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Evaluation of Salinity Tolerance of Alfalfa Genotypes during Germination Stage Using Multivariate Analysis

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ABSTRACT

Alfalfa is one of the most important forage plants with a special position among crops. Germination is one of the most sensitive stages to environmental stresses in crops. Therefore, to evaluate and select the most tolerant genotypes to salinity stress during germination stage, 20 genotypes of alfalfa were compared to each other in a completely randomized design. Four salinity levels including 0 (control), 75, 150, and 225 mM NaCl were applied. Analysis of variance showed significant difference for studied characteristics and indices among salinity stress levels and different genotypes. Principal component analysis using all measured characteristics under salinity stress showed that Nikshahri, Bami, Mesa-Sira, Gomi, Sahandava, Hamedani, Kodi, and Siriver were tolerant genotypes to salinity and Defi, Melissa, Kaiseri, Gargologh, and Diablo verdewere sensitive genotypes to salinity. Cluster analysis using the studied characteristics led to the classification of genotypes into two clusters. The first cluster (salinity tolerant) included Nikshahri, Hamedani, Yazdi, Baghdadi, Bami, Gomi, Kodi, Rahnani, Mesa-Sira, kf15, Sahandava, and Siriver genotypes and the second cluster (sensitive to salinity) involved Defi, Melissa, Kaiseri, Gargologh, Ramandi, Dastgerd, Harpinger, and Diablo verde genotypes.
Keywords: Alfalfa, Germination, Salinity stress, PCA, Cluster analysis.

1. INTRODUCTION

Alfalfa, *Medicago sativa L*, is the most important forage plant in Iran and many other parts of the world and is known as the Queen of forage plants. The general opinion is that alfalfa has been originated from Iran and was firstly transferred to north of America by Europeans [10]. Salinity is one of the major stresses especially in arid and semi-arid areas which severely limits the plant growth and reduces efficiency [3, 9]. This plant is moderately tolerant to salinity, as salinities more than 2 dS/m reduce its growth and performance [13, 16]. Compared to other forage plants, alfalfa has always been considered by researchers because of quick regrowth after harvesting, resistance to drought and heat, production of nutritious fodder, and biological fixation of molecular nitrogen of the air [7]. Salinity of water and soil resources is one of the factors that reduces the production of alfalfa and affects the quality of fodder [21]. Salinity affects seed germination with the increase in osmotic pressure and reduce in water absorption and also through ionic toxic effects. Therefore, the main problem for the production of crop in these farms is related to germination and appropriate establishment of shoot in the farm. Rapid and uniform emergence of shoot in the farm is an important factor in achieving the potential yield and quality of crops [23]. In many crops, germination and shoot early growth are of the most sensitive stages to environmental stresses such as salinity [12, 17]. Germination stage is very important for determining the final density of plan per unit of area and this density is obtained when the planted seeds have an appropriate percentage and speed of germination [22]. Several studies have been conducted on the effect of salinity stress on the germination stage of crops. In a study on 20 genotypes of Iranian alfalfa, it was shown that salinity stress in the germination stage reduces percentage of germination and other indices of germination, with the highest reduction in radicle length [18]. Monirifar *et al*. [15] evaluated 5 genotypes of alfalfa in the germination stage and found that there is difference between genotypes for germination percentage and germination indices under salinity condition. There are several multivariate statistical methods for determining the most tolerant and sensitive genotypes. Among these methods, cluster analysis and principal component analysis are the best [8]. Soltani *et al*. [20] used cluster analysis to classify 20 genotypes of Iranian alfalfa into two groups of tolerant and sensitive in the germination stage. Other researchers have also used principal component analysis for selection of drought tolerant genotypes of alfalfa and found that Gargologh, Malekkandy and Famentin genotypes are the most tolerant genotypes under moderate and severe drought stress [4].

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2. MATERIALS AND METHODS

To evaluate the effects of salinity stress on 20 genotypes of alfalfa (Yazdi, Melisa, Bami, Gomi, Nikshahri, Rahnani, Harpinger, *Mesa*-Sirsa, Dastgerd, Sahandava, Hamedani, Kf15, Ramandi, Siriver, Keri, Defi, Kaisari, Gargologh, Baghdadi and Diabloverde) obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran, an experiment was conducted as a completely randomized design in Safiabad Agricultural Research Center, Iran in 2014.The growth chamber conditions were: 60±3%, 16/8-hour light/dark photoperiod at25°C.Seeds were disinfected, with 5% sodium hypochlorite for 2 minutes and then the seeds were washed in double sterile water for three times. Germination test for evaluation of salinity stress was performed based on the standard test for salinity assessment in the germination stage [18]. To apply salinity stress, distilled water (as the control) and solution of 75, 150, and 225 mMNaCl for 4.5mL in 10-cm petri dishes containing Whatman Paper No. 1 were used. 25 seeds were put inside each petri dish and 4 replicates were established for each treatment. A seven-day trial was conducted in a completely randomized design (CRD) in form of factorial experiment. In order to measure the studied characteristics and indices, the number of germinated seeds (the seeds in which the radicle was 2 mm out of the cost) were counted and recorded on a daily basis. At the end of the experiment, germination indices for each of the genotypes were calculated based on the following formulas.

CVG: Coefficient of velocity of germination is an index for speed and acceleration of seed germination [11]. This index was calculated using following formula:

$$CVG = \frac{G_1 + G_2 + G_3 + ... + G_n}{(1\times G_1) + (2\times G_2) + (3\times G_3) + ... + (n\times G_n)} \text{ (seed/day)}$$

In which, G_1 to G_n are number of germinated seeds from the first to the last day of observations.

MTG: Means time to germination which is an index for germination rate. Ellis and Roberts [5] was calculated using following equation. In this equation, n, d and Σn are the number seeds germinated per day, number of days from the starting of the experiment and total germinated seeds, respectively.

$$MTG = \frac{\Sigma(nd)}{\Sigma n} \text{ (day}^{-1}\text{)}$$

MDG: Mean daily germination that is a display from daily germination rate. Scott [19] was used as

$$MDG = \frac{FGP}{d}$$

In the above formula, FGP is final germination percentage and d is the period of experiment.

SLVI: Seedling length vigor index was assayed according to Abbasian and Moemni [1].

SLVI= (mean shoot length + mean root length)× FGP.

DGS: Daily germination speed was calculated as:

$$DGS = \frac{1}{MDG} \qquad [6]$$

All statistical calculations were done by Excel, SAS 9.1 and MINITAB softwares.

3. RESULTS AND DISCUSSION

The results obtained from analysis of variance indicated that there is a significant difference among levels of salinity stress and genotypes for studied characteristics and indices, at an error level of 5%. Comparison of mean values of the studied characteristics and indices carried out by Duncan's Multiple Range Test.

According to this comparison, the highest germination percentage (89%), radicle length (30 mm), plumule length (4.9 mm), mean daily germination (12.77), mean germination speed (34.97), and seedling length vigor index (34.14) belonged to Mesa-Sirsa while Diabloverde showed the lowest germination percentage (35%), radicle length (10.8 mm), plumule length (2.86 mm), mean daily germination (5.05), coefficient of velocity of germination (0.145), daily germination speed (13.69), and seedling length vigor index (8.61). For mean time to germination, Harpinger genotype with 6.104 days had the highest MTG while Yazdi and Nikshahri genotypes had the lowest MTG (4.12 days). Genetic diversity is the foundation of plant breeding programs [14]. The high diversity among genotypes for the studied characteristics and indices showed that there is high response to the selection for tolerance improvement to salinity in all of genotypes. The study correlation between the different characteristics helps to plant breeders to determine the importance and value traits as selection criteria[2].To determine the correlation between the characteristics and indices affecting germination percentage, correlation coefficient between all studied characteristics was calculated (Table 1). The significant and positive correlation of germination percentage with shoot dry weight, radicle length, plumule length, MDG, CVG, and SLVI showed the parallel changes of these characteristics with germination percentage. In addition, the significant and

negative correlation of germination percentage with DGS and MTG indicates the inverse relationship of these characteristics with germination percentage. These results are in agreement with the findings of Soltani *et al*. [20]. In order to determine the genotypes tolerant and sensitive to salinity stress, principal component analysis was used. The results of principal component analysis for germination characteristics and indices under different salinity stress levels have been presented (Table 2). The first component (PCA1) had a higher share of total variance under salinity stress conditions. There is a positive and relatively high correlation between this component and shoot dry weight, radicle length, plumule length, MDG, CVG, and SLVI. Therefore, PCA1 can be considered as a potential performance index and salinity tolerance component (Table 2). The higher value of this component means the more favorable it is. This component separates salinity tolerance genotypes with high potential performance from genotypes sensitive to salinity with low potential performance. Principal component analysis showed that the highest changes of data are explained by two components of PCA1 and PCA2 (85% and 7%, respectively). Therefore, the use of these two components will lead to the loss of only a small amount of variance (8%) and interpretation of results based on them is of high efficiency. As a result, according to the values of PCA1 and PCA2, biplot diagram with vectors of measured characteristics and indices was plotted for the studied genotypes (Figure 1). According Figure 1, Nikshahri, Bami, Mesa-Sirsa, Gomi, Sahandava, Hamedani, Kodi, and Siriver genotypes, which had gained positive values of the first and second components, were considered salinity tolerant genotypes. On the other hand, Defi, Melissa, Kaiseri, Gargologh, and Diablo verde were identified as sensitive genotypes to salinity at germination stage. Obtuse angle, right angle, and acute angle between vectors in the biplot diagram suggest a strong negative relationship, a close-to-zero relationship, and a positive relationship between indices [24]. According to the angles between vectors of indices, it can be concluded that shoot dry weight, radicle length, plumule length, MDG, CVG, and SLVI have a positive relationship with germination percentage, while DGS and MTG have a negative relationship with germination percentage. Recent results were consistent with the results of correlation coefficients. Cluster analysis based on Ward's Minimum Variance method was used to determine genetic affinity and classification of the studied genotypes of alfalfa. Cutting dendrogram of cluster analysis between 5 and 24 euclidean distance were divided genotypes in two clusters (Figure 2). According to cluster analysis, the first cluster (salinity tolerant group) included Nikshahri, Hamedani, Yazdi, Baghdadi, Bami, Gomi, Kodi, Rahnani, Mesa-Sira, kf15, Sahandava, and Siriver genotypes and the second cluster (sensitive to salinity) involved Defi, Melissa, Kaiseri, Gargologh, Ramandi, Dastgerd, Harpinger, and Diabloverde genotypes (Figure 2). Mean and deviation percentage from total mean of both clusters are shown in Table 3. In genotypes of the first cluster, deviation percentage from total mean was positive and mean was higher than the mean of all genotypes for shoot dry weight, radicle length, plumule length, MDG, CVG, and SLVI. In this cluster for DGS and MTG, deviation percentage from total mean was negative and mean was lower than the mean of all genotypes. Therefore, genotypes of first cluster can be introduced as salinity tolerant genotypes of alfalfa (Table 3). By contrast, in genotypes of the second cluster, deviation percentage from total mean was negative and mean was lower than the mean of all genotypes for shoot dry weight, radicle length, plumule length, MDG, CVG, and SLVI and deviation percentage from total mean was positive and mean was higher than the mean of all genotypes for DGS and MTG. As a result, genotypes of this cluster can be identified as genotypes sensitive to salinity (Table 3).

Since the genotypes in each of the clusters have higher genetic affinity than genotypes in other clusters, so for genetic improvement with using heterosis and transgressive segregation, genotypes indifferent clusters can be used for hybridization programs. Finally, it should be mentioned that the results of the present study are only for determining salinity tolerance of the studied genotypes of alfalfa in the germination stage and because of different reactions of plants in various growth stages, in order to determine the salinity tolerant genotypes in the later growth stages of alfalfa, salinity stress evaluation studies, in addition to laboratory, should be also carried out in greenhouse and farm.

Table 1. Correlation coefficients between traits under salinity stress condition

Traits	Germination (%)	Shoot dry weight	Radicle length	Plumule length	MGT	MDG	DGS	CVG	SLVI
Germination (%)	1								
Shoot dry weight	0.721**	1							
Radicle length	0.876**	0.735**	1						
Plumule length	0.780**	0.912**	0.859**	1					
MGT	-0.929**	-0.854**	-0.894**	-0.868**	1				
MDG	1.000**	0.721**	0.876**	0.780**	-0.929**	1			
DGS	-0.944**	-0.773**	-0.894**	-0.821**	0.967**	-0.944**	1		
CVG	0.919**	0.841**	0.889**	0.851**	-0.994**	0.919**	-0.972**	1	
SLVI	0.929**	0.745**	0.981**	0.864**	-0.899**	0.929**	-0.903**	0.889**	**1**

ns, * and ** , non significant and significant at 1%and 5% probability level respectively

Table 2. The eigen values, variance and coefficients of two principle components obtained from all data under salinity stress condition

				Germination indices								
Component	Eigen value	Variance%	Cumulative variance	Shoot dry weight	Radicle length	Plumule length	MGT	Germination (%)	MDG	DGS	CVG	SLVI
1	7.6504	0.85	0.85	0.314	0.328	0.307	-0.350	0.338	0.338	-0.34	0.351	0.332
2	0.6159	0.068	0.918	-0.515	-0.040	-0.619	-0.082	0.401	0.401	0.01	0.064	0.132

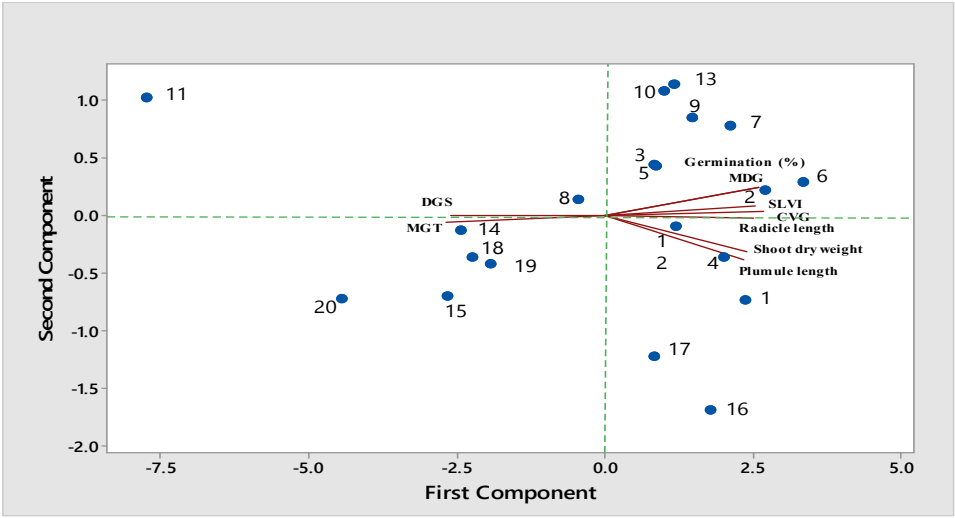

Figure 1. Graphical biplot show for 20 alfalfa genotypes and 9 germination indices on the basis of first and second components under salinity stress conditions (1-Yazdi 2-Nikshahri 3-Bami 4-Rahnani 5-Gomi 6-Mesa-Sirsa 7-Hamedani 8-Ramandi 9-Sahandava 10-Siriver 11-Harpinger 12-Kf15 13-Kodi 14-Defi 15-Kaiseri 16-Baghdadi 17-Dastgerd 18-Gargolog 19-Melissa 20-Diablo verde).

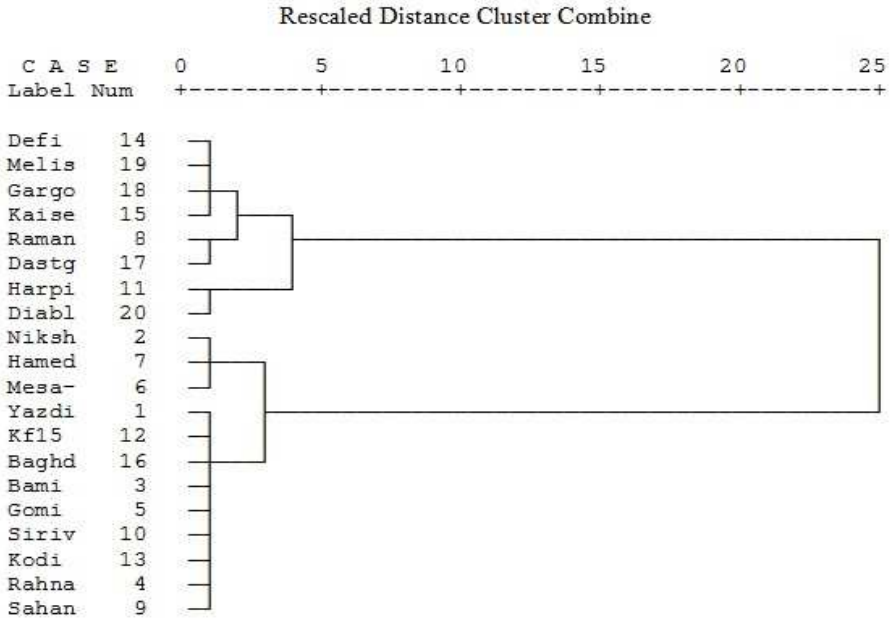

Figure 2. Cluster analysis of alfalfa genotypes under salinity stress conditions.

Table 3. Means and deviation percentage from total mean for difference indices in two cluster of cluster analysis

	Cluster 1	**Cluster2**	
Traits	Yazdi, Nikshahri, Bami, Rahnani, Gomi, Mesa-Sirsa, Hamedani, Sahandava, Siriver, Kf15, Keri and Baghdadi	Defi, Melisa, Gargologh, Kaisari, Ramandi, Dastgerd, Harpinger and Diabloverde	Total mean
Germination (%)	72.38 +27.5	33.27 -41.36	56.73
Shoot dry weight(mm)	1.56 +14.03	1.08 -21.05	1.37
Radicle length(mm)	15.12 +18.44	9.23 -27.66	12.76
Plumule length(mm)	3.23 +12.13	2.35 -18.19	2.88
MGT(day)	4.35 –8.39	5.35 +12.58	4.75
MDG	10.34 +27.57	4.75 -41.36	8.10
DGS	0.1 –39.67	0.26 +59.5	0.16
CVG	0.23 +15.22	0.15 -22.83	0.20
SLVI	14.99 +28.89	56.67 -43.33	11.63

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