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Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (*Zea mays* L.) hybrids

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Abstract In vivo production of maternal haploid plants and advancement in chromosome doubling technology has led to rapid production of doubled haploid homozygous lines. These in turn have boosted rapid advancement in most breeding programs. This has resulted in production of a large number of maize hybrids which need testing across production environments to select the most suitable hybrids for release and cultivation. The objective of this study was to assess the genotype × environment interactions (GE) for grain yield and other agronomic traits and evaluate the performance of 44 recently developed doubled haploids (DH) testcross hybrids along with six checks across five locations in Uganda. Significant mean squares for environment (E), genotype (G) and GE were observed for all studied traits. Environment explained 46.5 % of the total variance, while G and GE contributed 13.2 and 7.2 %, respectively. Genetic correlations among locations were high (0.999), suggesting little GE among environments. The 10 best testcross hybrids had a 49.2 % average grain yield advantage over the six checks at all locations. DH hybrids CKHDHH0887, CKDHH0878, CKDHH 0859, WM1210, CKDHH0858, and WM1214 were the most stable, across locations. The DH testcross hybrids produced higher grain yield and possessed acceptable agronomic traits compared to the commercial hybrids developed earlier. Use of the best DH testcross hybrids, well targeted to the production environments, could boost maize production among farmers.

Keywords Doubled haploids · East Africa · Genotype × environment · Grain yield · Maize · Stability

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Introduction

Doubled haploids (DH) technology has paved the way to rapidly generate large number of inbred lines. The technology involves in vivo haploid induction by specific inducers that lead to production of haploid seeds from the maternal plants (Beyene et al. 2011).

These haploid maternal plants then get their chromosomes doubled through use of colchicine. DH lines are highly efficient tools in genetic research and practical maize breeding (Thomas et al. 2003; Bordes et al. 2007; Beyene et al. 2011). Major advantages of DH lines compared to pedigree lines include (i) maximum genetic variance among lines for per se and testcross performance from the first generation; (ii) reduced length of breeding cycle; (iii) perfect fulfillment of distinctness, uniformity, and stability (DUS) for satisfying varietal status; (iv) reduced costs in maintenance breeding; (v) simplified logistics; and (vi) increased efficiency in marker-assisted selection, gene introgression, and gene stacking.

Since 2008, there has been an effort to enhance rapid development of elite lines for tolerance to drought and other stresses by the International Maize and Wheat Improvement Center (CIMMYT), the National Agricultural Research Systems (NARS) partners, and Monsanto Company through the Water Efficient Maize for Africa (WEMA) project. The partnership has developed DH lines from several drought tolerant maize source populations. Several DH hybrids have also been developed and tested for their performance in different drought-stress and non-drought stress environments in Kenya (Beyene et al. 2011; 2013). Results showed that the use of DH testcrosses performed much better than the commercial hybrids in both stressed and non-stressed environments (Beyene et al. 2011). These DH hybrids needed to be evaluated in different environments to assess their performance and adaptability, and to identify the major basis of the genotype adaptation.

The performance of a genotype can vary from one environment to another and genotypes that are superior in one environment may not be superior in other environments due to genotype-by-environment interactions (GE) (Makumbi et al. 2015). The presence of GE results in the failure of genotypes to achieve the same relative performance in different environments (Baker 1988; Beyene et al. 2011). This reduces the correlation between phenotypes and genotypes, complicating breeding and selection of superior cultivars (Kang 1993; Makumbi et al. 2015).

The WEMA project team formed WEMA-Wide trials (WWT) through which common maize trials are grown in different environments across five countries (Kenya, Mozambique, South Africa, Tanzania and Uganda) in eastern and southern Africa to identify high-yielding and adapted varieties for release and cultivation in the respective countries. WWT are grown tested in multi-environment that sample drought and non-stress locations.

Significant gains in grain yield (GY) performance have been reported in these CIMMYT Maize Regional Trials (Beyene et al. 2011). Earlier studies have suggested that by considering GE, superior genotypes were selected for commercial release to farmers in Africa (Pixley and Bjarnason 2002; Beyene et al. 2011). Genotype × environment interactions have been investigated through the use of statistical tools such as the additive main effects and multiplicative interaction (AMMI) analysis for grain yield and grain micronutrients' concentrations and stability (Zobel et al. 1988; Gauch 2006; Kassa et al. 2013); and genotype main effect plus genotype × environment interaction (GGE) for the analyses of grain yield and stability in tropical maize (Yan et al. 2000; Makumbi et al. 2015). These analytical methods provide an insight into the extent of GE present in a given study. Genetic correlations can be used to quantify the importance of GE (Falconer 1952); and have been used in GE studies (Cooper and DeLacy 1994). However, although DH maize hybrids developed by CIMMYT and its partners have been evaluated in eastern and southern Africa, the level of their GE has not been assessed. There is limited information available in the literature on the level of GE on grain yield performance and stability of maize testcrosses developed from DH lines. Therefore, the objectives of this study were to (i) assess GE for GY and other agronomic traits; and (ii) evaluate the performance and stability of 44 DH maize testcrosses across five locations in Uganda.

Materials and methods

Field evaluation and experimental design

The genotypes used for the study comprised 44 DH hybrids developed from the WEMA project and 6 checks that included 1 internal CIMMYT hybrid, 3 commercial hybrids and 2 Ugandan local hybrids. These were grown in trials sown at five locations: Namulonge, Serere, Bulindi, Ngetta and Kasese under different environmental conditions in Uganda in 2012 (Tables 1, 2). The experiment design was a 5 × 10

Table 1 List of maize DH testcross hybrid genotypes used in the study and their sources

Genotype	Pedigree	Source
G1	WM1206	Monsanto
G2	WM1207	Monsanto
G3	WM1208	Monsanto
G4	WM1209	Monsanto
G5	WM1210	Monsanto
G6	WM1211	Monsanto
G7	WM1212	Monsanto
G8	WM1213	Monsanto
G9	WM1214	Monsanto
G10	WM1215	Monsanto
G11	WM1216	Monsanto
G12	WM1219	Monsanto
G13	WM1220	Monsanto
G14	WM1221	Monsanto
G15	WM1222	Monsanto
G16	CKDHH0858	CIMMYT
G17	CKDHH0888	CIMMYT
G18	CKDHH0873	CIMMYT
G19	CKDHH0859	CIMMYT
G20	CKDHH0889	CIMMYT
G21	CKDHH0874	CIMMYT
G22	CKDHH0860	CIMMYT
G23	CKDHH0890	CIMMYT
G24	CKDHH0875	CIMMYT
G25	CKDHH0861	CIMMYT
G26	CKDHH0891	CIMMYT
G27	CKDHH0876	CIMMYT
G28	CKDHH0862	CIMMYT
G29	CKDHH0892	CIMMYT
G30	CKDHH0877	CIMMYT
G31	CKDHH0863	CIMMYT
G32	CKDHH0893	CIMMYT
G33	CKDHH0878	CIMMYT
G34	CKDHH0864	CIMMYT
G35	CKDHH0894	CIMMYT
G36	CKDHH0879	CIMMYT
G37	CKDHH0865	CIMMYT
G38	CKDHH0895	CIMMYT
G39	CKDHH0880	CIMMYT
G40	CKDHH0866	CIMMYT
G41	CKDHH0896	CIMMYT
G42	CKDHH0881	CIMMYT
G43	CKDHH0872	CIMMYT
G44	CZH0616	CIMMYT
G45	CKDHH0887	CIMMYT
G46	H513	Seed company
G47	PH 3253	Seed company
G48	DK 8053	Seed company
G49	Local Check 1	NARO
G50	Local Check 2	NARO

Alpha lattice with two replications at each location. Each entry was planted in a two-row plot of 5 m long and 0.75 m apart with the hills spaced 0.25 m apart. Two seeds were initially planted per hill but were subsequently thinned to one plant per hill at 4 weeks after emergence to give a plant population of 53,333 plants per hectare. In all the experiments, standard cultural practices including weeding control throughout the growing season were followed. Fertilizer application at each location consisted of 125 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}.

Data collection

The data recorded from each plot included: days to anthesis (AD), i.e. days from planting to when 50 % of the plants shed pollen, and days to silking (SD), i.e. days from planting to when 50 % of the plants had extruded silks. Anthesis–silking interval (ASI) was determined as the difference between SD and AD. Plant height (PH) measured in centimeters as the distance from the base of the plant to the height of the first tassel branch, number of ears per plant (EPP), determined by dividing the total number of ears per plot by the number of plants harvested per plot, husk cover (HC), obtained by dividing the number of ears with poor husk cover by the number of plants harvested per plot; and expressed as percentage; lower value indicates best husk cover), ear aspect (EA), rated on a scale of 1–5, where 1 = nice uniform ears with the preferred texture; and 5 = cobs with the undesirable texture), plant aspect (PA) (1–5) 1 = short plant with uniform and short ear placement; 5 = tall plants with high ear placement ear position or height (EP): the ear height is determined by measuring a representative plant from the ground to the insertion of the top ear and grain moisture.

Table 2 Agro-climatic description of the locations where maize DH testcross hybrids were evaluated during the study period in 2012

Site	Latitude	Longitude	Elevation (masl)	Mean rainfall (mm)	Temperature (°C)		Soil type
					Min	Max	
Namulonge	0.5297	32.6025	1150	1270	16	28	Sandy clay loam
Serere	1.5176	33.4579	1080	1419	19	31	Sandy clay loams and black clays
Ngetta	53.6947	22.9297	1300	1483	19	29	Sandy loam
Bulindi	−0.777	29.1402	1020	1338	19	29	Sandy loam
Kasese	0.1833	30.0833	960	1200	18	31	Peaty sands and clay

masl metres above sea level

All ears harvested from each plot at all locations were weighed and randomly selected representative samples of ears were shelled and weighed. Grain moisture was determined using a Dickey Jones moisture meter. Grain yield in tons per hectare (t ha^{-1}) was determined based grain moisture content of 12.5 %.

Statistical analysis

Analysis of variance (ANOVA) for all traits was done separately for each location, and combined across locations using PROC MIXED Model procedure from SAS (SAS Institute 2008). Genotypes were considered as fixed effects, and replications and blocks within replications as random effects. For the combined analysis, variances were partitioned into the relevant sources of variation to test for differences among genotypes and the presence of GE. Broad-sense heritability (H) was calculated as the proportion of genetic variance over the total phenotypic variance. Heritability estimates refer to entry means across environments and replicates (Hallauer and Miranda 1981). For comparing entries evaluated in different locations, the entry means were expressed as a percentage of the average performance of the best check hybrid in the respective locations.

Estimates of genotypic (σ^2_G), location (σ^2_L), genotype × location ($\sigma^2_{G\times L}$), and error variance (σ^2_E) were calculated using the PROC MIXED (option = REML) of SAS (SAS Institute 2008). Across environments, ANOVA for each trait was conducted using PROC GLM of SAS (SAS Institute 2008). In the across-environment ANOVA, genotype effects were tested for significance using the corresponding interaction with the environment as the error term, while the GE was tested using the pooled error.

Heritability and genetic correlations

Broad-sense heritability (H) for individual trials was estimated according to Hallauer et al. (2010):

$$H = \frac{\sigma^2_G}{\left[\sigma^2_G + \left\{\frac{\sigma^2_E}{r}\right\}\right]}$$

where σ^2_G is the genotypic variance, σ^2_E is the error variance, and r the number of replications.

H for traits across environments was estimated using the variance components according to Hallauer et al. (2010) as:

$$H = \frac{\sigma^2_G}{\left[\sigma^2_G + \frac{\sigma^2_{GxL}}{E} + \frac{\sigma^2_E}{ER}\right]}$$

where σ^2_G, $\sigma^2_{G\times L}$ and σ^2_E are genotypic, genotype × location, and residual variance components, respectively: E is the number of environments, and R is the number of replications. Genotypic correlations (r) between locations were estimated according to Cooper et al. (1996) as:

$$r_{g=}\frac{r_{p^{(12)}}}{(H_1 \times H_2)^{1/2}}$$

where $r_{p^{(12)}}$ is the phenotypic correlation between the traits measured in locations 1 and 2, H_1 and H_2 are the broad-sense heritabilities for the traits measured in locations 1 and 2, respectively.

Cluster analysis using Ward's minimum variance method (Ward 1963; Makumbi et al. 2015) was

performed to group environments based on genetic correlations among the environments. The SAS procedure PROC CLUSTER was used for cluster analysis. The PROC TREE procedure of SAS was used to generate the dendrograms.

Genotype main effect and genotype × environment (GGE) biplot analysis

Adjusted GY from ANOVA was subjected to GGE biplot analysis to decompose the GE of each experiment (Yan et al. 2000; Yan 2001) to compare genotype stability in performance across the various environments.

$$Yijl = m + Gi + Ej + \left(\sum \lambda k \alpha ik \gamma jk\right) + dij + eijl,$$

where λk = kth eigenvalue, αik = principal component score for the ith genotype for the kth principal component axis, γjk = principal component score for the jth environment for the kth principal component axis, dij = residual G × E not explained by model.

The GGE biplot shows the first two principal components (PC1 and PC2) derived from subjecting the environment-centered yield data (the yield variation due to GGE) to singular value decomposition (Yan et al. 2000). GGE biplots were constructed using R package named GGEBiplotGUI (Frutos et al. 2014).

Results

Analysis of variance

Combined analysis of variance across the five locations revealed that G, E, and GE were significant for all the traits except GE for SD, ASI, and HC (Table 3). There were, therefore differences in the performance of the test materials at the different locations. GE was highly significant ($P < 0.001$) for PA and EP and significant ($P < 0.05$) for GY, AD, grain moisture (MOI), ear per plant (EPP), and ear aspect (EA). Overall on the grain yield, the effect of environment explained 46.5 % of the total variance while genotype contributed 13.2 %, and GE contributed the least (7.2 %) to the total variation hence the environment

Table 3 Mean squares and degrees of freedom from ANOVA for grain yield and agronomic traits for 44 maize doubled haploids (DH) testcross hybrids, and six checks across five locations in Uganda in 2012

		Mean square									
Source	df	Grain yield (GY) ($t\ ha^{-1}$)	Days to anthesis (AD) (days)	Days to silking (SD) (days)	Anthesis silking interval (ASI) (days)	Grain moisture (MOI) (%)	Ear per plant (EPP) (#)	Husk cover (HC) (%)	Ear aspect (EA) (1–5)	Plant aspect (PA) (1–5)	Ear position (EP) (cm)
Environment (E)	4	1128.6***	12552***	15518***	426.3***	1375.8***	1.891***	99,528***	30,9***	164.87***	106,8136***
Genotype (G)	49	520.7***	1244***	1622***	219.6***	127.1*	1.891***	27,256***	14.32***	14.34***	70,263***
GE	196	590.5*	728*	896	428.7	459.9*	4.548*	43,597	24.8*	51.08***	185,958***
Error	245	557.7	685	962	463.6	459.7	4.477	55,511	24.58	35.92	148,432

Significance level: ns not significant, $P > 0.05$; * $P < 0.05$; ** <0.001; *** $P < 0.0001$

influences a lot on the performance of different germplasm (Table 4).

Genotypes performance at individual location

Grain yield of the test hybrids varied with locations. The lowest yield of 4.2–7.1 t ha^{-1} was obtained at Namulonge, and the highest yield of 7.2–12.1 t ha^{-1} was recorded at Serere (Table 5, Supplementary 1). The highest yielding testcross hybrids at Kasese, Namulonge, Serere, Bulindi, and Ngetta were 51, 48, 30, 17 and 5 % above the best commercial hybrid, DK 8053, respectively. At each location, the best testcross hybrid out-yielded the CIMMYT internal check, CZH0616, by 9–34 %.There were no significant yield advantage between the commercial check and the CIMMYT internal check at all locations. The environments could be ranked in terms of their grain yielding potential: Serere > Kasese > Ngetta > Bulindi > Namulonge; and the ranking of the environments in terms of heritability followed a similar pattern as grain yield.

Average genotypes performance across locations

The combined analysis for average performance across the five locations for the top 10 testcross hybrids showed 23 % yield advantage over the average of the six checks included in the trial (Table 6, Supplementary 2). The best performing hybrids (CKDHH0858 and WM1216) across five locations out-yielded the commercial check (DK8053) by 25 %.

All the genotypes had comparable maturity with SD ranging from 59.1 to 64.1 days except the testcross CKDHH0891 that significantly flowered 4–5 days earlier than the testcross CKDHH0877 and the Local Check 1 (Table 6). Therefore, the testcross CKDHH0891 could be categorized as an early maturing genotype that also had the least anthesis-silking interval (ASI) of 0.3 days (Table 6). This is an indication that this testcross hybrid and testcross hybrid, WM1209 with a similar low ASI of 0.4 days might be the most drought-tolerant hybrids among the genotypes evaluated. All the test materials had similar rating of husk cover (4–5.9 %) (Table 6). But the testcross CKDHH0881 had significantly better husk cover rating (4 %) than the commercial check DK8053 (8.7 %).

Medium to high heritability estimates were found in different traits except for grain moisture and plant aspect with heritability of 0.1–0.4. The highest heritability of 0.9 was recorded for AD and SD, and the lowest was for MO ($h^2 = 0.1$).

Genetic and phenotypic correlation among different test environments

Based on grain yield, genetic correlations among locations ranged from −0.2191 (between Ngetta and

Table 4 Variance decomposition, heritability of grain yield and agronomic traits 44 of maize doubled haploids (DH) testcross hybrids, and 6 checks across 5 locations in Uganda in 2012

Statistic	Grain yield (GY)	Days to anthesis (AD)	Days to silking (SD)	Anthesis silking interval (ASI)	Grain moisture (MOI)	Ear per plant (EPP)	Husk cover (HC)	Ear aspect (EA)	Plant aspect (PA)	Ear position (EP)
Genotypic variance	0.74	2.11	2.91	0.23	0.02	0.00	3.00	0.02	0.00	0.00
Location variance	2.60	31.07	38.48	1.03	3.39	0.00	0.47	0.07	0.41	0.00
Gen × env variance	0.40	0.42	0.25	0.15	0.23	0.00	9.42	0.01	0.06	0.00
Residual variance	1.85	2.24	3.02	1.89	1.60	0.02	5.87	0.09	0.12	0.00
Grand mean	8.01	60.54	61.37	0.84	15.30	0.99	5.17	2.80	3.20	0.52
$LSD_{0.05}$	2.67	2.93	3.41	2.70	2.48	0.26	4.75	0.58	0.69	0.06
Heritability	0.74	0.87	0.89	0.51	0.09	0.40	0.55	0.59	0.16	0.72

Table 5 Mean performance of 10 highest yielding from among 44 maize Doubled haploids (DH) testcross hybrids, and six checks across five locations in Uganda in 2012 combined across five locations in Uganda

	Namulonge			Serere			Bulindi			Ngetta			Kasese	
	Entry	Grain yield (GY) (t ha^{-1})		Entry	Grain yield (GY) (t ha^{-1})		Entry	Grain yield (GY) (t ha^{-1})		Entry	Grain yield (GY) (t ha^{-1})		Entry	Grain yield (GY) (t ha^{-1})
26	CKDHH0891	7.1	9	WM1214	12.1	11	WM1216	9.0	11	WM1216	8.5	17	CKDHH0888	11.9
41	CKDHH0896	7.0	4	WM1209	12.0	30	CKDHH0877	8.9	32	CKDHH0893	8.4	25	CKDHH0861	10.6
5	WM1210	6.6	10	WM1215	11.8	16	CKDHH0858	8.6	42	CKDHH0881	8.4	16	CKDHH0858	10.4
19	CKDHH0859	6.5	22	CKDHH0860	11.7	4	WM1209	8.5	10	WM1215	8.4	36	CKDHH0879	10.3
36	CKDHH0879	6.5	16	CKDHH0858	11.6	45	CKDHH0887	8.5	4	WM1209	8.3	22	CKDHH0860	10.3
16	CKDHH0858	6.2	17	CKDHH0888	11.5	8	WM1213	8.4	5	WM1210	8.3	18	CKDHH0873	10.3
11	WM1216	6.2	11	WM1216	11.2	22	CKDHH0860	8.4	21	CKDHH0874	8.3	9	WM1214	10.2
25	CKDHH0861	6.0	42	CKDHH0881	11.1	10	WM1215	8.3	34	CKDHH0864	8.3	5	WM1210	10.2
35	CKDHH0894	5.9	5	WM1210	11.0	26	CKDHH0891	8.3	29	CKDHH0892	8.3	7	WM1212	10.1
6	WM1211	5.9	6	WM1211	10.9	23	CKDHH0890	8.3	16	CKDHH0858	8.3	39	CKDHH0880	10.1
44	CZH0616	5.5	47	PH 3253	10.3	44	CZH0616	7.9	46	H513	8.1	44	CZH0616	9.2
50	Local check 2	5.2	46	H513	10.1	46	H513	7.7	50	Local check 2	8.1	49	Local check 1	8.5
49	Local Check 1	5.0	48	DK 8053	9.3	48	DK 8053	7.7	48	DK 8053	8.1	46	H513	8.3
48	DK 8053	4.8	44	CZH0616	9.0	50	Local check 2	7.4	49	Local check 1	7.9	48	DK 8053	7.9
47	PH 3253	4.6	49	Local check 1	8.3	47	PH 3253	7.2	44	CZH0616	7.8	50	Local check 2	7.9
46	H513	4.2	50	Local check 2	7.2	49	Local check 1	7.1	47	PH 3253	7.7	47	PH 3253	5.9
	Grand mean	5.4			9.8			7.8			8.1			9
	$LSD_{0.05}$	1.8			2.3			ns			ns			2
	CV(%)	16.9			11.8			26.1			16.9			11.5
	Heritability	0.7			0.7			0.3			0.2			0.8

Table 6 Mean Performance of the 10 highest yielding among 44 maize Doubled haploids (DH) testcross hybrids, and 6 checks across five locations in Uganda in 2012 combined across five locations in Uganda

Genotype no.	Entry	Grain yield (GY) (t ha^{-1})	Days to anthesis (AD) (days)	Days to silking (SD) (days)	Anthesis silking interval (ASI) (days)	Grain moisture (MOI) (%)	Ear per plant (EPP) (#)	Husk cover (HC) (%)	Ear aspect (EA) (1–5)	Plant aspect (PA) (1–5)
16	CKDHH0858	9.4	60.5	61.8	1.1	15.4	1.0	4.4	2.7	3.2
11	WM1216	9.4	61.0	62.1	1.0	15.3	1.0	5.3	2.8	3.2
17	CKDHH0888	9.2	59.9	60.1	0.6	15.3	1.0	4.2	2.8	3.2
4	WM1209	9.1	60.7	60.6	0.4	15.3	1.0	5.9	2.7	3.2
5	WM1210	9.1	59.2	60.2	1.1	15.3	1.0	5.2	2.6	3.2
26	CKDHH0891	9.0	59.4	59.1	0.3	15.3	1.0	5.3	2.6	3.2
22	CKDHH0860	8.9	59.7	60.5	0.8	15.3	1.0	5.1	2.7	3.2
9	WM1214	8.7	60.8	62.7	1.5	15.3	1.0	5.3	2.8	3.2
42	CKDHH0881	8.7	60.6	61.6	1.0	15.3	1.0	4.0	2.8	3.2
30	CKDHH0877	8.6	62.1	63.3	1.0	15.4	1.0	4.3	2.7	3.2
44	CZH0616	7.9	58.5	59.2	0.8	15.3	1.0	4.9	2.8	3.2
46	H513	7.7	59.1	60.5	1.2	15.3	1.0	7.0	2.8	3.3
48	DK 8053	7.5	59.4	60.6	1.2	15.3	1.0	8.7	2.7	3.2
49	Local Check 1	7.0	62.3	64.1	1.3	15.3	1.1	5.0	2.8	3.2
50	Local Check 2	6.9	60.7	62.3	1.2	15.3	1.0	4.8	2.7	3.2
47	PH 3253	6.7	59.0	59.8	0.9	15.3	1.0	6.0	2.8	3.2
	Min	6.7	59.0	59.8	0.9	15.3	1.0	6.0	2.8	3.2
	Max	9.4	60.5	61.8	1.1	15.4	1.0	4.4	2.7	3.2
	Grand mean	8.0	60.5	61.4	0.8	15.3	1.0	5.2	2.8	3.2
	$LSD_{0.05}$	2.7	2.9	3.4	2.7	2.5	0.3	4.7	0.6	0.7
	CV (%)	17.0	2.5	2.8	164.5	8.3	13.6	46.8	10.5	11.0
	Heritability	0.7	0.9	0.9	0.5	0.1	0.4	0.5	0.6	0.2

Kasese) to 0.999 between Bulindi and Serere, and between Ngetta and Bulindi locations (Table 7). The phenotypic correlations among locations for grain yield varied from −0.0909 between Ngetta and Kasese to 0.6106 between Bulindi and Serere locations (Table 8). Since genotypic correlations between Bulindi and Serere; and between Ngetta and Bulindi were high (r = 0.999), it implies a similar ranking of the genotypes in these pairs of locations.

The genetic correlation between locations for grain yield was used for cluster analysis to classify the environments. Clustering based on genetic correlation for grain yield revealed two clusters at 0.75 (Fig. 1). Cluster 1 consisted of the two locations (Namulonge and Serere) that were separated into individual environment and Cluster II consisted of one individual environment and two sub-clusters (Fig. 1).

Genotype main effect plus genotype × environment interaction biplot analysis of performance and stability

The GGE biplot analysis was used to identify the best entries at each location and assess the stability of the entries. The bi-plot analysis gave a good visual assessment of GE based on grain yield which explained 73 % (PC1 = 54.4 and PC2 = 18.6 %) of the total variation across the test environments (Fig. 2). The environmental vector bi-plot identified Kasese, Serere and Bulindi as highly discriminating for the genotypes tested, as evidenced by the large environment vectors (Fig. 2). Along environment vector represents a good discriminating ability for a given environment. Discriminant test environment accurately resolve genotype differences, thereby

Table 7 Genetic correlations for grain yield of 44 Doubled haploids (DH) testcross hybrids, 1 internal CIMMYT hybrid checks, 3 commercial hybrids and 2 local hybrid checks across five locations in Uganda

	Namulonge	Serere	Bulindi	Ngetta	Kasese
Namulonge	1				
Serere	0.4588	1			
Bulindi	0.9123	0.9999	1		
Ngetta	0.4314	0.5682	0.9999	1	
Kasese	0.708	0.6276	0.8396	−0.2191	1

Table 8 Phenotypic correlations for grain yield of 44 doubled haploids (DH) testcross hybrids, 1 internal CIMMYT hybrid checks, 3 commercial hybrids and 2 local hybrid checks across five locations in Uganda

	Namulonge	Serere	Bulindi	Ngetta	Kasese
Namulonge	1				
Serere	0.3227	1			
Bulindi	0.4013	0.6106	1		
Ngetta	0.167	0.2331	0.2945	1	
Kasese	0.5034	0.4728	0.3956	−0.0909	1

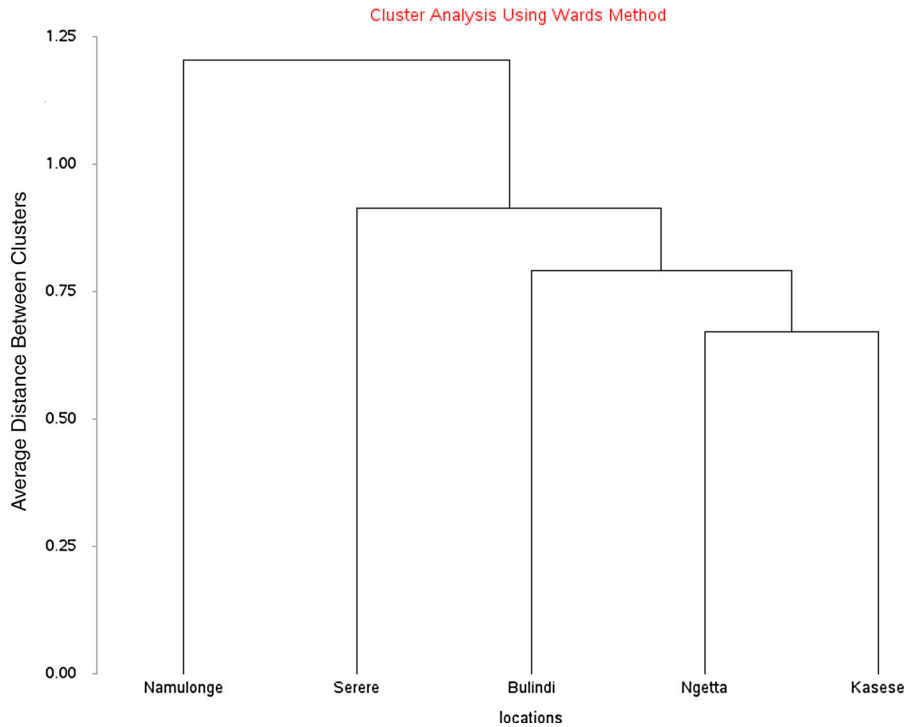

Fig. 1 Dendrogram of five environments based on grain yield, 2012

providing the necessary information for selection by a breeder (Tukamuhabwa et al. 2012). Namulonge was the least discriminating of the five environments, as evidenced by the short environment vector. It's advisable to evaluate genotypes in environments which are most representative and high discriminating

capabilities verses the environments with low discriminating capability and lack of representativeness which might give misleading results.

Based on the five locations used in this study, the results revealed five sectors with two mega environments with different "winning" genotypes identified using a scatter plot with polygon bisectors (Fig. 3). Mega environments are test environments with different winning genotypes located at the vertex of the polygon. Locations within mega environment 1 were Kasese, Namulonge, and Serere. Mega environment II comprised of Ngetta and Bulindi.

The vertex genotypes were G17 (CKDHH0888), G18 (CKDHH0873), G43 (CKDHH0872), G13 (WM1220), and G11 (WM1216) (Fig. 3). The vertex genotype in each sector represents the highest yielding genotype in the location that fell within that particular sector (Yan et al. 2000; Makumbi et al. 2015). Genotype G11 (WM1216) was the vertex entry in the sector where three (Serere, Bulindi, and Ngetta) of the five locations fell, indicating that this genotype was the highest yielding entry in these locations. Genotypes G18 (CKDHH0873), G43 (CKDHH0872) and G13 (WM1220), did not have any location falling in the sectors where they were located, suggesting that these entries were low yielding in some or all of the locations.

The mean versus stability view biplot (Fig. 4) was used to assess stability of the 50 genotypes across the five locations. This biplot accounted for 73 % of the

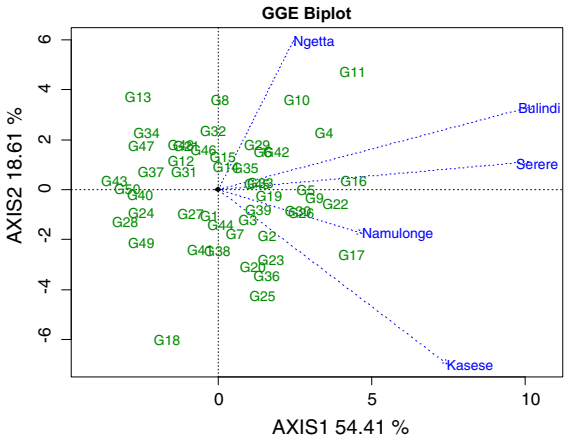

Fig. 2 The Environment vector bi-plot showing environmental differences in discriminating the 50 genotypes for grain yield at the five test environments during 2012 season in Uganda

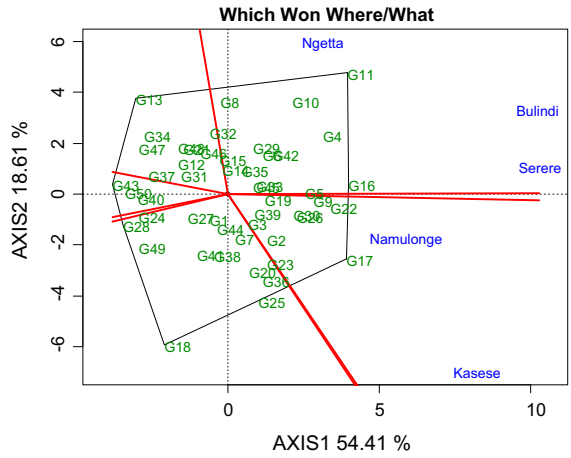

Fig. 3 An environment focused bi-plot showing "winning" genotypes for the two different mega environments for grain yield at the five environments during 2012 seasons in Uganda

variation in grain yield. In this biplot, the axis of the average environment coordinate (AEC) abscissa, or average environment axis, is the single-arrowed line that passes through the biplot origin and the average environment, which is at the center of the small circle. The axis of the AEC ordinate is the double-arrowed line that passes through the biplot origin and is perpendicular to the AEC abscissa (Yan et al. 2007; Makumbi et al. 2015). The cultivars were ranked along the average environment axis, with the arrow pointing to a greater value based on mean performance across all locations. The seven top ranking entries according to their projections onto the average environment axis were: G11 (WM1216), G16 (CKDHH0858), G17 (CKDHH0888), G22 (CKDHH0860), G4 (WM1209), G9 (WM1214) and G5 (WM1210) (Fig. 4). The stability of the cultivars was measured by their projections onto the AEC ordinates. Six entries G45 (CKHDHH0887), G33 (CKDHH0878), G19 (CKDHH0859, G5 (WM1210), G16 (CKDHH0858), and G9 (WM1214) were the most stable because their short projection onto the AEC ordinate. Among the most stable genotypes, testcross G16 (CKDHH0858) was the highest yielding across all locations. The second most high yielding G11 (WM1216), was not among the most stable, suggesting that this variety may have specific adaptation to some of the environments. The commercial check, G50 (Local Check 2) was among the lowest yielding genotype in this study but very stable in the test environments.

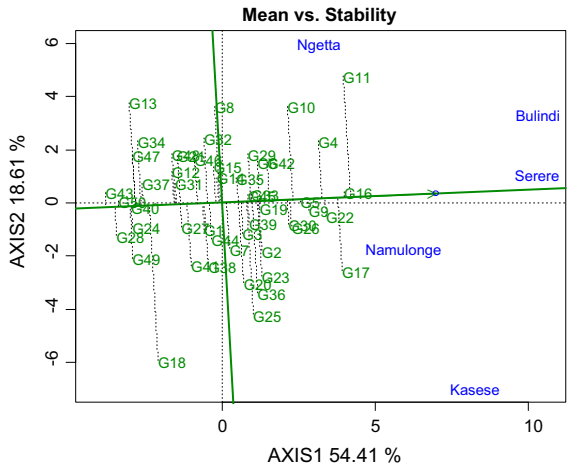

Fig. 4 The mean versus stability view of the genotype main effect plus genotype × environment interaction biplot based on yield data of 50 genotypes grown in five test environments in Uganda

Discussion

In this study we tested the agronomic performance and GE of recently developed DH hybrids in different environments of Uganda, East Africa. The study revealed that genotype, environment and genotype × environment interaction were significant for all the traits except GE for days to silking, Anthesis silking interval (ASI) and Husk cover (HC) suggesting differential responses of the genotypes across environments. This could be attributed to variations in terms of climatic and edaphic factors in the test environments. Similar observations were reported by Butron et al. (2002) in which they indicated that G × E effects for grain yield in maize were mainly due to environmental yield-limiting factors such as the mean minimum temperature and relative humidity. Also, Makumbi et al. (2015) reported similar significant genotypic differences for grain yield, emerged *Striga* plants and other agronomic traits except ASI across *Striga*-infested locations. GE effects for grain yield were found to be lower than the genotype effect as earlier reported by Van Eeuwijk et al. (1995) who found that variation due to the G × E interaction was smaller than the genotypic variation for silage dry matter content of 18 Dutch maize varieties. Also, Beyene et al. (2011) reported that variation due to the G × E interaction was smaller than the genotypic variation while testing for the performance of double haploid maize lines from tropical adapted backcross population. But our results are in contrast to earlier studies were G × E effects were higher than the genotypic effect in a study of early-maturity maize variety trials in France (Epinat-Le et al. 2001).

The DH hybrids used in this study exhibited a broad range of variation in grain yield and other agronomic traits under contrasting environments. Similar observations were reported by Munyiri et al. (2010) who characterized Kenyan maize landraces for drought tolerance; and Odiyo et al. (2014) who examined the performance and adaptability of DH maize testcross hybrids under drought stress and non-stress conditions in East Africa.

According to the results in this study, DH hybrids outperformed the commercial hybrids for grain yield and other agronomic traits assessed. Similar to the present study, Beyene et al. (2011) and Odiyo et al. (2014) reported superiority in performance by DH hybrids over the commercial checks in their studies. The best DH hybrid G16 (CKDHH0858) in our study produced 22 % over the best commercial check G46 (H513) across the five locations. This implied that DH lines were superior in performance over the commercially available hybrids that farmers use. Therefore, the performance of the DH testcross hybrids indicated that the DH lines used in creating them offered potential new sources for rapidly producing high yielding and drought tolerant maize hybrids.

Broad-sense heritability is defined as an estimate of the upper boundary of narrow-sense heritability (Robinson 1963). The moderate broad-sense heritability (0.51–0.59) for Anthesis-Silking interval (ASI), husk cover (HC) and ear aspect (EA) in this study suggested that the actual heritability estimates might be lower (Falconer and Mackay 1996), which may lead to low genetic gain from selection for these traits in the five test environments. Conversely, the broad-sense heritability estimate for grain yield was 0.74, suggesting that actual heritability estimates might be high (Falconer and Mackay 1996), which might lead to high genetic gain when selecting for this trait. For a trait measured from the same genotype in different environments, indirect selection can be applied given information on the heritability and the genetic correlation for the trait in the two environments (Makumbi et al. 2015).

In this study, the majority of the genetic correlations among locations were positive and highly

significant for grain yield. There were some low genetic correlations between some pairs of locations suggesting that these environments are very different (Falconer 1952; Malla et al. 2010; Makumbi et al. 2015). This also indicates that GE has a strong influence (Falconer 1952; Cooper and DeLacy 1994) and hence different systems operate in the two environment (Falconer 1952; Eisen and Saxton 1983). Genotypes × environment interactions are of importance where there are environmental extremes that induce stress conditions (Eisen and Saxton 1983). The Ngetta location had low genetic correlations with other locations in this study. Burdon (1977) pointed out that locations with low genetic correlations between them should be treated separately thus, based on our results Ngetta should be considered a unique environment for evaluations of genotypes for high yield potential.

Genetic correlations can also be used to evaluate similarities among locations. In this study, cluster analysis using genetic correlations based on GY revealed different groups of locations. Similar grouping pattern among locations were reported by Makumbi et al. (2015) while examining the agronomic performance and genotype × environment interaction of Imidazolinone-resistant (IR) open-pollinated maize varieties (OPVs) under *Striga*-infested and *Striga*-free conditions in East Africa. But in contrast, Malla et al. (2010) reported that locations used to evaluate wheat germplasm were not clustered according to geographical location. Results showed that Ngetta and Kasese with different altitudes were distinct from the rest locations. The presence of locations (Ngetta and Kasese) that clustered separately suggested the presence of GE and the effect of different crop management practices (Makumbi et al. 2015). These results provided further support that the presence of GE was due to the low genetic correlations between some locations.

Utilization of GGE biplot analysis gave us good visual information on variety performance and stability. An ideal genotype should have both high mean grain yield and high stability within a mega environment (Yan and Tinker 2006; Makumbi et al. 2015). The most stable test genotypes and the check in this study were: G45 (CKHDHH0887), G33 (CKDHH 0878), G19 (CKDHH0859, G5 (WM1210), G16 (CKDHH0858), and G9 (WM1214) because their short projection onto the AEC ordinate. Among the most stable genotypes testcross G16 (CKDHH0858) had the highest yield across all the five locations. The second most high yielding G11 (WM1216) was not among the most stable, suggesting that this variety may have specific adaptation to some of the environments as previously reported by Badu-Apraku et al. (2012) who identified high yielding but unstable varieties in West Africa. The commercial check, G50 (Local Check 2) was among the lowest yielding genotype in this study but very stable in the test environments (Badu-Apraku et al. 2012).

A number of DH testcross hybrids showed superiority in GY and other agronomic traits compared to all the checks used by the farmers and in the breeding programs. This suggested there would be increase in production and productivity if these hybrids are eventually released and adopted by farmers. Also, the GGE biplot approach used in this study could help breeders to make better decisions on what genotypes should be recommended for release in the region based on adaptation and stability. Experimental hybrids with more than 20 % yield advantage over the commercial check (e.g. CKDHH0858) and stable across environments should be recommended for release in Uganda and other similar environments in East Africa for adoption.

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