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Genetic distance among doubled haploid maize lines and their testcross performance under drought stress and non-stress conditions

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Abstract In contrast to conventional inbreeding that takes up to seven generations to develop inbred lines, the doubled haploid (DH) technology allows production of inbred lines in two generations. The objectives of the present study were to: (a) evaluate testcross performance of 45 doubled haploid lines under drought stress and non-stress conditions (b) estimate heritabilities for grain yield and other traits and (c) to assess the genetic distance and relationship among the DH lines using 163,080 SNPs generated using genotyping-by-sequencing (GBS). The 45 hybrid and five checks were evaluated using a 10 × 5 alpha lattice in six drought stress and nine well-watered environments in Kenya, Uganda, and Tanzania. Differences in trait means between the drought stress and well-watered conditions were significant for all measured traits except for anthesis date. Genetic variances for grain yield, grain moisture, plant height and ear height were high under well-watered environments while genetic variance for anthesis date, root lodging and stalk lodging were high under drought stress environments. Combined analyses across drought stress and well-watered environments showed that ten top hybrids produced 1.6–2.2 t/ha grain yield under well-watered condition and 1–1.4 t/ha under drought stress condition higher than the mean of the commercial checks. Genetic distance between pairwise comparisons of the 38 of the 45 DH lines ranged from 0.07 to 0.48, and the overall average distance was 0.36. Both cluster and principal coordinate analysis using the genetic distance matrix calculated from 163,080 SNPs showed two major groups and the patterns of group was in agreement with their pedigree. Thirteen (13) of the best hybrids are currently in National Performance Trials testing, an important step towards commercialization in Kenya, Tanzania and Uganda.

Keywords Doubled haploid · Genetic distance · Drought stress · Maize

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Introduction

Maize is among the most important food crops in the world and together with rice and wheat, provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al. 2011). Compared to other regions, in sub-Saharan Africa (SSA) countries maize yield variability is extremely high, though maize is the most important staple food for over 300 million people. For example, between 2005 and 2008 the average maize yield was estimated at 1.4 tons per hectare (t/ha) compared to 2.5 t/ha in the Philippines, 3.1 t/ha in Mexico, and 3.9 t/ha in Thailand (Smale et al. 2011). Although several factors could contribute for this low production, drought has been cited as one of the major factors that frequently limit maize production in the SSA region. Heisey and Edmeades (1999) estimated that 20–25 % of the global maize production area is affected by drought in any given year. Crop yields are more sensitive to changes in climatic conditions and yields are impaired by relatively small changes in growing season temperatures (Lloyd et al. 2011; Lobell and Field 2007).

The performance and genetic variability in maize for grain yield and other traits under water stress and non-stress conditions have been investigated by various workers (Bänziger et al. 1997; Campos et al. 2004; Duvick et al. 2004; Duvick 1997; Duvick and Cassman 1999; Edmeades et al. 1999, 2006; Lafitte and Edmeades 1994; Tollenaar and Wu 1999). These studies reported that during drought maize produced significantly lower grain yield, increase anthesis-silking-interval, fewer ears per plant, and lower grain moisture percentage than the non-stressed site. Maize varieties developed to be drought tolerant have also shown good level of tolerance to low nitrogen (N) conditions, suggesting the presence of common adaptive mechanisms for both water and low N stresses (Bänziger et al. 1999; Lafitte and Bänziger 1997). Weber et al. (2012) have reported high genetic correlations between drought tolerant and low N tolerance (0.73–0.79 between managed drought and low N, and 0.95–0.98 between random drought and low N), respectively.

Through the Water Efficient Maize for Africa (WEMA) project, the International Maize and Wheat Improvement Center (CIMMYT) has developed DH lines from several drought tolerant source populations (Beyene et al. 2011). Progress on in vivo haploid induction technology has made it possible to produce large numbers of maternal haploid plants. It is estimated that time to develop new cultivars may be reduced by 50 % when DH technology is used for fixation of traits following F1 crosses compared to classical pedigree breeding (Forster and Thomas 2004).Various studies have been conducted to compare the performance of DH hybrids with other conventional breeding methods. In some studies (Bordes et al. 2007; Seitz 2005), the performance of the DH lines was found to be similar to those produced by single seed descent (SSD) and pedigree methods. Another study (Wilde et al. 2010) reported that the mean testcross performance of the DH lines developed from three European landraces yielded 22–26 % less than the corresponding elite lines. Beyene et al. (2011) evaluated DH hybrids derived from tropical adapted backcross populations and reported some DH hybrids produced more grain yield than the commercial checks developed through pedigree breeding. The earlier study (Beyene et al. 2011) provided an insight on the usefulness of DH lines derived from tropical germplasm but there was little information on the testcross performance of the DH lines under drought stress and non-stress conditions.

In maize, the two main tasks of breeders include developing improved inbred lines and identifying the best parental combinations for creating hybrids that are phenotypically superior with significantly higher yield compared to their parents (Duvick 2001). Depending on the objectives of the breeding program, breeders use different methods in selecting the best parents for making crosses, including: (a) pedigree relationships (b) phenotypic performance for specific traits (c) adaptability and yield stability (d) top crosses (e) diallel crosses and (f) genetic distances estimated from morphological and molecular markers (Bertan et al. 2007). Genetic distance can be estimated from various types of molecular markers but single nucleotide polymorphisms (SNPs) have emerged as a powerful tool for many genetic applications. Elshire et al. (2011) of the Ed Buckler group at Cornell University has recently published a protocol for genotyping-by-sequencing (GBS) that is suitable for a wide range of purposes by generating over half a million SNPs at a relatively low per sample cost (http://www.maizegenetics.net/gbs-overview).

The objectives of the present study were to: (a) evaluate testcross performance of 45 doubled haploid lines under water stressed and non-stress conditions (b) estimate heritabilities for grain yield and other traits and (c) assess the genetic distances and genetic relationship among the DH lines using GBS.

Materials and methods

Germplasm selection

The DH lines were derived from BC_1F_1 of eight tropical maize backcross populations (Table 1) by means of in vivo haploid induction at the Monsanto facility in Mexico. The eight source populations were obtained by crossing four drought tolerant (DT) donor lines with four recurrent parents (CML312SR, CML395, CML444 and CML488). Three of the DT donor lines were extracted from La Posta Seq C7, a drought-tolerant population developed at CIMMYT Mexico through recurrent selection among full sib/S1 families (Edmeades et al. 1999). The fourth donor parent was developed from M37W, a temperate high yielding line. The recurrent parents are drought tolerant lines, have good combining abilities and are adapted across several environments in SSA (Magorokosho et al. 2008). Two hundred and fifty BC_1F_1 seeds from each of the eight populations were submitted for DH induction. After in vivo induction, treatment with colchicines and selfing, a total of 806 DH lines were received from Monsanto. The DH lines were grown at Kiboko, Kenya Agricultural Research Institute, during the 2009/2010 short rains season. Based on the results of *per se* evaluation (germination and good stand establishment, plant type, low ear placement, and well-filled ears) and stage I multi-location yield trials, the best 45 DH lines were selected for advanced yield trials (Table 1).

Testcross evaluations under drought stress and well-watered conditions

The 45 DH lines were crossed with a single cross tester from opposite heterotic group. The 45 DH hybrids along with three commercial checks and two local checks were evaluated in nine well-watered and six drought stressed locations in Kenya, Uganda and Tanzania in 2011/2012 (Table 2). The experimental design was a 10 × 5 alpha lattice design with three replications per location. The managed drought stress trials were conducted during the dry season by withdrawing irrigation 2 weeks before flowering and no irrigation applied until harvest. In the well-watered experiments, supplemental irrigation was given as required to avoid moisture stress. At all locations, each entry was planted in two-row plots of 5 m long rows spaced at 0.75 m between rows and 0.25 or 0.30 m between hills. Two seeds per hill were initially planted then thinned to one plant per hill 3 weeks after emergence to get a plant population density of 53,333 plants per hectare in Kenya and Tanzania and 44,444 in Uganda. Fertilizers were applied at the rate of 60 kg N and 60 kg P_2O_5 per ha as recommended for the area. Nitrogen was given in two applications: at planting and 6 weeks after emergency. The fields were kept free of weeds by hand weeding.

For each plot, the following traits were recorded; days to silking, as the number of days from planting to when 50 % of the plants had emerged silks, and days to anthesis, when 50 % had shed pollen. The anthesis-silking interval was computed as the difference between days to silking and anthesis. Plant height was measured as the distance from the base of the plant to the height of the first tassel branch and ear height as the distance from the base to the node bearing the upper ear. Root lodging (percentage of plants leaning more than 30° from the vertical) and stalk lodging (percentage broken at or below the highest ear node) were also recorded. In drought stress condition, ears were harvested from each plot and shelled to determine the percentage grain moisture. In the well-watered experiments harvested ears of each plot were weighed and the grain yield was estimated based on (800 g grain/kg ear weight) and adjusted to 125 g/kg moisture content.

DNA extraction and genotyping

DNA for 38 of the 45 DH lines was extracted, quantified and normalized as described by Semagn et al. (2012). The DNA samples were shipped to the Institute of Genomic Diversity, Cornell University, and they were genotyped with genotyping-by-sequencing as described elsewhere (Elshire et al. 2011).

Data analysis

Analysis of variance for all recorded traits was done separately for each location, and combined across locations using PROC MIXED procedure from SAS

Table 1 List of sub-tropical maize backcross source populations, pedigree, characteristics and number of DH lines used in study

Population code	Pedigree	Characteristic	Number of DH lines phenotyped	Number of DH lines genotyped	Heterotic group	Tester used
1	La Posta Seq C7-F96-1-2-1-1-B–B–B/CML395//CML395	Drought tolerant donor lines crossed with an elite line adapted to tropical climate, resistance to maize streak virus and widely used tester in CIMMYT	1	1	B	CML312 x CML442
2	La Posta Seq C7-F96-1-2-1-1-B–B–B/CML444//CML444	Drought tolerant donor lines crossed with an elite line tolerant to low nitrogen and widely used tester in CIMMYT	5	2	B	CML312 x CML442
3	La Posta Seq C7-F96-1-2-1-1-B–B–B/CML488//CML488	Drought tolerant donor lines crossed with an elite line tolerant to low nitrogen	1	1	AB	CML312 x CML442
4	La Posta Seq C7-F71-1-2-1-2-B–B–B/CML312SR//CML312SR	Drought tolerant donor lines crossed with an elite line partially resistance to maize streak virus	13	12	A	CML395 x CML444
5	La Posta Seq C7-F71-1-2-1-2-B–B–B/CML395//CML395	Drought tolerant donor lines crossed with an elite line resistance to maize streak virus and widely used tester in CIMMYT	13	11	B	CML312 x CML442
6	La Posta Seq C7-F71-1-2-1-2-B–B–B/CML444//CML444	Drought tolerant donor lines crossed with an elite line tolerant to low nitrogen and widely used tester in CIMMYT	4	4	B	CML312 x CML442
8	CML395/[M37 W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-2-2 x [KILIMA ST94A]-30/MSV-03-101-08-B–B-1 x P84c1 F27-4-1-4-B-3-B] F2-1-2-1-1-1-B x CML486]-1-1/CML395	An elite line resistance to maize streak virus and widely used tester in CIMMYT crossed with line derived from temperate genetic background	7	7	B	CML312 x CML442
9	CML395/La Posta Seq C7-F102-1-3-1-2-B–B–B/CML395	An elite line resistance to maize streak virus and widely used tester in CIMMYT crossed with drought tolerant donor lines	1	0	B	CML312 x CML442

version 9.2 (SAS 2003). Genotypes were considered as fixed effects, and replications and blocks within replications as random effects. For the combined analysis, variances were partitioned into relevant sources of variation to test for differences among genotypes and the presence of G×E interaction. Broad-sense heritability was calculated as the proportion of genetic variance over the total phenotypic variance. Heritability estimates refer to entry means across environments and replicates. For comparing entries evaluated in different locations, the entry means were expressed as a percentage of the average performance of the mean of the check hybrids in the respective locations.

Table 2 Characteristics of the experimental locations, average yield, heritability, and CV for 45 DH hybrids and 5 checks evaluated in Kenya, Uganda and Tanzania in 2011/2012 season

S/N	Country	Location	Years	Type of environment	No. of replications	Plot size (m^2)	Plant density per ha	Grain yield (t/ha)	Heritability	CV (%)
1	Kenya	Alupe	2011/12	Well-watered	3	7.13	53,333	5.39	0.54	9.42
2	Kenya	Bukura	2011/12	Well-watered	3	7.13	53,333	7.55	0.00	26.84
3	Uganda	Bulindi	2011/12	Well-watered	3	6.75	44,444	8.64	0.21	19.59
4	Tanzania	CFT Makutupo	2011/12	Drought stress	3	7.13	53,333	3.75	0.35	19.25
5	Kenya	KARI-Embu	2011/12	Well-watered	3	7.13	53,333	6.30	0.65	14.20
6	Kenya	KARI-Kakameg	2011/12	Well-watered	3	7.13	53,333	10.18	0.66	9.24
7	Kenya	KARI-Kiboko_	2011/12	Well-watered	3	6.38	53,333	9.93	0.60	6.17
8	Kenya	KARI-Mtwapa	2011/12	Drought stress	3	7.13	53,333	3.80	0.37	14.42
9	Kenya	Kiboko_KARI	2011/12	Drought stress	3	7.13	53,333	3.18	0.65	14.29
10	Kenya	Kiboko_RD	2011/12	Drought stress	3	7.13	53,333	4.04	0.48	16.38
11	Kenya	Malava Chemu	2011/12	Drought stress	3	7.13	53,333	1.99	0.52	43.19
12	Uganda	Masaka	2011/12	Drought stress	3	6.75	44,444	3.95	0.47	18.83
13	Uganda	Namulonge	2011/12	Well-watered	3	6.75	44,444	9.09	0.80	8.50
14	Uganda	Ngeta	2011/12	Well-watered	3	6.75	44,444	9.20	0.35	12.42
15	Tanzania	Nraramtoni	2011/12	Well-watered	3	7.13	53,333	5.03	0.56	11.93
16	Uganda	Serere	2011/12	Well-watered	3	6.75	44,444	9.17	0.69	9.79

Each DH line was genotyped with 681,257 SNPs but 518,177 SNPs (76 %) were excluded from the datasets because they had a minor allele frequency <0.05. The remaining 163,080 SNPs (24 %) had a minor allele frequency >0.05 and were used for statistical analyses. Genetic distance among the lines was calculated using TASSEL version 4.0.11 (Bradbury et al. 2007). The resulting distance matrix was used as input file to construct phylogenetic tree using the neighbor-joining algorithm implemented in MEGA version 5.0 (Tamura et al. 2007) and factorial analysis implemented in Darwin version 5.

Results

Comparison of means and ranges under well-watered and drought stressed conditions

Differences between the drought stressed and non-stressed conditions were significant for all traits except anthesis date (Table 3). Mean grain yield across locations in the drought stressed condition was 58 % lower than the mean grain yield in the non-stress environments. Water stress had also significantly reduced grain moisture by 10 %, plant height by

23 %, and ear height by 32 % but it increased ASI by 64 %, both root lodging and stalk lodging by 116 % as compared with non-stress conditions (Table 3). Anthesis date was the only trait that didn't show statistical difference between drought stress and well-watered locations.

The mean grain yield of the testcrosses evaluated in well-watered environments varied from 7.2 to 9.3 t/ha (Table 4). The top 15 testcrosses produced 1.3–2.2 t/ha higher grain yield than the mean of the commercial checks. Five of the highest yielding testcrosses produced significantly higher grain yields than the best commercial check. More than 90 % of testcrosses did not differ significantly from the mean of the best commercial check for moisture content, plant height and ear height.

Mean grain yields across drought stress environments ranged from 2.7 to 4.1 t/ha (Table 4). All of the DH hybrids had equal or higher grain yield than the mean of commercial checks; while the top 10 DH hybrids produced 1.0–1.4 t/ha higher than the mean of commercial checks. Combined across six drought stressed locations, the top three DH hybrids produced more than 4 t/ha and were comparable with the mean of the best commercial check in terms of anthesis date, plant height, moisture content and ear height.

Variance components and heritabilities

Variance of entry (*V*ENT) for grain yield, grain moisture, plant height and ear height was 7–61 % larger in the well-watered condition than in drought stressed condition. For anthesis date and root lodging, *V*ENT was 13–87 % larger in the water stress condition than in the well-watered condition (Table 5). The error variance (*V*Error) estimate for grain yield, grain moisture and ear height was higher in the well-watered; comparable between the two systems for plant height, and smaller in the well-watered condition for anthesis date, ASI and stalk lodging. Estimates of heritability for grain yield were slightly higher in the well-watered condition (0.8) than in the stress condition (0.7). For grain moisture, heritability estimates were high (>0.60) for both stress and well-watered conditions. The heritability estimates for anthesis date, ASI, and plant height were considerably higher in the well-watered than in the stress condition, whereas estimates of heritability for ear height, root lodging were comparable between systems. Overall, the results for genetic variances and heritability estimates indicated that genetic differences for grain yield may be better expressed under a well-watered condition, whereas genetic differences for root lodging, stalk lodging and ear height may be better expressed under drought stress condition.

Genetic distance and relationship

Genetic distance between pairwise comparisons of the 38 DH lines ranged from 0.070 to 0.475, and the overall average distance was 0.355 (Fig. 1). Only five of the 703 pairwise comparisons showed a genetic distance <0.100 that involved 6 DH lines (CKDHL0326, CKDHL0571, CKDHL0590, CKDHL0601, CKDHL0602, CKDHL0621). The majority of the genetic distances (69 %) fell between 0.300 and 0.475 (Fig. 1).

The neighbor-joining (NJ) tree generated from the genetic distance matrix grouped the 38 lines into two major groups (Fig. 2). Group 1 consisted of 19 DH lines extracted from populations 1, 5 and 8 that have

Table 3 Trait means of DH testcrosses evaluated in drought stress and well-watered environments in Kenya, Uganda and Tanzania in 2011/2012

Management	Grain yield (t/ha)	Anthesis date (days)	Anthesis-silking interval (days)	Grain moisture (%)	Plant height (cm)	Ear height (cm)	Root lodging (%)	Stalk lodging (%)
Well-watered	8.1	69.6	1.4	19.8	243.6	131.0	3.8	3.1
Drought stress	3.4	69.3	2.3	17.8	188.6	89.1	8.2	6.7
Difference	4.7	0.3	−0.9	2.0	55.0	41.9	−4.4	−3.6
	**	ns	**	*	**	**	**	**
Difference in %	58	0.00	−64	10	23	32	−116	−116

*,** significant at 0.05 and 0.001, respectively

Table 4 Mean performance of the 45 DH testcross and check hybrids evaluated at nine well-watered and six water stressed environments in Kenya, Uganda and Tanzania, in 2011/12 growing season

Entry	Well-watered									Drought stressed								
	Yield (t/ha)	Rank	AD	ASI	EH	MOC	PH	RL	SL	Yield	Rank	AD	ASI	EH	MOI	PH	RL	SL
CKDHH0232	9.3	1	70.5	1.6	130.1	21.0	250.1	5.4	3.3	3.5	26	71.3	1.8	98.4	19.7	183.4	3.9	6.7
CKDHH0157	9.1	2	69.5	0.5	128.1	19.1	250.9	2.3	2.7	3.6	16	67.7	1.2	100.8	17.6	197.0	2.4	7.6
CKDHH0214	9.1	3	71.3	0.8	126.0	20.3	244.0	4.8	2.6	3.7	12	71.5	1.8	95.7	18.9	186.8	4.0	4.6
CKDHH0202	9.0	4	69.9	1.5	148.1	20.2	251.5	5.4	2.5	3.4	35	69.5	2.7	100.9	18.4	187.7	10.1	9.7
CKDHH0342	8.9	5	71.7	1.1	129.9	21.0	248.1	1.8	2.1	4.1	2	71.7	2.1	99.2	19.1	195.1	0.6	3.9
CKDHH0373	8.8	6	70.1	1.8	137.5	20.9	244.9	6.7	3.1	3.8	10	70.4	2.3	102.3	19.1	192.0	3.0	16.5
CKDHH0426	8.8	7	71.4	0.7	130.4	19.9	242.9	2.1	1.7	3.4	32	71.5	1.3	95.6	18.6	190.5	2.6	7.6
CKDHH0213	8.8	8	70.1	0.8	128.0	21.0	229.3	4.1	4.1	3.9	6	69.9	2.0	92.2	18.4	178.3	4.7	15.9
CKDHH0086	8.7	9	70.5	0.9	132.8	19.8	246.1	3.4	2.4	3.7	13	70.2	1.4	95.7	18.2	192.1	-0.2	5.3
CKDHH0303	8.7	10	70.1	1.7	129.4	19.9	246.1	1.9	2.0	3.4	29	70.2	1.8	98.1	18.3	188.8	1.1	6.3
CKDHH0430	8.7	11	70.5	0.9	129.0	19.8	243.1	3.6	0.6	3.5	23	69.6	1.8	101.8	18.0	191.8	1.7	7.7
CKDHH0294	8.6	12	69.6	1.3	130.2	19.9	251.1	2.6	1.1	3.7	14	69.8	2.5	99.1	17.5	197.2	1.1	6.3
CKDHH0207	8.6	13	70.5	0.8	137.9	19.9	262.8	2.2	2.7	4.1	1	71.7	1.5	105.9	17.6	198.6	6.6	7.7
CKDHH0203	8.6	14	69.1	1.1	139.4	19.3	247.8	1.5	1.4	3.8	9	69.8	1.5	102.4	17.3	194.7	3.2	5.7
CKDHH0319	8.5	15	69.2	2.1	124.2	20.3	246.7	1.8	2.1	3.5	19	68.5	3.0	95.8	17.9	194.0	2.7	3.1
CKDHH0151	8.3	16	69.6	0.7	126.4	19.5	243.9	1.1	2.5	3.2	40	68.9	1.6	96.8	17.7	188.1	1.5	7.5
CKDHH0103	8.3	17	69.9	0.5	137.0	18.7	247.9	2.1	2.3	3.2	42	69.4	1.3	96.9	16.5	185.4	1.9	6.2
CKDHH0387	8.3	18	70.8	0.8	135.2	19.7	248.8	2.3	2.0	3.4	34	70.0	1.3	94.7	17.7	184.4	1.7	6.1
CKDHH0254	8.3	19	70.1	2.4	133.4	20.4	243.1	2.8	1.4	3.5	22	70.4	2.6	96.6	17.9	192.4	2.7	10.3
CKDHH0274	8.3	20	69.4	1.3	125.9	20.3	237.6	2.6	1.8	3.8	8	68.2	2.6	102.2	19.0	187.5	4.9	6.2
CKDHH0249	8.3	21	70.3	1.8	132.1	19.7	243.9	4.1	1.3	3.4	28	70.0	2.0	95.0	17.8	180.8	9.8	5.8
CKDHH0267	8.3	22	70.3	1.3	133.5	20.1	239.7	2.0	1.4	3.9	4	69.9	1.8	99.3	18.3	195.7	3.0	6.2
CKDHH0333	8.3	23	71.8	1.4	141.0	20.6	248.1	3.8	2.0	4.0	3	71.8	2.1	96.6	18.5	191.2	4.8	6.3
CKDHH0341	8.3	24	71.1	1.7	127.4	21.3	241.3	3.0	2.5	3.8	7	71.5	2.3	101.6	18.5	199.2	2.6	3.2
CKDHH0640	8.2	25	69.7	1.6	122.8	20.2	234.5	2.2	1.6	3.4	31	68.3	2.7	94.7	17.5	184.4	6.5	7.5
CKDHH0466	8.2	27	70.5	0.7	137.8	18.7	243.5	2.5	1.6	3.2	38	70.2	2.1	96.8	17.9	188.3	3.7	6.5
CKDHH0204	8.2	28	69.8	1.0	138.2	20.2	238.8	2.8	2.5	3.9	5	69.2	1.4	98.4	19.1	188.4	2.9	6.9
CKDHH0612	8.1	29	68.9	1.7	118.5	18.5	246.1	3.0	2.9	3.5	24	69.7	2.3	98.2	16.5	195.9	3.2	9.0
CKDHH0659	8.0	30	69.5	0.9	137.9	20.6	246.1	4.8	1.0	3.7	11	68.4	2.2	97.2	18.2	191.1	2.2	11.6
CKDHH0298	8.0	31	69.6	2.5	118.3	20.2	236.4	1.2	0.8	3.6	18	69.7	3.2	90.6	19.4	184.2	2.0	5.3
CKDHH0297	7.9	32	69.3	2.0	134.3	19.8	247.1	1.8	1.5	3.6	15	69.8	2.9	98.2	17.5	181.4	5.6	4.2

Table 4 continued

	Well-watered									Drought stressed								
Entry	Yield (t/ha)	Rank	AD	ASI	EH	MOC	PH	RL	SL	Yield	Rank	AD	ASI	EH	MOI	PH	RL	SL
CKDHH0673	7.9	33	69.8	2.1	126.2	19.3	244.5	3.9	1.8	3.2	41	68.9	2.4	98.9	17.1	194.0	3.6	3.1
CKDHH0240	7.8	34	70.7	0.9	143.1	20.4	234.2	6.8	5.6	3.5	25	68.8	3.4	101.2	17.2	187.7	10.6	11.1
CKDHH0205	7.8	35	68.1	1.3	126.5	19.9	232.9	3.4	2.0	3.4	27	67.8	2.1	83.1	17.4	177.7	3.3	3.3
CKDHH0217	7.8	36	68.5	1.8	128.1	20.1	234.4	3.4	2.9	3.5	21	67.1	2.3	97.0	17.9	181.7	3.2	6.8
CKDHH0630	7.7	37	69.6	1.1	135.8	20.3	255.8	4.4	3.6	3.6	17	69.4	1.8	100.8	18.2	198.5	1.7	5.0
CKDHH0245	7.7	38	70.7	1.5	136.5	19.6	248.3	2.4	3.2	3.1	45	71.5	1.8	97.7	17.4	191.0	3.8	6.7
CKDHH0037	7.6	39	67.9	1.8	121.5	20.3	238.0	2.7	1.5	3.4	33	67.5	2.4	93.4	18.2	183.2	1.9	8.1
CKDHH0192	7.6	40	68.4	1.1	129.2	19.2	237.2	1.6	2.4	3.2	39	67.0	2.1	93.5	17.2	188.6	3.7	5.3
CKDHH0250	7.5	41	69.8	1.3	138.2	19.4	245.4	2.4	3.2	3.1	44	69.5	1.8	95.9	17.7	177.4	0.6	8.0
CKDHH0352	7.5	42	68.2	2.2	115.6	20.8	233.8	0.7	3.6	3.2	43	68.6	3.0	89.8	18.0	178.0	0.4	5.0
CKDHH0080	7.4	43	69.3	1.5	128.7	19.8	244.1	1.2	3.3	3.0	46	68.8	2.9	97.4	17.5	187.5	1.6	14.9
CKDHH0622	7.3	44	67.7	1.5	131.3	18.8	251.1	3.5	1.9	3.4	30	67.0	3.8	100.1	17.0	198.0	10.5	13.0
CKDHH0629	7.3	45	67.7	1.5	124.0	18.7	245.0	4.6	4.1	2.7	47	67.0	2.2	96.4	17.7	190.4	2.3	8.4
CKDHH0641	7.2	46	67.8	2.3	124.8	19.4	234.4	1.6	1.4	3.5	20	67.9	2.9	94.6	17.1	185.6	0.6	13.2
Local Check 2	7.1	47	67.4	2.1	121.8	18.1	242.7	3.3	3.6	2.5	48	70.4	4.7	103.2	17.2	193.8	5.0	13.3
Local Check 1	6.9	48	67.8	1.5	136.2	18.3	245.9	6.2	4.0	2.3	50	67.8	2.4	96.1	16.5	186.8	4.4	9.3
Commercial check 1	8.2	26	71.0	1.3	126.9	19.6	242.4	4.2	3.3	3.3	37	70.9	1.9	92.7	18.6	192.3	4.0	10.5
Commercial check 2	6.7	49	68.3	1.9	145.4	19.6	249.4	3.2	3.6	2.4	49	67.4	4.4	99.8	16.2	188.0	5.3	13.1
Commercial check 3	6.7	50	66.8	0.5	127.1	17.3	229.3	4.0	3.5	3.3	36	67.5	0.9	93.3	16.1	181.3	14.5	6.0
Mean	8.1		69.6	1.4	131.0	19.8	243.6	3.1	2.4	3.4		69.3	2.3	97.1	17.8	188.6	3.7	7.8
LSD (0.05)	0.8		1.2	0.7	14.4	1.3	9.1	3.8	3.1	0.6		2.1	1.7	7.4	1.5	11.2	8.2	6.7
CV	4.9		0.9	26.3	5.6	3.3	1.9	62.9	64.7	9.6		1.6	37.9	3.9	4.2	3.0	113.0	43.5
Heritability	0.8		0.9	0.8	0.5	0.7	0.8	0.1	0.0	0.7		0.7	0.3	0.6	0.6	0.6	0.1	0.5

AD anthesis date, *ASI* anthesis-silking interval, *EH* ear height, *MOI* moisture index, *PH* plant height, *RL* root lodging, *SL* stalk lodging

Table 5 Variance component and heritability estimates in 45 DH testcrosses and five checks evaluated under water stress and optimal rain-fed environments in Kenya, Uganda and Tanzania in 2011/2012

	Grain yield (t/ha)	Anthesis date (days)	Anthesis-silking interval (days)	Grain moisture (%)	Plant height (cm)	Ear height (cm)	Root lodging (%)	Stalk lodging (%)
*V*ENT								
Non-stress	0.3 (0.07)	1.2 (0.35)	0.2 (0.06)	0.5 (0.18)	33.8 (10.1)	21.9 (12.1)	0.1 (0.45)	0 (0.33)
Water stress	0.1 (0.03)	1.4 (0.39)	0.2 (0.11)	0.4 (0.14)	22 (7.8)	8.6 (3.1)	1 (2.1)	5.3 (2.2)
*V*ENT X *V*LOC								
Non-stress	0.2 (0.05)	0.9 (0.11)	0.2 (0.05)	0.4 (0.16)	20.7 (8.1)	21.9 (21.8)	0	1.8 (0.59)
Water stress	0.1 (0.03)	1.4 (0.32)	0.4 (0.21)	0.3 (0.14)	14.4 (9.5)	10.9 (3.97)	13 (3.4)	4.2 (2.8)
*V*Error								
Non-stress	1.2 (0.07)	2.4 (0.09)	1.3 (0.06)	3.4 (0.2)	201.7 (11)	420.7 (26)	32.7 (1.3)	15.7 (0.66)
Water stress	0.6 (0.04)	5.1 (0.33)	4.8 (0.3)	2.8 (0.2)	199.5 (13)	71.6 (4.7)	28.1 (2.6)	44.9 (3.6)
Heritability								
Non-stress	0.8	0.9	0.8	0.7	0.8	0.5	0.1	0
Water stress	0.7	0.7	0.3	0.6	0.6	0.6	0.1	0.5

SE was given in parenthesis

VENT entry variance, *VENT X VLOC* location by entry variance, *VError* residual variance

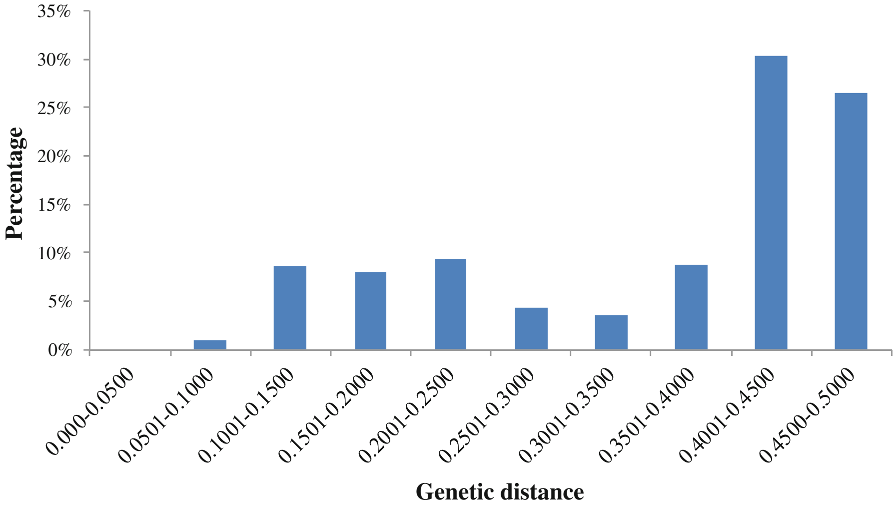

Fig. 1 Distribution of pairwise genetic distance calculated for 38 maize DH lines genotyped with 163,080 SNPs

CML395 as a common parent. The remaining 19 DH lines from populations 2, 3 4 and 6 were assigned in group 2. Populations 2 and 6 have CML444 as a common parent; populations 4 and 6 share LaPosta-SeqC7-F71-1-2-1-2-B–B–B while population 2 and 3 share LaPostaSeqC7-F96-1-2-1-1-B–B–B. Group 2 can further be subdivided into two subgroups, with DH lines from populations 2 and 6 forming the first subgroup and those from population 4 forming the second subgroup.

The first two principal components (PCs) from factorial analysis explained 63.7 % of the total SNP variations among samples (Fig 3). A plot of PC1 (43.4 %) and PC2 (20.4 %) revealed three groups and the patterns of grouping remained the same as that of the cluster analysis.

Discussion

The top 10 DH hybrids from the combined analyses of the well-watered and drought stressed environments produced 1.6–2.2 t/ha and 1.0–1.4 t/ha higher grain yield than the mean of commercial checks,

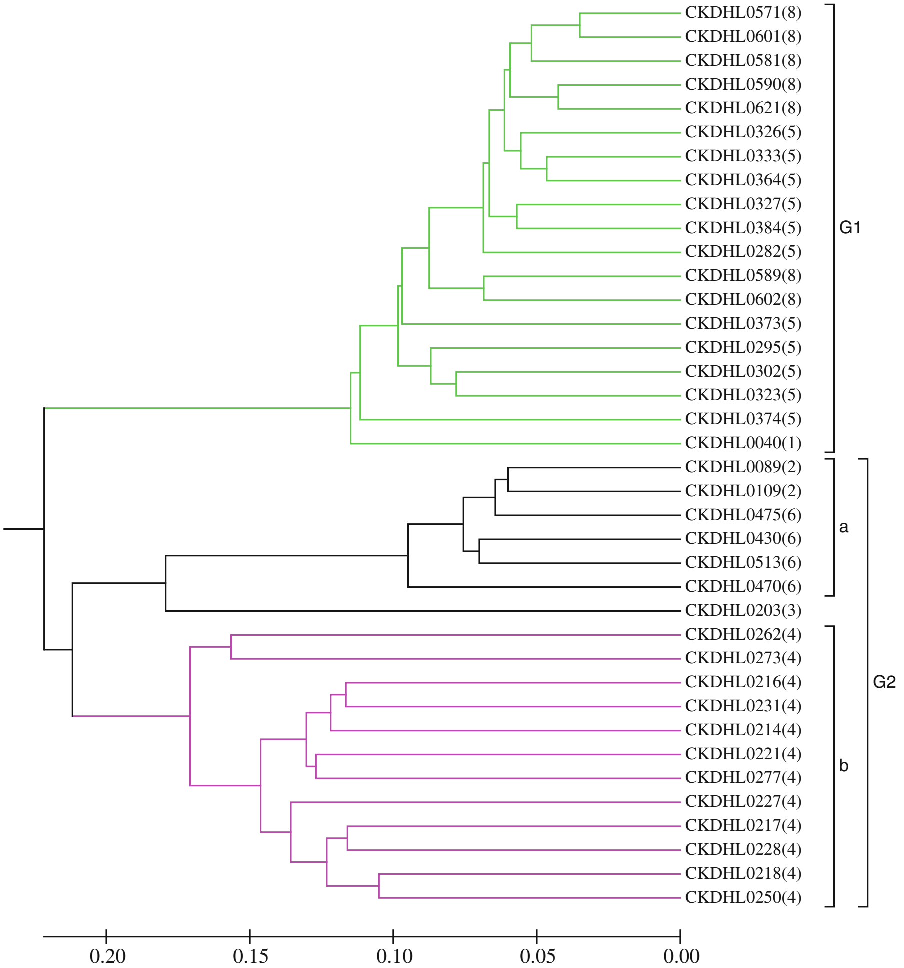

Fig. 2 Neighbor-joining tree for 38 DH lines based on genetic distance calculated from 163,080 SNP markers. The different subgroups (*G1*, *G2a* and *G2b*) are indicated with different colors. The numbers *1*, *2*, *4*, *5*, *6* and *8* within bracket refer to the base population that was used for developing the DH lines

respectively. These hybrids were similar to or better than the commercial checks for all other agronomic traits. These superior hybrids were derived from all of the eight populations suggesting that the donor parents used in developing the DH lines are excellent sources of germplasm to improve drought tolerance and combining ability of sub-Saharan Africa adopted germplasm. Hybrids CKDHH0214, CKDHH0342, CKDHH0373 CKDHH0213, CKDHH0086, CKDHH 0294, CKDHH0207 and CKDHH0203 performed well across optimum and stress locations (Table 5) indicating that it is possible to combine stress tolerance and yield potential in tropical doubled haploid maize hybrids. The eight hybrids were derived from three source populations (La Posta Seq C7-F71-1-2-1-2-B–B–B/CML312SR//CML312SR; La Posta Seq C7-F71-1-2-1-2-B–B–B/CML395//CML395 and La Posta Seq C7-F96-1-2-1-1-B–B–B/CML444//CML 444. Therefore, the three source populations could be good sources for improving maize for both drought tolerance and high yield potential in sub-Saharan Africa.

Mean grain yield across locations in the water stress environments was 58 % lower than the well-watered environments. However, ASI increased by 64 % under drought stressed than well-watered condition. Drought stress had also significant effect on all measured traits except anthesis date indicating that drought stress has significant impacts on those traits. Menkir et al. (2006) reported that moisture deficit reduced grain yield by 58 %, plant height by 16 %, ears per plant by 30 % and ear height by 19 %, while increasing days to

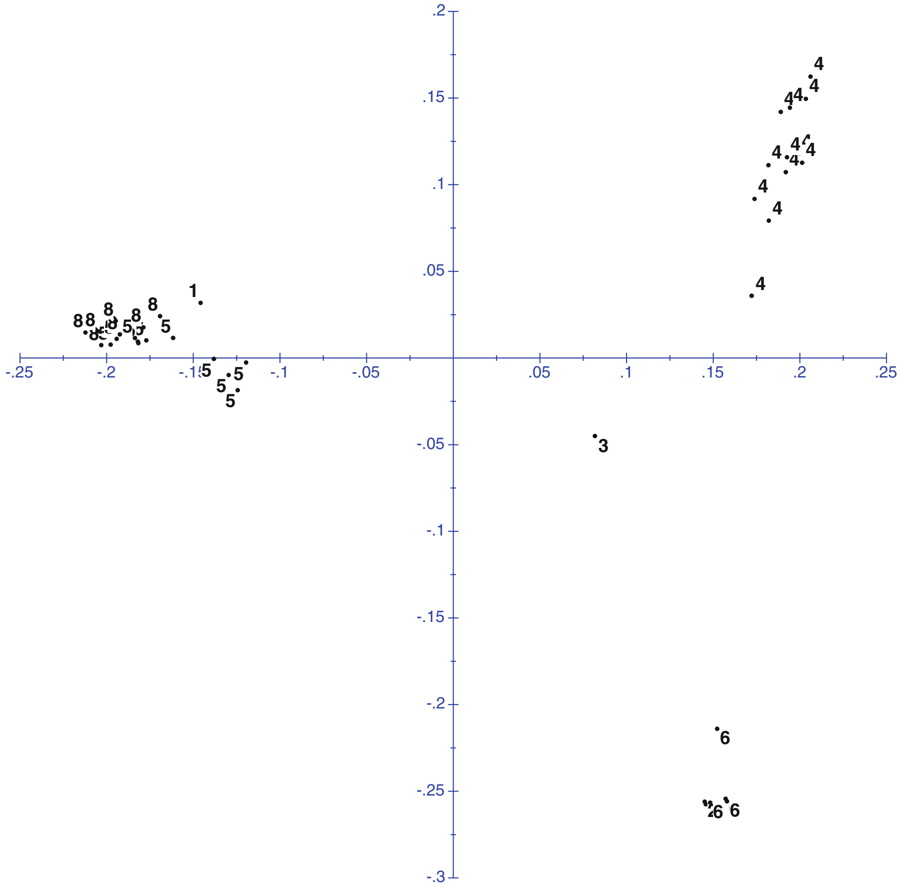

Fig. 3 Plot of Axis-*1* (43.4 %) and Axis-*2* (20.4 %) from factorial analysis using the genetic distance matrix calculated from 163,080 SNP markers. The numbers *1*, *2*, *4*, *5*, *6* and *8* refer to the base population that was used for developing the DH lines

silking by 6 % and ASI by 144 % in comparison with well-watered condition while drought stress had little effect on days to anthesis compared to well-watered condition. However, drought stress had little effect on anthesis date. Drought stress at flowering does not greatly affect days to pollen shed, but it often slows silk elongation and results in large ASI for drought-susceptible genotypes (Westgate et al. 1997). Most of the hybrids had shorter ASI suggesting that the DH lines had favorable genes from drought tolerant parents. The reduced ASI in well-watered conditions is an indication of the expected better synchronisation of the anthesis and silking periods, which allows for better fertilisation and good cob fill. Increased flowering synchronization and reduced kernel and ear abortion were closely related to grain yield improvements in maze under drought condition (Bänziger and Araus, 2007).

Selection for reduced ASI in tropical open-pollinated varieties has been shown to be correlated with improved yields under drought stress (Ribaut et al. 1996). Genetic gains under stressed conditions were significantly greater than those observed in conventionally selected counterpart populations without loss of yield potential (Byrne et al. 1995). Bolaños and Edmeades (1996) observed significant and linear phenotypic correlations between grain yield and ASI.

For breeders interested in developing inbred lines, the number of superior lines which are common in stress and non-stress environments is of great importance. In the current study, eight of 15 instances, the top DH testcrosses performed well under drought stress and well-watered environments indicating that the DH line that makes these hybrids could have favorable alleles for grain yield under drought and optimum conditions. Betrán et al. (2003) reported that lines with different selection history produced high yielding hybrids under contrasting environments, indicating favorable allele combinations between optimum selected inbred lines and inbred lines selected for drought tolerance. However, not all hybrids responded similarly to well-watered and drought stressed conditions. Out of the top 15 hybrids that performed well under optimum condition only eight were on the top 15 under drought conditions (Table 4). Thus, there is no guarantee that a genotype selected for optimum rain-fed trial will produce high under drought stress condition. It is important, therefore, to differentiate between genotypes that produce high yields under drought stress because of high inherent yield potential and those that produce high yield under drought stress condition because of their inherent drought tolerance.

Genetic variance and heritability for grain yield were larger in the well-watered condition than in the drought stressed condition. These findings were in agreement with (Blum 1988; Bolaños and Edmeades 1996; Rosielle and Hamblin 1981) who reported that genetic variance and heritability of grain yield often decline with increasing moisture stress. Several earlier studies reported that genetic variance and heritability of maize grain yield were generally lower under stress than under optimal conditions (Johnson and Gaedelmann 1989; Lafitte and Edmeades, 1994; Lafitte and Bänziger 1997a; Bänziger and Cooper, 2001). Badu-Apraku et al. (2011a) reported that the most reliable traits for selection for improved grain yield under drought stress in the early maturing germplasm were ear aspect, ears per plant, anthesis—silking—interval, and plant aspect.

Genetic variance, heritability, and genetic correlation estimates are influenced by gene frequencies and are population-specific (Falconer and Mackay 1996). Therefore, the results of this study apply to the DH lines derived from the eight populations and not necessarily to other populations. Despite huge environmental differences among locations, heritabilities for grain yield and other agronomic traits were fairly consistent across drought stress and well-watered conditions indicating that the DH lines were adapted to wide environmental conditions.

Substantial molecular variation was observed among DH lines. Similarly, high genetic variability among diverse CIMMYT maize lines have been reported (Semagn et al. 2012). Among pairwise comparisons of the 38 DH lines, none of them fell within a genetic distance less than 0.05, indicating a lack of redundant lines among the DH lines studied. The high mean genetic distance among the DH lines indicated that the donor parents contributed new alleles into adapted germplasm. This may be expected, because the parent lines are elite inbreds, which are adapted to the SSA while the donor parents were developed at Mexico for drought tolerance and expected to have different alleles. More than 500 DH lines derived from the same sources populations have been phneotyped and genotyped by GBS (Beyene et al. unpublished), and could be used in QTL detection. These lines also find application in association and/or genome wide association studies to identify the genomic regions/genes associated with grain yield and drought tolerance.

This research confirms that DH lines developed from tropical adapted BC populations had favorable gene for improving yield under stress and non-stress conditions. DH lines represent homozygous and true-breeding lines, which can be repeatedly phenotyped. It also increases speed and efficiency to produce new product to the market. The results showed that these newly developed DH hybrids maize are significantly better than the best commercials checks developed through pedigree breeding in grain yield and comparable in other major agronomic traits. Thirteen of the best DH hybrids are currently in National Performance Trials (NPT) testing, an important step towards commercialization in Kenya, Tanzania and Uganda.

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