Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.9, 2015
www.iiste.org

Genetic Variations of Leaf Trait in Maize (Zea mays L.) under Drought Stress in Different Growth Stages

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Abstract
Maize is the third most important food crop worldwide, and it is more sensitive to drought. Two field experiments were conducted under drought in different growth stages at two locations, the Demonstration Farm, Faculty of Agriculture, University of Khartoum, at Shambat, and the Gezira Research Station Farm, at Medani, in season (2003/2004). To estimate the pattern of inheritance, determine the relative magnitude of genetic variation effects for a number of leaves/plant and leaf area index in fifteen maize genotypes. A split-plot design, layout within randomized complete block design with three replications was used for the experiment. Significant differences among genotypes were detected in all traits, except, leaf area index (30 and 60 days) and number of leaves/plant (45 days). High genotypic coefficient of variation, genetic advance and heritability were exhibited by a number of leaves/plant for 60 days. Grain yield was significantly and positively associated at the phenotypic level with the leaf area index and a number of leaves/plant at 60 days. Thus the characters leaf area index and number of leaves/plant to be the important traits which would be an effectual in selection for maize improvement under drought stress at different growth stages. Based on their positive association with grain yield, the traits leaf area index and number of leaves/plant would be the exploited for improving grain yield and facilitate further efforts in the maize improvement program in the country.
Keywords: correlation; heritability; morphological traits; vegetative and reproductive phases; water stress

1. Introduction

Maize is the third most important food crop worldwide (Frova et al., 1999). In the Sudan, maize is normally grown as a rain-fed crop in Kordofan, Darfur and Southern States or in small-irrigated areas in the Northern States (Ahmed and El Hag, 1999). Recently, there has been an increased interest in maize production in the Sudan (Abdalla et al., 2010). Maize is used in many ways than any other cereal. It is considered as a multi-purpose crop and has been put to a wider range of uses such as human food, animal and poultry feed and for hundreds of industrial purposes. It is satisfactory and nutritious, higher in fats (4%) than rice and wheat and also contains about 10% protein (Timothy et al., 1988). Maize can be cooked in a variety of ways to make very palatable and popular foods. Every part of the plant has economic value. The grain, leaves, stalk, and even the cob, are used to produce hundreds of food and nonfood products (Skerman and Riveros, 1990; Dowswell et al., 1996; Meseka et al., 2003).

The lower leaf water potential completely inhibited photosynthesis and reproductive development (Westgate and Boyer, 1986). Bãnziger et al., (2000) reported that drought led to decreased leaf, root and grain development. Incomplete ground cover results from reduced leaf area expansion. Leaf senescence is accelerated (from the bottom of the plant first, while in conditions of high-potential evapotranspiration, in order it can occur at the top of the plant as well) moreover, reduces radiation interception. Leaf elongation rate and photosynthetic capacity per unit leaf area depend on the anatomy and physiology of the developing tissue. Both are important in grassland management and are affected by genotype and environment (Schnyder and Nelson, 1989). The leaf area adjustment has been suggested as one of the most powerful means of avoiding stress (Seetharama et al., 1982). Bãnziger et al., (2000) found that plants avoid low tissue water potentials by one or more quite discrete mechanism, such as a change in rooting pattern and adjustment in leaf area.

The genetic variances were larger under stress conditions (Ceccarelli et al., 1992; Hohls, 2001). Abundant secondary traits for drought tolerance were of low heritability (Bãnziger et al., 2000). These characters are leaf and stem elongation rate, canopy temperature, leaf photo-oxidation, leaf chlorophyll concentration, predawn leaf water potential, and seedling survival under drought. Nyuetta and Cross, (1997) reported that maize genotypes of the high leaf number tend to produce longer leaves and correlated positively with grain yield. De Souza et al., (1997) demonstrated that moisture stress during seed filling reduced yield by accelerating senescence and shortens the seed filling period. The main objectives were (1) to estimate the genetic variability for the leaf traits under drought stress in different growth stages. (2) To determine the correlations between yield and leaf traits under normal and stress conditions.

1.1 Materials and methods

Study site and experimental design

Two field experiments were used to achieve the objectives of this study. The experiments were conducted during the 2003/04 season at two sites in Sudan, was previously described (Sabiel et al., 2014). The first was Gezira Research Station Farm, at Wad Medani. The second was the Demonstration Farm of the Faculty of Agriculture, University of Khartoum, at Shambat. Fifteen genotypes of maize provided by the Gezira Research Station (Wad Medani) of the Agricultural Research Corporation were used in the study. Different drought stress levels were induced; control: watering every 14 days during the growing season, water stress throughout the vegetative stage: irrigation was every 21 days till the end of vegetative period, and then followed by well watering every 14 days till harvest and water stress during the reproductive stage: irrigation was every 14 days till the end of flowering, and then irrigating every 21 days till mature stage. The experiment was laid out as a split plot design with three replications. Each replication consisted of three main plots in which the water treatments were distributed randomly. 15 genotypes were grown as subplots and were assigned erratically. Each genotype was planted on 3 meters length of two ridges at a rate of two seeds per hole, spaced at 80 cm between ridges and 25 cm between holes. The seedlings were thinned to one plant per hole, two weeks after germination. Sown date was 7 July 2003 at Shambat and 15 July 2003 at Medani. At both locations, weeding was devastated by hand two times per season.

1.1.1 Data collection

Data were recorded on the following: Leaf area index was measured at 30, 45 and 60 days from planting. The numbers of leaves per plant were counted at 30, 45 and 60 days from planting. Grain yield (kg ha-1), this was estimated from the grain yield per subplot.

1.1.2 Statistical analysis

Analysis of variance (ANOVA) was carried out for each character using the computer system PLABSTAT version (2N of 1997/09 /15), to found significant effects among the genotypes and environment. The mean, standard error, LSD5%, coefficient of variation (CV%), coefficient of correlation and the combined analysis of variance also calculated for further interpretation of the results.

Results

Phenotypic variability

Analysis of variance depicts highly significant differences ($P \leq 0.01$) among the genotypes for the number of leaves/plant in Shambat at 30 days and 60 days in Medani. Significant difference was detected for the leaf area index in Shambat at 45 days. However, non-significant differences were found in other's traits (Table 1).

The combined analysis showed highly significant differences ($P \leq 0.01$) among the genotypes for 30 days and non-significant for 45 and 60 days. The variation due to genotype x locations, interaction was significant for 60 days and non-significant for (30 and 45 days) (Table 2). Non significant differences between the genotypes and genotype x location's interactions were found (Table 2).

The overall means of leaf area index at 30, 45 and 60 days were (2.75, 3.18 and 2.49) at Shambat, (2.68, 3.62 and 3.18) at Medani and (2.71, 3.4 and 2.83) for the average of both locations (Table 3). For the leaf area index at 30 days, the greatest genotypes were D-3 at Medani, G-3 at Shambat and for the average of both locations, while the slightest genotypes were D-3 at Shambat, G-4 at Medani and the average of both locations (Table 3). For the leaf area index at 45 days, the greatest genotypes were D-3 at Shambat, E-7 at Medani and for the average of both locations, while the smallest genotypes were V-113 at Shambat, PR-1 at Medani and for the average of both locations (Table 3). For the leaf area index at 60 days, the largest genotypes were E-7 at Medani, M-45 at Shambat and for the average of both locations, while the smallest genotype was V-113 at both locations (Table 3).

The overall means of the number of leaves/plant in 30, 45 and 60 days were (12, 13 and 11) at Shambat, (10, 13 and 11) at Medani and (11, 13 and 11) for the average of both locations (Table 4). For the number of leaves/plant at 30 days, the lowest and the highest genotypes' number ranged between (10-13) at Shambat, (9-11) at Medani and (10-11) for the average of both locations (Table 4). For the number of leaves/plant at 45 days, the smallest and the highest genotype's number ranged between (12-14) at S (12-13) at Medani and for the average of the locations (Table 4). For the number of leaves/plant at 60 days, the lowest and the highest genotypes the number ranged between (11-12) at Shambat, (10-12) at Medani and (10-11) for the average of both locations (Table 4).

Genotypic variability

High genotypic variance relative to phenotypic variance was recorded for a number of leaves/plant at 30 days at Shambat. However, at Medani it was recorded for leaf area index at 60 days and the number of leaves/plant at 60 days (Table5). At Medani, slightly high genotypes x treatment's interaction variance relative to phenotypic variance was obtained from a number of leaves/plant at 30 days. Whereas at Shambat it was recorded for a number of leaves/plant at 60 days (Table 5).

Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all characters at both locations (Table 6). Most of the characters showed high values of phenotypic coefficient of variation at Shambat than Medani, except number of leaves/plant (Table 6). The highest phenotypic coefficient of variation (58.6%) at Shambat was found through the leaf area index at 30 days. There was a wide range of genotypic coefficient of variation among the different characters (Table 6). Values of heritability estimated at Shambat were slightly greater than those at Medani for most of the characters. The highest values (57% and 61%) were found in the number of leaves/plant (30 and 60 days) at Shambat and Medani, respectively. However, the lowest values (8% and 17%) were found in the number of leaves/plant (45 days) and leaf area index (45 days) at Shambat and Medani, correspondingly (Table 6).

Grain yield (kg/ha) was positively correlated with leaf area index at 60 days (r = 0.589 and 0.569), number of leaves/plant at 60 days (r = 0.577and 0.612) at Shambat and Medani, respectively (Table 7).

Discussion

Significant differences between the genotypes were found at both locations for the leaf traits. This indicated the existence of a high degree of genetic variability in the materials to be preferable for the maize improvement program in Sudan. This study is in agreement results found by Salami et al., (2007) and Hajibabaee et al., (2012). The effect of stress at vegetative stage reduced vegetative traits slightly at the two locations. These findings are in agreement with studies by Mangobe et al., (1996) and Ribaut et al., (1997). The high genotypic coefficient of variation was recorded for a number of leaves per plant, have been also reported in previous studies by Alan et al., (2013).

Leaf area index was reduced by inducing drought stress during vegetative and reproductive stages at Shambat. Stress during pre-flowering period decreased leaf development and photosynthesis rate (Bãnziger et al., 2000). Therefore, leaf area adjustment has been suggested as one of the most powerful means of avoiding stress (Seetharama et al., 1982). This indicates that the leaf area index was one of the most important physiological traits under drought stress. The number of leaves per plant was not affected by stress during vegetative and reproductive stages at both locations. This indicates that the number of leaves/plant was stable for a given genotype. The timing and intensity of stress determine the giant reduction in grain yield. Water stress applied at the reproductive stage resulted in high reduction in grain yield at Medani. This may be due to accelerating leaf senescence and shortening the seed filling period (De Souza et al., 1997). This reveals that soil moisture stress at any stage of growth decreased grain yield substantially. Similar results were observed by Ahmed, (2002). Drought stresses affect's maize grain yield to some degree at almost all growth stages (Grant et al., 1989). The effect of drought on yield at Shambat was more pronounced than that at Medani. This may be due to differences in the monthly temperature and relative humidity during the growing season. Ahmed, (2002) reported that heavy losses in yield may occur in maize growing under water-limited conditions and high temperatures.

Grain yield showed significant and positive correlations with the leaf area index (60 days) and number of leaves/plant (60 days) at both locations. Similar results were also observed by Nyuetta and Cross, (1997). Selection for the correlated traits like leaf area index and number of leaves/plant will simultaneously improve the potential yield and accumulate desirable genes (Guei et al., 1993).

Conclusions

It could be concluded from this study that there are convenient genetic variations in the material examined. The broad sense heritability, genotypic coefficient of variation and genetic advance were found that the selection of leaf area index and number of leaves/plant will simultaneously improve potential yield in maize. There were correlations between grain yield and leaf area index at 60 days and the number of leaves per plant at 60 days, suggesting that these characters may be important in determining yield under normal and stress conditions in the Sudan.

Acknowledgment

We gratefully acknowledge the Agronomy Department, Faculty of Agriculture, University of Khartoum and the Agricultural Research Corporation (ARC), Sudan for providing the necessary funds and research facilities required for this study.

Disclosure statement

The authors have no conflict of interest to declare.

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Table 1. Mean squares from the analysis of variance due to Treatments (T), Genotypes (G) and their Interactions (G x T) for leaf area index and number of leaves/plant characters of fifteen maize genotypes, evaluated over three water treatments at two locations (Shambat and Medani) during the 2003/04 season.

Characters		Shambat			Medani		
		T	G	G x T	T	G	G x T
		d. f = 2	d. f =14	d. f =28	d. f =2	d. f =14	d. f =28
Leaf area index	30 days	1.51^{ns}	2.12^{ns}	2.91^{ns}	4.44^{ns}	0.53^{ns}	0.54^{ns}
	45 days	0.19^{ns}	0.41*	0.28^{ns}	1.16^{ns}	0.20^{ns}	0.40**
	60 days	1.85^{ns}	0.23^{ns}	0.32_{ns}	0.98^{ns}	0.40^{ns}	0.29^{ns}
No. of leaves / plant	30 days	0.85^{ns}	3.12**	1.18^{ns}	2.11^{ns}	1.55^{ns}	1.60^{ns}
	45 days	0.53^{ns}	0.77^{ns}	0.64^{ns}	4.87^{ns}	1.32^{ns}	1.36^{ns}
	60 days	11.85^{ns}	1.49^{ns}	1.36^{ns}	1.57^{ns}	2.38**	1.48^{ns}

Note: *, ** Significant at 0.05 and 0.01 levels, respectively. ns = Non significant.

Table 2. Mean squares from combined analysis due to Locations (L), Treatments (T), Genotypes (G) and their Interactions for leaf area index and number of leaves/plant characters in fifteen maize genotypes evaluated over three water treatments at two locations (Shambat and Medani) during the 2003/04 season.

Characters		L	T	T x L	G	G x T	G x L
		d. f = 1	d. f = 2	d. f = 2	d. f = 14	d. f = 28	d. f = 14
Leaf area index	30 days	0.33^{ns}	1.25^{ns}	4.70^{ns}	1.22^{ns}	1.87^{ns}	1.43^{ns}
	45 days	13.05*	0.94^{ns}	0.42^{ns}	0.42^{ns}	0.35^{ns}	0.20^{ns}
	60 days	31.63**	2.19^{ns}	0.63^{ns}	0.32^{ns}	0.34^{ns}	0.31^{ns}
No. of leaves/plant	30 days	243.24**	1.13^{ns}	1.48^{ns}	3.79**	1.56^{ns}	0.87^{ns}
	45 days	13.16*	2.27^{ns}	3.13^{ns}	1.45^{ns}	0.88^{ns}	0.63^{ns}
	60 days	26.70*	10.76^{ns}	2.64^{ns}	1.87^{ns}	1.72^{ns}	2.00*

Note: *, ** Significant at 0.05 and 0.01 level, respectively. ns = Non significant

Table 3. Means of leaf area index for fifteen genotypes of maize evaluated under three water treatments at Shambat, Medani and over two locations during the 2003/04 season.

Serial No.	Genotypes	Shambat			Medani			Combined		
		30 days	45 days	60 days	30 days	45 days	60 days	30 days	45 days	60 days
1	G-1	2.42	3.12	2.44	2.54	3.57	3.31	2.48	3.35	2.88
2	G-2	2.68	3.10	2.54	2.53	3.73	3.08	2.60	3.42	2.81
3	G-3	4.31	2.88	2.34	2.40	3.48	3.15	3.36	3.18	2.74
4	G-4	2.56	3.28	2.46	2.32	3.50	3.01	2.44	3.39	2.73
5	V-113	2.47	2.83	2.25	2.68	3.67	2.80	2.57	3.25	2.52
6	Z-2	2.47	3.40	2.65	3.02	3.66	3.27	2.88	3.53	2.96
7	M-45	2.72	3.34	2.71	2.58	3.65	3.38	2.65	3.49	3.05
8	PR-1	2.76	2.92	2.56	2.81	3.27	3.06	2.79	3.09	2.81
9	PR-2	3.10	3.15	2.63	2.97	3.76	3.33	3.04	3.45	2.98
10	D-2	2.49	3.41	2.70	2.44	3.58	3.16	2.47	3.49	2.93
11	D-3	2.32	3.48	2.30	3.09	3.75	3.43	2.70	3.61	2.87
12	D-6	2.55	3.25	2.59	2.59	3.58	3.09	2.57	3.42	2.84
13	D-7	2.42	3.15	2.58	2.48	3.46	2.83	2.45	3.31	2.71
14	E-7	3.04	3.39	2.31	2.96	3.86	3.56	3.00	3.63	2.93
15	C-12	2.61	2.92	2.30	2.72	3.71	3.17	2.66	3.31	2.73
Mean		2.75	3.18	2.49	2.68	3.62	3.18	2.71	3.40	2.83
LSD5%		1.54	0.43	0.43	0.56	0.38	0.44	0.85	0.32	0.40
CV%		6.0	4.3	8.2	22.5	11.3	14.9	5.6	12.7	16.4

Table 4. Means of the number of leaves/plant for fifteen genotypes of maize evaluated under three water treatments at Shambat, Medani and over two locations during the 2003/04 season.

Serial No.	Genotypes	Shambat			Medani			Combined		
		30 days	45 days	60 days	30 days	45 days	60 days	30 days	45 days	60 days
1	G-1	12	13	12	10	12	11	11	13	11
2	G-2	12	13	11	10	12	10	11	12	10
3	G-3	10	12	11	9	12	10	10	12	10
4	G-4	12	13	12	9	12	10	11	13	11
5	V-113	11	13	11	10	12	10	11	12	10
6	Z-2	12	13	11	11	13	11	11	13	11
7	M-45	12	13	12	10	13	11	11	13	11
8	PR-1	13	13	12	10	13	11	11	13	11
9	PR-2	12	13	12	10	13	11	11	13	11
10	D-2	11	14	12	9	12	10	10	13	11
11	D-3	11	13	11	10	13	11	11	13	11
12	D-6	11	13	12	10	13	11	10	13	11
13	D-7	12	13	11	9	12	11	11	13	11
14	E-7	12	13	11	10	13	12	11	13	11
15	C-12	12	13	11	10	13	11	11	13	11
Mean		12	13	11	10	13	11	11	13	11
LSD5%		1.03	0.79	0.99	0.96	0.91	0.95	0.67	0.57	1.01
CV%		9.4	6.5	9.3	10.4	7.7	9.5	9.9	7.1	9.4

Table 5. Phenotypic (σ^2 ph), genotypic (σ^2 g), experimental (σ^2 e) and genotypes x treatment's interactions (σ^2 gt) variances for leaf area index and number of leaves/plant characters in fifteen maize genotypes evaluated under three water treatments at two locations (Shambat and Medani) during the 2003/04 season.

Characters		σ^2 ph		σ^2 g		σ^2 e		σ^2 g t	
		Shambat	Medani	Shambat	Medani	Shambat	Medani	Shambat	Medani
Leaf area index	30 days	2.60	-1.03	-0.09	-1.39	2.69	0.36	0.08	0.06
	45 days	0.22	0.15	0.01	-0.02	0.21	0.17	0.02	0.08
	60 days	0.20	0.23	-0.01	0.01	0.21	0.22	0.04	0.02
No. of leaves/plant	30 days	1.37	-4.86	0.15	-5.91	1.22	1.05	-0.01	0.19
	45 days	0.72	-4.53	0.01	-5.47	0.71	0.94	-0.02	0.14
	60 days	1.12	1.12	0.01	0.10	1.11	1.02	0.08	0.15

Table 6. Estimates of phenotypic and genotypic coefficient of variation, broad sense heritability (h^2B), genetic advance for leaf area index and number of leaves/plant characters measured on fifteen maize genotypes evaluated under three water treatments at two locations (Shambat and Medani), during the 2003/04 season.

Characters		PCV		GCV		h^2 B		GA	
		Shambat	Medani	Shambat	Medani	Shambat	Medani	Shambat	Medani
Leaf area index	30 days	58.6	#	#	#	54	32	#	#
	45 days	14.8	10.7	3.1	#	49	17	0.1	#
	60 days	18.0	14.1	#	3.1	9	44	#	0.1
No. of leaves/plant	30 days	10.0	#	3.3	#	61	33	0.3	#
	45 days	6.5	#	0.8	#	8	26	0.1	#
	60 days	9.4	9.9	0.9	3.0	25	57	0.1	0.2

Note: # = The value were not calculated because their variance was negative.

Table 7. Simple linear correlation coefficients between seven pairs of traits in maize using locations Shambat (above the diagonal) and Medani (below the diagonal) averaged over three water treatments in season 2003/2004.

Traits	GY	LA 1	LA 2	LA 3	LsNo 1	LsNo 2	LsNo 3
GY	1	-0.180	0.017	0.589*	0.197	0.116	0.577*
LA 1	0.363	1	-0.346	0.237	0.611*	0.390	0.496
LA 2	0.463	0.656**	1	-0.549*	0.750**	0.627*	0.508
LA 3	0.569*	0.475	0.723**	1	-0.382	0.247	0.305
LsNo 1	0.441	0.671**	0.159	0.865**	1	-0.146	0.378
LsNo 2	0.432	0.583*	0.466	0.396	0.554*	1	-0.337
LsNo 3	0.612*	0.797**	0.687**	0.401	0.549*	0.491	1

Note: *, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively. GY, grain yield; LA 1, Leaf area index at 30 days; LA 2, Leaf area index at 45 days; LA 3, Leaf area index at 60 days; LsNo 1, Number of leaves/plant at 30 days; LsNo 2, Number of leaves/plant at 45 days; LsNo 3, Number of leaves/plant at 60 days.