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RESEARCH PAPER

Assessment of genetic variability of inbred lines and their F_1-hybrids of grain maize (*Zea mays* L.) under drought stress conditions

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Abstract

This study was carried out to assess the genetic variability of seven inbred lines of grain maize (*Zea mays* L.) and their F_1-hybrids under drought and irrigated conditions and to identify the most drought tolerance genotypes, using drought tolerance parameters. A field experiment was executed during the winter and summer of 2009 and 2010 at two locations, Shambat and Elrawakeeb (only during summer season 2010). A split-plot design with three replications was used to layout the experiment. The inbred lines and their F_1-hybrids were evaluated in the field under normal (D_0) irrigation and stress conditions (D_1). The results showed that, drought stress caused significant reduction in yield and most of the studied characters. Significant differences were detected among the genotypes for most of the studied characters. Awide range for values of drought tolerance parameters were exhibited by the inbred lines and F_1-hybrids. The F_1-hybrids showed high estimates of genotypic coefficient of variation, heritability and genetic advance for grain yield/ha and its components. It concluded that, drought tolerance parameters were used in this studied, as most suitable in indicators for screening drought tolerant genotypes and the hybrid 160 × 405 had the highest tolerance to drought in the conditions of this study.

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Introduction

Maize grows over a wider geographical and environmental range than any other cereals. It is grown at latitudes varying from the equator to slightly north and south of latitude 50°, from sea level to over 3000 meters elevation, under heavy rainfall and semi-arid conditions, cool and very hot climates (Abdemula *et al.*2007) . In Sudan, although maize is of less importance than sorghum, wheat and millet as a staple human food. However, the crop plays a great role in food security for the people in Blue Nile and South Kordofan States. The crop is grown in the two states by traditional farmers in small-holdings under rain-fed. Nowadays, different companies and individuals started to grow the crop at a large scale under irrigation or under rainfall in different parts of Sudan. However, the total cultivated area of maize in the Sudan increased from 17 thousand hectares in 1971 to 37 thousand hectares in 2010 (Ahmed, 2011).). Recently, there has been an increased interest in maize Production in the Sudan (Nour *et al*, 1997 and Abdemula *et al.*2007). Maize is more sensitive to drought. It is exposed to more hazards and it is a higher risk crop in general (Misovic, 1985 and Abdemula *et al.*2007). Drought is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression (Leopold *et al.*, 1990). Low water availability is one of the major causes for crop yield reductions affecting the majority of the farmed regions around the world. As water resources for agronomic uses become more limiting, the development of drought-tolerant varieties of maize becomes increasingly more important. Over the years, maize breeders have aimed at generates hybrids with higher grain yield potentials, better grain yield stability and improved grain quality for consumers (Duvick, 1997). On the other hand, Maize produces the highest yields when water is abundant and soil fertility is high, but maize is least tolerant to stress among cereals (Muchow, 1989). However, an estimated 80% of the maize crop suffers periodic yield reduction due to drought stress (Bolanos and Edmeades, 1993). Drought may occur at any stage of maize growth, but when it coincides with the flowering and grain filling periods it causes yield losses of 40-90% (Nesmith and Ritchie, 1992). Understanding of genetic basis of drought tolerance would be used in developing maize genotypes for drought prone areas. However, the objectives of this study were to assess the genetic variability among inbred lines and their F_1- hybrids of grain maize under normal and water stress conditions, using agro-morphological traits and to identify the most tolerance genotype under drought stress condition.

Material and methods

Twenty two genotypes of grain maize (Table 1); were evaluated under two levels of water treatments, namely normal irrigation every 7 days and water stress every 21 days and under four different environments namely: [Shambat winter season 2009 (SW09), Elrawakeeb summer season 2010 (ERS10), Shambat summer season 2010 (SS10) and Shambat winter 2010 (SW10)]. Four field experiments was conducted to achieve the objective of this study. The first field experiments were carried out during the winter and summer seasons of the two years 2009 and 2010 at the Experimental Farm of the Faculty of Agriculture, University of Khartoum at Shambat (32°:32´ E. Longitude, 15°:40´ N. Latitude and 380 meters above the sea level). The second field experiment (summer 2010) was carried out at Elrawakeeb Dry lands and Desertification Research Station, National Centre for Research, about 35 Km west of Khartoum (32°:15´ E. Longitude, 15°:25´ N.Latitude and 420 meters above the sea level), Sudan.

A split- plot design with three replications was used to execute the experiments. The water treatments were assigned to the main-plots and genotypes to the sub-plots. Each genotype was grown in a 4×5 meters/plot at seed rate of 3 – 4 seeds/hill on ridges during the last week of July for summer season and the first week of November for winter season. Thinning was carried out after a week from sowing to raise two plants/hill. Hill-to hill and ridge-to ridge spacing was 20 and 70cm, respectively. Weeding was carried out by hand hoeing twice for each experiment.

Appropriate amount of chemical fertilizer, urea N\ha (1N= 43kg) applied after four weeks from sowing. Before planting, seeds were treated with a protective fungicide (Thiram). Systematic insecticide (Furidan) was used twice, one of them at sowing for protection against stem borer. In addition, the plants were sprayed with "Durispan" against termites. Ten randomly selected plants were used to record the data. Different plant characters were measured, which included plant height, leaf area index, Days to 50% tasseling and date to maturity, cob length, cob diameter, number of kernels/row, number of kernels/cob, cob weight ,100 kernels/cob, Grain yield/plant and grain yield (kg/ha). Data from each site was subjected to ANOVA separately to detect the significance of genotypic differences (Gomez and Gomez, 1984) before a combined ANOVA. To estimate the extent of variability, genotypic coefficient of variation were estimated based on the following formula.

Genotypic coefficient of variation (GCA %)

They were estimated according to formula suggested by Burton and Devane (1953) as follows:

Genotypic coefficient of variation

$$(GCV\ \%) = \frac{\sqrt{\sigma^2 g}}{\text{Grand mean}} \times 100$$

Broad sense heritability (h^2)

It was estimated in each location separately, from the analysis of variance according to Johnson *et al.* (1955) by the formula:

$h^2 = \sigma^2 g / \sigma^2 ph$

$\sigma^2 g$ = genotypic variance

$\sigma^2 ph$ = phenotypic variance

Expected genetic advance as percentage of the mean (GA %)

It was estimated using the formula of Robinson *et al*, (1949) as follows:

$$GA = K\frac{\sigma^2 g}{\sqrt{\sigma\ Ph}} \quad \text{and } GA\% = \frac{GA}{G} \times 100$$

Where:

G= the grand mean

K = selection differential and it was 2.06 as defined by Lush (1943) at selection intensity of 5%.

Drought tolerance parameters

Grain yield of both irrigated and drought stress experiments were determined after physiological maturity and used as Y_w and Y_d, respectively. Where Y_d and Y_w are the mean yields of all genotypes under stress and non-stress conditions, respectively, and 1– ($\hat{Y}_d\ \hat{Y}_w$) is the stress intensity (SI). Drought resistance indices were calculated using the following relationships:

1. **Y_d**/Y_w % = Ratio of grain yield /plant (under stress) to grain yield /plant (normal irrigation) as a percent.

(2) SSI (stress susceptibility index of Fisher& Maurer (1978)).

It was determined using the formula $= 1 - \frac{(\hat{Y}_d - \hat{Y}_w)}{SI}$

Values of SSI < 1 describe below average drought susceptibility (=above drought tolerance, as average reaction is defined by SSI = 1, and values of SSI >1 describe above drought susceptibility (= below average drought tolerance).

(3) STI Stress tolerance index (Fernadez, 1993 Stress tolerance index (Fernadez, 1993= It is measured as $(Yd)\ (Yw)/(yw)^2$. Where yw is the mean yield under well –watered conditions over all genotypes.

(4) GMP (Geometric mean of productivity) as suggested by Fernandez (1993) = $\sqrt{(Y_d \times Y_w)}$.

Results and discussion

Genetic variability

The inbred lines and their F_1- hybrids showed significant effects for all characters were studied (Table 2).These differences indicate the existence of great amount of variability among the tested genotypes. This variability might be attributed to genetic and environmental factors as well as their interactions. Similarly, Sokolov and Guzhva (1997) reported pronounced variation for different morphological traits among inbred lines. Inbred lines

× environments interaction was also highly significant for most of the characters. Indicating that inbred lines were not equally effect with environmental factors. Furthermore, Mean square of hybrids and hybrids × environmental interaction were significant for all characters. Variations in response of maize hybrids to environmental stress were previously reported by several researchers (Betran *et al.*, 2003; Mosisa *et al.*, 2007; Derera *et al.*, 2008). The inbred lines as well as F_1-hybrids showed highly significant interactions with water treatments for traits such as grain yield/plant and grain yield (kg/ha) (Table 2). Similar results were recorded by Grant *et al.,* (1989) and Ahamed (2002).

Table 1. The maize genotypes used in the study of the extent of variability under normal and water stress conditions.

Genotype code	*Genotypes*	*Type*
1	66y	Inbred Line
2	277	″
3	3	″
4	6	″
5	2	″
6	160	″
7	405	″
8	Hudieba-1	Improved Open pollinated
9	Hudieba-2	Improved Open pollinated
10	66y× 405	Hybrid
11	66y × 277	Hybrid
12	66y × 6	Hybrid
13	160 × 277	Hybrid
14	160 × 3	Hybrid
15	160 × 66y	Hybrid
16	160 × 6	Hybrid
17	66y × 2	Hybrid
18	405 × 160	Hybrid
19	405 × 6	Hybrid
20	6 × 3	Hybrid
21	2 × 160	Hybrid
22	66y × 3	Hybrid

Genotypic Coefficient of variation, Heritability and Genetic Advance

In this study, a wide range of genetic variability among the evaluated genotypes was detected for the studied characters (Table 6). The highest estimate of GCV was shown by number of kernels/cob for inbred lines and grain yield/plant for F_1- hybrids, whereas the lowest one was shown by days to maturity. Similar results were reported by Katerji *et al.* (1994). Based on these results, the number of kernels/cob exhibited high genetic coefficients of variation, high heritability and high genetic advance (Table 6). This indicates that this trait is highly genetically controlled and less affected by environments. Therefore, it could be used in improving productivity of maize under drought conditions.

The heritability value estimated in broad sense for the studied traits are shown in Table 6. High heritability estimates ($h^2>0.60$) were recorded for most of the measured characters (Table 6). This result indicates that these traits are highly genetically controlled and less affected by environments. This finding is in agreement with that reported by Baenziger *et al.* (2000). On the other hand, grain yield (kg/ha) was quantitative character, controlled by many genes and much affected by environmental conditions. Thus it has low values of heritability. Furthermore, the improvement of grain yield (kg/ha) could be improving by mass selection. Consequently, the genetic advance was counted for grain yield and its components and the lowest one was for days to 50% tasselling. This result indicates that the amount of genetic advance from selection for trait, in general, depends largely on the amount of genetic variability presented in the material under study. This was probably due to the fact that genetic advance as a percentage of the mean for any character depends mainly upon the genetic variability of the character.

Table 2. Variance components due to genotypes (G), of Inbred lines (I) and F_1- hybrids (II), and their interactions (G× T) and (G× E) with water treatments (well-watered and dry) and with environments (Elrawakeeb- Summer -2010, Shambat-Summer-2010, Shambat-winter-2009 and Shambat, winter-2010).

Characters	Genotypes	E	T	E×T	G	G×E	G×T
	I	d.f: I = 3, II=3	d.f: I = 1, II=1	d.f: I = 3, II=3	d.f: I = 6, II=12	d.f: I = 18, II=36	d.f: I = 6, II=12
Plant height (cm)		25420.7**	8536.0**	880.8	678.9*	2322.4*	195.6
	II	4606.8**	23182.3**	485.4	453.0*	710.6**	199.3
Leaf area index	I	7.26**	3.93**	0.17*	1.17**	0.37**	0.04
	II	15.4**	9.74**	0.18	0.58**	0.32**	0.18
Days to 50% tassel ling (days)	I	8389.0**	463.3**	39.1	44.8**	19.4**	1.88
	II	15522.0**	557.3**	26.9	79.4**	35.6**	4.5
Days to maturity (days)	I	17990.3**	314.9**	2.33	36.4**	26.2**	3.01
	II	38095.2**	696.0**	31.6	42.2	32.9	7.94
Cob length (cm)	I	25.1*	116.0**	5.07	30.4**	7.02**	2.47
	II	163.0**	220.5**	2.89	9.42**	4.38*	3.05
Cob diameters (mm)	I	977.0**	413.4**	3.74	11.0**	3.76**	2.75*
	II	1626.9**	1010.9**	109.6	48.4**	25.7**	6.72
Cob weight (g)	I	1119.4**	1687.5**	94.5*	62.9**	22.2	23.4
	II	5746.0**	11715.4**	1954.3**	539.9**	293.9**	287.7**
No of rows per cob	I	28.5**	36.2**	4.15	16.3**	8.07**	2.37
	II	24.3**	114.6**	23.0**	13.3**	8.62**	5.97**
No of kernels /cob	I	395643.1**	167654.8**	5772.0	27857.0**	12459.2**	4472.8*
	II	639599.9**	674813.8**	12654.9	16553.4**	17153.8*	5198.8
100- kernels weight (g)	I	105.3*	85.6**	10.6	20.3**	16.9**	6.63
	II	630.8**	495.9**	114.8**	29.8**	19.1**	4.22
Grain yield/plant	I	3114,0**	7123.8**	154.1	218.2**	148.5**	105.7**
	II	24220.3**	24376.4**	1389.3**	2025.3**	947.8**	266.4*
Grain yield (kg/ha)	I	12048641.0**	1676547.8**	5772.0	27857.0**	2459.2**	4472.8**
	II	14641022.2**	1112751.0**	1315330.5	6241911.1**	1330501.5**	1657285.5**

ns= not significant, * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$.E= Environments; T= Treatment (four levels of water stress); G=Genotypes.

Table 3. Estimates of genotypic coefficient of variation (GCV%), phenotypic coefficient of variation (PCV%), broad sense heritability (h^2) and genetic advance (GA%) for different characters measured on Inbred lines (I) and F_1-hybrids (II) of maize, evaluated under two water treatments (well-watered and dry) and across four environments (ERS10, SS10, SW09 and SW10).

Characters	Genotype	GCV (%)	h^2	GA (%)
Plant height (cm)	I	8.09	0.32	9.41
	II	5.56	0.24	5.64
Leaf area index	I	27.9	0.71	48.4
	II	18.6	0.53	20.2
Days to 50% tasselling (days)	I	7.51	0.91	14.1
	II	12.7	0.85	14.7
Days to maturity (days)	I	4.48	0.85	7.82
	II	4.46	0.62	5.73
Cob length (cm)	I	32.1	0.84	60.5
	II	11.5	0.46	16.2
Cob diameter (mm)	I	22.8	0.77	41.2
	II	10.2	0.43	13.8
Cob weight (g)	I	16.0	0.71	23.9
	II	38.1	0.83	69.6
No. of rows/ cob	I	22.0	0.74	39.0
	II	11.5	0.68	16.2
No. of kernels/cob	I	113.4	0.84	214.5
	II	19.6	0.36	24.3
100-kernel weight (g)	I	26.1	0.63	42.7
	II	30.4	0.59	48.9
Grain yield/plant (g)	I	26.9	0.52	46.9
	II	54.1	0.79	101.7
Grain yield (kg/ha)	I	15.9	0.57	24.6
	II	34.2	0.79	62.4

Drought tolerance parameters

Awide range for values of drought tolerance parameters were exhibited by parental lines and F_1-hybrids (Table 3). The F_1- hybrids expressed higher general means for drought tolerance parameters than parental lines (Table 3). Furthermore, some hybrids performed well across stress levels, indicating that it is possible to combine stress tolerance and yield potential in maize hybrids. Similar results were reported with temperate maize hybrids, where improvements for tolerance to a biotic and biotic stresses have been associated with the ability to maximize grain yield under non- stress growing conditions (Carlone and Russell, 1987., Castleberry *et al.*, 1984 and Duvick, 1997). On the other hand, analysis of variance for various quantitative criteria of drought tolerance showed highly significant differences for most of the indices, except SSI and STI for F_1-hybrids (Table 3), indicating the presence of genetic variation and the possibility of selection for drought-tolerant genotypes based on Y_W, Y_d, and GMP. Genetic variation between maize genotypes for drought tolerance was reported by Bolanos and Edmeades (1996) and Morris *et al.* (1991) and for Y_w, Y_d, and GMP by Ahmadzadeh (1997) and Afarinesh (2000). The effect due to genotype × environments was highly significant for Y_w, Y_d and GMP drought tolerance parameters, indicating the genetic variance in stress environment more than non-stress. The inbred lines and F_1-hybrids with high SSI and STI are sensitive to water stress. However; selection must be based on low rates of these indices. Therefore, the use of SSI and STI indices lead the selection toward tolerant and low yielding genotypes. It is better to use these indices for omission of susceptible genotypes, but not for the selection of both stress tolerant and high yielding genotypes. Maghaddam and Hadizadeh (2001) have got similar results on this subject.

Table 4. Variance components due to genotypes (G) and their interaction with environments (G×E) among inbred lines (I) and F_1-hybrids (II) for drought tolerance parameters, across four environments (ERS10, SS10, SW09 and SW10).

Parameters	Variance components due to			
	G		G × E	
	I	II	I	II
Yw	1.50ns	10.5**	0.89ns	3.62**
Yd	1.28ns	6.58**	1.06ns	5.61**
Yd/Yw	1.33ns	1.47ns	0.65ns	1.42ns
GMP	2.79*	13.5**	1.83*	6.97**
SSI	0.99ns	1.29ns	0.86ns	1.40ns
STI	1.33ns	1.47ns	0.65ns	1.42ns

ns= not significant, * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$.

Correlation between drought tolerance parameters

In the present study, a positive correlation was detected between yield under stress (Yd) and yield under non- stress (Table 4). This relationship was more pronounced and determined in the F_1-hybrids than the inbred lines. These were in agreement with Sallah *et al.*, (2002) who reported that yield in the stress environment was positively associated with yield in the non-stress environment. The strong positive correlation between Y_d and Y_w indicates that some of the genes controlling grain yield under both stress and non- stress environments was probably common (Alza and Fernandez-Martinez, 1997). According to the nature of their association (positive and negative) with yield potential (Y_w), the investigated drought tolerance parameters can be classified into two groups. Group one, including Y_d/Y_w and STI showed negative relationship with (Y_w). Selection for improving these parameters decreases yield potential. Similar results were found by other workers (Fisher and Maurer, 1978; Rosielle and Hamblin, 1981; Riemer, 1995; Schneider *et al.*, 1997; Abdelmula and link, 1998). The other group of parameters exhibited positive relationship with Yw and Yd, e.g., GMP and SSI. Selection for high values of these parameters improves yield under stress and non-stress environments. Similar results were found by Ceccarelli *et al.,* (1992).

Table 5. Means of the inbred lines, check cultivars and F_1-hybrids under drought tolerance parameters, averaged over three replications and across four environments (ERS10, SS10, SW09 and SW10).

	Drought tolerance parameters					
Genotypes	Yw	Yd	Yd/Yw	GMP	SSI	STI
Parental lines						
66y	31.4	19.8	0.63	24.9	0.94	0.63
277	36.9	24.0	0.65	29.7	0.89	0.65
3	30.8	22.3	0.72	26.2	0.71	0.72
6	26.3	17.2	0.65	21.2	0.89	0.65
2	40.8	20.0	0.49	28.6	1.30	0.49
160	33.1	20.7	0.63	26.2	0.96	0.62
405	33.7	17.9	0.53	24.6	1.20	0.53
Mean	33.3	20.3	0.61	25.9	0.98	0.61
Checks						
Huediba I	49.8	44.1	0.89	46.8	1.44	0.89
Huediba II	47.7	45.7	0.96	46.7	0.54	0.96
F_1-hybrids						
66y×405	48.2	27.9	0.58	36.7	1.22	0.58
66y×277	56.0	38.6	0.69	46.5	0.90	0.69
66y×6	56.4	33.6	0.60	43.6	1.17	0.60
66y×2	39.6	28.8	0.73	33.8	0.79	0.73
66y×3	66.5	45.1	0.68	54.8	0.93	0.68
160×405	73.5	47.7	0.65	59.2	1.02	0.65
160×277	41.3	33.5	0.81	37.2	0.55	0.81
160×6	64.0	40.2	0.63	50.8	1.08	0.63
160×2	45.0	24.4	0.54	33.1	1.33	0.54
160×3	56.7	30.3	0.53	41.4	1.35	0.53
160×66y	40.9	27.4	0.67	33.5	0.96	0.67
6×405	37.1	29.5	0.80	33.1	0.59	0.80
6×3	41.0	29.0	0.71	34.5	0.85	0.71
Mean	45.3	30.35	0.67	37.0	1.00	0.67
SE±	2.67	2.04	0.02	2.23	0.07	0.02

Table 6. Correlation coefficients between several drought tolerance parameters inbred lines (Below the diagonal) and F_1-hydrids (above the diagonal), across four environments (ERS10, SS10, SW09 and SW10).

	Yw	*Yd*	*Yd/Yw*	*GMP*	*SSI*	STI
Yw	-	0.860**	-0.412	0.966**	0.412	-0.410
Yd	0.428	-	0.105	0.963**	-0.106	0.108
Yd/Yw	-0.648	0.407	-	-0.167	-1.000**	0.997**
GMP	0.871**	0.816**	-0.193	-	0.166	-0.163
SSI	0.637	-0.417	-0.999**	0.182	-	-0.999**
STI	-0.638	0.415	0.999**	-0.184	-0.999**	-

ns= not significant, * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$.

Conclusion

It concluded that, awide range of genetic variability was detected among genotypes for drought tolerance. The genotypes expressed different degree of relative response to drought with respect to 50% tasselling, date to maturity and yield and its components. Grain yield and its components were more sensitive to drought stress than vegetative characters. Highly significant positive correlation for GMP and STI under the different water stress with Yw. That means selection for high values of these parameters improves yield under stress and non stress environments.

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