Effect of Drought Stress Induced by Polyethylene Glycol on Seed Germination of Four Wild Almond Species

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Abstract: Effects of drought stress induced by polyethylene glycol (PEG) on germination of four wild almonds were evaluated. The seeds of *Prunus scoparia* Spach, *P. eleagnifolia* Spach, *P. lycioides* Spach and *P. dulcis* Mill (No. 24 Zarghan) were collected and stratified at 4±1 °C for 4 weeks. Stratified seeds were incubated in PEG solution with various osmotic potentials (0, -0.05, -0.1 and -0.5 MPa). The experiment was arranged in a completely randomized design with 4 replications, and germination capacity, mean and time of germination, germination rate, length of hypocotyls and radicles and also fresh and dry weight of seedlings were determined. Germination capacity, germination rate and growth parameters of all species were decreased by increase in PEG concentration and germination onset was also delayed. Furthermore the results indicated that osmotic stress depressed the hypocotyls growth more than radicle; and root systems were more drought tolerant. Although PEG adversely affected the germination of all 4 species, they responded differently to drought stress. *P. scoparia* showing higher germination capacity, germination rate and seedling length had the highest tolerance to drought stress and *P. eleagnifolia* in early phase of its seedling growth could not completely cope with drought stress. The germination results revealed the presence of drought resistant almonds with rich germplasm in Iran.

Key words: Almond, Drought Stress, Germination, PEG

INTRODUCTION

Iran is one the main origins of almond and also one of the major almond producers in the world (FAO, 2006; Rom, R.C. and R.F. Carlson, 1987). This country has one of the richest germplasm of almonds and after screening them for their effects on scion productivity, nut quality and tolerance to soil born diseases, these species can be used as rootstock for commercial almond growing (Baninasab, B. and M. Rahemi, 2007). These trees are currently grown in their native habitats and their products are used locally. For example, the kernel of *P. scoparia* is used after debittering and roasting or *P. eleagnifolia* is used as rootstock for plum. Furthermore in pastures and range lands, grafting various almond cultivars on these trees is a common practice.

However, the potentials of using these species as rootstock and their nuts for pharmaceutical and cosmetic purposes are high (Rouhi, V., R. Samson, 2007). It has been stated that it is possible to improve almond rootstocks by screening these species and make hybridization among them (Kester, D.E. and T.M. Gradiziel, 1996). Because of their high adaptation to unfavorable environmental conditions, they can be used in semi arid areas to control soil erosion (Baninasab, B. and M. Rahemi, 2007). They can also be used for afforestation and dedesertification only if they can survive under adverse conditions. To recommend these species for afforestation and dedesertification they should be able to reproduce in such environments. Their inability to regenerate under artificial conditions is the most important factor causing their loss after some years (Zhu, J., H. Kang, 2006). Vickers and Palmer (2000) reported that suitable conditions for seed germination and seedling growth are the most important factors that affect the natural regeneration of forests. Decrease in water potential in germination medium because of drought or salinity prevent water absorption needed for germination process to start (Almansouri, M., J.M. Kinet, 2001). According to Qkcu *et al.*, (2005) seeds supplied with an insufficient moisture will show unsynchronized seedling emergence which is an important problem for nurserymen. Drought is one of the most important factors that limit trees growth in Iran. Therefore successful tree culture depends on the ability of seeds to germinate under low soil moisture conditions.

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The damage caused by drought or water stress is different among various crops and their growth stages (Wilhite, D.A., 2001). In many crops seed germination and early seedling growth are the most sensitive stages to environmental stresses (Foolad, M.R., P. Subbiah, 2003). Evaluation of seed germination may be useful to identify resistant seeds and genotypes that are capable of generating sufficiently under low soil moisture conditions (Bouslama, M. and W.T. Schapaugh, 1984).

In spite of a very long history of wild almond species growth in Iran, few studies have been made to find their responses to environmental stresses and in particular no study has been made in relation to the effect of drought stress on wild almond seed germination in order to grow their seedlings and use them as rootstocks for almond cultivation. This research aims to evaluate the germination of four wild almond seeds in germination media with different water potentials.

MATERIALS AND METHODS

Seeds of *P. scoparia*, *P. eleagnifolia* and *P. lycioides* (wild species) were collected from Beneh forest of Firoozabad and those of *P. dulcis* (No. 24 Zarghan) were provided by Research Center of Agriculture and Natural Resources of Fars province. Some of their traits are listed in Table 1.

Table 1: Some quantitative nut traits of wild almonds [*]

Species	Seed length (cm)	Seed width (cm)	Seed weight (g)	Kernel weight (g)	Shell thickness (mm)
P. scoparia	1.45	1.06	0.65	0.22	1.34
P. eleagnifolia	2.00	1.09	0.79	0.23	1.61
P. lycioides	1.10	0.98	0.46	0.13	1.23
P. dulcis (No.24 z)	2.50	1.87	2.75	0.58	2.62

[*]: mean of 20 seeds

Seed preparation. Normal nuts were separated from non- viable ones using flotation method. Seed shells were removed and their kernels were soaked in distilled water for 24 h. Soaked seed were mixed with sand: vermiculate (2: 1) and kept at 4±1 °C for 4 weeks and treated with 0.2% benomyl solution during stratification. *Germination.* Four replicates of 180 seeds of each species were used for each treatment and placed in plastic dishes (5 × 10 × 7 cm) on Whatman filter paper No. 1 moistened with different concentration of polyethylene glycol (PEG 6000). A series of drought stresses with water potentials of -0.05, -0.1 and -0.5 MPa were prepared by different concentration of PEG according to Michel and Kaufmann equation (Michel, B.E. and M.R. Kaufmann, 1973). Distilled water was used as control (0 MPa). Distilled water was added to covered plastic petri dishes to make up for the water post by evaporation everyday. Plastic petri dishes were placed in a germinator at 21±1 °C in the dark for 20 days. A seed was considered to have germinated when the length of its emerging radicle was 2 mm.

Calculations. Germination capacity, mean germination time and germination rate were calculated from equations 1-3 (Zhu, J., H. Kang, 2006).

$$GC = S_{NG} / S_{NO} \times 100 \tag{1}$$

Where GC is germination capacity, S_{NG} is the number of germinated seeds and S_{NO} is the number of viable seeds.

$$MGT = \Sigma\, D.n / \Sigma\, n \tag{2}$$

Where MGT is the mean germination time (day), D is the time in days from the starting / sowing day and n is the number of germinated seeds on a given day

$$GR = S_{Nm} / S_{NO} \times 100 \tag{3}$$

Where GR is the germination rate and S_{Nm} is the number of germinated seeds at germination peak.Besides GC, NGT and GR, hypocotyl and radicle length and fresh and dry weight were measured on day 20. Dry weight was determined after drying samples at 80 °C for 48h.

Experimental design and statistical analysis. Treatments were arranged in a completely randomized design with 4 replications and 180 seeds per replicate. Data were presented as percentage and were statisticaly analyzed by arcsin transformation. Analysis of variance was performed using the SPSS software package and significant differences among the mean values were compared by LSD test (P< 0.05).

RESULTS AND DISCUSSION

Germination parameters were severely affected by drought. The effects of various osmotic potentials on germination capacity are shown in Fig. 1. In all species seeds in control media (zero osmotic potential), had the highest germination capacity (GC) but decreased significantly by decreasing water potential (more negative). Germination capacities of control seeds of *P. scoparia* and *P. dulcis* (No. 24 Zarghan) were 100% while those of *P. eleagnifolia* and *P. lycioides* were 71.11 and 74.56%, respectively. The mean germination time (MGT) of all species increased with the increase in PEG concentration (Fig. 2). The highest increases were about 1.48 and 4.13 days for *P. scoparia* and *P. eleagnifolia,* respectively. Germination rate (GR) decreased with decreasing osmotic potential (Fig. 3). The lowest GR for all species occurred at -0.5 MPa of PEG, with *P. eleagnifolia* being the lowest (8.82) of all.
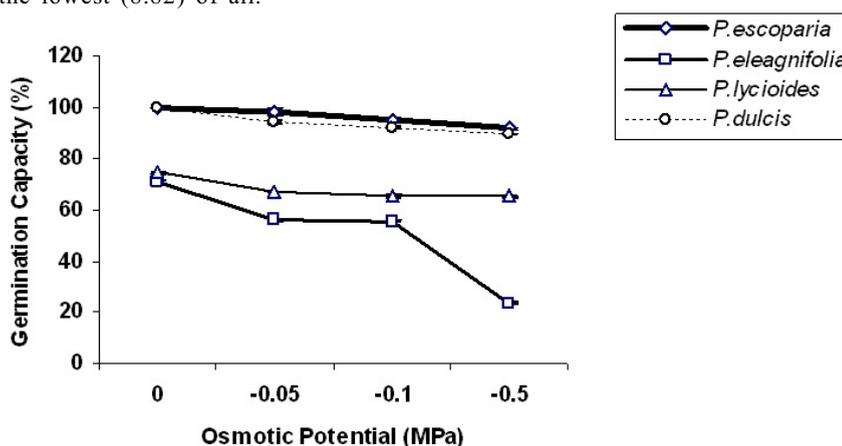
Fig. 1: Effect of osmotic potential on germination capacity in wild almond species. Vertical bars are standard deviation (SD) of means.
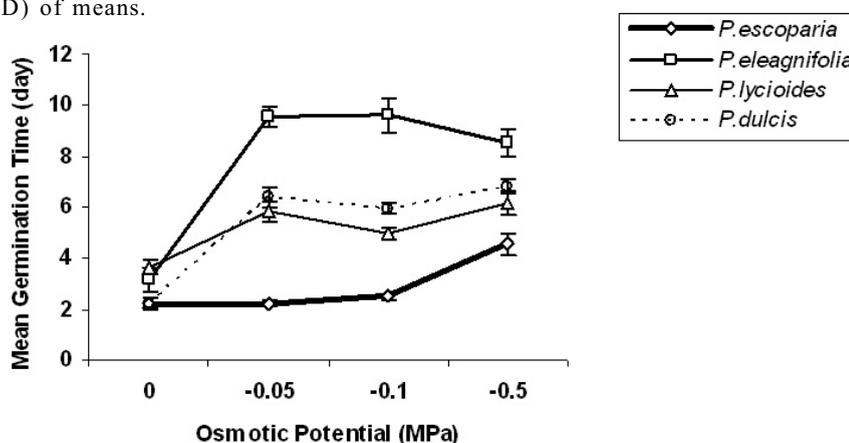
Fig. 2: Effect of osmotic potential on mean germination time in wild almond species. Vertical bars are standard deviation (SD) of means.

In *P. scoparia,* decreasing water potential down to -0.1 MPa of PEG had no significant effect on radicle length compared to control (Fig. 4-a) however in this species hypocotyls length decreased significantly by decreasing water potential (Fig. 4-b). The fresh weight of radicles and hypocotyls decreased parallel with the hypocotyls length (Table 2). In *P. eleagnifolia,* radicles length decreased significantly at -0.5 MPa osmotic potential as compared to control while its hypocotyls length at -0.05 MPa osmotic potential was significantly lower than control. Responses of hypocotyls and radicles fresh weights to various treatments in this species were more or less similar with those of hypocotyls and radicles length while the decrease in hypocotyls and radicles dry weight with increasing PEG concentrations were not significant. In *P. lycioides*, radicles lengths at -0.05 and -0.1 MPa of PEG were significantly higher than control. On the other hand, hypocotyls lengths like the other species were the highest in the control. There were no significant differences in radicles fresh
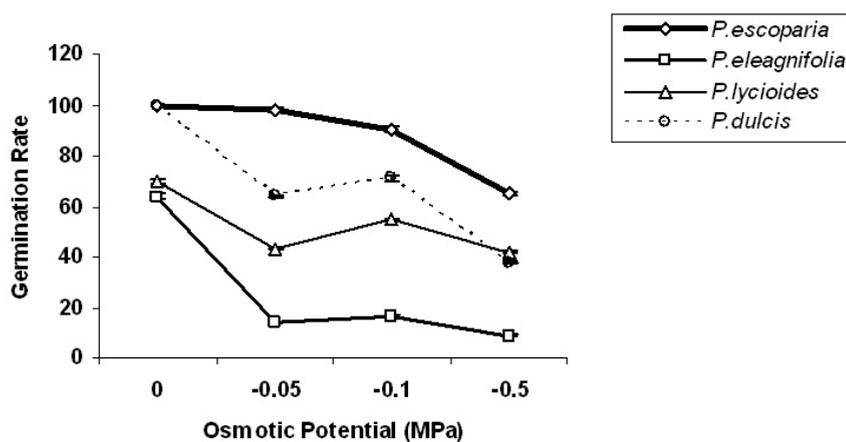
Fig. 3: Effect of osmotic potential on germination rate in wild almond species. Vertical bars are standard deviation (SD) of means.
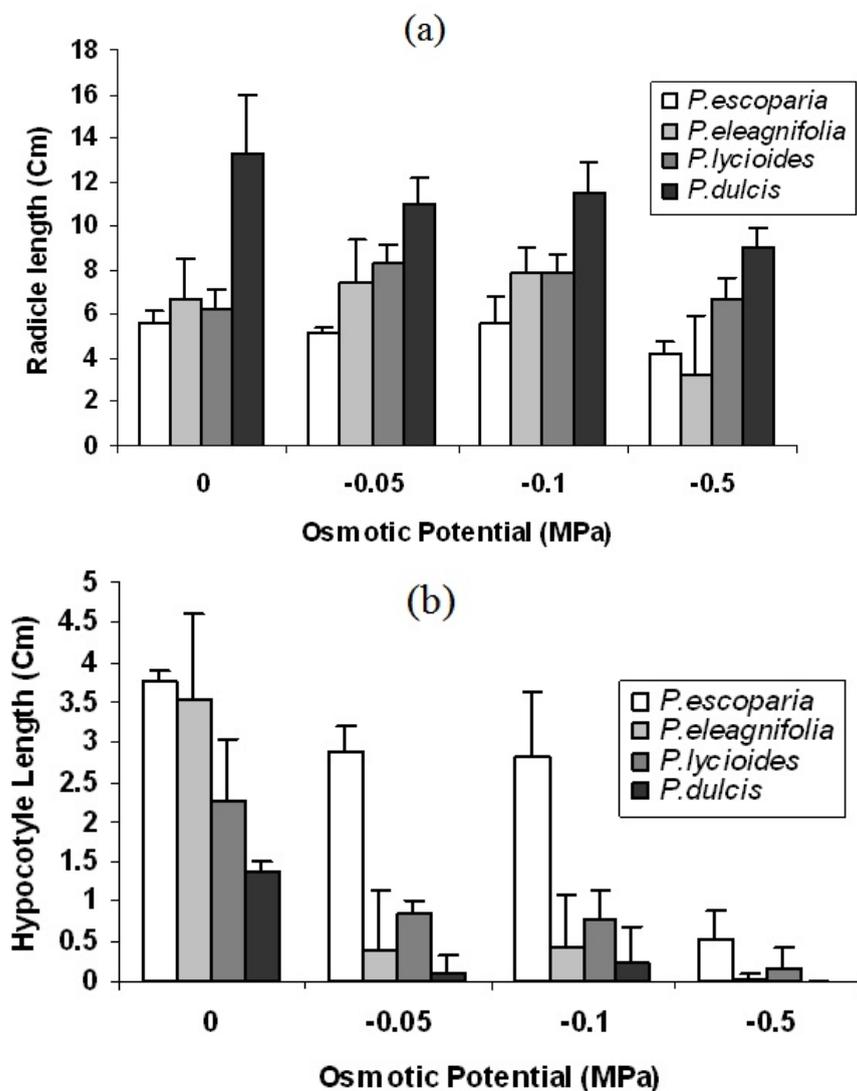
Fig. 4: Effect of osmotic potential on a) radicle and b) hypotoyl length in wild almond species. Vertical bars are standard deviation (SD) of means.

Table 2: Effect of osmotic stress on fresh and dry weight of radicles and hypocotyls of almond species

Species	ψ_{op} (MPa)	Dry Wight (g)		Fresh Weight (g)	
		Radicle	Hypocotyl	Radicle	Hypocotyl
	0	3.6	3	0.47	0.36
	-0.05	2.54	1.81	0.34	0.24
P. scoparia	-0.1	2.36	1.66	0.34	0.22
	-0.5	1.02	0.43	0.21	0.02
	LSD$_{.05}$	0.94	0.56	0.13	0.13
	0	3.18	1.7	0.36	0.2
	-0.05	1.88	0.09	0.19	0.02
P. eleagnifolia	-0.1	1.82	0.16	0.19	0.03
	-0.5	0.57	0.02	0.08	0.003
	LSD$_{05}$	1.66	0.94	0.48	0.23
	0	2.55	1.12	0.29	0.14
	-0.05	2.37	0.78	0.29	0.08
P. lycioides	-0.1	2.33	0.39	0.25	0.05
	-0.5	1.45	0.03	0.2	0.005
	LSD$_{05}$	1.85	0.87	0.45	0.17
	0	10.18	1.95	1.7	0.37
	-0.05	7.01	0.15	0.99	0.03
P. dulcis	-0.1	6.79	0.14	0.92	0.03
	-0.5	5.36	0	0.69	0
	LSD$_{05}$	1.61	0.67	0.94	0.21

Mean separation within columns by LSD, at 5% level.

and dry weights and also in hypocotyl dry weights. In *P. dulcis* (No. 24 Zarghan), PEG decreased radicles length significantly at -0.5 MPa relative to the control, but the other three PEG solutions did not have significant effects on radicle lengths. Hypocotyl lengths of this species declined in all treatment solutions relative to the control and no hypocotyls growth occured at -0.5 MPa of PEG. Increasing PEG concentrations resulted in a decrease in hypocotyls and radicles fresh and dry weights.

In all wild almond species, hypocotyls emergence occurred only in low PEG concentrations and with decreasing water potential, in spite of long radicles, no hypocotyl growth was observed. i.e., water stress depressed the shoot growth more than the root growth. For example *P. dulcis* (No. 24 Zarghan) had the highest (9.1 cm) root length at -0.5 MPa osmotic potential, while failed to produce shoot at this osmotic potential (Fig. 4-a and 4-b).

Discussion:

Water stress in germination media induced by PEG affected both the percent and the rate of seed germination in all four species. These results are in agreement with those reported by Dahal *et al.* (1996), Zayed *et al.* (1998), Lopez *et al.* (2000), Almansouri *et al.* (2001) and by Zhu *et al.* (2006).

PEG not only delayed the germination but also affected the final germination percentages. Our results are not in agreement with those reported by Bradford (1990) and Almansouri *et al.* (2001) who stated that moderate osmotic stresses only delayed germination while high stresses reduced the final germination percentages.

PEG which is a non-penetrating osmoticum prevent water uptake by plant cells. The decrease in fresh weight in the presence of PEG is in support of this contention (Almansouri, M., J.M. Kinet, 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 radicle growth occurs rapidly, but at higher osmotic potential (more negative) seeds water content increases gradually (Bradford, K.J., 1986).

According to Bradford (1990), at high osmotic potential the degree of seeds endosperm weakening which primarily controls the time of radicle emergence due to lowered seed water potential or pressure potential (turgor) is delayed. Higher germination rate allows the young seedlings to produce more biomass. *P. scoparia* which had higher germination rate had also more total fresh weight than *P. eleagnifolia* and *P. lycioides* (Table 2).

Growth was affected greatly by water stress since radicles and hypocotyls lengths were decreased by low water potential. 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, P., N.S. Kim, 1996; Zayed, M.A. and I.M. Zeid, 1998). Thus, it seeds that the cell expansion under osmotic stress is inhibited mainly by metabolic factors rather than by insufficient pressure potential (Stout, D.G., G.M. Simpson, 1980).

In this study radicles began to grow faster than hypocotyls. Zhu *et al.* (2006) stated that under drought conditions, the underground organs (roots or radicles) will develop faster than the aboveground organs (stems or hypocotyls) to acclimatize with the water stresses. Also osmotic stress had more inhibiting influence on hypocotyls growth than radicles growth (Fig. 4-a and 4-b). The decrease was frequently dependent on species and PEG concentration and as has been reported by other studies (Bayuelo-Jimenez, J.S., R. Craig, 2002; Foolad, M.R., 1996; Huang, J. and R.E. Redmann, 1995).

P. scoparia with the highest germination rate, better germination capacity and seedling growth was identified as the most tolerant species in this study. The differences in its radicles growth, fresh and dry weight between *P. scoparia* and *P. dulcis* (No. 24 Zarghan), could be due to higher *P. dulcis* (No. 24 Zarghan) seed size resulting in higher nutrient reserve. Decrease in germination capacity of about 66.9% in *P. eleagnifolia* with decreasing water potential from 0 to -0.5 MPa of PEG might be due to deficient in genetic potential required for coping with drought (Foolad, M.R., 1996).

In conclusion, in this study drought tolerance in early phase of seedling growth of four wild almonds was investigated by evaluating growth and germination process under PEG-induced water stress. It was observed that water stress causes delay and also inhibition of germination process which could be due to various physicals and metabolic parameters. Our results show that there were variations in response to water stress and this is an indication of rich genetic resources of wild almond in Iran that provide the possibilities of using them in breeding programs. This study shows that *P. scoparia* had an exceptional ability in germination and early growth under drought conditions. It is recommended that this species be used in almond rootstock improvement programs. However, drought tolerance at this stage may not be compatible with other growth stages and also with other characteristics such as freezing and frost stresses which have to be investigated.

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