Evaluation of different alfalfa genotypes to drought stress and selection drought tolerant genotypes

Rahmati Hoshang *[1] and Farshadfar Mohsen [2]

1- Department Of Agriculture Payame nor University,Po Box19395-3697 Tehran, Iran
2- Department Of Agriculture Payame nor University,Po Box19395-3697 Tehran, Iran

Corresponding: Hoshang.Rahmati@yahoo.com

Abstract: Forage plants play an important role in animal feed and subsequently in human life. Water is the most limiting factor in plant growth and crop production. Therefore study on drought tolerant genotypes in arid and semi arid regions like Iran is significant. Alfalfa as one of the most important forage plants needs to high among of water during growing stages. On the other hand optimum irrigation leads to increase in crop yield. In this study 12 different alfalfa (*Medicago sativa*) genotypes were evaluated in response to drought tolerance. Alfalfa genotypes were considered as first factor and three osmotic potential (0, -0.4 and -0.8 Mpa) were considered as second factor in factorial arrangement experiment based on completely randomized design. Alfalfa seeds were germinated on Petri dishes under controlled conditions. Germination percentage, germination rate and germination vigor index were calculated. The results showed that ES178 genotype was superior genotypes regarding germination rate, germination percentage and germination vigor index. In addition, there was significant and positive correlation.
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Introduction

Increase in dry forage yield along with its quality has considerable effect on animal products. In order to increase in yield and forage palatability, in addition to breeding methods there is some approaches for producing and introducing compatible genotypes for each weather conditions. Alfalfa is one of the most important forage crops because of its palatability and quality. Alfalfa is rich in proteins and minerals such as calcium. In addition, A and C vitamins are found in alfalfa. Regardless alfalfa nutritional value, alfalfa cultivation improves soil permeability and increases soil organic matter. Water is the most limiting factor in plant growth and crop production. Since a huge part of Iran is located in arid and semiarid regions, relative drought tolerance has specific importance in crops. One of the basic solutions is that, breeders evaluate genotypes having sum of desirable and heritable traits and are able to produce acceptable yield under drought stress conditions. Alfalfa as a one of the forage crops has high water consumption; however irrigation at suitable time and at optimum amount leads to improve in alfalfa growth and production. Polyethylene glycol is flexible and nontoxic polymer so that is used to induce osmotic pressure in biological experiments. Polyethylene glycol and other inorganic salts have been used in seed germination tests (tomato by Mauromicale and Cavallaro., 1995; melon by Nascimento, 2003; celery by Prez- Garcia et al., 1995 and Echinacea by Pill et al., 1994) previously. Their results showed that germination percentage and germination uniformity was increased due polyethylene glycol application. This method is useful for seeds having weak germination vigor or should be cultivated in unfavorable soil conditions.

Material and methods

In this study, 12 different alfalfa genotypes were used. Seeds were provided from gene bank of pasture and forest research institute (Table 1). The experiment was conducted in Payame-Noor University, Kermanshah, Iran in 2011. The experimental design was a completely randomized design arranged in factorial with three replications. Genotypes were considered as first factor and three osmotic potential (0, -0.4 and -0.8 Mpa, induced by polyethylene glycol 6000) were considered as second factor. Data were collected from 5 germinated seed from each genotype randomly. Averages were used for each genotype. Germination percentage, germination rate and germination vigor were studied. Following formula were used for Germination percentage, germination rate and germination vigor calculation.

Germination rate $=nd_2$ (1.0) $+nd_4$ (0.8) $+nd_6$ (0.6) $+nd_8$ (0.4) $+nd_{10}$ (0.2)

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 (Bouslama and Schapaugh, 1984).
Germination percentage = germinated seeds till i^{th} days/ number of total seeds × 100
Germination vigor = (GP% × MSH) × 100 (Abdolbaghi and Anderson, 1970)
Where MSH; sum of shoot and root length

Results and discussion

Analysis of variance of studied traits is shown in tables 2, 3 and 4. There was significant difference among stress levels at 0.01 probability level. In this study significant difference was only observed for germination percentage at 0.05 probability level. Coefficient of variation was obtained 22.44, 18.68 and 16.74 for germination vigor, germination percentage and germination rate, respectively.

Germination vigor:
The highest germination vigor was observed in Es178 (control) and Es058 genotypes while the lowest vigor was related to Es056 and Es040 (Table 5).
Germination percentage:
The highest germination percentage was found in Es178, Es058, Es052 and Es096 genotypes. Es056 with 53% germination percentage had the lowest germination (Table 5). Previous results have shown that germination percentage in basil plants is stable at -1.4 Mpa but less than -1.4 Mpa germination percentages would significantly decreased so that at -1.35 germination stops completely (Hasani, 2005). In dill and fennel with increasing drought stress germination percentage significantly decreased (Rezazadeh and Kochaki, 2005).
Germination rate:

The highest germination rate was observed in Es178. On the contrary, Es056 had the lowest germination rate (Table 5). Similar results were obtained by Rezazadeh and Kochaki (2005) in dill and fennel plants grown under drought stress conditions. Decrease in germination because of drought stress might be due to decrease in water absorption by seeds. Low water absorption interrupts metabolic activities during germination and decreases germination. Subsequently it takes more time to radicle emergence and germination rate would decrease (Hoseini and Rezvani moghadam, 2006). Germination percentage and germination rate decreased due to -0.4 Mpa by 8 and 9% compared with non stress conditions. This decrease was more pronounced when -0.8 Mpa stress was applied so that germination percentage and germination rate decreased by 96.5 and 98% compared with non stress conditions. Similar results were obtained by Song and Park (1990). They have reported that the highest germination was related to control conditions while decreasing in water potential decreased germination in *Astragalus spp*.

Correlation coefficients are given in table 6. There was significant and positive correlation between germination rate and germination percentage, germination vigor, sum of root and shoot length as well as shoot length to root length ratio. In addition, positive and significant correlation was found between germination vigor and sum of root and shoot length, germination percentage and shoot length to root length ratio. Results indicated that germination percentage had significant and positive correlation with sum of root and shoot length and shoot length to root length ratio.

Cluster analysis:

Cluster analysis was performed based on germination stress index under -0.4 and -0.8 Mpa. Also cluster analysis for germination rate was done under normal and stressed conditions. Cluster analysis of genotypes based on germination rate under -0.4 Mpa is shown in figure 1.

Es178 and KR2197 genotypes were classified in first cluster while KR2421 and Es012 were classified in third cluster. Our results demonstrated that Es058, Es052 and Es008 were classified in fourth and rests of genotypes were classified in second cluster.
Cluster analysis of genotypes based on germination rate under -0.8 Mpa is shown in figure 2. Similarly Es178 and KR2197 genotypes were classified in first cluster while KR2421 and Es012 were classified in third cluster. Our results demonstrated that Es008, Es052 and Es058 were classified in fourth and rests of genotypes were classified in second cluster.
Confidence interval (0.95) and standard error for germination percentage of alfalfa genotypes are given in table 8. Standard error for this trait was 4.133 and the lowest average based on Duncan test (0.05% probability level and confidence interval 95%) was related to Es056 genotype with lower confidence of 45.65 and upper confidence of 62.12. The highest average was observed from Es178 (78.79) with confidence interval 95% with lower confidence of 70.65 and upper confidence of 87.12.
Confidence interval (0.95) and standard error for germination rate of alfalfa genotypes are given in table 8. Standard error for this trait was 1.665 and the lowest average based on Duncan test (0.05% probability level and confidence interval 95%) was related to Es056 genotype with lower confidence of 25.83 and upper confidence of 32.47. The highest average was observed from Es178 (41.26) with confidence interval 95% with lower confidence of 37.94 and upper confidence of 44.58.

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 germination vigor of alfalfa genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19585.603[a]	35	559.589	33.639	.000
Intercept	35701.157	1	35701.157	2.146E3	.000
Gen	544.825	11	49.530	2.977	.003
level	18478.550	2	9239.275	555.412	.000
level * Gen	562.228	22	25.556	1.536	.089
Error	1197.720	72	16.635		
Total	56484.480	108			
CV %			22.44		

Table 3: Analysis of variance on germination rate of alfalfa genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42556.043[a]	35	1215.887	48.711	.000
Intercept	137245.370	1	137245.370	5.498E3	.000
Gen	956.167	11	86.924	3.482	.001
level	40754.047	2	20377.024	816.339	.000
level * Gen	845.828	22	38.447	1.540	.088
Error	1797.227	72	24.961		
Total	181598.640	108			
CV %			16.74		

Table 4: Analysis of variance on germination percentage of alfalfa genotypes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	80863.657[a]	35	2310.390	15.031	.000
Intercept	475344.676	1	475344.676	3.093E3	.000
Gen	4730.324	11	430.029	2.798	.004
level	70061.574	2	35030.787	227.911	.000
level * Gen	6071.759	22	275.989	1.796	.034
Error	11066.667	72	153.704		
Total	567275.000	108			
CV %			18.68		

Table 5: Comparison of means of different germination traits of 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

Table 6: Correlation coefficient of alfalfa traits

	gp	pi	r.l	s.l	msh	vi	s.r	r.s
gp	1							
pi	.961**	1						
r.l	.758**	.825**	1					
s.l	.670**	.715**	.819**	1				
msh	.759**	.821**	.978**	.921**	1			
vi	.832**	.875**	.947**	.917**	.978**	1		
s.r	.605**	.632**	.572**	.872**	.707**	.723**	1	
r.s	-.240*	-.327**	-.234*	-.373**	-.295**	-.336**	-.401**	1

Table 7: Cluster Membership- pi0.8

Case	4 Clusters
1:ES178(co	1
2:KR2197	1
3:ES056	2
4:KR2421	3
5:ES058	4
6:ES052	4
7:ES051	2
8:ES040	2
9:ES012	3
10:ES008	4
11:ES096	2
12:ES014	2

Table 8: Confidence interval and standard error of germination percentage

fac.a	Mean-gp	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
ES008	63.333	4.133	55.095	71.571
ES012	63.333	4.133	55.095	71.571
ES014	66.667	4.133	58.429	74.905
ES040	62.222	4.133	53.984	70.460
ES051	63.333	4.133	55.095	71.571
ES052	72.222	4.133	63.984	80.460
ES056	53.889	4.133	45.651	62.127
ES058	75.000	4.133	66.762	83.238
ES096	71.667	4.133	63.429	79.905
ES178(co	78.889	4.133	70.651	87.127
KR2197	61.111	4.133	52.873	69.349
KR2421	64.444	4.133	56.206	72.683

Table 9: Confidence interval and standard error of germination rate

fac.a	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
ES008	35.222	1.665	31.902	38.542
ES012	34.800	1.665	31.480	38.120
ES014	34.422	1.665	31.102	37.742
ES040	32.578	1.665	29.258	35.898
ES051	35.422	1.665	32.102	38.742
ES052	37.222	1.665	33.902	40.542
ES056	29.156	1.665	25.836	32.475
ES058	39.511	1.665	36.191	42.831
ES096	36.689	1.665	33.369	40.009
ES178(co	41.267	1.665	37.947	44.587
KR2197	34.711	1.665	31.391	38.031
KR2421	36.778	1.665	33.458	40.098

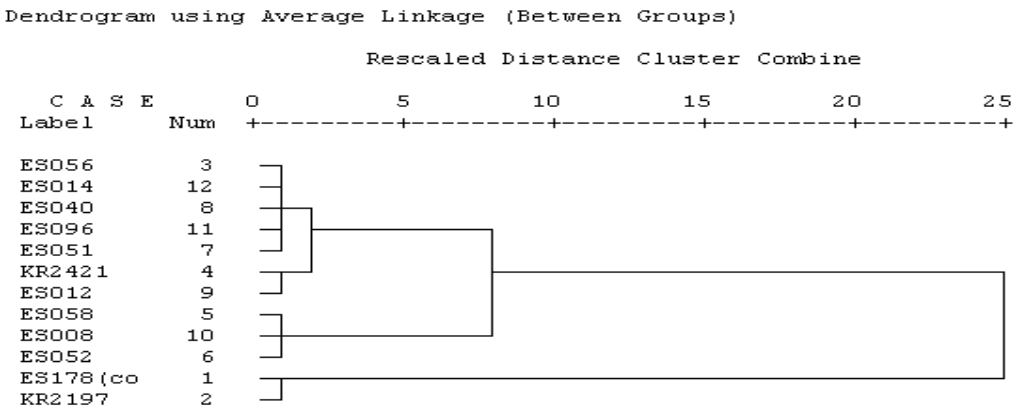

Figure 1: Cluster analysis o genotypes based on germination rate under conditions of -0.4 Mpa

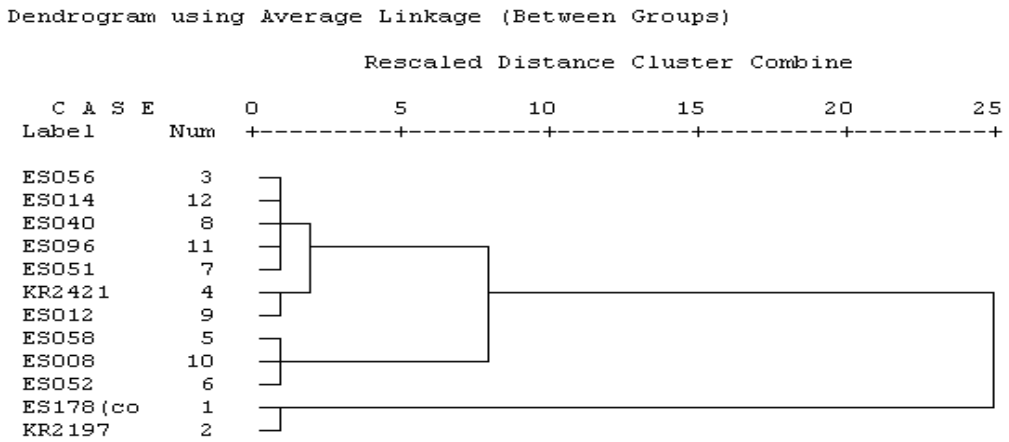

Figure 2: Cluster analysis o genotypes based on germination rate under conditions of -0.8 Mpa

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