Performance-based grouping of adapted and exotic drought-tolerant maize (*Zea mays* L) inbred lines under stressed and non-stressed conditions

Moses A Adebayo[1,2,3], Abebe Menkir[2,*], Essie Blay[1], Vernon Gracen[1], Eric Y Danquaha[1]

[1]West Africa Centre for Crop Improvement (WACCI), University of Ghana, Legon
[2]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[3]Ladoke Akintola University of Technology, Ogbomoso, Nigeria
*Corresponding author: E-mail: amenkir@iita.org

Abstract

Knowledge of the heterotic responses of elite adapted and exotic maize inbred lines can facilitate their utilization for population improvement and hybrid development. In the present study, a line x tester mating design was used to determine the combining ability of 20 elite drought-tolerant maize inbred lines developed at CIMMYT and IITA and to classify them into heterotic groups under diverse growing conditions. The 20 lines were crossed each with two inbred line testers representing the tropical and temperate heterotic pattern established in West and Cental Africa (WCA), to generate 40 testcrosses. A trial comprising the 40 testcrosses along with the cross between the two testers and three hybrid checks were evaluated at two environments in the dry season and at six environments in the rainy season. GCA effects were more important than SCA effects in controlling grain yield in both seasons. Two exotic lines in the dry season and four exotic lines in the rainy season had significantly positive GCA effects. Only EXL22 was identified as a superior line in the two seasons. Only two adapted lines had significantly positive GCA effects in either or both seasons while three adapted lines consistently had significantly negative GCA effects in both seasons. Hybrid between EXL22 and tester 9071 showed broad adaptation to all test environments. The two testers separated some of the lines into two main heterotic groups. the lines in each heterotic group and the good combiners will be utilized for developing populations for extracting new improved inbred lines.

Keywords: heterotic grouping, maize, exotic germplasm, combining ability, tester

Introduction

Lack of adequate knowledge about the heterotic responses of elite maize inbred lines may limit their utilization for population improvement, hybrid development and impede progress in generation of new inbred lines.

Several maize inbred lines that were developed over time have been used as parents of successful commercial hybrids and synthetic varieties (Kim, 1997; Kim et al, 1999) and as repositories of favorable alleles for population improvement (Menkir et al, 2003). Inbred lines should thus be evaluated in crosses with known testers to determine the breeding values of the lines in hybrid breeding programs (Hallauer et al, 2010).

There is widespread concern among maize breeders that the genetic base of their germplasm has become too narrow to guarantee progress in the development of cultivars that can withstand increasing impact of biotic and abiotic stresses (Lu and Bernardo, 2001; Shanbao et al, 2009). This has prompted the utilization of «exotic x adapted» heterotic patterns (Li et al, 2004) in hybrid maize development and for identification of useful introduced lines for population improvement. Inbred lines of temperate origins have been utilized in crosses with tropical germplasm and new inbred lines with better adaptation and good tolerance/resistance to biotic and environmental stresses have been developed from resulting populations (Adetimirin et al, 2008). Inbred lines containing exotic germplasm may serve as sources of favorable characters that are free from deleterious recessive alleles, which have been eliminated through several generations of inbreeding and selection (Menkir et al, 2006; Shanbao et al, 2009).

Clearly defined heterotic groups of maize inbreds with diverse genetic backgrounds will help maximize exploitation of heterosis in hybrids and identify new productive inbred lines (Librando and Magulama, 2008; Kanyamasoro et al, 2012); it will also assist in identifying lines that possess novel alleles for introgression. When a large number of inbred lines that cannot be conveniently handled with a factorial mating scheme to generate hybrids are developed (Menkir et al, 2003; Hallauer et al, 2010), the breeding values and heterotic relationships of the lines can be determined by their relative performance in crosses

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with known testers (Hallauer et al, 2010). As heterosis is primarily controlled by epistatic or dominance genetic components, line x tester crosses can be used to identify new lines with good specific combining ability in a relatively smaller number of field crosses (Xia et al, 2005) than using several inbred lines in diallel and factorial crosses (Menkir et al, 2003; Hallauer et al, 2010). By keeping one half of the genetic component of a series of crosses constant, the combining ability of new lines can be assessed very rapidly and efficiently to separate the lines into different heterotic groups. Also, lines that perform well in testcrosses would nearly always produce excellent single cross hybrids (Johnson and Hayes, 1936).

Line x tester mating design (Kempthorne, 1957) has been applied to identify heterotic patterns in tropical and subtropical maize inbred lines (Vasal et al, 1992a, 1992b). This design is efficient for estimating breeding values of maize inbred lines and for determining the gene action that controls a quantitatively inherited trait (Sofi and Rather, 2006) such as drought tolerance. Several other workers have successfully used line x tester design to study the combining ability of their lines, classify them into heterotic groups, and identify useful heterotic patterns (Menkir et al, 2003; Sofi and Rather, 2006; Librando and Magulana, 2008; Kanyamasoro et al, 2012). This mating scheme can also be used to determine the heterotic patterns of introduced and adapted lines to create complementary source populations for development of new inbred lines with superior combining abilities for drought tolerance. A set of elite maize inbred lines developed at CIMMYT and IITA with appreciable levels of drought tolerance from diverse genetic backgrounds were assembled for a series of study at IITA, Ibadan in Nigeria. Before these lines can be judiciously used for germplasm improvement and hybrid breeding, their alignments or otherwise with one another must first be determined. Twenty of the lines, 10 each from CIMMYT and IITA, selected for a line x tester analysis with two inbred line testers were evaluated under stress managed conditions in the dry season and across diverse locations in the rainy season with the objectives to (i) identify those with good combining ability for grain yield, and (ii) classify the lines into heterotic groups under well-watered and drought stress in the dry season, and across the environments in the rainy season.

Materials and Methods

Germplasm

The twenty tropical lowland late-maturing maize inbred lines selected for this study (Table 1) comprised ten lines each from CIMMYT and IITA that were hereafter referred to as «exotic lines» (EXL) and «adapted lines» (ADL), respectively. These lines were a subset of an association panel of a diverse collection of 359 advanced drought tolerant maize inbred lines genotyped recently using 1,260 single nucleotide polymorphism (SNP) markers (Wen et al, 2011). All the inbred lines included in this association panel were evaluated for per se performance under controlled drought stress at Ikenne in Nigeria in 2008. More than 70 promising inbred lines with desirable agronomic characters and good levels of drought tolerance were selected for further pre-breeding program. Having achieved satisfactory level of adaptation to Nigerian growing conditions 10 exotic lines along with 10 adapted lines were selected for this study based on high levels of drought tolerance and other desirable

Table 1 - Line code, abbreviated pedigree, adaptation, and breeding center of 20 lowlands late-maturing exotic and adapted maize inbred lines along with 2 testers evaluated in testcrosses at diverse test environments in Nigeria.

No	Line code	Pedigree	Adaptation	Center
1	EXL09	Cuba/Guad C3 F85-3-3-1-B*6	Exotic	CIMMYT
2	EXL11	La Posta Seq C7-F103-2-2-2-1-B*5	Exotic	CIMMYT
3	EXL12	La Posta Seq C7-F12-2-3-1-1-B*5	Exotic	CIMMYT
4	EXL13	La Posta Seq C7-F152-1-1-2-1-B*3	Exotic	CIMMYT
5	EXL18	La Posta Seq C7-F31-2-3-1-1-B*5	Exotic	CIMMYT
6	EXL19	La Posta Seq C7-F32-2-1-1-2-B*4	Exotic	CIMMYT
7	EXL20	La Posta Seq C7-F64-1-1-1-1-B*5	Exotic	CIMMYT
8	EXL21	La Posta Seq C7-F64-2-6-2-2-B-B	Exotic	CIMMYT
9	EXL22	La Posta Seq C7-F86-3-1-1-1-B*5	Exotic	CIMMYT
10	EXL23	La Posta Seq C7-F97-3-1-1-2-B*5	Exotic	CIMMYT
11	ADL25	P43SRC9FS100-1-1-8-#1-B1-13-B1-B*7	Adapted	IITA
12	ADL30	(TZMI501xKU1414x501)-1-4-3-1-B*7	Adapted	IITA
13	ADL32	161-B-B-B-B-B	Adapted	IITA
14	ADL33	ACR-86-8-1-2-1-1-1-B-1-B*6	Adapted	IITA
15	ADL34	TZL-COMP3-C2-S2-34-4-1-2-B*6	Adapted	IITA
16	ADL35	DTPL-W-C7-S2-7-1-1-1-1-B-5-B*6	Adapted	IITA
17	ADL36	DTPL-W-C7-S2-1-2-1-1-5-B-1-B*6	Adapted	IITA
18	ADL38	Babangoyo x MO17LPA x Babangoyo-23-4-3-4-B*8	Adapted	IITA
19	ADL42	(GT-MAS:Gk x BABANGOYO x GT-MAS:Gk)-1-1-3-1-B*6	Adapted	IITA
20	ADL48	GT-MAS:gk x 9450 x GT-MAS:gk -1-1-2-3- B*9	Adapted	IITA
21	1368	Across 7721 x TZSR	Tester	IITA
22	9071	N28 x TZSR	Tester	IITA

Table 2 - Agro-ecological characteristics of the test environments in Nigeria.

Environment	Altitude (masl)	Latitude	Longitude	Annual rainfall (mm)	Agro-ecology	Agronomic Characteristic
Ikenne	60	60°87’N	30°70’E	1,421	Rainforest	High disease incidence during rain
Saminaka	730	100°40’N	80°77’E	1,200	Northern Guinea Savanna	Highly productive
Bagauda	800	120°00’N	80°22’E	1,245	Sudan Savanna	Random drought

agronomic attributes. All lines had white endosperm except ADL42 that had a yellow endosperm. The lines were carefully selected to ensure that they do not have common parentages with the two testers used. The two inbred testers, 1368 and 9071, which represent the two major heterotic patterns of tropical and temperate-derived lines, respectively (Kim, 1990; Menkir et al, 2003), are the parents of a successful commercial hybrid marketed by Premier Seed Coy in Nigeria. The twenty lines were crossed to the two testers in the growing season of 2010 to generate 40 testcrosses that were evaluated under drought stress managed conditions in the dry seasons of 2010/11 and 2011/12, and in diverse growing environments the rainy seasons of 2011 and 2012.

Evaluation under drought stress and well-watered conditions

The first trial composed of the 40 testcrosses, a hybrid between the two testers, and three hybrid checks was planted under managed drought stress conditions at Ikenne (Table 2) on November 24 in 2010 and on November 22 in 2011. Ikenne receives little rainfall from November to March of every year, making the location suitable for conducting drought stress tolerance evaluation during the dry season. The soil at this site is eutricnitrosol (FAO classification). The experimental fields are flat and reasonably uniform, with high water-holding capacity (Menkir et al, 2009). The three hybrid checks include Oba Super 1 and two synthetic drought tolerant hybrids – M1026-7 and M1026-8 – developed at IITA. Experiments were planted in two adjacent blocks that received different irrigation treatments. The first block (Block 1) received irrigation until the crop had attained physiological maturity, whereas the second block (Bock 2) received irrigation only for 28 days which was approximately three to four weeks before anthesis. The blocks were separated by four ranges, each 4.25 m wide, to restrict lateral movement of water from the fully irrigated block to the drought stress block. Irrigation water was supplied with an overhead sprinkler irrigation system that dispenses 12 mm of water per week. All field management practices were uniform for both the well-watered and water-stressed experiments. The hybrids were arranged in a 4 x 11 lattice design with three replications in each block in single 4 m rows, with 0.75 m spacing between rows and 0.50 m spacing between plants within a row. Three seeds were sown per hill and later thinned to two plants per hill after emergence to attain a population density of 53,333 plants ha^{-1}. A compound fertilizer was applied at the rates of 60 kg N, 60 kg P, and 60 kg K ha^{-1} at the time of sowing. An additional 60 kg ha^{-1} N was applied in the form of urea as top dressing four weeks later. Gramoxone and primextra were applied as pre-emergence herbicides at 5.0 l ha^{-1} each of paraquat (N,N’-dimethyl-4,4’-bipyridinuim dichloride) and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) to control weeds. Subsequently, manual weeding was done to keep the trial weed-free.

PVC access tubes were installed in December 2010 and 2011 in both well-watered and drought stress blocks to monitor soil moisture content during the growing cycle of the crop. Soil profiles were dug in each block by inserting a 2-m long PVC access tube into each soil profile using specialized installation kits and procedures contained in access tube installation guide version 1.0 (Sentek Sensor Technologies, 2003).

Soil moisture content measurements commenced at Ikenne 35 days after planting (DAP) in each year using a portable soil moisture monitoring system known as Diviner 2000 which had a display unit and a portable 1.6 m long probe with a Diviner Cable. With the 1.6 m probe soil moisture readings were taken at regular intervals of 10 cm down through the soil profile. Soil moisture content scores were taken through the wall of a PVC access tube by remote sensing following the procedures described in Diviner 2000 Portable Soil Moisture Monitoring Solution User Guide Version 1.5 (Sentek Pty Ltd). Data were recorded first on weekly basis and later on daily basis when the impact of water stress became very critical in each year. Data were downloaded from the Diviner 2000 display unit on a desktop computer

In each plot under drought stress and full irrigation days to 50% anthesis (DTA) and days to 50% silking (DTS) were recorded as the number of days from planting to when 50% of plants in a plot had shed pollen, and had emerged silks, respectively. Anthesis-silking interval (ASI) was computed as the difference between days to 50% silking and 50% anthesis. Leaf death (LFDTH) score which is an indication of leaf senescence was rated in drought stress block at 10 weeks after planting (WAP) on a scale of 1 to 9, where 1 = almost all leaves are green and 9 = virtually all leaves are dead. At the time of harvest, ear aspect (EASP) was visually rated on a scale of 1 to 5, where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small, and partially filled ears. The total number of plants and ears were counted in each. A cob was counted if it had at least one kernel set. The number of ears per plant (EPP) was then computed as the proportion of the total number of ears at harvest divided by the total number of plants harvested. All ears harvested from each plot were weighed and shelled to determine grain weight and a representative sample was taken to determine percent moisture. Grain yield (GY), measured in kg ha^{-1} adjusted to 15% moisture content was calculated from grain weight and percent moisture.

Evaluation during rainy seasons

A second trial composed of the 41 hybrids and the hybrid checks used during the dry seasons were evaluated at Saminaka, Ikenne, and Bagauda (Table 2) during the rainy seasons of 2011 and 2012. The hybrids were arranged in 4 x 11 alpha lattice design and were planted in single row plots, 5 m long with spacing of 0.75 m between rows and 0.50 m between plants in a row. At each test environment, 60 kg ha^{-1} N, 60 kg ha^{-1} P, and 60 kg ha^{-1} K were applied at planting, and another 60 kg ha^{-1} N was applied four weeks after as top dressing. Standard cultural practices were applied to keep the trial weed-free.

At each test environment, DTS, DTA, ASI, PLHT, EASP, and EPP were recorded in each plot as earlier described. All ears harvested from each plot were shelled to determine percent moisture. GY adjusted to 15% was then computed using grain weight and percent moisture.

Statistical Analyses

Each location-year under drought stress and well-watered conditions as well as in the rainy season was considered a test environment. Analyses of variance (ANOVA) were computed for each location-year combination within each growing condition (drought stress, well-watered, rainy season) to entry means adjusted for block effects according the lattice design (Cochran and Cox, 1960; Menkir et al, 2003). The pooled error mean square was calculated by dividing the sum of the error sums of square by the corresponding sum of the error degrees of freedom for each test environment. Combined analysis of variance was then computed across years using the adjusted means. In the combined analysis, location-year combinations were treated as random effects while testcrosses were considered as fixed effects. All analyses were carried out with PROC GLM in SAS (SAS Institute, 2009) using a RANDOM statement with TEST option. Line x tester analysis was done to partition the genotype source of variation into that due to parental line and tester general combining ability (GCA) effects as well as that due to specific combining ability (SCA) effects from the adjusted means using the method described by Kempthorne (1957). The mean squares for GCA and SCA effects were tested for significance using their interactions with environment as error terms. The mean squares due to GCA x environment and SCA x environment effects were tested using the line x tester x environment mean square as error term while the mean square due to environment x line x tester was tested using the pooled error mean square. The estimates of GCA and SCA effects for grain yield which is main trait of interest were computed using a line x tester model. SPEARMAN’S correlation coefficient was calculated between pairs of combining ability effects for grain yield estimated in different growing conditions.

Results

Results of the soil moisture content monitored with Diviner 2000 during the flowering periods of the maize crop under both well-watered and drought stress conditions in 2010/11 and 2011/12 dry seasons at Ikenne were reported in Adebayo et al (2013). As expected, the soil moisture content at every 10 cm depth down the soil profile in fully irrigated block was always higher than that of drought stress blocks. The volumetric water content assumes downward trend at every data point in the drought stress block while it fluctuates in the well-watered block because of weekly irrigation.

Trait means of testcrosses of exotic and adapted drought tolerant inbred lines and two testers evaluated under well-watered and drought stress conditions

Table 3 - Means (±SE) of grain yield and other traits averaged over two years for 40 testcrosses of adapted and exotic maize inbred lines crossed to two testers and evaluated under well-watered and drought stress environments at Ikenne, Nigeria.

Traits	Well-watered environment				Drought stress environment			
	1368		9071		1368		9071	
	adapted	exotic	adapted	exotic	adapted	exotic	adapted	exotic
Grain yield (kg ha^{-1})	4777±482	5807±179	5542±382	6829±260	1201±148	1784±114	1441±138	2188±153
Days to anthesis	57±0.6	56±0.5	58±0.4	57±0.5	61±0.7	60±0.5	60±0.5	60±0.5
Days to silking	59±0.6	57±0.3	59±0.3	58±0.4	65±0.8	62±0.6	65±0.5	63±0.6
Anthesis-silking-interval (days)	1.4±0.1	0.8±0.2	1.7±0.2	1.1±0.3	4.3±0.3	2.8±0.2	4.8±0.4	3.5±0.3
Plant height (cm)	197±4.1	202±3.0	209±3.2	212±3.4	128±5.1	135±3.2	138±2.9	145±3.1
Ear aspect (1-5)	3.1±0.2	2.8±0.1	3.1±0.1	2.9±0.1	3.4±0.2	3.0±0.1	3.2±0.1	2.9±0.1
Ear per plant number	0.9±0.02	1.0±0.02	0.9±0.02	1.0±0.02	0.6±0.03	0.7±0.02	0.7±0.02	0.7±0.02
Leaf death score(1-9)	-	-	-	-	7.3±0.2	6.9±0.2	6.7±0.1	6.7±0.2

Table 4 - Means (±SE) of grain yield and other traits averaged over two years for 40 testcrosses of adapted and exotic maize inbred lines crossed to two testers and evaluated in 3 test environments in Nigeria.

Traits	Rainy season			
	1368		9071	
	adapted	exotic	adapted	exotic
Grain yield (kg ha^{-1})	3409 ± 147	4196 ± 146	4146 ± 173	4712 ± 192
Days to anthesis	61 ± 0.3	60 ± 0.3	61 ± 0.4	60 ± 0.3
Days to silking	62 ± 0.4	60 ± 0.4	62 ± 0.4	610 ± 0.4
Anthesis-silking-interval (days)	1.5 ± 0.5	1.2 ± 0.1	2.2 ± 0.1	1.7 ± 0.1
Plant height (cm)	178 ± 2.1	184.5 ± 2.2	188 ± 2.3	189 ± 2.2
Ear aspect (1-5)	3.3 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
Ear per plant number	0.9 ± 0.01	1.0 ± 0.01	0.9 ± 0.01	0.9 ± 0.01

and in three diverse growing conditions are presented in Tables 3 and 4. Testcrosses of exotic lines involving 9071 as a tester produced the highest mean grain yield under well-watered conditions and testcrosses of adapted lines involving 1368 as a tester produced the lowest grain yield under drought stress conditions (Table 3). Testcrosses involving exotic lines had better scores for ear aspect, number of ears per plant, antheisi-silking-interval, days to anthesis and silking than those involving adapted lines under both irrigation treatments, and also for leaf death score under drought stress in dry season (Table 3).

Dry season

In the analysis of variance combined across years and irrigation treatments, environment (E) and genotype (G) effects significantly affected all the measured traits (Table 5). Significant differences were also detected among the two water regimes (Wat_Reg) and G x Wat_Reg for almost all the traits. Testcross trait means averaged over two years under well-watered and drought stress conditions showed that drought stress reduced grain yield by 71%, plant height by 33%, and number of ears per plant by 30% but increased days to anthesis by 5%, days to silk by 10%, and anthesis-silking-interval by 200% (data not shown). G x E interaction was significant for grain yield and plant height only. The variation among lines (GCA) was highly significant for all measured traits while significant differences were observed between the testers for plant height only. Line x tester interaction (SCA) was highly significant for days to anthesis, days to silking, and ear aspect. Mean squares for GCA$_{line}$ x E, GCA$_{tester}$ x E, and SCA x E were all significant for grain yield and few other traits (Table 5).

Testcross means, estimates of GCA and SCA effects of the 20 DT lines for grain yield, and the heterotic classes under each irrigation regime are presented in Table 6. Under full irrigation, the GCA effect of 447 kg ha^{-1} and 161 kg ha^{-1} for tester 9071 under well-watered and drought stress, respectively, were significantly different from zero (Table 6). On the average, testcrosses involving 9071 as a tester out-yielded those involving 1368 as a tester by 17% under well-watered conditions and by 20% under drought stress. The number of testcrosses involving 1368 and 9071 having mean grain yields that exceeded the mean yield of the hybrid involving the two testers (1368 x 9071) by at least 10% are five and ten, respectively, under well-watered condition. Under drought, 11 testcrosses of 1368 and 15 of 9071 produced mean yields that are 10% higher than the mean yield of 1368 x 9071 (Table 6). Among the 12 lines that had positive GCA effects under well-watered condition,

Table 5 - Mean squares of traits from the combined analysis of variance for 10 adapted and 10 exotic maize inbred lines evaluated in testcrosses with 2 testers across well-watered and drought stress conditions in the dry seasons of 2010 and 2011 at Ikenne in Nigeria.

Source of variation	Df	§GY (kg ha^{-1})	DTA (days)	DTS (days)	ASI (days)	PLHT (cm)	EASP (1-5)	EPP (no)	†LFDTH (1-9)
Environment (E)	3	745878304***	35.5***	1506.5***	318.9***	224649***	4.8**	3.1***	239.1*
‡Water_Reg	1	2212391584***	1301.6**	4421.9***	925.4***	611593***	1.4	9.4***	-
Genotype (G)	43	8452641***	30.9***	38.9***	8.7***	767.0***	1.1***	0.1**	2.0***
G x E	129	1671477***	2.7	4.7	2.3	272.7*	2.3	0.02	0.9
G x Wat_Reg	43	2534127***	2.8	8.3**	3.8**	355.4	0.4**	0.03*	-
GCA$_{line}$	19	14577067***	58.9***	71.0***	14.5***	1850.8***	1.9***	105**	3.1**
GCA$_{tester}$	1	38477215	0.5	21.8	15.7	10585*	0.3	0.02	5.7
SCA	19	1869629	5.4**	8.6**	2.3	300.3	0.4**	0.04	0.6
GCA$_{line}$ x E	19	2070279**	2.3	5.7*	3.3**	317.6	0.3*	0.03	0.9
GCA$_{tester}$ x E	1	3937657*	6.6*	4.3	1.6	380.9	0.8*	0.1*	1.9
SCA x E	19	1072819**	2.4	3.3	1.5	206.9	0.2	0.02	0.6

*,**,*** Data significant at $p < 0.0001$, 0.01, and 0.05, respectively. §GY= Grain yield, DTA = Days to 50% anthesis, DTS = Days to 50% silking, ASI = Anthesis-silking interval, PLHT = Plant height, EASP = Ear aspect where 1=clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears, EPP = Number of ears per plant calculated as ratio of plants harvested to ears harvested, †LFDTH = Leaf death score measured in drought stress blocks in the two seasons only, where 1 = almost all leaves are green and 9 = virtually all leaves are dead, ‡Wat_Reg = Water or irrigation regimes which are well-watered environment and drought stress environment.

Table 6 - Mean grain yield, general combining ability (GCA) and specific combining ability (SCA) effects, and heterotic group of 10 adapted and 10 exotic DT lines evaluated in testcrosses with two testers under well-watered (WW) and drought stress (DS) conditions in dry seasons of 2010 and 2011 at Ikenne in Nigeria.

Inbred	Grain yield (kg ha^{-1}) under				GCA effects (kg ha^{-1})		SCA effects (kg ha^{-1}) under				Heterotic Group	
	Full irrigation (WW)		Drought stress (DS)				Full irrigation (WW)		Drought stress (DS)			
	1368	9071	1368	9071	WW	DS	1368	9071	1368	9071	WW	DS
ADL25	6725	7439	1732	1930	1343*	177	90	-90	62	-62	1	1
ADL30	6176	7441	1648	1592	1070*	-34	-185	185	189	-189	2	1
ADL32	5738	5006	1591	958	-367	-379	813	-813	478	-478	1	1
ADL33	4345	5679	702	1763	-727*	-421	-220	220	-370	370	2	2
ADL34	2462	4738	633	1002	-2139*	-836*	-691	691	-23	23	0	2
ADL35	2818	3862	801	1135	-2398*	-686*	-75	75	-6	6	0	2
ADL36	3061	5113	679	915	-1652*	-857*	-579	579	43	-43	0	1
ADL38	5744	4264	1761	2164	-735*	309	1187	-1187	-41	41	1	2
ADL42	5754	6017	1348	1355	147	-303	316	-316	157	-157	1	1
ADL48	4948	5859	1117	1595	-335	-298	-9	9	-78	78	2	2
EXL09	4865	5574	1993	2247	-519	466*	92	-92	34	-34	1	1
EXL11	6161	6638	1903	2634	661	615*	208	-208	-205	205	1	2
EXL12	5856	7173	1426	1465	776*	-208	-212	212	142	-142	2	1
EXL13	5840	6846	2098	2582	604	687*	-56	56	-81	81	2	2
EXL18	5730	7579	1878	2365	916*	468*	-478	478	-83	83	2	2
EXL19	5838	7056	1352	1654	708*	-151	-162	162	10	-10	2	1
EXL20	5337	6198	1758	1826	29	138	16	-16	127	-127	1	1
EXL21	5230	7152	1360	2118	453	86	-514	514	-218	218	2	2
EXL22	6744	8292	2476	3040	1780*	1104*	-327	327	-121	121	2	2
EXL23	6465	5784	1600	1953	385	123	787	-787	-16	16	1	2
1368	0	5556	0	1307	-447	-161						
9071	5556	0	1307	0	447	161						
Mean	5292	6186	1493	1790	0	0	0	0	0	0		
SE	96	96	59	59	351	216	269	269	187	187		

*GCA effects significantly different from zero, standard error (SE) for tester GCA under WW and DS are 0.81 and 0.50 kg ha^{-1}, respectively; LSD(0.05) estimated in kg ha^{-1}

only two adapted (ADL25 and ADL30) and four exotic (EXL12, EXL18, EXL19, and EXL22) lines had positive and significant GCA effects. Under drought stress, out of 10 lines with positive GCA effects, five exotic lines (EXL09, EXL11, EXL13, EXL18, and EXL22) had significantly positive GCA effects. Three adapted lines (ADL34, ADL35, and ADL36) under both irrigation treatments, and one other adapted line (ADL38) under well-watered condition, had significantly negative GCA effects (Table 6). Although the SCA effect was not significant for grain yield (Table 5), five testcrosses (ADl32 x 1368, ADL38 x 1368, EXL23 x 1368, ADL34 X 9071, and ADL36 x 9071) had significantly positive SCA effects under well-watered (Table 6).

The procedures described earlier (Menkir et al, 2003; Menkir et al, 2004; Librando and Magulama, 2008) with some modifications were used to classify the adapted and exotic lines into groups. Inbred lines whose testcross mean grain yields were significantly lower than the mean grain yield of the hybrid between the two testers under an irrigation treatment were placed in Group 0 (Table 6). Inbred lines showing positive SCA effects with tester 1368 but having negative SCA effects with 9071 and with similar or significantly higher testcross mean yields in comparison to the mean grain yield of the hybrid between the two testers were included in heterotic Group 1 under each irrigation treatment (Table 6). Also, the inbred lines showing negative SCA effects with 1368 but having positive SCA effects with 9071 and with similar or significantly higher mean grain yields in comparison to the mean grain yield of the cross between the two testers were placed in heterotic Group 2 under each irrigation treatment (Table 6). Using this scheme, three adapted lines were included in Group 0 under full irrigation. Four each of adapted and exotic lines were included in Group 1 whereas three adapted and six exotic lines were included in Group 2 under full irrigation. Five adapted lines were not grouped under full irrigation. Under drought stress conditions, five adapted and four exotic lines were included in Group 1 whereas five adapted and six exotic lines were included in Group 2. Five adapted (ADL25, ADL32, ADL33, ADL42, and ADL48) and six exotic (EXL09, EXL13, EXL18, EXL20, EXL21, and EXL22) lines were classified in the same group under the two irrigation treatments.

Rainy season

In the combined analysis of variance, environment, genotype, and G x E interaction were significant sources of variation for all measured traits (Table 7). The variation among the DT lines in testcrosses (GCA) was also highly significant for all traits whereas the two testers (GCA) in crosses with the DT lines differed significantly only for antheis-silking-interval and plant height. The line x tester interaction (SCA) was highly significant for all traits except anthesis-silking-interval and number of ears per plant. The mean squares for line x environment and tester x environment interactions were significant for grain yield and most other traits whereas the mean squares for line x tester x environment interaction was significant only for anthesis-silking-interval (Table 7).

Testcross means, estimates of GCA and SCA effects for grain yield, and the heterotic classes across the three environments are presented in Table 8.

Table 7 - Mean squares of traits from the combined analysis of variance for 10 adapted and 10 exotic DT inbred lines evaluated in testcrosses with 2 testers across 3 test environments in the growing seasons of 2011 and 2012 in Nigeria.

Source of variation	Df	§GY (kg ha^{-1})	DTA (days)	DTS (days)	ASI (days)	PLHT (cm)	EASP (1-5)	EPP (no)
Environment (E)	5	477541587***	2003.2***	2565.6***	60.7**	72952***	11.4**	0.7**
Genotype (G)	43	8967563***	42.1***	41.5***	5.0***	1524.0***	1.2***	0.04***
G x E	215	1616245***	3.3**	4.0***	1.2***	225.6**	0.3***	0.02*
GCAline	19	12626845***	75.3***	74.2***	5.1***	1944.3***	1.1**	0.1***
GCAtester	1	59845544	12.3	35.1	61.9*	7494.5**	0.001	0.01
SCA	19	2930498***	13.7***	12.0***	0.8	448.6**	1.4***	0.03
GCAline x E	95	1976782***	3.3	4.4*	1.3**	284.6**	0.4**	0.02
GCAtester x E	5	10365399***	9.4**	9.4**	5.7***	137.3	0.9**	0.02
SCA x E	95	831430	2.9	2.8	0.7*	167.1	0.2	0.02

*,**,*** Data significant at $p < 0.0001$, 0.01, and 0.05, respectively. §GY= Grain yield, DTA = Days to 50% anthesis, DTS = Days to 50% silking, ASI = Anthesis-silking interval, PLHT = Plant height, EASP = Ear aspect where 1=clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears, EPP = Number of ears per plant calculated as ratio of plants harvested to ears harvested.

Mean grain yields of testcrosses in this trial ranged between 1,963 and 5,323 kg ha^{-1}. The severe droughts at Bagauda during the two rainy seasons under consideration and the excessive rainfall at Saminaka in 2012 were suspected to be responsible for the relatively low mean grain yields for testcrosses recorded in the rainy season. Mean grain yield of testcrosses and checks at Saminaka in 2012 (2,854 kg ha^{-1}) was just 36% of the mean yield in 2011 (7,911 kg ha^{-1}). Mean yields of 10 hybrids of 1368 and 13 of 9071 exceeded the mean yield of the hybrid between the two testers (1368 x 9071) by at least 10%. Among the 12 lines that had positive GCA estimates for grain yield, only one adapted (ADL30) and four exotic (EXL11, EXL19, EXL22, EXL23) lines had significant GCA effects (Table 8). Three adapted (ADL34, ADL35, and EDL36) and one exotic (EXL09) lines had significantly negative GCA effects for grain yield. Although the mean squares for SCA was significant for grain yield, only three testcrosses (ADL32 x 1368, ADL33 x 9071, and ADL35 x 9071) showed significantly positive SCA estimates (Table 8).

In classifying the 20 lines into heterotic groups, the estimates of the combining ability effects and the mean grain yields of the lines in testcrosses with the two testers were considered. 11 lines showing positive SCA effects with 1368 and negative SCA effect with 9071 and whose mean grain yields were similar to or significantly higher than the mean yield of 1368 x 9071 were placed in group 1. Six lines having positive GCA effect with 9071 and negative GCA effect with 1368 and whose mean grain yields were similar to or significantly higher than the mean yield of 1368 x 9071 were placed in group 2. Three lines that had mean grain yield that was lower than the mean yield of 1368 x 9071 were not grouped.

Performance of DT lines across diverse growing environments

Although the line x environment, tester x environment, and line x tester x environment interactions were mostly significant for grain yield in the trials (Tables 5 and 7), some lines still showed consistency in their heterotic groups. In the trials for dry and rainy seasons, four adapted (ADL25, ADL32, ADL42, and ADL48) and one exotic (EXL11) lines were in group 1 whereas two adapted (ADL30 and ADL33) and one exotic (EXL22) were in group 2. In both trials, three lines (ADL34, ADL35, and EXL09) could not be grouped (Tables 6 and 8).

Discussion

Maize breeders classify inbred lines into heterotic groups based on testcross performance with testers of known genetic backgrounds and heterotic relationships (Vasal et al, 1992a, 1992b; Menkir et al, 2004; Librando and Magulama, 2008). The drought tolerant maize inbred lines developed at CIMMYT and IITA were classified into heterotic groups using the two inbred testers, 1368 and 9071. Menkir et al (2003) had earlier used the same inbred testers to classify 34 lowland white inbred lines, and reported satisfactory results. These testers represent the heterotic patterns of tropical and temperate-bred inbred lines mostly used in West and Central Africa (WCA) for determining the heterotic response of new inbred lines so that those with good combining ability can be identified (Menkir et al, 2003). Although the two testers were not specifically bred for drought tolerance, most of the hybrids involving the two testers expressed significant differences in grain yield and other agronomic traits. Most of the hybrids that out-yielded the cross between the two testers were those involving 9071 as a tester, indicating that 9071 possess more complementary alleles to the lines used in this study than 1368. This finding was contrary to that reported by Menkir et al (2003) for a different set of lowland white inbred lines.

The consistently lower volumetric soil moisture content in the drought stress block compared to the well-watered block in each year's dry season experiment, the significant differences detected between the two irrigation (or water) regimes over the two years, the trial mean grain yield reduction of over 70% revealed that severe drought stress which elicited differential reactions from the tested drought tolerant maize inbred lines was simulated in the dry season. Testcrosses of exotic lines with each tester were more productive than those of adapted lines in trials

Table 3 - Mean grain yield, general combining ability (GCA) and specific combining ability (SCA) effects, and heterotic group of 10 adapted and 10 exotic DT lines evaluated in testcrosses with two inbred testers at three locations in rainy seasons of 2011 and 2012 in Nigeria.

Inbred	Mean grain yield (kg ha^{-1}) with		GCA effects (kg ha^{-1})	SCA effects (kg ha^{-1})		Heterotic Group
	1368	9071		1368	9071	
ADL25	3867	4375	-68	49	-49	1
ADL30	4382	5323	730*	-55	55	2
ADL32	4300	3871	93	499	-499	1
ADL33	3118	5075	-5	-719	719	2
ADL34	1963	3378	-1435*	-333	333	0
ADL35	2042	3610	-1167*	-459	459	0
ADL36	2575	4017	-936*	-418	418	2
ADL38	3853	3494	-490	446	-446	1
ADL42	3904	3843	-240	333	-333	1
ADL48	4082	4474	255	57	-57	1
EXL09	3374	3944	-568*	24	-24	0
EXL11	4367	4700	532*	83	-83	1
EXL12	4179	4334	94	161	-161	1
EXL13	4149	4690	370	7	-7	1
EXL18	4562	4624	434	250	-250	1
EXL19	4282	5124	552*	-80	80	2
EXL20	4038	4978	278	-23	23	2
EXL21	3877	4376	166	56	-56	1
EXL22	4366	5132	576*	-32	32	2
EXL23	4764	5220	830*	154	-154	1
1368		3710	-312			
9071	3710		312			
Mean	4115	4115	0	0	0	
SE	80	80	257	229	229	

*GCA effects significantly different from zero, standard error (SE) for tester GCA is 58 kg ha^{-1}, respectively.

under the two irrigation treatments suggesting that the exotic lines possess favorable and unique alleles that can be used to improve the adapted germplasm for grain yield and drought tolerance. Other authors have earlier reported the benefits of using CIMMYT-bred germplasm for population improvement at IITA, and vice versa (Dhiliwayo et al 2009; Adebayo et al, 2013). The adapted and exotic lines having significant additive genetic effects for grain yield under each irrigation treatment would be exploited for population improvement in order to develop new inbred lines with higher levels of drought tolerance and productivity. The two exotic lines, namely EXL18 and EXL22 possessing superior general combining ability effects under both irrigation regimes were originated from the La Posta Sequia population that has undergone several cycles of recurrent selection for drought tolerance improvement (Guei and Wassom, 1996).

The different agro-ecological conditions that are peculiar to test environments that were used for the rainy season trials coupled with the excessive rainfall at Saminaka in 2012 that introduced more heterogeneity among the three environments accounted for the significant genotype x environment, line x environment, and tester x environment interactions which suggest that the testcrosses, lines, and testers are inconsistent in their performance across the environments. Less than 50% of lines tested had good or poor general combining ability under drought stress condition in the dry season and in the rainy season. Only inbred line EXL22 had good general combining ability in the rainy season as well as under well-watered and drought stress conditions. Across all environments (well-watered, drought stress, and rainy season), the cross between EXL22 and 9071 consistently produced the highest mean grain yield and had positive though non-significant SCA estimates. Hence, testcross hybrid EXL22 x 9071 most probably possesses broad adaptation to the diverse growing conditions used in this study. Testcross mean yields of ADL34, ADL35, and ADL36, particularly with tester 1368, were consistently the poorest in all the environments and therefore, the lines could not be grouped by the two testers. These three lines that were developed from some tropical populations most probably shared the same genetic backgrounds with one another and also with 1368.

Our results show that the two inbred testers fairly classified the set of adapted and exotic lines evaluated in this study into two main heterotic groups that can be exploited further for the creation of complementary reciprocal populations for developing more productive drought tolerant inbred lines.

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