Effects of osmotic stress on alfalfa germination and determine superior genotypes with regard to radicle and shoot length using polyethylene glycol

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Abstract: Consider to critical role of pastures in soil erosion prevention providing livestock forage we evaluated osmotic stress induced by polyethylene glycol on alfalfa germination and determined superior genotypes in response to the stress. Experimental design was completely randomized design arranged in factorial with three replications. Twelve alfalfa genotypes were considered as min factor and different osmotic stresses (0, -0.4 and -0.8 MPa) were considered as second factor. Different osmotic potential was imposed using polyethylene glycol 6000. Alfalfa seeds were placed in 9 cm Petri dishes and put in germinator under controlled conditions. In this study, germination percentage, shoot and radicle length, germination stress index and radicle number were measured. There was significant variation between studied traits at 0.01 probability level. In addition, there was significant difference between osmotic stress level and genotypes. The highest shoot length was observed from KR2197 genotypes. On the other hand, KR2197 and ES058 produced the longest radicles. Furthermore, we found positive and significant correlation between studied traits. The highest germination stress index was related to ES0178 and ES008. In conclusion, ES0178, ES058 and KR 2421 genotypes were known as superior genotypes compared with other genotypes.

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Introduction

Given the critical role of pastures in preventing soil erosion and provide livestock forage, reclamation of pastures and improve their productivity is unavoidable. Annual medics are considered as the most important pasture plants to pastures renovation. Direct cultivation of this plant in pastures leads to optimum use of pastures and increases forage production in inefficient and dry lands. Environmental stresses cause some sort of morphologic and physiologic changes in plants. It has been reported that increase in drought stress tolerance is in parallel with increase in heat stress injuries (Gauch and Zobel.1988). Polyethylene glycol is a flexible and nonpoisonous polymer which is able to induce osmotic pressure. Polyethylene glycol does not react with other chemical and biologic substances, so this polymer is known as the most applicable substance to induce osmotic pressure in biology (Blum, 2005; Macar et al., 2009). Moreover polyethylene glycol is mentioned as suitable osmolite in biological experiments because of its specific characteristics such as immobility and non toxicity (Al- Baharany, 2002).

Material and methods

Current experiment was laid out in Payame-Noor University, Kermanshah, Iran in 2012. Experimental design was completely randomized design arranged in factorial with three replications. Twelve alfalfa genotypes (Table 1) were considered as min factor and different osmotic stresses (0, -0.4 and -0.8 MPa) were considered as second factor. Data collection was performed on at least five germinated seed from each genotype group randomly. Different osmotic potential was imposed using polyethylene glycol 6000. Polyethylene glycol amount was calculated using Michel and Kaufmann equation (Michel and Kaufmann, 1972). Alfalfa seeds were surface sterilized by 96% ethanol for 10 second and 15% hypochloride sodium for 40 second and then rinsed with distilled water. Twenty seeds were placed in 9 cm Petri dishes with filter paper and then 10 ml of osmotic solutions was added to the plates. Distilled water was used for control treatments. After that plates were labeled and put in germinator under controlled conditions (20± 0.5 C, 16/8 day/night photoperiod) for ten days. At the end of germination period, germination percentage, radicle and shoot length were measured. Germination stress index was calculated using Bouslama and Schapaugh (1984) equation.

PI=nd2 (1.0) + nd4 (0.8) + nd6 (0.6) + nd8 (0.4) +nd10 (0.2)

GSI= [PI, under stress condition / PI, under non-stress condition]

Where nd_2, nd_4, nd_6, nd_8 and nd_{10}: number of germinated seed on second, fourth, sixth, eighth and tenth days, respectively.
Germination percentage = germinated seeds till i^{th} days/ number of total seeds × 100
All data were subjected to SPSS and analysis of variance and phenotypic correlation between traits and also cluster analysis was performed.

Results and discussion

Analysis of variance:
Analysis of variance results are shown in table 2 to 9. Based on obtained results, shoot length showed some variations at 0.05 probability level while radicle to shoot length ration did not show any variation. Other traits indicate significant variation at

0.01 probability level. The results demonstrated that, germination percentage and radicle length were significant at 0.05 probability level while sum of radicle and shoot length were significant at 0.01 probability level. Coefficient variation for germination percentage, radicle length and shoot length was 18.68, 20.48 and 24.35, respectively. According to table 7 regarding to comparison of means following cases can be mentioned.

Sum of radicle and shoot length:

The highest radicle and shoot length was related to Es058 and KR2421 genotypes with value of 27 and 25.7 mm, respectively. By contrast, the lowest value (18.6 mm) was observed in Es05 genotype (Table 7) .

Radicle length:

Es05 and KR2421 genotypes produced the longest radicles (41.9 and 41.2 mm respectively) while Es05 and Es056 represented the shortest ones (28.4 and 28.9 mm respectively) (Table 7). It has been reported that root growth in basil is affected by water deficit less than shoot growth (Hasani, 2006). In addition, in dill and fennel increase in stress intensity is parallel with decrease in root length (Boromand Rezazadeh and Kochaki, 2006).

Shoot length:

Es052 and KR2197 genotypes produced the longest shoot (12.1 and 11.7 mm respectively) while Es014 and Es056 represented the shortest ones (8.47 and 8.7 mm respectively) (Table 7). Similar results were found in dill and fennel due to water deficit stress (Boromand Rezazadeh and Kochaki, 2006).

Radicle to shoot length ratio:

Es040 and Es012 genotypes showed the highest radicle to shoot length ratio while Es052 and Es056 represented the lowest ratio (Table 7).

Shoot to radicle length ratio:

Es052 and Es008 genotypes showed the highest and lowest shoot to radicle length ratio respectively (Table 7).

Comparison between stress and non stress conditions:

Radicle and shoot length reduction due to -0.4 MPa was 25 and 72% compared with control treatment. This reduction was more pronounced (75 and 96.7%) when -0.8 MPa stress was applied. Song and Park (1990) have shown that decrease in water potential germination and shoot length would decrease in *Astragalus spp*.

Phenotypic correlation:

There was positive and significant correlation between radicle length and all studied traits. However, we found a negative correlation (-0.234) between radicle length and radicle to shoot length ration. The highest correlations were related to sum of radicle and shoot length (0.978), germination vigor index (0.947), shoot length (0.819), germination rate (0.825), and germination percentage (0.758). In addition, there was positive and significant correlation with shoot to radicle length ration (0.572).

Radicle to shoot length ratio showed negative and significant correlation with shoot length (-0.373), shoot to radicle ratio (-0.230), germination percentage (-0.240), germination rate (-0.327), sum of shoot and radicle length (-0.295) and germination vigor index.

Shoot length had positive and significant correlation with germination rate (0.715), germination vigor index (0.917), germination percentage (0.670), sum of shoot and radicle length (0.921) and shoot to radicle length ratio (0.872).

Table 1: Alfalfa genotypes

Number	Genotypes code
1	ES178(control)
2	KR2197
3	ES056
4	KR2421
5	ES058
6	ES052
7	ES051
8	ES040
9	ES012
10	ES008
11	ES096
12	ES014

Table 2: Analysis of variance on sum of shoot and radicle length in alfalfa genotypes

Source	Type III Sum o Squares	df	Mean Square	F	Sig.
Corrected Model	19109.917[a]	35	545.998	36.106	0.000
Intercept	56302.433	1	56302.433	3.723E3	0.000
Gen	524.672	11	47.697	3.154	0.002
level	17807.547	2	8903.773	588.787	0.000
level * Gen	777.698	22	35.350	2.338	0.004
Error	1088.800	72	15.122		
Total	76501.150	108			
CV%			17.03		

Table 3: Analysis of variance on radicle to shoot length ration in alfalfa genotypes

Source	Type III Sum of Squ	df	Mean Square	F	Sig.
Corrected Model	4652.225[a]	35	132.921	2.160	0.003
Intercept	4812.275	1	4812.275	78.213	0.000
Gen	1095.513	11	99.592	1.619	0.112
level	1534.388	2	767.194	12.469	0.000
level * Gen	2022.325	22	91.924	1.494	0.104

Error	4429.985	72	61.528		
Total	13894.484	108			

Table 4: Analysis of variance on root length of alfalfa genotypes

Source	Type III Sum of Squ	df	Mean Square	F	Sig.
Corrected Model	33378.234[a]	35	953.664	18.091	.000
Intercept	135759.322	1	135759.322	2.575E3	.000
Gen	1865.677	11	169.607	3.217	.001
level	29052.003	2	14526.001	275.553	.000
level * Gen	2460.555	22	111.843	2.122	.009
Error	3795.533	72	52.716		
Total	172933.090	108			
CV%			20.48		

Table 5: Analysis of variance on shoot to radicle length ratio of alfalfa genotypes

Source	Type III Sum of Squ	df	Mean Square	F	Sig.
Corrected Model	2.868[a]	35	0.082	7.274	.000
Intercept	5.801	1	5.801	515.003	.000
Gen	0.201	11	0.018	1.624	.110
level	2.460	2	1.230	109.181	.000
level * Gen	0.207	22	0.009	0.834	.675
Error	0.811	72	0.011		
Total	9.480	108			

Table 6: Analysis of variance on shoot length of alfalfa genotypes

Source	Type III Sum of Squ	df	Mean Square	F	Sig.
Corrected Model	10369.182[a]	35	296.262	48.001	0.000
Intercept	11242.441	1	11242.441	1.822E3	0.000
Gen	131.807	11	11.982	1.941	0.048
level	10035.702	2	5017.851	812.998	0.000
Error	444.387	72	6.172		
Total	22056.010	108			
CV%			24.35		

Table 7: comparison of means on different alfalfa genotypes

Genotypes	Gp%	vi	Msh(mm)	pi	Root.l(mm)	Shoot.L(mm)	r/s	s/r
ES178(control)	78.89 a	21.27 a	24.2 abc	41.2 a	37.4 ab	11.1667 abc	5.3578 ab	.2267 abc
KR2197	61.11 cd	19.05 abc	23.6 abc	34.7 bc	35.1 abc	12.1a	3.0711 b	.3189 ab
ES056	53.88 d	13.5 d	18.6 d	29.1 d	28.9 c	8.4 d	3.5778 b	.2367 abc
KR2421	64.4 bcd	19.57 ab	25.7 a	36.7 abc	41.2 a	10.1 abcd	4.8533 ab	.2100 abc
ES058	75.0 ab	21.39 a	26.0 a	39.5ab	41.9 a	10.0 abcd	9.3256 ab	.2067 bc
ES052	72.2 abc	16.4 bcd	20.1 cd	37.2abc	28.4 c	11.7 ab	2.6578 b	.3244 a
ES051	63.3 bcd	18.8 abc	24.6 ab	35.4 bc	38.6 ab	10.5 abcd	4.7989 ab	.2178 abc
ES040	62.2 bcd	14.7 dc	20.6 bcd	32.5 c	32.1 bc	9.2 bcd	12.8844 a	.2267 abc
ES012	63.3 bcd	17.53 abcd	21.4 bcd	34.8 bc	34.0 abc	8.7 cd	10.0544 ab	.1867 bc
ES008	63.3 bcd	19.12 abc	24.0 abc	35.2 bc	38.6 ab	9.5 abcd	9.2311 ab	.1856 c
ES096	71.6 abc	18.74 abc	22.1 abcd	36.6 abc	34.5 abc	9.6 abcd	4.8589 ab	.2067 bc
ES014	66.6 bcd	17.91 abc	22.5 abcd	34.4 bc	34.2 abc	10.9 abcd	9.4311 ab	.2344 abc

Reference

Abdul-Baki, A. A., and Anderson, J. D. 1970. Viability and leaching of sugars from germinating barley. Crop Science 10: 31 – 34.

Almasouri, M., Kinet, J.M. and Lutts, S., 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil, 231: 243-254.

Bagheri Kamal, M., 1996. Study of effective physiological traits for evaluating of wheat Species that are resistant to drought stress, MSC thesis of agriculture faculty, Azadi Eslami university of Karaj, 122p.

Bauchan, G. R., Diwan, N., and McIntosh, M. 1993. Development and evaluation of a core germplasm collection of annual Medicago species in the United States. Pp. 265-266. In: Proceedings of the XVII International Grassland Congress. New Zealand.

Bouslama, M., Schapaugh, W.T., 1984. Stress tolerance in soybean. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci. 24, 933-937.

Blum A. 2005. Use of PEG to induce and control plant water deficit in experimental hydroponics culture. ww.plantstress.com/method/PEC.htm.

Crawford, E. J. 1983. Selecting cultivars from naturally occurring genotypes: Evaluating annual Medicago species. Pp. 203-215. In: J. G. Mclvor and R. A. Bray (eds). Genetic resources of forage plants. Melbourne, Australia: CSIRO.

Cowett, E., and Sprague, M. A. 1962. Factors affecting tillering in alfalfa. Agron. J. 54: 294-297.

De, R. and Kar, R.K., 1995. Seed Germination and seedling growth of Mung (*Vigna radiata*) under water stress induced by PEG-6000. Seed Science and Technology, 23:301-308.

Francis, C. M. 1981. The distribution and ecology of annual Medicago species in North West Libya. A report based on a plant collection tour. Australian Plant Introduction Review 13: 3-14.

Gauch,H.G. and Zobel, R.W. 1989. Accuracy and selectlon success in yield trials analyses. Theoretical and Applied Genetlcs, 77:473 – 481.

Ghanavati, F., Mozaffari, H., Masoumi, A. A., and Kazempour, S. 2007. Morphological studies of pollen grains of Medicago species in Iran. Iranian Journal of Crop Sciences 9: 136-145.

Gintzburger, G. 1987. The effect of soil pitting on establishment and growth of annual *Medicago spp*. on degraded rangeland in western Australia. Australian Rangeland Journal 9: 49-52.

Gupta, P. C. and Otoole. J. C., 1986. In: Upland rice, global perspective. IRRI.

Hedayat, N. (2001). *Feeding value in 3 species of annual alfalfa and digestibility of them in sheep*. M.Sc. Thesis. Tarbiat Modares University.

Itabari, J.K., P.J. Gregory. and R.K. Jones. 1993. Effects of temperature, soil water status and depth plating on germination and emergence of maize (*Zea mays* L.) adapted to semi-arid eastern Kenya. Exp. Agriculture. 29: 351- 36.

Karimi, H. 1988. Alfalfa. Tehran University Press. Tehran, Iran. 269pp.

Kaya, M. D., G. Okcu, M. Atak, Y. Cıkılı and O. Kolsarıcı. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur. J. Agron. 24: 291- 295.

Lehman W F., M D. Rumbaugh. 2001 . Lucern biology and genetic improvement. *Austra J. Agr. Res.* 52: 699-712.

Levitt. j., 1972. Responses of Plants to Environmental Stresses. Academic. Press. Newyork.

Liu, Y.O., R.Y. Bino., W.J. Vander Burg., S.P.C. Groot. and H.W.M. Hilhorst. 1996. Effects of osmotic priming on dormancy and storability of tomato (Lycopersicon esculentum Mill) seeds. Seed Science Research.6: 49- 5

Macar, T. K., T. Ozlem and Y. Ekmekci. 2009. Effect of deficit induced by PEG and NaCl on chickpea (*Cicer arientinum* L.) cultivars and lines at early seedling stages. Gazi Univ. J. Sci. 2009. 22(1): 5-14.

Mauromicale, G. and Cavallaro, V., 1995. Effects of seed osmopriming on germination of tomato at different water potential. Seed Science and Technology, 23: 393-403.

McCoy, T. J., and E. T. Bingham. 1988. Cytology and cytogenetics of alfalfa. In: A. A. Hanson, D. K. Barnes, and R. R. Hill (eds), Alfalfa and Alfalfa Improvement, Monogr. 29,PP: 737-776. Soc. Agron., Madison

Michel, B.E. and Kaufmann, M.R., 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology, 51: 914-916.

Moghadam. M. 2000. Range and rangeland management. Publishment University of Tehran. Pp. 470.

Mohammadian, R., M. Moghadam, H. Rahimian and S. Y. Sadeghian. 2005. Effect of early season drought stress on growth characteristics of sugar beet genotypes. Turk. J. Agric. Forest. 29: 357-368.

Moneim, A. A. M., and Cocks, P. S. 1986. Adaptation of *Medicago rigidula* to a cereal-pasture rotation in north-west Syria. Journal of Agricutral Science (Cambridge) 107: 179-180.

Nascimento, W.M., 2003. Muskmelon seed germination and seedling development in response to seed priming. Scientia Agricola, 60: 71-75.

Neto, N.B.M., Saturnino, S.M., Bomfim, D.C. and Custodio, C.C., 2004. Water stress induced by Mannitol and Sodium chloride in Soybean cultivars. Brazilian Biology and Technology, 47: 521-529.

Okcu, G., Demir, K.M. and Atak, M., 2005. Effect of salt and drought stress on germination and seedling growth of Pea (*Pisum sativum* L.). Turkish Journal of Agriculture, 29: 237-242.

Perez-Garcia, F., Pita, J.M., Gonzalez-Benito, M.E. and Iriondo, J.M., 1995. Effects of light, temperature and seed priming on germination of celery seeds (*Apium graveolens* L.). Seed Science and Technology, 23: 377-383.

Peng L. Z., L. S. Gong and C. Y. Qing. 2007. A novel statistical method for assessing SSR variation in autotetraploid alfalfa (*Medicago sativa* L.). Genet. Mol. Biol. 30: 385-391.

Pill, W.G., Crossan, C.K., Frett, J.J. and Smith, W.G., 1994. Matric and osmotic priming of *Echinacea purprea* (L.) Moench seeds. Scientia Hotriculturae,

Radwan, M. S., Al- Fakhray, A. K., and Al-Hasan, A. M. 1978. Some observation on the performance of annual medics in northern Iraq. Mesopotania Journal of Agriculture 13: 55-67.

Rahimian Ashhadi, H., A. Bagheri, A. Paryab, 1991. The effect of various degree of PEG and NACL with temperature to germination of wheat. Science and Agriculture Technology magazine, No 1, Agriculture faculty of Ferdousi, 29, 12- 18.

Sanadgol, A., 1992. Shrub plants in salt lands, Magazine number 93, Research institute of Forests and Rangelands, 40, 73-77.

Shanehchi, M. (1990). *Cultivation and Management of Forage Crops*. Astane Ghdse Razavi Publication. pp: 278.

Small, E., and Jomphe, M. 1988. A synopsis of the genus Medicago (Leguminosae). Canadian Journal of Botany 67: 3260-3294.

Seong, R.C., Y. Park & J.Y. Chol, 1990. Effects of temperature, Polyethylen glycol and Sulphuric acid treatments on germination of Chinese milkvetch. Korean Journal of Crop Science, (35) 248-253.

Weisz, P. R., Denison, R. F., and Sinclair, T. R. 1985. Response to drought stress of nitrogen fixation (acetylen reduction) rates by field grown soybean. Plant physiol. 78: 525-530.