5): -8 bar of water potentials.

Discussion

Stress treatments applied in this study significantly reduced germination percentage, germination speed, germination energy and vigor index and a significant increase observed in mean germination time rather than control in both species. Decreasing the water potential in the substrate decreased germination, indicating that water stress inhibits germination. Similar trends have also been observed in some other conifers, lowering the water potential to -8 bars reduced the germination of Maritime pine (*Pinus pinaster* Aiton) (Falleri, 1994), Lodgepole pine (*Pinus contorta* Douglas) and Engelmann spruce (*Picea engelmanii* Parry ex Engelm) by approximately 50% (Kaufmann and Eckard, 1977), Lebanon cedar (*Cedrus libani* A.Rich) (Dirik, 2002), *P. brutia* by more than half (Boydak et al., 2003) and Scots pine (*Pinus sylvestris* L.) (Tilki and Dirik, 2007). Also results Maraghni et al. (Maraghni et al., 2010) on Lotus jujube (*Ziziphus lotus* (L.) Lam.) seeds in water stress levels by polyethylene glycol (PEG)-6000 solutions of different osmotic potentials (0 to −1 MPa) showed that seeds germinated to 95% and less than 5% in PEG-6000 solutions of −0.4 and −1 MPa, then when tested for germination in distilled water, after PEG treatments, seeds germinated to the same extent as when fresh. In general, the physical process of water absorption increases metabolic activity within the seed, the synthesis of proteins, enzymes, hydration and seed germination rate (Zhu et al., 2006). Reduction in the seeds water content due to low media water potential will decrease the activity of hydrolytic enzymes such as α-amylase, proteases and lipases responsible for hydrolyzing cotyledons reserves required for providing energy in the early stages of seeds growth by respiration (Dahal et al., 1996; Zayed and Zeid, 1998). Falleri et al. (2004) reported that direct effects due to slower decomposition of endosperm or slower transition of decomposed materials to seedlings is one of factors reducing the percentage of germination in the water stress conditions. Van Gastel et al. (Van Gastel et al., 1996) found hardening the cell wall as cause loss or lack of water absorption by seed shell in osmotic stress environments. In the present study decreased germination speed and germination energy in treatments of water stress can be due to seed deterioration and degradation of cell membrane (Falleri, 1994). Germination speed is one of seed quality parameters and as seed has a higher germination percentage in less time, causes the seedling established earlier and to use more resources and environment conditions and have higher quality and vigor. Similarly, Zhu et al. (2006) also have reported that increasing the osmotic concentration decreased speed and energy of seeds germination.

In the present study also time of initial germination was delayed with increasing osmotic concentration. Gholami et al. (Gholami et al., 2010) achieved similar results on four wild almond species. PEG which is a non-penetrating osmoticum prevents water uptake by plant cells. The decrease in fresh weight in the presence of PEG is in support of this contention (Almansouri et al., 2001). In seeds that are soaked in pure water, their water content reaches a plateau and up to just before radicle emergence changes very little. When the water potential is reduced outside the seeds media, the rate of water uptake decreases and the onset of germination are delayed. The major reason for germination delay is increase in the length of the lag phase between imbibition and radicle growth, since the increase in seeds water content proceeds slowly during this period. In pure water, seeds are saturated (100%) with water and as a result radical growth occurs rapidly, but at higher osmotic potential (more negative) seeds water content increases gradually (Bradford, 1986). Cumulative germination percentage was not adversely affected when moisture stress reached -6 bars, lowering the water potential to -8 bars decreased cumulative germination percentage. About this parameter, the seeds of *C. sempervirens* were more sensitive to deficiency of water availability (-8 bars) in comparison with *C. arizonica* seeds.

Also, seed vigor index showed more reaction to moisture loss than other parameters by increase in PEG concentration and was adversely affected when moisture stress reached -6 bars. Lo'pez et al. (Lo´pez et al., 2009) also while achieving the same results on seeds Canary island pine (*Pinus canariensis* C. Sm.), expressed that probably plumule and radicle growth compared with the germination process need more turgor pressure. The results Gholami et al. (Gholami et al., 2010) with various osmotic potentials (0, -0.05, -0.1 and -0.5 MPa) indicated that PEG adversely affected the plumule of four wild Almonds. Decreasing seed vigor index is probably due to decreasing moisture availability, which causes enzyme activity by some problems in the transfer of endosperm reserves in the form used for the growth of embryonic axes and synthesis compounds of seed (Van Gastel et al., 1996). Organs growth also depends to speed produce new cells and rapidly growing cells and both processes are sensitive to cell swelling, but the amount sensitivity probably depending on tissue, species or stress intensity. So that when the seeds are exposed to drought, flexibility decreased in cells wall growing, that reduces cell expansion and consequently organs growth (Natale et al., 2010).

In the present study revealed that the rate, speed and energy of germination both of species decreased at -8 bars by approximately 85% that is very close to the threshold stopping. By experimental transfer to deionized water after 20 days of water stress simulated by PEG-6000 solutions, seeds of both of species were able to initiate germinate that show osmotic effects on germination. Seeds that do not germinate in the presence of water stress constitute a persistent seed bank helping the species in spreading germination over the year. Comparison of recovery of germination when treated with various concentrations of PEG showing about 15% germination in *C. arizonica* and about 18% germination in *C. sempervirens* at the highest osmotic potential. Similar instances were also found by Maraghni et al. (2010) on *Z. lotus* and recovery was significantly affected by water stress levels.

In the other hand, result interaction between water stress *species showed that response *C. sempervirens* was better than *C. sempervirens* can probably concluded that *C. sempervirens is* more resistant than drought stress and may be differences related to the ecology of the species. Of course, reaction species than water stress is related to quality mother tree and its impact on fruitification, genetic factors, duration and seed storage conditions and difference in resistance to drought stress (Beardmore et al., 2008). From the study, it is concluded that for optimum germination these species, it is needed to keep the desired seed moisture content from harvesting date to sowing date. In general, the results of this research support the idea that both of species are drought-resistance with respect to several physiological characteristics and the results presented in this paper can be applied in forestation programs.

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