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Evaluation of forage sorghum [*Sorghum bicolor* (L.) Moench] lines for drought tolerance at germination stage

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Abstract

Drought is a major factor which limits growth and development of higher plants. This study was conducted to evaluate the effect of drought 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 contained 15 lines and 5 levels of drought (control, -3, -6, -9 and -12 bar) with PEG 6000. Results showed that the most negative and significant correlation had between germination rate and mean germination time (r=-0.98**) and the most positive and significant correlation had between root length and seedling length (r=0.99**). Factor analysis based on principal component analysis showed that three independent factors, respectively, 52, 21 and 10% of all variation data determined. In the first component germination percentage, germination rate, root length, seedling length, index seed vigour and dry weight of seedling positive factor coefficients were shown. In the second component mean germination time, root/shoot length ratio and dry/wet weight ratio negative factor coefficients and shoot length positive factor coefficient were shown. In the three component reduced germination negative factor coefficient was shown. Based biplot, lines of KFS_2, KFS_3, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10}, KFS_{11} and KFS_{12} were low sensitive to drought stress and lines KFS_1, KFS_{13}, KFS_{15}, KFS_{16}, KFS_{17} and KFS_{18} were high sensitive to drought stress.

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Introduction

Plant growth and productivity are greatly affected by environmental stresses such as drought, high salinity, and low temperature (Zheng *et al*., 2010). Drought is one of the most important abiotic stress factor (Bruce *et al*., 2002), which affects almost every aspect of plant growth (Aslam *et al*., 2006).

Iran is an agro-based country and has a variety of important crops. There are a number of crops whose genetic diversity is required to be found out due to their importance in different fields. One such crop includes *Sorghum bicolor* (L.) Moench, which is ranked the 5th most important cereal crop in the world. Sorghum is a drought resistant low input cereal grain grown throughout the world. In most countries it is used primarily as animal feed, but in Africa and India it is used as human feed where it is stable food for millions of people (Agrama and Tuinstra, 2003). The more presence of different lines of sorghum is a valuable source for screening and identifying the tolerant types with regard to environmental stresses such as drought.

Among these critical stages, water stress induced during seedling stage has been exploited in various crop species to screen germplasm or breeders populations i.e. sorghum (Gill *et al*., 2002; Bibi *et al.,* 2010; Bibi *et al.,* 2012). Rauf (2008) narrated several benefits of screening genotypes at seedling stages such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest. Furthermore seedling traits have also shown moderate to high heritability with additive type of genetic variance within and over environments (Rauf *et al.,* 2008).

Seed sowing is generally considered the first critical and most sensitive stage in the live cycle of plants and seeds are frequently exposed to unfavourable environmental conditions that may compromise the establishment of seedling (Figueiredo-e-Albuquerque and De Carvalho, 2003).

An artificially created water-stress environment is used to provide the opportunity in selecting superior lines out of a population. On the basis of these grounds, experiment was carried out to categorize sorghum lines against drought stress; to select suitable lines for drought tolerance and also to determine the suitability of various seedling traits for selection of tolerant or susceptible lines to drought stress.

Materials and methods

Experimental treatments and experimental design

Effect of drought stress induced by different osmotic potential levels (control, -3, -6, -9 and -12 bar) PEG 6000 (Fisher, 1985) 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%) 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 drought 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 drought 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, germination percentage, reduced germination, mean germination time, germination rate, root length, shoot length, seedling length, root/shoot 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 and factor analysis based on principal component 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 drought stress, lines and interaction between drought stress and lines had for all traits (Table 1).

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

Source of variance	Df	Germination	Reduced Germination	Mean germination time	Germination rate	Root length	Shoot length
Drought levels	4	** 5282.493	**2762.562	**1.522	**0.170	** 483.591	**38.925
Lines	14	** 1513.125	641.184 **	1.454 **	0.184 **	** 56.423	** 0.835
Drought levels×Lines	56	**179.891	80.713 **	0.175 **	0.019**	19.058 **	0.728**
Error	150	15.62	3.822	0.006	0.001	0.143	0.007

**: Significant at P=0.01 probability level.

Continued Table 1.

Source of variance	Df	Seedling length	Root/shoot length ratio	Wet weight of seedling	Dry weight of seedling	Dry/wet weight ratio	Index seed vigour
Drought levels	4	**764.982	**2817.320	**79211.611	**8774.229	**9.399	**380.331
Lines	14	** 55.685	** 450.894	**5273.324	**1505.103	** 0.613	** 45.397
Drought levels×Lines	56	20.454**	254.999**	*3368.447	**476.507	0.256**	10.633**
Error	150	3.390	0.051	0.009	14.662	0.003	0.048

** and *: Significant at P=0.01 and P=0.05 probability level, respectively.

Simple correlation

Simple correlation coefficients between studied traits are illustrated in Table 2. Results showed that germination percentage had positive and significant correlation with germination rate, root length, seedling length, index seed vigour and dry weight of seedling. But, germination percentage had negative and significant correlation with reduced germination and mean germination time. The most negative and significant correlation had between germination rate and mean germination time (r=-0.98**) and the most positive and significant correlation between had between root length and seedling length. The results of correlation analysis revealed some important associations among the germination traits as well as between the seedling related characters. However, Matsuura *et al.*, (1996) reported a positive relation between drought tolerance and root length in sorghum and millet. This suggested that these characters could be selected simultaneously with their positive effects on drought tolerance in different stages of crop growth in sorghum. Khodarahmpour (2013) with study on drought stress of alfalfa cultivars reported that germination percentage had the most positive and significant correlation with radicle length. Germination rate had the most positive and significant correlation with seed vigour and seedling length had the most positive and significant correlation with radicle length. 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.

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

Traits	1	2	3	4	5	6	7	8	9	10	11
1.Germination (%)	1										
2. Reduced germination (%)	**0.740 -	1									
3.Mean germination time (day)	**-0.720	0.353	1								
4.Germination rate (number in day)	**0.791	-0.410	-0.980**	1							
5.Root length (cm)	**0.692	-0.459	-0.500	*0.613	1						
6.Shoot length (cm)	0.152	0.261	0.346-	0.314	0.205	1					
7.Seedling length (cm)	**0.702	-0.407	* 0.515-	* 0.626	** 0.992	0.300	1				
8.Root/shoot length ratio	0.403	-0.452	0.046	0.086	* 0.573	-0.425	*0.545	1			
9. Index seed vigour	**0.865	*-0.549	* 0.627-	0.733 **	0.939**	0.198	0.947**	0.588 *	1		
10. Wet weight of seedling (mg)	0.449	-0.171	-0.535*	0.531*	0.337	0.458	0.377	-0.067	0.416	1	
11. Dry weight of seedling (mg)	*0.590	-0.277	-0.470	*0.571	**0.724	0.332	** 0.747	0.305	0.689 **	0.641*	1
12. Dry/wet weight ratio	0.090	-0.323	0.172	-0.062	0.404	-0.360	0.366	** 0.696	0.269	-0.027	0.477

* and **: Significant at P=0.05 and =0.01 probability levels, respectively.

Principal component analysis

Factor analysis based on principal component analysis showed that three independent factors, respectively, 52, 21 and 10% of all variation data determined (Table 3). In the first component germination percentage, germination rate, root length, seedling length, index seed vigour and dry weight of seedling positive factor coefficients were shown. With attention to significant traits in the first component, this component named germination characteristics, seedling length, index seed vigour and dry weight of seedling. Furthermore, with attention to be desirable of traits with positive factor coefficients, to be high first component should be considered. In the second component mean germination time, root/shoot length ratio and dry/wet weight ratio negative factor coefficients and shoot length positive factor coefficient were shown. Therefore, named mean germination time and seedling characteristics. With attention to be desirable of traits with negative factor coefficients, to be low second component should be considered. In the three component reduced germination negative factor coefficient was shown. Therefore, named reduced germination. With attention to be undesirable of this trait with negative factor coefficient, to be high second component should be considered.

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). Principal component analysis (PCA) has been widely used in the studies of variability in germplasm collections of forage plants under drought stress (Bibi *et al.,* 2010; Bibi *et al.,* 2012; Khodarahmpour, 2013).

Biplot display

A better approach than a correlation analysis such as a biplot is needed to identify superior lines for stress condition, as the lines in biplot analysis are compared simultaneously for all the traits. The first two principal component accounted for about 73.6% 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 basis on the first and second components in drought 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}, KFS_{11} and KFS_{12} in region with high first component were located. Therefore, these lines were low sensitive to drought stress. The lines KFS_1, 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 drought stress.

The presence of large genotypic variation for tolerance to drought reported in sorghum (Gill *et al.*, 2002; Bibi *et al.*, 2010 and Bibi *et al.*, 2012) offers a good scope for integrating tolerance characteristics into appropriate breeding programs to improve crop productivity on drought regions.

Table 3. Results of principal component analysis all traits studied in forage sorghum lines under drought stress.

Traits	Components		
	1	2	3
Germination (%)	<u>0.354</u>	0.028	0.314
Reduced germination (%)	-0.237	0.215	<u>-0.487</u>
Mean germination time (day)	-0.292	<u>-0.312</u>	-0.286
Germination rate (number in day)	<u>0.328</u>	0.244	0.241
Root length (cm)	<u>0.361</u>	-0.115	-0.170
Shoot length (cm)	0.101	<u>0.461</u>	-0.398
Seedling length (cm)	<u>0.364</u>	-0.073	-0.215
Root/shoot length ratio	0.193	<u>-0.497</u>	-0.025
Index seed vigour	<u>0.383</u>	-0.060	0.012
Wet weight of seedling (mg)	0.224	0.291	-0.200
Dry weight of seedling (mg)	<u>0.391</u>	0.015	-0.321
Dry/wet weight ratio of seedling	0.126	<u>-0.479</u>	-0.311
Eigen value	6.2905	2.5377	1.2530
Relative variance	0.524	0.211	0.104
Cumulative variance	0.524	0.736	0.840

The use of biplot graph in selecting tolerant genotypes under droght stress has already been used by Bibi *et al.*, (2010) and Bibi *et al.*, (2012) in sorghum genotypes, Basafa and Taherian (2010) in alfalfa ecotypes, Khodarahmpour (2013) in alfalfa genotypes, Jaber Hosseini *et al.*, (2012) in rice genotypes and Zaheri and Bahraminejad (2012) in oat genotypes.

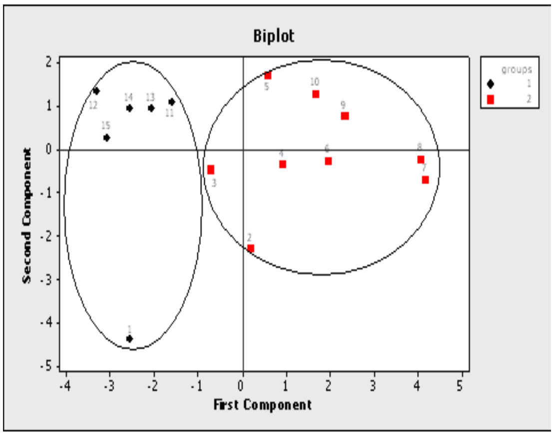

Fig. 1. The biplot display of forage sorghum lines on the first and second components in drought stress condition (1: KFS_1, 2:KFS_2, 3:KFS_3, 4: KFS_6, 5: KFS_7, 6: KFS_8, 7: KFS_9, 8: KFS_{10}, 9: KFS_{11}, 10: KFS_{12}, 11: KFS_{13}, 12: KFS_{15}, 13: KFS_{16}, 14: KFS_{17}, 15: KFS_{18}).

Discussion

With attention to results of correlation, traits germination rate, root length, seedling length, index seed vigour and dry weight of seedling had positive and significant correlation with germination percentage and were located in first component that 52% 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 growth seedling for drought tolerance in forage sorghum lines. According to positive and significant correlation between germination rate and germination percentage, it could be stated that genotypes selection based on high germination percentage will cause fast germination and better seedling establishment. Also, mean germination time had the negative and significant correlation with germination percentage. Therefore, with increased water stress germination percentage and germination rate decreased but mean germination time increased.

The information about significant correlation among the traits is important for initiation of any breeding program because it provides a chance for selection of desirable genotypes with desirable traits simultaneously (Ali *et al.*, 2009).

We observed that there exist some tolerant lines to drought stress. Indeed, KFS_2, KFS_3, KFS_6, KFS_7, KFS_8, KFS_9, KFS_{10}, KFS_{11} and KFS_{12} expressed a good tolerance to drought stress and in region with high first component were located. Therefore, lines were low sensitive to drought stress. Valuable plants from the most promising materials could be used for future activities in future sorghum breeding programme.

Evaluation of crop tolerance to environmental stresses during seed germination and seedling emergence is a main measure to choose them for cropping in different circumstances. Since common investigations in field conditions are time consuming and influenced by many companion variables of soil, climate and agricultural practices, so a fast and precise evaluation of crop response to stress would be achieved using an experiment in controlled environment conditions.

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