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# Testcross performance of doubled haploid maize lines derived from tropical adapted backcross populations

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## Abstract

Doubled haploid (DH) lines produced by in vivo induction of maternal haploids are routinely used in maize breeding. The present study was carried out to assess the performance of 75 doubled haploid maize testcrosses and six checks tested across four locations in Kenya for grain yield, agronomic traits and reaction to major leaf diseases. The 75 DH lines were derived from the backcross (BC1) plants of two CIMMYT bi-parental crosses. Significant location, genotype and genotype x location effects were observed for grain yield and anthesis-silking interval (ASI). Genotypes were significantly different for reaction to leaf blight and gray leaf spot. Location explained 69% of the total phenotypic variance while both genotype and genotype by environment interaction effects contributed 4% each. Fifteen DH testcross hybrids yielded better than the best commercial check, WH505 (5.1 t $ha^{-1}$). The best DH testcross hybrid (CKDHH0223) averaged over the four locations yielded 29.5% higher than WH505. These results indicate that maize testcrosses developed from DH lines produced as high a grain yield and as acceptable agronomic traits as the commercial hybrids developed through conventional pedigree methods. The DH lines identified in the study may be useful for improving yield and disease resistance in maize breeding programs in eastern and southern Africa.

**Keywords**: doubled haploid, maize, Genotype x Environment interaction

## Introduction

Progress on *in vivo* haploid induction by specific pollinators (inducers) has made it possible to produce large numbers of maternal haploid plants. In maize, some genotypes produce pollen that is able to induce maternal haploids (Chase, 1949). Coe (1959) reported the maternal haploid inducer Stock-6, with an induction rate of about 1%. The haploid-inducing capacity of the inducer can be increased by selection (Sarkar et al, 1972) and new inducers with higher rates of haploid induction have been obtained (Eder and Chalyk, 2002; Röber et al, 2005). The pollination results in regular $F_1$ kernels, haploid kernels (maternal embryo) and a regular triploid endosperm. The haploid kernels display a normal germination rate and lead to viable haploid seedlings (Röber et al, 2005; Geiger, 2009). Treating haploid plants with colchicine and selfing them leads to doubled haploid (DH) lines which are highly efficient tools in genetic research and practical breeding (Thomas et al, 2003; Bordes et al, 2007).

The use of doubled haploids has two advantages over inbred lines developed through classical breeding methods. Firstly, it saves time because homozygous plants are produced in one generation, with 100% homozygozity. The conventional inbreeding method results in an average level of homozygozity of 96.9% after five generations of selfing (Briggs and Knowles, 1967). Secondly, the genetic variation of a segregating population can be exploited more rapidly than classical breeding methods such as pedigree selection or single seed descent (Bordes et al, 2007). The efficiency of recurrent selection can also be improved by the use of DH, specifically for low heritability traits (Gallais, 1993; Bouchez and Gallais, 2000; Bordes et al, 2007).

In an effort to develop drought-tolerant maize, the Water Efficient Maize for Africa (WEMA) project was launched among the African Agricultural Technology Foundation (AATF), Monsanto, International Maize and Wheat Improvement Center (CIMMYT) and the National Agricultural Research Systems in Kenya, Mozambique, South Africa, Tanzania and Uganda. The WEMA project uses DH technology to develop drought-tolerant inbred lines and hybrids.

Newly developed cultivars need to be tested in many locations for several years to determine the performance and adaptability of the cultivar before commercial release. Inconsistent genotypic responses to environmental factors such as temperature, soil moisture, soil type or fertility level from location to location and year to year are a function of genotype x environment interactions (GEI). Genotype x environ-

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ment interactions have been defined as the failure of genotypes to achieve the same relative performance in different environments (Baker, 1988). The large GEI variation usually impairs the accuracy of yield estimation and reduces the relationship between genotypic and phenotypic values (Nachit et al, 1992). Previous research suggests that selection of superior genotypes for grain yield and agronomic traits in maize hybrid performance trials is affected by G × E (Pixley and Bjarnason, 2002; Lee et al, 2003; Butron et al, 2004; Beyene et al, 2011). However, little information is available in the literature on the performance of maize testcrosses developed from DH lines. Murigneux et al (1993) and Marhic et al (1998) did not find large differences between DH lines derived by anther culture and lines from pedigree selection or single seed descent (SSD) evaluated for their per se value or their testcross performances. Bordes et al (2007) found that maize lines generated by the doubled haploid method from a broad-base population were as good as those produced by SSD methods for grain yield, kernel moisture, plant height, and ear height and leaf length. Seitz (2005) compared testcross performance of DH lines with conventionally derived lines, and found similar variation. Wilde et al (2010) found that mean testcross performance of the three DH-line groups developed from three European landraces did not differ significantly from the average testcross performance of their parental landraces, but yielded 22-26% lower than that of present elite flint lines. The objective of the present study was to evaluate the performance of doubled haploid maize testcrosses across four locations in Kenya.

## Materials and Methods

### *Genetic materials*

The DH lines were derived from two CIMMYT backcross populations: La Posta Seq C7-F96-1-2-1-1-B-B-B/CML488//CML488 (here afterward referred as Pop1) and La Posta Seq C7-F71-1-2-1-2-B-B-B/CML539//CML539 (here afterward referred as Pop2). The DH lines were developed by means of in vivo haploid induction and later doubled at the Monsanto facility in Mexico. La Posta Seq C7 is a drought-tolerant population developed in CIMMYT Mexico through recurrent selection among full sib/S1 families during a rain-free season where the timing and intensity of stress was managed by irrigation (Edmeades et al, 1999). Selection of the best family was based on high grain yield, small ASI and low level of leaf senescence (stay green) under stress conditions, and adequate yield, small tassel and upright leaf under well-watered conditions (Beck et al, 1997). CML539 and CML488 are drought-tolerant lines, good combiners and are adapted across environments in eastern and southern Africa. Two hundred and fifty BC1 seeds from each population were used for doubled haploid production. After induction, treatment with colchicines and selfing, 47 DH lines were obtained from Pop1 and 124 from Pop2 and grown at Kiboko farm of the Kenya Agricultural Research Institute, during the 2009/2010 short rains season. Based on the results of preliminary per se evaluation (standability, short plant type, low ear placement, and well-filled ears) the best 32 lines were selected from Pop1 and 43 from Pop2 for testcross formation and subsequent field evaluation.

### *Formation of DH Testcrosses*

During the 2010/11 season, all the 75 DH lines were crossed with one single- cross hybrid tester (CML312 x CML442) from the opposite heterotic group in a nursery at Kenya Agricultural Research Institute, Kiboko. The tester has proved useful in hybrid formation for subtropical and mid- altitude environments and has been used in many hybrids in CIMMYT and sub-Saharan national maize breeding programs. The DH lines were used as the females while the single- cross tester was used as the male parent. Seeds were harvested and bulked within each female row plot for use in the testcross evaluations.

### *Field evaluations of the DH Testcrosses*

Seventy-five hybrids and six checks were evaluated in a 9 x 9 alpha lattice field experimental design with three replications per location during the 2010/2011 short rains season (October-February). The trials were planted in four different locations [Kiboko, Embu, Kakamega, and Kirinyaga Technical Institute (KTI) farm in Kenya] (Table 1). The trials at Embu, KTI, and Kakamega were grown under optimum rain-fed conditions whereas the trial at Kiboko was grown under managed drought-stress conditions. At Kiboko, irrigation was withdrawn about two weeks before flowering. Each entry was planted in two-row plots of 5 m length 0.75 m apart, and the hills were spaced 0.25 m apart. Two seeds per hill were planted and later thinned at 3 weeks after emergence to one plant per hill to give a plant population of 53,333 plants per hectare. Fertilizers were applied

Table 1 - Agro-climatic description of the site where the DH testcross hybrids were evaluated.

| Site | Longitude | Latitude | Elevation (masl) | Rain fall (mm) | Temperature (°C) Min | Max | Soil texture |
|---|---|---|---|---|---|---|---|
| Kiboko | 37°75'E | 2°15'S | 975 | 530 | 14.3 | 35.1 | Sandy clay |
| Embu | 37°42'E | 0°449'S | 1510 | 1200 | 14.1 | 25.0 | Clay loam |
| Kakamega | 34°45'E | 0°16'N | 1585 | 1916 | 12.8 | 28.6 | Sandy loam |
| KTI | 37°19'E | 0°34'S | 1282 | 1500 | 18.0 | 24.0 | Clay loam |

Table 2 - Mean squares and degrees of freedom from ANOVA for grain yield, and agronomic traits of 75 doubled haploid maize lines testcrosses and six checks evaluated across locations in Kenya.

| | | Mean Square | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Source | df | Grain Yield (t ha$^{-1}$) | Days to Anthesis | ASI (days) | Plant Height (cm) | Ear Height (cm) | Gray* leaf spot (1-5) | Leaf * blight (1-5) | Moisture (%) |
| Environment (E) | 3 | 435.83** | 5977.76** | 142.83** | 43059.50** | 9909.95** | . | . | 539.19** |
| Genotype (G) | 80 | 4.17** | 27.51** | 6.35** | 700.84** | 502.41** | 0.09** | 0.25** | 13.61** |
| G x E | 240 | 2.65* | 3.70ns | 2.09* | 295.79ns | 127.09ns | . | . | 5.33** |
| Error | 647 | 2.12 | 3.49 | 1.71 | 348.86 | 164.62 | 0.04 | 0.13 | 3.01 |

*Data for gray leaf spot and leaf blight was only from one location

at the rate of 60 kg N and 60 kg $P_2O_5$ ha$^{-1}$ as recommended for the area. Nitrogen was given in two applications. The fields were kept free of weeds by hand weeding.

### *Data collection*

Data from each plot was recorded on number of days to 50% pollen shed, number of days to 50% female flowering, plant height and ear height. Gray leaf spot caused by Cercospora zeae maydis, and leaf blight caused by *Exerohilium turcicum*, were recorded for disease severity on all plants per plot using a 1-5 scale where 1 = no symptoms on leaves, 2 = light disease symptoms on 20-40% of the leaf area, 3 = moderate symptoms on 40-60% of the leaf area, 4 = severe symptoms on 60% of the leaf area, 5 = severe symptoms on 75% or more of the leaf area. For both the diseases, using the visual scale, a plant showing < 1-1.0 was considered highly resistant; 1.1-2.0 resistant; 2.1-3.0 moderately resistant; 3.1-4.0 susceptible; 4.1-5.0 highly susceptible. Grain yield in tons per hectare (t ha$^{-1}$) adjusted to 12.5% moisture content was calculated using unshelled grain weight.

### *Data analysis*

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

## Results

### *Analysis of variance*

Significant (P<0.01) genotype and location effects

Table 3 - Variance decomposition, heritability of grain yield and agronomic traits of 75 doubled haploid maize lines testcrosses and six checks combined across four locations.

| Statistic | Yield | AD | ASI | PH | EH | ET* | GLS* | MOI |
|---|---|---|---|---|---|---|---|---|
| Replication | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Location | 4 | 4 | 4 | 4 | 4 | 1 | 1 | 3 |
| Variance _Location | 3.3 | 24.3 | 0.6 | 149.0 | 25.8 | 0.0 | 0.0 | 2.1 |
| Variance _Entry | 0.2 | 2.0 | 0.4 | 30.7 | 27.9 | 0.05 | 0.02 | 0.8 |
| Variance –Loc x Entry | 0.2 | 0.4 | 0.1 | 3.2 | 7.3 | 0.0 | 0.0 | 0.8 |
| Variance _Residual | 1.1 | 2.1 | 1.5 | 140.1 | 75.6 | 0.1 | 0.03 | 2.2 |
| Grand mean | 5.2 | 74.7 | 1.9 | 214.4 | 115.9 | 3.0 | 1.6 | 14.7 |
| LSD | 1.1 | 1.5 | 1.1 | 10.3 | 8.3 | 0.5 | 0.3 | 2.1 |
| CV | 10.4 | 1.0 | 30.7 | 2.4 | 3.6 | 8.9 | 9.1 | 7.1 |
| Heritability | 0.6 | 0.9 | 0.7 | 0.7 | 0.8 | 0.5 | 0.7 | 0.6 |

*data only from one location; AD = Number of days to anthesis ASI = Anthesis silking interval; PH = Plant height (cm); EH = Ear height (cm); ET= leaf blight; GLS= gray leaf spot; MOI= Moisture index in %

Table 4 - Mean performance of the highest yielding 15 and lowest yielding five doubled haploid maize lines testcrosses and check hybrids evaluated at Embu, Kakamega, KTI and Kiboko in Kenya in 2010.

| Embu | | Kakamega | | KTI | | Kiboko | |
|---|---|---|---|---|---|---|---|
| Entry | Yield (t ha$^{-1}$) | Entry | Yield (t ha$^{-1}$) | Entry | Yield (t ha$^{-1}$) | Entry | Yield (t ha$^{-1}$) |
| CKDHH0249 | 9.9 | CKDHH0191 | 8.4 | CKDHH0223 | 8.6 | CKDHH0192 | 5.6 |
| CKDHH0214 | 8.6 | CKDHH0204 | 7.3 | CKDHH0210 | 8.1 | CKDHH0176 | 5.2 |
| CKDHH0203 | 8.2 | CKDHH0223 | 7.3 | CKDHH0220 | 7.8 | CKDHH0213 | 5.0 |
| CKDHH0204 | 8.1 | CKDHH0235 | 7.1 | CKDHH0186 | 7.8 | CKDHH0236 | 4.7 |
| CKDHH0198 | 7.8 | CKDHH0237 | 6.8 | CKDHH0207 | 7.6 | CKDHH0235 | 4.6 |
| CKDHH0199 | 7.3 | CKDHH0202 | 6.7 | CKDHH0214 | 7.3 | CKDHH0250 | 4.4 |
| CKDHH0250 | 7.3 | CKDHH0248 | 6.7 | CKDHH0235 | 7.3 | CKDHH0238 | 4.3 |
| CKDHH0188 | 7.3 | CKDHH0220 | 6.7 | CKDHH0202 | 7.3 | CKDHH0207 | 4.3 |
| CKDHH0223 | 7.2 | CKDHH0213 | 6.7 | CKDHH0213 | 7.2 | CKDHH0170 | 4.3 |
| CKDHH0245 | 7.2 | CKDHH0241 | 6.7 | CKDHH0248 | 7.1 | CKDHH0245 | 4.3 |
| CKDHH0210 | 7.1 | CKDHH0198 | 6.6 | CKDHH0218 | 7.0 | CKDHH0214 | 4.2 |
| CKDHH0241 | 7.0 | CKDHH0211 | 6.5 | CKDHH0251 | 6.9 | CKDHH0240 | 4.2 |
| CKDHH0206 | 6.9 | CKDHH0218 | 6.4 | CKDHH0206 | 6.9 | CKDHH0243 | 4.1 |
| CKDHH0230 | 6.9 | CKDHH0203 | 6.4 | CKDHH0211 | 6.8 | CKDHH0187 | 4.1 |
| CKDHH0211 | 6.9 | CKDHH0171 | 6.3 | CKDHH0237 | 6.7 | CKDHH0217 | 4.1 |
| CKDHH0179 | 5.0 | CKDHH0247 | 4.1 | CKDHH0216 | 4.4 | CKDHH0194 | 2.1 |
| CKDHH0215 | 5.0 | CKDHH0174 | 3.8 | CKDHH0205 | 4.3 | CKDHH0171 | 2.0 |
| CKDHH0218 | 4.9 | CKDHH0239 | 3.5 | CKDHH0177 | 4.3 | CKDHH0169 | 2.0 |
| CKDHH0229 | 4.6 | CKDHH0222 | 3.4 | CKDHH0183 | 3.4 | CKDHH0226 | 2.0 |
| CKDHH0239 | 4.4 | CKDHH0216 | 2.9 | CKDHH0239 | 2.9 | CKDHH0241 | 1.8 |
| Checks | | | | | | | |
| CML312/CML442 | 7.6 | | 5.4 | | 4.9 | | 2.5 |
| CML395/CML444 | 6.0 | | 6.3 | | 6.9 | | 4.1 |
| H513 | 4.7 | | 4.7 | | 4.9 | | 2.3 |
| WH505 | 6.7 | | 5.2 | | 6.3 | | 2.0 |
| Local Check 1 | 5.2 | | 5.6 | | 3.1 | | 3.6 |
| Local Check 2 | 5.2 | | 4.0 | | 3.3 | | 1.9 |
| Var_Entry | 0.4 | | 0.5 | | 0.6 | | 0.1 |
| Var_Resid | 1.2 | | 0.8 | | 0.9 | | 1.6 |
| Gmean | 6.3 | | 5.5 | | 5.8 | | 3.3 |
| LSD | 1.9 | | 1.5 | | 1.6 | | 2.1 |
| CV | 15.3 | | 14.0 | | 14.2 | | 33.3 |
| Heritability | 0.5 | | 0.6 | | 0.7 | | 0.2 |

were observed for all traits (Table 2). The Genotype x location interaction effect was highly significant ($P<0.01$) for moisture content and significant ($P<0.05$) for grain yield and ASI. Location alone accounted for 69% of the total variance for grain yield (Table 3) while genotype and genotype x location interaction were comparable (each about 4% of the total variance).

*Mean performance in individual locations*

Grain yields for the hybrids ranged from 4.4 to 9.9 t ha$^{-1}$ at Embu, 2.9 to 8.4 t ha$^{-1}$ at Kakamega, 2.9 to 8.6 t ha$^{-1}$ at KTI and 1.8 to 5.6 t ha$^{-1}$ at Kiboko (Table 4). The highest yielding experimental hybrids at Embu, Kakamega, KTI and Kiboko were 47.76%, 61.54%, 36.50% and 180% above the best commercial hybrid, WH505, respectively. At Kiboko (under managed drought-stress conditions) the mean grain yield of hybrids was 51%, 56% and 59% of their mean grain yield at Embu, KTI and Kakamega, respectively. The high-yield locations were Embu (6.3 t ha$^{-1}$) followed by KTI (5.8 t ha$^{-1}$). The lowest mean yield was obtained at Kiboko (3.3 t ha$^{-1}$). The highest heritability for grain yield was obtained at KTI ($h^2$ = 0.68) and the lowest heritability for grain yield was recorded at Kiboko ($h^2$ = 0.19).

*Mean performance averaged across locations*

Averaged across four locations, 15 DH testcross hybrids yielded equivalent to or more than the commercial check, WH505 (Table 5). Three hybrids, CKDHH0223 (6.6 t ha$^{-1}$), CKDHH0214 (6.5 t/h) and CKDHH0213 (6.3 t ha$^{-1}$) yielded significantly higher than the best commercial check, WH505 (5.1 t ha$^{-1}$). The worst performing hybrids were CKDHH0239 (3.6 t ha$^{-1}$), and CKDHH0216 (3.6 t ha$^{-1}$). The best hybrid, (CKDHH0223) averaged over the four locations yielded 29.5% better than the best commercial check. The number of days to 50% female flowering ranged from 70.8 to 78.2 days with hybrid CKDHH0216 being the earliest to flower. Plant height ranged from 200 to 233.7 cm with an average of 214.4 cm. By com-

parison, the commercial check hybrid had an average plant height of 211.4 cm, ear height of 114.2 cm and an average of 75.6 days to 50% female flowering. The most resistant hybrids to leaf blight were CKDHH0213 and CKDHH0315 with scores of 2.4 and 2.6, respectively. Hybrid CKDHH0216, however, was the most susceptible to leaf blight with a disease score of 4.0. The remaining hybrids were moderately resistant with disease scores of 2.4 to 4.0. All the experimental hybrids and checks were resistant to GLS with a disease score below 2.0. Medium to high heritability estimates were found in different traits. Heritability ($h^2$) of days to 50% female flowering, ASI, ear and plant heights were higher than the heritability of grain yield and moisture content (Table 3). The highest heritability ($h^2$ = 0.9) was recorded for days to 50% female flowering and the lowest was for leaf blight disease score ($h^2$ = 0.5).

All sites were positively correlated with each other with the highest between KTI and Kiboko (0.37) and the lowest at Kakamega and Kiboko (r = 0.07), (Table 6). The correlation between Kakamega and Embu was high (r = 0.31) indicating a similar ranking of the genotypes in these two locations.

## Discussion

Efficient production of maize DH lines through the in vivo method provided breeders with a tool for the production of pure lines within one to two generations and an increase in genetic advance per unit of time at the level of hybrid development. Combined analysis of variance showed that all sources of variations were significant. This study first underlines the magnitude of the environmental effect which accounts for about 69% of the total variance and equal contributions of genotypic (4%) and genotypic x environment interaction (4%), (Table 3). G x E effects were, however, found to be higher than the genotypic effect from a study of early maize variety trials in France (Epinat-Le Signor et al, 2001). Van Eeuwijk et al (1995) studied silage dry matter content data from the Dutch maize variety trials evaluated at four locations and found that variety x environment interaction was small in comparison with the variety main effect.

A significant genotype x environment interaction effect observed for grain yield, kernel moisture and ASI (Table 2) indicates the differential response of genotypes across different environments. This is in agreement with findings of Burger et al (2008) for Central European breeding materials. Beyene et al ( 2011) found a similar effect for tropical insect- resis-

Table 5 - Mean performance of the highest yielding 15 and lowest yielding five doubled haploid maize lines testcrosses and check hybrids combined across four locations.

| Entry | Yield | AD | ASI | PH | EH | ET | GLS | MOI |
|---|---|---|---|---|---|---|---|---|
| CKDHH0223 | 6.6 | 76.6 | 1.8 | 226.6 | 127.4 | 3.2 | 1.5 | 16.0 |
| CKDHH0214 | 6.5 | 77.0 | 1.3 | 223.6 | 118.2 | 3.2 | 1.5 | 15.6 |
| CKDHH0213 | 6.3 | 74.5 | 1.1 | 218.4 | 118.1 | 2.4 | 1.5 | 17.2 |
| CKDHH0203 | 6.2 | 74.7 | 1.4 | 222.0 | 123.2 | 3.1 | 1.5 | 14.9 |
| CKDHH0235 | 6.2 | 78.2 | 1.5 | 219.9 | 125.9 | 2.6 | 1.5 | 15.3 |
| CKDHH0249 | 6.2 | 75.8 | 1.4 | 217.3 | 116.1 | 3.0 | 1.5 | 13.9 |
| CKDHH0204 | 6.2 | 74.3 | 1.3 | 225.2 | 120.4 | 2.8 | 1.5 | 16.4 |
| CKDHH0198 | 6.1 | 76.7 | -0.2 | 213.5 | 123.3 | 3.0 | 2.0 | 14.7 |
| CKDHH0210 | 6.1 | 76.0 | 0.6 | 218.4 | 129.1 | 2.9 | 1.5 | 14.8 |
| CKDHH0207 | 6.1 | 74.9 | 1.0 | 233.7 | 124.2 | 2.9 | 1.5 | 15.7 |
| CKDHH0247 | 4.4 | 74.7 | 2.0 | 212.0 | 106.3 | 2.7 | 1.5 | 13.9 |
| CKDHH0179 | 4.2 | 73.3 | 1.2 | 204.6 | 106.6 | 3.0 | 1.7 | 13.4 |
| CKDHH0174 | 4.1 | 74.4 | 1.3 | 203.9 | 110.4 | 3.5 | 1.7 | 14.2 |
| CKDHH0239 | 3.6 | 75.6 | 2.6 | 200.0 | 108.2 | 3.4 | 1.5 | 14.1 |
| CKDHH0216 | 3.6 | 70.8 | 1.3 | 211.9 | 109.0 | 4.0 | 1.8 | 12.4 |
| WH505 (check 1) | 5.1 | 75.6 | 2.4 | 211.4 | 114.2 | 3.1 | 1.8 | 13.3 |
| CML395/CML444 | 5.8 | 76.9 | 1.5 | 233.5 | 126.5 | 3.3 | 1.5 | 15.7 |
| CML312/CML442 | 5.1 | 72.5 | 2.5 | 223.3 | 112.7 | 3.4 | 1.7 | 14.3 |
| Local Check (DT) | 4.4 | 72.4 | 3.1 | 215.5 | 113.5 | 3.4 | 1.8 | 10.5 |
| H513 | 4.2 | 73.0 | 2.7 | 207.9 | 117.4 | 3.3 | 2.2 | 13.4 |
| | | | | | | | | |
| Min | 3.6 | 70.8 | -0.2 | 200.0 | 104.6 | 2.4 | 1.5 | 10.5 |
| Max | 6.6 | 78.2 | 3.8 | 233.7 | 130.3 | 4.0 | 2.2 | 17.2 |
| $G_{mean}$ | 5.2 | 74.7 | 1.9 | 214.4 | 115.9 | 3.0 | 1.6 | 14.7 |
| LSD | 1.1 | 1.5 | 1.1 | 10.3 | 8.3 | 0.5 | 0.3 | 2.1 |
| CV | 10.4 | 1.0 | 30.7 | 2.4 | 3.6 | 8.9 | 9.1 | 7.1 |
| Heritability | 0.6 | 0.9 | 0.7 | 0.7 | 0.8 | 0.5 | 0.7 | 0.6 |

AD = Number of days to anthesis; ASI = Anthesis silking interval; PH = Plant height (cm); EH = Ear height (cm); ET= leaf blight; GLS= gray leaf spot; MOI= Moisture index in %

Table 6 - Genetic correlation for grain yield of 75 doubled haploid maize lines testcrosses evaluated at four locations in Kenya.

| | Embu | Kakamega | Kiboko | KTI |
|---|---|---|---|---|
| Embu | 1.00 | 0.31 | 0.21 | 0.23 |
| Kakamega | | 1.00 | 0.07 | 0.31 |
| Kiboko | | | 1.00 | 0.37 |
| KTI | | | | 1.00 |

tant maize hybrids. This deviates, however, from the results of Wilde et al (2010) for doubled haploid testcrosses developed from temperate landraces, and of Lorenzana and Bernardo (2008) for Northern Corn Belt germplasm.

Hybrids CKDHH0235, CKDHH0213 and CKDHH0250 performed well across optimum and stress locations (Table 4) indicating that it is possible to combine stress tolerance and yield potential in tropical doubled haploid maize hybrids. Similar results have been reported with temperate maize hybrids where improvements for tolerance to abiotic and biotic stresses have been associated with the ability to maximize grain yield under non-stress growing conditions (Castleberry et al, 1984; Carlone and Russell, 1987; Duvick, 1997). Genetic variance and heritability for grain yield were higher for optimum environments (Kakamega, Embu and KTI) than for a stress environment (Kiboko) indicating that expected breeding progress during the development of maize inbred is generally lower under stress than under optimal conditions (Bànziger et al, 1997). The genetic correlation for grain yield between Kakamega (optimal) and Kiboko (stress) was low (r = 0.07) suggesting that hybrids which perform well under optimal conditions may not perform well under stress conditions. These results were supported by our findings where only two of the top ten hybrids averaged across three optimum environments were in the top ten under managed stress (Table 4). These results were in agreement with previous maize studies where the genetic correlation for grain yield between stress and optimal environments seems to decrease as stress intensity increases (Bànziger et al, 1997; Cooper et al, 1997; Fukai et al, 1999).

The best DH testcross hybrid (CKDHH0223) averaged over the four locations produced 29.5% higher grain yield than the best commercial check WH505 (Table 5). These hybrids were comparable with the best check hybrids in terms of flowering, plant height, ear height, reaction to leaf blight and gray leaf spot but had slightly higher moisture content. Therefore the performance of these DH testcross hybrids relative to that of WH505 (commercial check) indicated that the superior lines identified in this study are useful sources for improving yield in maize-growing areas of Kenya and other similar environments in eastern and southern Africa. Unlike in the present study, Wilde et al (2010) found that the mean testcross performance of the three DH-line groups developed from three European landraces did not differ significantly from the average testcross performance of their parental landraces, but was 22–26% lower than that of present elite flint lines. This might be due to the fact that in our study, the DH lines were derived from the most elite tropical adapted lines while in the previous study they were generated from landraces.

The 81 genotypes tested presented a range of variability for grain yield and other agronomic traits with opportunities for selecting maize genotypes for high yield and acceptable agronomic traits. Averaged across four locations, 10 DH testcross hybrids yielded over 6 t $ha^{-1}$ (Table 5). These hybrids had a 1 t $ha^{-1}$ yield advantage over the best commercial check hybrid (WH505 and were comparable to the check in terms of agronomic traits and reaction to gray leaf spot and leaf blight, indicating that they could be suitable for growing in a wide range of environments. All the experimental hybrids and checks appeared to be resistant to GLS and had a varying level of resistance to leaf blight with a disease scoring range from 2.4 to 4.0 (Table 5). Gray leaf spot and leaf blight are the cause of significant yield losses in maize (Bosque-Perez, 2000; Pratt and Gordon, 2006). Yield losses of 60% or more have been reported from the two diseases (Raymundo and Hooker, 1981; Ward et al, 1997). Therefore the resistant DH lines identified could be incorporated in maize breeding programs in eastern and southern Africa in efforts directed towards developing high yielding and disease resistant varieties.

Our results indicate that maize testcrosses developed from DH lines produce as high a grain yield and as acceptable agronomic traits as commercial hybrids developed through conventional pedigree methods. DH technology provides the opportunity to shorten the time required for the development of homozygous lines through pedigree breeding and to increase the genetic gain per unit of time.

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