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Multivariate analysis of forage sorghum [*Sorghum bicolor* (L.) Moench] lines for salinity tolerance at germination stage

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Abstract

This study was conducted to evaluate the effect of salinity on the germination and early growth of forage sorghum lines. In order to an experiment was performed as factorial form under completely randomized design (CRD) with 3 replications. Line factor was contains of 15 lines and 5 levels of salinity (control, -3, -6, -9 and -12 bar) with NaCl. Results of correlation showed that seedling length had the most positive and significant correlation with radicle length (r=0.99**). Factor analysis based on principal component analysis showed that three independent factors 79% of all variation data determined. The first component named germination percentage, seedling length and index seed vigour. The second component named germination characteristics and dry/wet weight ratio of seedling. The three component named mean germination time, plumule length and dry weight of seedling. Cluster analysis (Ward's minimum variance method) and bipot arranged lines into two groups. Based biplot, lines of KFS_2, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10} and KFS_{11} were low sensitive to salinity stress. The lines KFS_1, KFS_{12}, KFS_{13}, KFS_{15}, KFS_{16}, KFS_{17} and KFS_{18} were high sensitive to salinity stress.

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Introduction

Salinity is a common abiotic stress factor seriously affecting the crop production in different regions, particularly in arid and semi-arid regions of the world. It is estimated that over 800 million hectares of land in the world is affected both by salinity and sodicity (Munns, 2005).

Sorghum is rated as moderately salt tolerant and can produce profitable crops on saline soils (Chauhan *et al.*, 2012). Its cultivation area is expanding to areas having high potential for accumulation of salts in the soil profile, such as Khuzestan province in Iran. It is, therefore, important to develop new sorghum lines with high genetic capacity to tolerate salt stress. The first important step in breeding new lines with high salt tolerance is to have a useful and substantial genetic variation in tolerance to salinity stress. Breeders seek to develop and identify cultivars that are more tolerant towards salinity and water stress (Janmohammadi *et al.,* 2008).

Multivariate analysis is a very useful method because it reveals the relationships and correlation among variables studies. This type of analysis applied to studies of germplasm collection allows a better understanding of the structure of the collection, identification of more relevant variables, detection of the relationships among accession, as well as identification of possible groups (Martines-Calvo *et al.*, 2008).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Germination percentage, germination rate and seedling growth are most suitable criteria for selection of cultivars. One of the commonest experiments in germination of the seeds is the application of NaCl. Seed response to salinity can be simulated by NaCl induced ionic stress in the germination experiments. Ionic stress is caused by a toxic accumulation of NaCl in plant tissues. Germination rates decrease with an increase in NaCl concentration (Murillo-Amador *et al.,* 2002).

The goal of this research was to determine the importance of traits using factor analysis in the salinity stress condition. Hereby, morphological patterns in a number of sorghum lines are identified and determined. Using this pattern, leading to planned breeding programmes more successful and useful for the preparation of figures is desirable. Also, other goal of this research was classification of sorghum lines under salinity stress.

Materials and methods

Experimental treatments and experimental design

Effect of salinity stress induced by different osmotic potential levels (control, -3, -6, -9 and -12 bar) NaCl treatments on germination and early seedling development of 15 forage sorghum lines (KFS_1, KFS_2, KFS_3, KFA_6, KFA_7, KFA_8, KFA_9, KFA_{10}, KFA_{11}, KFA_{12}, KFA_{13}, KFA_{15}, KFA_{16}, KFS_{17} and KFA_{18}) was studied. This investigation was performed as factorial experiment under completely randomized design with three replications at Seed Laboratory, Islamic Azad University, Shoushtar Branch, Iran in the year 2013.

Germination test and studied traits

The selected seeds of each line were first sterilized in sodium hypochlorite (1.5%) solution and then washed and the washed twice in deionized distilled water. Then petri-dishes containing one layer filter paper were moistened by respective prepared salinity solutions. Thereafter, 25 seeds of each line were soaked in these petri-dishes and then kept in an incubator (40% relative humidity) at 25°C. Daily germination rate was measured and filter papers were replaced, when needed. Similarly, respective salinity solutions were added when required. Seeds were considered germinated when the emergent radicle reached to 2 mm length. After 7 days, germination percentage was measured by ISTA (1996) standard method. By the end of the 7th day, the germination percentage, reduced germination, mean germination time, germination rate, radicle length, plumule length, seedling length, radicle/plumule length ratio, dry weight of seedling, wet weight of seedling, dry/wet weight ratio of seedling and index seed vigour were also measured.

Statistical analysis

For statistical analysis, the data of germinating percentage were transformed to arcsin $\sqrt{\frac{X}{100}}$.

Analysis of the variance, correlations, factor analysis based on principal component analysis and cluster analysis using Minitab software was carried out. Variables assigned to different components and independent component with regard to the coefficient value after operating Varimax took turns. Component coefficient greater than 0.3, regardless of its mark as a significant component for any independent components was considered.

Results

Analysis of variance

Results of ANOVA showed significant differences among different levels of salinity stress for all traits. In the lines and interaction between salinity stress and lines had significant differences for all traits except mean germination time and dry weight of seedling (Table 1).

Table 1. Analysis of variance on mean of squares of studied traits forage sorghum lines under salinity stress.

Source of variance	Df	Germination	Reduced Germination	Mean germination time	Germination rate	Radicle length	Plumule length
Salinity levels	4	** 5653.336	**11357.203	**7.455	**0.289	** 519.477	**30.814
Lines	14	**943.322	995.356**	2.016^{ns}	0.075**	**32.658	**0.357
Salinity levels×Lines	56	**182.542	202.986**	1.789^{ns}	0.025**	21.595**	0.506**
Error	150	40.665	68.995	1.588	0.006	5.470	0.085

ns and**: non significant and significant at P=0.01 probability level.

Continued table 1.

Source of variance	Df	Seedling length	Radicle/plumule length ratio	Wet weight of seedling	Dry weight of seedling	Dry/wet weight ratio	Index seed vigour
Salinity levels	4	**751.281	**49.988	**360.873	83109.50**	6245.073**	**0.268
Lines	14	**35.553	**13.718	**33.000	4279.54^{ns}	136.145**	**0.031
Salinity levels×Lines	56	21.270**	7.386**	11.384**	3823.74^{ns}	99.340**	0.028**
Error	150	3.515	3.087	1.741	4677.81	34.347	0.009

ns and**: non significant and significant at P=0.01 probability level.

Simple correlation

Simple correlation coefficients between studied traits are illustrated in Table 2. Results showed that germination percentage had positive and significant correlation with radicle length (r=0.81**), seedling length (r=0.82**), radiclel/plumule length ratio (r=0.71**) and index seed vigour (r=0.94**). Seedling length had the most positive and significant correlation with radicle length (r=0.99**). Khodarahmpour and soltani (2013) with study on alfalfa in salinity stress reported that seedling length had the most positive and significant correlation with radicle length (r=0.96**). But, Khalesro and Aghaalikhani (2007) with study on forage sorghum [*Sorghum bicolor* (L.) cultivar speedfeed] and pearl millet [*Pennisetum americanum* (L.) cultivar nutrifeed] to salinity and water deficit stress reported positive and the highest correlation (r=0.98**) between germination rate and germination percentage.

Principal component analysis

Factor analysis based on principal component analysis showed that three independent components, respectively, 49, 16 and 13% of all variation data determined (Table 3). In the first component germination percentage, radicle length, seedling length, radicle/plumule length ratio and index seed vigour positive factor coefficients were shown. With attention to significant traits in the first component, this component named germination percentage, seedling length and index seed vigour. Furthermore, with attention to be desirable of traits with positive factor coefficients, to be high first component should be considered. In the second component, reduced germination and germination rate had positive factor coefficients but dry/wet weight ratio negative factor coefficient were shown. Therefore, named germination characteristics and dry/wet weight ratio of seedling. With attention to be undesirable of trait reduced germination with positive factor coefficient and desirable dry/wet weight ratio with negative factor coefficient, to be low second component should be considered.

Table 2. Correlation coefficients of studied traits forage sorghum lines under salinity stress.

Traits	1	2	3	4	5	6	7	8	9	10	11
1.Germination (%)	1										
2. Reduced germination (%)	0.508 -	1									
3.Mean germination time (day)	0.159 -	0.120	1								
4.Germination rate (number in day)	0.288	0.187	-0.482	1							
5.Radicle length (cm)	**0.814	*-0.528	-0.165	0.150	1						
6.Plumule length (cm)	0.501	-0.143	0.173	0.121	**0.671	1					
7.Seedling length (cm)	**0.824	-0.489	0.113-	0.097	**0.992	**0.704	1				
8.Radicle/plumule length ratio	**0.710	*-0.553	-0.066	-0.176	**0.795	0.262	** 0.811	1			
9. Index seed vigour	**0.938	-0.425	0.131-	0.166	**0.916	0.569 *	**0.932	**0.820	1		
10. Wet weight of seedling (mg)	0.275	-0.407	-0.111	0.121	0.406	0.363	0.405	0.248	0.337	1	
11. Dry weight of seedling (mg)	0.455	-0.026	0.144	0.387	*0.528	**0.777	* 0.536	0.136	0.482	0.451	1
12. Dry/wet weight ratio	-0.432	-0.028	0.437	*-0.536	-0.487	-0.354	-0.499	-0.297	-0.469	-0.322	-0.150

In the three component mean germination time, plumule length and dry weight of seedling negative factor coefficients was shown. Therefore, named mean germination time, plumule length and dry weight of seedling. Principal component analysis (PCA) has been widely used in the studies of variability in germplasm collections of forage plants (Martines-Calvo *et al.*, 2008; Ashraf Jafari and Zeiaei- Nasab, 2004; Bibi *et al.*, 2012 and Khodarahmpour and soltani, 2013).

Biplot display

A better approach than a correlation analysis such as a biplot is needed to identify superior lines for stress stress, as the lines in biplot analysis are compared simultaneously for all the traits. The first two principal component accounted for about 65.3% of total variation of data set. Therefore, the first two PCAs were employed to generate biplot. PCA indicated that the traits could discriminate the forage sorghum lines. Biplot analysis (Fig. 1) confirmed correlation analysis between all studied traits. Biplot basis on the first and second components in salinity stress condition (Fig. 1) sorghum lines to two groups was divided. This plot showed that lines of KFS_2, KFS_3, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10} and KFS_{11} in region with high first component were located. Therefore, these lines, with attention of results of principal components analysis were low sensitive to salinity stress. The lines KFS_1, KFS_{12}, KFS_{13}, KFS_{15}, KFS_{16}, KFS_{17} and KFS_{18} in region with low first component were located. Therefore, lines were high sensitive to salinity stress.

The presence of large genotypic variation for tolerance to salinity reported in sorghum (Taylor *et al.*, 1975; Hassanein 1985; Azhar and McNeilly 1987, 1988; Maiti *et al.* 1994; Krishnamurthy *et al.* 2007; Chauhan *et al.*, 2012) offers a good scope for integrating tolerance characteristics into appropriate breeding programs to improve crop productivity on saline soils.

Table 3. Results of factor analysis all traits studied in forage sorghum lines under salinity stress.

Traits	Components		
	1	2	3
Germination (%)	0.366	-0.014	0.098
Reduced germination (%)	-0.213	0.341	-0.282
Mean germination time (day)	-0.071	-0.410	-0.520
Germination rate (number in day)	0.098	0.634	0.009
Radicle length (cm)	0.398	-0.061	0.029
Plumule length (cm)	0.287	0.040	-0.471
Seedling length (cm)	0.400	-0.081	-0.002
Radicle/plumule length ratio	0.315	-0.295	0.265
Index seed vigour	0.389	-0.058	0.062
Wet weight of seedling (mg)	0.209	0.057	-0.107
Dry weight of seedling (mg)	0.245	0.160	-0.549
Dry/wet weight ratio of seedling	-0.226	-0.426	-0.176
Eigen value	5.9191	1.9229	1.5950
Relative variance	0.493	0.160	0.133
Cumulative variance	0.493	0.653	0.786

The use of biplot display in selecting tolerant genotypes has already been used by Basafa and Taherian (2010) in alfalfa ecotypes under drought stress, Bibi *et al.*, (2010) and Bibi *et al.*, (1012) in sorghum under drought stress, Zaheri and Bahraminejad (2012) in oat genotypes under drought stress, Scasta (2012) and Khodarahmpour and soltani (2013) in alfalfa genotypes under salinity stress.

Cluster analysis

Results of cluster analysis (Ward's minimum variance method) arranged lines into two groups (Fig. 2). The results of the first cluster which included eight of the lines KFS_2, KFS_3, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10} and KFS_{11}. The results of the second cluster which included seven cultivars KFS_1, KFS_{12}, KFS_{13}, KFS_{15}, KFS_{16}, KFS_{17} and KFS_{18}. Soltani *et al.* (2012) with study on alfalfa cultivars under salinity stress reported that cultivars KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdi Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri as well as KFA_3, KFA_6,

KFA_7, KFA_9, KFA_{17}, KFA_8, KFA13, KFA_{10}, KFA_{14} and KFA_{15} were placed in the first and second clusters, respectively. Cultivars in the first cluster were found to be tolerant, while those in the second cluster were sensitive to salt.

Cluster analysis has been widely used for description of genetic diversity and grouping based on similar characteristics under stress conditon (Zaheri and Bahraminejad, 2012 and Scasta, 2012).

Discussion

The main purpose of this study was to assess the range of variation for salinity tolerance in forage sorghum lines, as a first step to future breeding efforts.

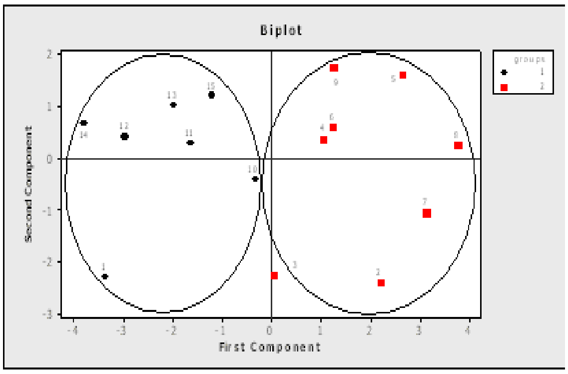

Fig. 1. The biplot display of forage sorghum lines on the first and second components in salinity stress condition (1: KFS_1, 2:KFS_2, 3:KFS_3, 4: KFS_6, 5: KFS_7, 6: KFS_8, 7: KFS_9, 8: KFS_{10}, 9: KFS_{11}, 10: KFS_{12}, 11: KFS_{13}, 12: KFS_{15}, 13: KFS_{16}, 14: KFS_{17}, 15: KFS_{18})

With attention to results of correlation, traits radicle length, seedling length, radicle/plumule length ratio and index seed vigour had positive and significant correlation with germination percentage and were located in first component that 49% of all variation data determined and with attention to be desirable of this traits, to be high first component should be considered, therefore this traits are the best germination indices and seedling growth for salinity tolerance in forage sorghum lines.

We observed that there exist some tolerant lines to salinity stress. Indeed, KFS_2, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10} and KFS_{11} expressed a good tolerance to salinity stress and in region with high first component were located. Also, in cluster analysis in one group were located. Therefore, lines were low sensitive to salinity stress. Valuable plants from the most promising materials could be used for future activities in our sorghum breeding programme.

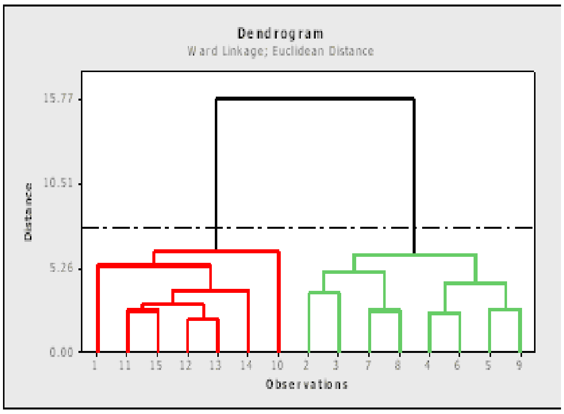

Fig. 2. Cluster analysis of forage sorghun lines under different levels of salinity stress using Ward's minimum variance method (1: KFS_1, 2:KFS_2, 3:KFS_3, 4: KFS_6, 5: KFS_7, 6: KFS_8, 7: KFS_9, 8: KFS_{10}, 9: KFS_{11}, 10: KFS_{12}, 11: KFS_{13}, 12: KFS_{15}, 13: KFS_{16}, 14: KFS_{17}, 15: KFS_{18})

These results can be related to some earlier studies in which lines identified as salinity tolerant at the earlier growth stages showed tolerance when tested at the later growth stages. Although, a considerable magnitude of variation for salinity tolerance was observed in the 15 lines of sorghum while screening them at germination stages, further studies need to be carried out to assess whether the lines marked as salinity tolerant at the initial growth stages maintain their degree of salinity tolerance when tested as adult plants.

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