# Relationship between hybrid performance and AFLP based genetic distance in highland maize inbred lines

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# Abstract

The objectives of this study were to determine the crossing performance of highland maize inbred lines for grain yield, days to silk and plant height; estimate genetic distance (GD) among the inbred lines and in association with tester parents, and to investigate the relationship of GD with hybrid performance and midparent heterosis (MPH). A total of 26 inbred lines were crossed with six (population and line) testers in a factorial-mating scheme. The $F_1$'s and the parents were evaluated at five locations in Ethiopia. Nine amplified fragment length polymorphism (AFLP) primer pairs were used to genotype all the parents. The $F_1$'s were found to vary widely for grain yield and other traits measured. Yield superiority of more than 30% over the best hybrid check was obtained for some testcross hybrids. Midparent heterosis on average was moderate for grain yield and, plant height. And for days to silking, MPH values were mostly negative. Mean GD values determined from the inbred lines by population tester (0.680) and line tester (0.661) combinations were not significantly different. Cluster analysis separated the tester parents from the corresponding inbred lines. AFLP grouping of the inbred lines was in agreement with their pedigree records. Genetic distances derived from the inbred lines × all testers and from the population testers' sub-group were not positively correlated with hybrid performance and MPH for most traits. In contrast, correlations of GDs involving the line testers' sub-group with $F_1$'s and MPH were significantly positive but with low magnitude to be of predictive value.

**Abbreviations** *AFLP* Amplified fragment length polymorphism - *GD* Genetic distance - *MPH* Midparent heterosis

# Introduction

Maize (*Zea mays* L.) is one of the important cereals broadly adapted worldwide. In Ethiopia, it is grown in the lowlands, the mid-altitudes and the highland regions. It is an important field crop in terms of area coverage, production and utilization for food and feed purposes. However, maize varieties mostly grown in the highlands (altitude = 1,700–2,400 masl.) of Ethiopia are local cultivars. They are low yielding, vulnerable to biotic and abiotic constraints and also exhibit undesirable agronomic performances such as late maturity and susceptibility to root and stalk lodging (EARO *2000*). Enhancement of maize production and productivity can be achieved through identification of potentially superior inbred line combinations in the form of hybrids (Bernardo *1999*; Saleh et al. *2002*).

In maize, hybrid breeding remains the method of choice for attaining maximum genetic gain from the effects of heterosis. Nevertheless, identification of parental inbred lines leading to superior hybrid combinations is a crucial factor (Hallauer et al. *1988*). Such activities using conventional breeding methods are expensive and time consuming. Furthermore, the large number of possible hybrid combinations to be produced from a relatively small number of inbred lines, render the evaluation of all possible combinations unfeasible (Bernardo *1992*; Betran et al. *2003*). In addition, morphological markers have shortcomings to detect differences among closely related genotypes and are influenced by prevailing environmental conditions. The efficiency of hybrid breeding program could be increased if the inbred lines per se could be screened for genetic diversity using molecular markers and superior crosses are accurately predicted prior to field evaluation (Melchinger et al. *1991*).

Molecular markers are not influenced by environmental factors and are also fast, efficient and more sensitive than field testing to detect large numbers of distinct differences between genotypes at the DNA level (Melchinger *1999*). However, one should not overlook the importance of field testing across years and locations to identify phynotypically desirable hybrid combinations.

Molecular markers use to meet a number of objectives including genetic diversity analysis and prediction of hybrid performances in different crop species (Melchinger *1999*). Currently several molecular marker techniques are available serving various purposes in crops. Amplified fragment length polymorphism (AFLP) is one of the well-known molecular marker systems relying on polymerase chain reaction (PCR) technique (Mullis et al. *1986*) for DNA amplification. It requires no prior sequence knowledge and can detect large number of genetic loci than restrict fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers (Pejic et al. *1998*). On the other hand, single nucleotide polymorphism (SNP) markers have highly automated DNA scoring potential than AFLPs (Suliman-Pollatschek et al. *2002*); however, they are expensive and demand high technology input and special instruments are required for many SNP genotyping technologies (Ching and Rafalski *2002*). It is also known that the AFLP technique had lower cost and is more transferable across species than SSR markers (Garecia et al. *2004*). In maize, AFLP

techniques have been applied to genome mapping (Ajmone-Marsan et al. *2001*), DNA fingerprinting (Oliveira et al. *2004*), genetic diversity studies (Garecia et al. *2004*) and hybrid performance prediction (Sheng and Rui *2000*; Barbosa et al. *2003*).
Previous studies conducted to assess genetic diversity and to predict hybrid performance in maize were mostly focused on temperate germplasm (Melchinger *1999*). Using AFLP markers, some information on tropical maize germplasm is present but the genotypes studied were of lowland tropical origin (Sheng and Rui *2000*; Barbosa et al. *2003*; Garecia et al. *2004*). No such information is available on tropical highland maize gerplasm serving breeding programs. The objectives of this study were to (1) estimate genetic distance (GD) values for AFLPs among highland maize inbred lines and in association with their tester parents, (2) determine the crossing performances of the inbred lines for grain yield, days to silk and plant height, and (3) investigate the relationship of GD with hybrid performance and heterosis for grain yield, days to silk and plant height.

# Materials and methods

**Field evaluation**
The study materials involved 26 maize inbred lines derived from three different populations, 1) Kitale Synthetic II × N3-type inbred lines, 2) Ecuador-573 × SC-type inbred lines, and 3) Pool9A × IITA's mid-altitude streak resistant population by the CIMMYT regional program in Zimbabwe. They were introduced to Ethiopia and selected for tolerance to common foliar diseases [northern leaf blight (NLB, caused by *Exserohilum turcicum*); common rust (*Puccinia sorghi*); and gray leaf spot (GLS, *Cercospora zeae-maydis* Tehon)], vigour and general adaptation to the highland regions (Twumasi-Afriyie *2001*). The pedigree and genetic background of the study materials is given in Table 1.

**Table 1** Identification and pedigree of highland maize inbred lines and testers assayed for genetic distance using AFLP markers and evaluated for hybrid performances

| No | Genotypes | Pegigree | Source |
|---|---|---|---|
| 1 | AMBOON6-1 | KIT/SNSYN ((N3) TUXC1F1 # ## (GLS = 1) 6-1 | CIMMYT |
| 2 | AMBOON6-4 | KIT/SNSYN ((N3) TUXC1F1 # ## (GLS = 1) 14-1 | CIMMYT |
| 3 | AMBOON6-8 | KIT/SNSYN ((N3) TUXC1F1 # ## (GLS = 1) 7-3 | CIMMYT |
| 4 | AMBOON6-9 | KIT/SNSYN ((N3) TUXC1F1 # ## (GLS = 1) 11-1 | CIMMYT |
| 5 | AMBOON6-14 | KT/SNSYN ((N3) TUXC1F1 # ## (GLS = 1) 11-2 | CIMMYT |
| 6 | AMBOON6-15 | KIT/SNSYN ((N3) TUXC1F1 # ## | CIMMYT |

| No | Genotypes | Pegigree | Source |
|---|---|---|---|
| | | (GLS = 1) 14–2 | |
| 7 | AMBOON6-20 | SRSYN95 ((KIT/N3) TUXF1 # ## (GLS = 1) 6–1 | CIMMYT |
| 8 | AMBOON6-21 | ECU/SNSYN (SC/ETO) C1 F1 ### (GLS = 1.5) 16-1 | CIMMYT |
| 9 | AMBOON6-22 | ECU/SNSYN (SC/ETO) C1 F1 ### (GLS = 2.0)-3-1 | CIMMYT |
| 10 | AMBOON6-23 | ECU/SNSYN (SC/ETO) C1 F1 ### (GLS = 2.0)-8-2 | CIMMYT |
| 11 | AMBOON6-25 | ECU/SNSYN (SC/ETO) C1 F1 ## # (GLS = 2.5)-24-2 | CIMMYT |
| 12 | AMBOON6-26 | ECU/SNSYN (SC/ETO) C1 F1 ## # (GLS = 2.5)-42-3 | CIMMYT |
| 13 | AMBOON6-27 | ECU/SNSYN (SC/ETO) C1 F1 ## # (GLS = 3.0)-23-1 | CIMMYT |
| 14 | AMBOON6-29 | ECU/SNSYN (SC/ETO) C1 F1 ### (GLS = 3.5)-41-1 | CIMMYT |
| 15 | AMBOON6-37 | SRSYN95 ((ECU/SC/ETO) F1# # # (GLS = 3)-21-1 | CIMMYT |
| 16 | AMBOON6-38 | SRSYN95 ((ECU/SC/ETO) F1# # # (GLS = 3.5)-40-1 | CIMMYT |
| 17 | AMBOON6-39 | SRSYN95 ((ECU/SC/ETO) F1# ## (GLS = 3.5)-4-2 | CIMMYT |
| 18 | AMBOON6-40 | SRSYN95 ((ECU/SC/ETO) F1# # # (GLS = 3.5)-39.1 | CIMMYT |
| 19 | AMBOON6-41 | POOL9AC-7-SR (BC2) FS-1-1-3-1 | CIMMYT |
| 20 | AMBOON6-42 | POOL9AC-7-SR (BC2) FS-1-4-2-3 | CIMMYT |
| 21 | AMBOON6-44 | POOL9AC-7-SR (BC2) FS-4-3-SR-1-1 | CIMMYT |
| 22 | AMBOON6-47 | POOL9AC-7-SR (BC2) FS-50-1-2-3 | CIMMYT |
| 23 | AMBOON6-49 | POOL9AC-7-SR (BC2) FS-89-2SR-1-1 | CIMMYT |
| 24 | AMBOON6-54 | POOL9AC-7-SR (BC2) FS-170-2-1-3 | CIMMYT |
| 25 | AMBOON6-59 | POOL9AC-7-SR (BC2) FS-232-4-1-3 | CIMMYT |
| 26 | AMBOON6-60 | POOL9AC-7-SR (BC2) FS-48-1-1-3 | CIMMYT |
| 27 | KITALE Syn II (Pop. tester) | Tuxpeò o derived germplasm | Kenya |
| 28 | ECUADOR 573(Pop. tester) | Montana race | Kenya |

| No | Genotypes | Pegigree | Source |
|---|---|---|---|
| 29 | KULENI (Pop. tester) | Pool 9A | Ethiopia |
| 30 | 142 B1-e (Inbred line tester) | Derived from Ecuador 573 | Ethiopia |
| 31 | F7215 (Inbred line tester | Derived from Kitale Syn II) | Ethiopia |
| 32 | POOL9A-MHM (Inbred tester) | Derived from Poo9A | Ethiopia |

The inbred lines were crossed with six local testers, three populations [Kitale Syn. II, Ecuador 573, and Kuleni (Pool9A)] and three inbred testers (142-1-e, F7215, Pool9A-MHM); in a factorial mating design (Design II) that resulted in 156 $F_1$ progenies. The 156 $F_1$ crosses, plus two hybrid checks (BH540, BH660) and their 32 parents were evaluated in separate trials across wide range of environments representing mid-altitude and highland of Ethiopia namely: Ambo, Awassa, Bako, Holeta and Kulumsa in 2002. Awassa and Bako lie in the mid-altitudes, between 1,650 m and 1,700 m. above sea level and receive 1,250 and 1,110 mm annual rainfall, respectively. Ambo, Kulumsa and Holeta are found in the highland ranging from 2,200 m to 2,400 m above sea level. The average annual rainfall at Ambo, Holeta and Kulumsa are 1,115, 1,250 and 830 mm, respectively. The soil at Awassa and Kulumsa is andosol and at Bako it is characterized as nitosol, and at Ambo and Holeta the dominant soil type is vertisol. The experimental design was an alpha (0, 1) lattice (Patterson and Williams *1976*) with two replications at each location. The trials of parental inbred lines were grown directly adjacent to the $F_1$ progenies. The experimental unit consisted of a single five-metre long row with 75 cm spacing between rows. Planting was done using two seeds per hill and 25 cm apart between hills. Thinning was performed at the three to five leaf stages to attain a final plant density of 53,000 plants $ha^{-1}$. All other management practices including planting, fertilization, weeding and harvestings were performed as per the recommendations for each location. Grain yield (Mg $ha^{-1}$) adjusted to 12.5% moisture, numbers of days to silking (days) and plant height (cm) were recorded on a per plot basis.

**DNA extraction and AFLP analysis**

For AFLP analysis, leaf tissue from each genotype was harvested from 3 to 4-week-old seedlings grown in the greenhouse at the University of Pretoria, South Africa. Genomic DNA for each inbred line was isolated from the leaf tissue following Hexa-decyltrimethyl-ammonium bromide (CTAB) DNA extraction procedure (Doyle and Doyle *1987*). AFLP reactions were performed according to the protocol of Vos et al. (*1995*) except that in selective amplification, *Eco*RI primers were 5′ labelled with infrared dye (IRDye 700 or IRDye 800, LI-COR, Lincoln, NE, USA). The reactions were performed in 11 µl volumes containing 5 µl diluted pre-selective amplification reaction product, 10 × PCR buffer (1.5 mM Mg $Cl_2$), 2.5 mM of each dNTP, 0.5 mM $MgCl_2$, 1 µM IRDye 700/800-labelled *Eco*RI primers, 10 µM *Mse*I primer, and 5 U Ampli-Taq DNA polymerase (Promega).

**Electrophoresis and image analysis**
AFLP fragments were resolved in polyacrylamide gels containing 8% Long Ranger gel solution (BMA, Rockland, ME, USA), 7.0 M urea and 0.8 × TBE (71.2 mM Tris, 71.2 mM boric acid, and 1.6 mM EDTA) using LI-COR $IR^2$ automated DNA analysers (LI-COR, Lincoln, NE USA). The gel images were scored in a binary system that recorded the presence of band as plus (+) and absence of band as minus (−). Semi-automated scoring was performed with the $SAGA^{MX}$ software (Version 3.2, LI-COR). Scores were manually edited to make corrections to the automated score where necessary.

**Data analysis**
The data matrix was used to perform cluster analysis on the basis of average linkage method, known as the Unweighted Pair Group Method using Arithmetic averages (UPGMA) as applied in NCSS software package (Hintze *1998*). The average polymorphic information content (PIC) was calculated across each primer combination according to Riek et al. (*2001*). Estimates of genetic similarity between pairs of inbred lines and in association with each tester genotype were calculated in the form of dissimilarity and expressed as Euclidean GD. The "goodness of fit" of the clustering algorithm to the data matrix was determined by calculating the cophenetic correlation coefficient between the dissimilarity matrix and the cophenetic matrix derived from the dendrogram (Sneath and Sokal *1973*).

Analyses of variances (ANOVA) were performed for grain yield, days to sliking and plant height on data collected from the $F_1$s and the parental trials at each location and across locations. (Agrobase *2001*). Midparent heterosis (MPH) manifested in the hybrids for all the traits were computed (Betran et al. *2003*). Simple correlation coefficients were calculated for AFLP GD of various groupings, all testers, population testers and inbred testers with their respective $F_1$s and MPH values determined for each trait.

# Results

**Hybrid performance and heterosis**
In maize, promising hybrid varieties express desirable mean performances for yield and other agronomic attributes. The magnitude of heterosis manifested by such hybrids is also of interest in a breeding program. The means and ranges of hybrid performances and MPH determined for all traits are presented in Tables 2 and 3. Grain yield of all $F_1$s ranged from 5.2 (AMBOON6-22 × Kuleni) to 11.6 Mg $ha^{-1}$ (AMBOON6-20 × 142-1-e) with an overall mean of 7.6 Mg $ha^{-1}$. Mean yield of $F_1$s resulted from crosses of population testers and inbred line testers, varied between 7.4 Mg $ha^{-1}$ and 7.9 Mg $ha^{-1}$, respectively. Mean yield of $F_1$s resulted from inbred line tester combinations differed significantly ranging from 7.1 Mg $ha^{-1}$ to 9.1 Mg $ha^{-1}$, and among population testers the value ranged between 7.3 Mg $ha^{-1}$ and 7.8 Mg $ha^{-1}$ (Table 2).

**Table 2** Mean and ranges of hybrid performances for grain yield (Mg $ha^{-1}$), days to silking (days) and plant height (cm) for all testers, population testers, line testers and individual tester crosses

| Cross combinations | No. | Grain yield | | Days to silking | | Plant height | |
|---|---|---|---|---|---|---|---|
| | | Mean (SE) | Range | Mean (SE) | Range | Mean (SE) | Range |
| All male × Female[a] | 156 | 7.6 (0.1) | 5.2–11.6 | 85.6 (0.2) | 81.1–91.5 | 245.0 (1.2) | 213.2–294.6 |
| Pop. Tester crosses[b] | 78 | 7.4 (0.1) | 5.2–9.4 | 84.6 (02) | 81.1–89.5 | 241.4 (1.3) | 218.5–274.2 |
| Line Tester crosses[c] | 78 | 7.9 (0.1) | 5.3–11.6 | 86.7 (0.2) | 82.7–91.5 | 248.7 (2.0) | 213.2–294.6 |
| Kit. Syn.II × Female[d] | 26 | 7.3 (0.1) | 5.5–8.3 | 84.3 (0.3) | 81.1–87.2 | 239.1 (2.2) | 220.2–257.9 |
| Ecud. 573 × Female[d] | 26 | 7.8 (0.2) | 6.4–9.4 | 84.7 (0.3) | 82.0–88.0 | 242.2 (2.3) | 223.0–274.2 |
| Kuleni × Female[d] | 26 | 7.3 (0.2) | 5.2–8.8 | 84.6 (0.4) | 81.9–89.5 | 242.9 (2.3) | 218.5–270.1 |
| I42-1-e × Female[e] | 26 | 9.1 (0.2) | 7.2–11.6 | 87.9 (0.3) | 85.4–91.5 | 266.6 (2.6) | 246.5–294.6 |
| F7215 × Female[e] | 26 | 7.4 (0.1) | 5.3–8.6 | 86.4 (0.4) | 82.7–89.5 | 246.4 (2.1) | 226.7–269.4 |
| P9a-mhm × Female[e] | 26 | 7.1 (0.1) | 5.7–8.4 | 85.9 (0.4 | 82.5–89.6 | 232.7 (2.0) | 213.2–257.0 |

[a] all inbred lines (female) × all line and population testers (male)
[b] all inbred lines (female) × all population testers
[c] all inbred lines (female) × all line testers
[d] all inbred lines (female) × a population tester
[e] all inbred lines (female) × a line tester

**Table 3** Mean and range of midparent heterosis (MPH) for grain yield, days to silking and plant height for all testers, population testers, line testers and individual tester crosses

| Cross combinations | No | Grain yield (MPH %) | | Days to silking (MPH %) | | Plant height (MPH%) | |
|---|---|---|---|---|---|---|---|
| | | Mean (SE) | Range | Mean (SE) | Range | Mean (SE) | Range |
| All male × Female | 156 | 28.3 (2.6) | −24.2–121.7 | −3.1(0.2) | −9.8–4.2 | 27.7 (0.7) | 10.6–55.0 |
| Pop. Tester crosses | 78 | 5.7 (1.5) | −24.2–44.2 | −0.7 (0.3) | −5.7–4.2 | 24.0 (0.8) | 10.6–43.0 |
| Line Tester crosses | 78 | 51.0 (3.2) | 10.9–121.7 | −5.5 (0.2) | −9.8–0.3 | 31.4 (0.9) | 16.2–55.0 |
| Kit. Syn.II × Female[a] | 26 | 0.9 (2.0) | −16.1–23.3 | 0.8 (0.4) | −5.3–2.4 | 26.4 (1.3) | 14.1–36.3 |
| Ecud. 573 × Female[b] | 26 | 6.4 (2.4) | −24.2–33.2 | −8.0 (0.6) | −5.7–4.2 | 21.2 (1.1) | 10.6–33.7 |
| Kuleni × Female | 26 | 9.8 (3.1) | −17.1–44.2 | −0.4 (0.4) | −3.7–3.1 | 24.4 (1.5) | 10.6–43.0 |
| I42-1-e × Female | 26 | 77.2 (5.2) | 21.5–121.7 | -6.1 (0.4) | −8.8–1.6 | 31.1 (1.3) | 17.8–43.7 |
| F7215 × Female | 26 | 33.7 (2.9) | 10.9–59.0 | −5.2 (0.3) | −7.6–1.0 | 25.1 (1.0) | 16.2–35.2 |
| P9a-mhm × Female[c] | 26 | 41.9 (4.7) | 11.3–82.7 | −5.3 (0.4) | −9.8–0.3 | 37.8 (1.5) | 25.2–54.9 |

[a] Kitale Synthetic II
[b] Ecuador 573
[c] Pool9a-HMH

Midparent heterosis determined for grain yield across all $F_1$ hybrid crosses ranged from 24.2% to 121.7%. Significantly different average $F_1$ yield heterosis between crosses of population testers (5.7%) and line testers (51.0%) were determined. Such differences were profoundly greater when considering values determined for crosses of each individual tester; MPH varied between 0.9% (Kitale Syn. II) and 77.2% (142-1-e) (Table 3).

Mean performance of days to silking for all $F_1$s differed from 81.1 to 91.5 days across locations (Table 2). Plant height ranged from 213.2 cm to 294.6 cm between the shortest and the tallest hybrids. Non-significant mean $F_1$ values for days to silking and plant height across all tester parents were recorded. Midparent heterosis averaged for days to silkings were low and mostly negative for all $F_1$s (Table 3). Estimates of MPH for plant height was moderate and ranged from 10.6% (AMBOON6-38 × Ecuador 573) to 55.0% (AMBOON6-22 × Pool9A-MHM) across $F_1$s and locations.

The performances of six highest yielding crosses relative to the two hybrid checks (BH660 and BH540), three-way and single cross commercial maize hybrids widely produced in the mid-altitude and highland-transition zones in Ethiopia, are summarized in Table 4. Yield superiority of the inbred line crosses (single crosses) ranged from 14.0% to 36.5% over the two hybrid checks (Table 4). The checks, in spite of their differences in genetic compositions, did not differ for grain yield, however, BH660 is relatively taller and later maturing compared to the best crosses.

**Table 4** Grain yield, yield heterosis (MPH), days to silking (days) and plant height of best line × tester crosses evaluated across four locations in Ethiopia in 2002

| Crosses | Grain yield | | Difference % Check[a] | | Days to silking | Plant height (cm) |
|---|---|---|---|---|---|---|
| | Mg ha$^{-1}$ | MPH % | Check1 | Check2 | | |
| AMBOON6-4 × 142-1-e | 10.4 | 94.9**[b] | 122.3 | 120.9 | 88.0 | 261.4 |
| AMBOON6-8 x142-1-e | 10.0 | 104.8** | 117.6 | 116.3 | 85.6 | 273.1 |
| AMBOON6-15 × 142-1-e | 10.4 | 97.3** | 122.3 | 120.9 | 85.6 | 284.4 |
| AMBOON6-20 × 142-1-e | 11.6 | 83.3** | 136.4 | 136.5 | 88.0 | 267.4 |
| AMBOON6-39 × 142-1-e | 9.8 | 121.7** | 115.3 | 114.0 | 89.3 | 268.0 |
| AMBOON6-41 × 142-1-e | 10.2 | 95.0** | 120.0 | 118.6 | 86.0 | 294.6 |
| BH660 (Check1) | 8.5 | _ | 100 | | 90.5 | 293.0 |
| BH540 (Check2) | 8.6 | _ | _ | 100 | 85.2 | 259.0 |
| S.E[c] | 1.0 | _ | _ | | 2.3 | 15.3 |

[a] percentage differences of the crosses over two hybrid checks
[b] highly significant difference at ≤0.01 probability levels
[c] standard error

**Molecular polymorphism and Genetic distance**

AFLP analysis of 32 parental genotypes produced a total of 601 bands, of which 80.5% were polymorphic. Polymorphism ranging from 42 (AGG/CGA) to 66 (ACA/CCC) bands with mean of 50 was detected across nine primer combinations. Polymorphic information content values ranged from 0.25 to 0.40 (Table 5). Genetic distance calculated in terms of dissimilarity for all possible combinations among 32 genotypes ranged from 0.40 to 0.72 with an average of 0.59 units. Genetic distance estimates for the

26 female and six male parent combinations varied from 0.63 to 0.72 with a mean of 0.67. With further sub-groupings of the pairwise combinations into population testers and line testers, mean GD values for population tester and line tester combinations were 0.68 and 0.66, respectively (Table 6). Cluster analysis provided a fairly good resolution of the inbred lines from the tester parents. The inbred lines clustered into three groups, reflecting available pedigree records. The testers were distinctly separated among each other in the dendrogram as it is expected based on their genetic backgrounds (Fig. 1). The dendrogram constructed based on the AFLP data matrix demonstrated a high cophenetic correlation coefficient (0.88), and therefore, showed an excellent fit with the GD values.

**Table 5** Number of scored bands, degree of polymorphism and Polymorphic Information Content (PIC) for nine AFLP primer combinations applied to 26 female lines, six male lines and population parents

| Primer pairs[a] | No. of bands | Polymorphic bands | Polymorphism % | PIC |
|---|---|---|---|---|
| AAC/CGG (800) | 63 | 51 | 80.9 | 0.30 |
| ACA/CAC (700) | 74 | 59 | 79.7 | 0.35 |
| ACA/CCC (700) | 81 | 66 | 81.5 | 0.34 |
| ACA/CTG (700) | 73 | 60 | 82.2 | 0.40 |
| ACG/CCG (700) | 62 | 48 | 77.4 | 0.32 |
| AGG/CAG (800) | 57 | 45 | 78.9 | 0.31 |
| AGG/CGA (800) | 53 | 42 | 79.2 | 0.34 |
| AGG/CAC (800) | 78 | 64 | 84.6 | 0.37 |
| AGG/CCC (800) | 60 | 48 | 80.0 | 0.25 |
| Total | 601 | 483 | | |
| Mean | 66.8 | 53.8 | 80.5 | 0.33 |

[a] Selective nucleotides of *Eco*RI/*Mse*I adapter primers and IRD 700 or IRD 800 labelled primers

**Table 6** Mean minimum, maximum and standard deviation (SD) of Euclidean based genetic distance coefficients between male parents and female lines calculated from AFLP data of nine primer combinations

| Cross combinations Type | Number of pairs | Genetic distance | | | |
|---|---|---|---|---|---|
| | | Mean (SE) | Minimum | Maximum | SD |
| All genotypes | 496 | 0.594 (0.003) | 0.410 | 0.722 | 0.061 |
| All male × Female | 156 | 0.671 (0.001) | 0.625 | 0.723 | 0.020 |
| Pop. Tester crosses | 78 | 0.680 (0.002) | 0.631 | 0.723 | 0.018 |
| Line Tester crosses | 78 | 0.661 (0.001) | 0.625 | 0.697 | 0.016 |
| Kit. Syn.II × Female | 26 | 0.676 (0.003) | 0.645 | 0.708 | 0.016 |
| Ecud. 573 × Female | 26 | 0.693 (0.003) | 0.667 | 0.723 | 0.018 |
| Kuleni × Female | 26 | 0.672 (0.003) | 0.631 | 0.706 | 0.016 |
| I42-1-e × Female | 26 | 0.671 (0.003) | 0.647 | 0.698 | 0.014 |
| F7215 × Female | 26 | 0.651 (0.002) | 0.625 | 0.673 | 0.013 |
| P9a-mhm × Female | 26 | 0.660 (0.002) | 0.631 | 0.679 | 0.023 |

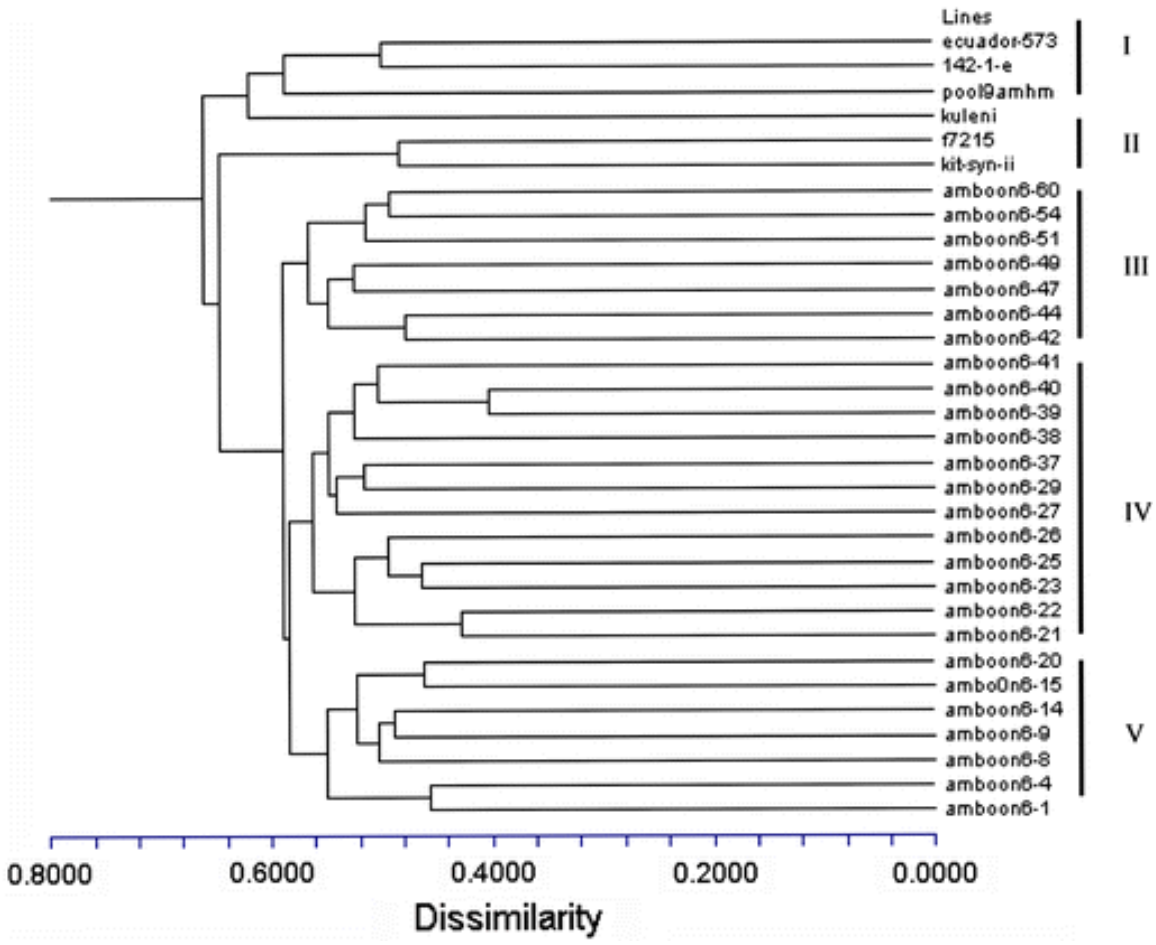


**Fig. 1** Dendrogram derived using UPGMA cluster analysis based on genetic distance data of 32 maize inbred lines and populations. I, II, III, IV & V indicate major cluster groups

**Relationship of hybrid performance and heterosis with genetic distance**

Associations between GDs of the inbred lines × all tester parents with the $F_1$s and MPH for grain yield and plant height were significantly negative in most cases. Significantly positive association between GDs and F1 performances for days to silking was determined. Associations between GDs of population testers sub-group and the respective $F_1$ performances and MPH for grain yield and plant height were mostly negative. In contrast, correlations between GDs of inbred line tester sub-group with $F_1$ performances and MPH were mostly significant for each trait (Table 7).

**Table 7** Simple correlation of genetic distance with grain yields, days to flowering and plant height

| Crosses | No | Grain yield | | Days to silking | | Plant height | |
|---|---|---|---|---|---|---|---|
| | | Performance | MPH+ | Performance | MPH | Performance | MPH |
| All male × Female | 156 | 0.031 | −0.225** | −0.037 | 0.340** | −0.290** | −0.239** |
| Pop. Tester crosses | 78 | 0.045 | −0.650 | 0.210* | −0.028 | −0.031 | −0.270* |
| Line Tester crosses | 78 | 0.276** | 0.363** | 0.343** | −0.064 | 0.229* | 0.199* |

*, ** Significantly different from zero at the 0.05 and 0.01 level of probability, respectively

+ Midparent heterosis

# Discussion

In maize, breeding for hybrid varieties is a well-recognized approach for yield increment through the exploitation of heterosis. The role of genetically divergent germplasm is of primary importance for these phenomena to occur. The wide range of average grain yield performance between the lowest and the highest yielding hybrids in this study could be explored based on the genetic divergence of the parental genotypes and the role of dominant favourable gene effects that may be cumulated in the hybrids. (Hallauer et al. *1988*). The parents of the lowest yielding hybrid (topcross) were known to have some degree of genetic relatedness, since both have Ecuador 573 background in their pedigree. In turn, the parents of the highest yielding hybrid (single cross) also differ to each other in their genetic background, hence indicating occurrence of heterotic pattern between the two genotypes. Such phenomena have been reported repeatedly in maize that the more parents are genetically unrelated the better will be their crossing performance (Hallauer et al. *1988*; Ordas *1991*; Saleh et al. *2002*), however, there will be exceptions due to mutually exclusive adaptation problems (Moll et al. *1965*).

In addition, performance of grain yield in maize hybrids is the cumulative contribution of favourable dominant gene effects; nonetheless, the role of additive and epistasis gene action is not ruled-out (Arunachalam et al. *1984*). Consequently, single crosses, unlike topcross hybrids, commonly give higher yields due to homozygousity advantages of their parents and the interaction of the gene in favour of cumulative dominant alleles useful for the expression of heterosis (Hallauer et al. *1988*; Falconer and Mackay *1996*).

The level of heterosis in the study showed variation from trait to trait and from population crosses to inbred line crosses. On the average, grain yield manifested the

highest MPH, which is consistent with other reports in maize (Legesse *1994*; Saleh et al. *2002*). Higher level of mean yield heterosis was shown for crosses with line testers than with population testers, indicating preponderance of dominance gene dispersion in the genomes of the parental inbred lines (Falconer and Mackay *1996*). The negative heterosis revealed by days to flowering suggests the effects of dominance gene action for earliness in a desirable direction.

The range of pairwise GDs determined for the inbred lines by all testers (26 × 6) were found narrow relative to the values determined for all possible combinations among the 32 parental genotypes (Table 6). However, higher mean GD value was determined for the inbred line × tester combinations (0.671) relative to the value (0.594) determined for all pairs of parents inclusive. On the other hand, mean GDs of population tester combinations (0.680) were slightly larger than the values of inbred line tester sub-group (0.661). This was consistently observed while considering the pairwise GD values determined for each tester combinations (Table 6). Such difference is to be expected between crosses of population testers and inbred line testers. Because populations commonly possess broader genetic bases than inbred lines, and hence exhibit larger genetic differences (Dubreuil and Charcosset *1999*). However, the female inbred lines were not completely homozygous, but in the $S_4$ inbreeding stages, which eventually influenced the mean GD values of the two groups to become very close to each other due to residual heterozygosity effects.

The negative associations of GDs with hybrid performance, and MPH for grain yield and plant height in reference to all the tester combinations and with a sub-group of population testers, imply little importance for prediction of hybrid performance. In contrast, the significantly positive correlations manifested between GDs and hybrid performance, GDs and MPH for most of the traits in reference to line tester combinations (Table 7) is good evidence to the hypothesis suggested by Bernardo (*1992*) who indicated the importance of strong dominance effects for effective prediction of hybrid performance using molecular markers. However, the magnitude of correlation coefficients determined between GDs of line tester combinations with $F_1$ yield and yield MPH in this study was not large enough; therefore, it is of less utility for the perdition of hybrid performance. Similar results were reported by a number of investigators with different types of crops including maize (Boppenmaier et al. *1992*; Martin et al. *1995*; Xu et al. *2002*; Barbosa et al. *2003*; Oliveira et al. *2004*).

A number of possible reasons could be enumerated for weak correlations of GD predicted by molecular markers versus hybrid performance and heterosis. These include lack of linkage between genes controlling the traits measured, unequal genome coverage, random marker distribution and diversified effect of dominance (Charcosset et al. *1991*; Bernardo *1992*). Effective prediction of hybrid performance using molecular marker as suggested by Bernardo (*1992*) would be only feasible when a significant portion (50%) of the selected markers are linked with quantitative trait loci (QTL). Other more recent strategies for predicting hybrid performances, based on best linear unbiased prediction (BLUP) (Bernardo *1994*), and based on the principle that two hybrids with parents similar at the marker level, should display similar specific combining ability (SCA)

values (Charcosset et al. *1998*) especially for unrelated inbred lines, were suggested. Moreover, Vuylsteke et al. (*2000*) proposed a novel approach relying on AFLP markers, hybrid performance and SCA across a set of hybrids. The efficiency of this method may be enhanced by detection of more marker alleles tightly linked to specific QTL and yield data of hybrids, available from multiple trials carried out across different locations and years (Vuylsteke et al. *2000*).

Molecular markers generally are claimed to be more efficient and accurate than morphological markers to identify and to generate variability. However, the final and more applied aspect of genetic variation is to maximize the efficiency of using genetic variation to develop improved composites and hybrids. Efficiently capitalizing on the use of genetic variation continues to be an enormous challenge. Genetic variability is created and/or identified, but that is not an end by itself in the course of cultivar development. The remaining challenge lies on plant breeders to separate the desirable variability from undesirable. Such activities involve testing of new combinations across years and locations to rigorously select and identify high yielding and stable cultivars (Lee *1995*; Gepts *2002*). The process all together indicates coherency between molecular markers and classical plant breeding in accelerating crop improvement activities.

Overall, the inbred line crosses (single crosses) showed yield performance as high as 11.5. Mg $ha^{-1}$. Yield superiority of top single crosses (15–36%) over the best commercial hybrid check is a promising indication deserving close attention in the breeding program. Value of mean grain yield MPH determined for the single cross hybrids was relatively larger than the value determined for the topcross hybrids. Negative heterosis manifested for days to silking implies favourable dominant gene effects to breed for earliness. AFLP clustering of the inbred lines into different groups are in agreement with their pedigree records indicating the effectiveness of AFLP marker for diversity analysis and heterotic groupings. The relationships between GDs of population tester combinations with their corresponding $F_1$ grain yield; plant height and MPH were negatively correlated. On the contrary, GDs of inbred line tester combinations showed positive and significant correlation coefficients with $F_1$ performances and MPH for most traits but with low magnitude to warrant prediction of hybrid performance.

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