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# Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers

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**Abstract** The challenge to maize breeders is to identify inbred lines that produce highly heterotic hybrids. In the present study we surveyed genetic divergence among 13 inbred lines of maize using DNA markers and assessed the relationship between genetic distance and hybrid performance in a diallel set of crosses between them. The parental lines were assayed for DNA polymorphism using 135 restriction fragment length polymorphisms (RFLPs) and 209 amplified-fragment polymorphisms (AFLPs). Considerable variation among inbreds was detected with RFLP and AFLP markers. Moreover AFLPs detect polymorphisms more efficiently in comparison to RFLPs, due to the larger number of loci assayed in a single PCR reaction. Genetic distances (GDs), calculated from RFLP and AFLP data, were greater among lines belonging to different heterotic groups compared to those calculated from lines of the same heterotic group. Cluster analysis based on GDs revealed associations among lines which agree with expectations based on pedigree information. The GD values of the 78 $F_1$ crosses were partioned into general (GGD) and specific (SGD) components. Correlations of GD with $F_1$ performance for grain yield were positive but too small to be of predictive value. The correlations of SGDs, particularly those based on AFLP data, with specific combining-ability effects for yield may have a practical utility in predicting hybrid performance.

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## Introduction

In maize the prediction of hybrid performance is of considerable importance and has attracted much interest over the years (reviewed in Hallauer et al. 1988). Recently, genetic linkage maps based on molecular markers have been constructed in this crop (Coe et al. 1995), with the hope that they will provide effective means for predicting hybrid performance and heterosis.

Restriction-fragment length polymorphisms (RFLPs) overcome many of the constraints associated with the study of the inheritance of morphological traits (reviewed in Stuber 1994). These polymorphisms are abundant (Evola et al. 1986) and allow precise measurement of the genetic similarity of genotypes (Messmer et al. 1991; Ajmone Marsan et al. 1992). Several studies have demonstrated that RFLP-based estimates of genetic relationship can be used to assign maize inbreds to heterotic groups (Livini et al. 1992; Messmer et al. 1992; Mumm and Dudley 1994). These determinations of genetic distances have been used to predict hybrid performance with mixed results. For example, Godshalk et al. (1990) and Melchinger et al. (1990a) found a low correlation between RFLP distance and hybrid performance, whereas Lee et al. (1989), Smith et al. (1990), and Melchinger et al. (1992) observed a high correlation. Furthermore, Melchinger et al. (1990a) indicated that the correlation between marker-based genetic distance and $F_1$ performance is dependent on the origin of the lines studied.

More recently, a novel DNA fingerprinting technique, called amplified fragment length polymorphism (AFLP™)[1], has been developed by Zabeau and Vos

[1] AFLP is a trademark filed by Keygene n.v.

(1993), and Vos et al. (1995). AFLP markers are genomic restriction fragments detected after selective amplification using the polymerase chain reaction (PCR) (Saiki et al. 1988). AFLPs are Mendelian markers with a number of appealing features relative to RFLPs. Thus, they provide a novel and very powerful tool for DNA fingerprinting of genomes of any origin or complexity, including those of maize (Vos et al. 1995).

Although the efficiency of RFLP and AFLP technology for determining the genetic relationship of maize germplasm pools has been discussed, estimates of genetic relationship based on these two marker types have not yet been compared. We have therefore, conducted a study to compare the use of RFLP and AFLP markers in order (1) to investigate the genetic diversity for RFLPs and AFLPs within a set of maize elite inbred lines and; (2) to examine the association of RFLP- and AFLP-based genetic distances of these inbreds in predicting the performance of single-cross hybrids.

## Materials and methods

### Plant materials and field experiments

Six inbred lines from Iowa Stiff Stalk Synthetic (BSSS), five from Lancaster Sure Crop (LSC) and two of different origin (Table 1) were crossed to form a diallel set design without reciprocals. The 78 single cross hybrids were evaluated in 1996 at three different locations (Bergamo, Luignano, and Turano), using randomized complete block designs with three replications. Plots consisted of four rows that were 5 m long, with 0.75 m between rows. Plots were overplanted and thinned to 61 000 plants $ha^{-1}$. Recommended crop-management techniques were used in all experiments. The plots were machine-harvested on the middle rows of each plot to reduce competition effects and grain yield (t $ha^{-1}$ at 15.5% moisture) was recorded.

### Laboratory analysis

The 13 inbred lines were assayed for their respective RFLP profiles. DNA extraction, purification, separation, blotting and hybridization were as described in Livini et al. (1992). Eighty two genomic clones from the UMC and BNL collections and two restriction enzymes (*Eco*RI and *Hin*dIII) were used to characterise 149 RFLP loci. A total of 508 RFLP bands were binary coded by 1 or 0 for their respective presence or absence in each line.

AFLP marker analysis was performed as described by Vos et al. (1995). Briefly, in these experiments genomic DNA of maize (400 ng) was digested using 5 U of *Eco*RI (rare cutter) and 5 U of *Mse*I (frequent cutter) (Pharmacia) in a final 40-µl volume of 10 mM Tris-Ac buffer, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/ml BSA, pH 7.5 for 1 h at 37°C. To the restricted DNA, 10 µl of a mixture containing 5 pmol of *Eco*RI adapter, 50 pmol of *Mse*I adapter and 1 U of T4 DNA Ligase (Pharmacia), in the same buffer as before, plus 1 mM of ATP, were added. The structure of the adapter sequences was:

*Eco*RI: 5-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5
*Mse*I: 5-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5

The primers used for pre-amplification and amplification were similar to those described by Vos et al. (1995) with the following extensions: AAG/CAA, ACA/CAG, ACA/CAT, ACA/CGT, ACT/CAA and ACT/CCA.

The image plates were scanned with a Fujix Bas 2000 phosphorimager (Fuji Photo Films Co, Ldt). Polymorphic bands were binary scored by 1 or 0 for their respective presence or absence in each line.

### Statistical analysis

Genetic distances (GD) between pairs of inbred lines were calculated from RFLP and AFLP data for all possible pairs of inbreds by the following equation:

$$GD(i, j) = 1 - \{2\ N(i, j)/[N(i) + N(j)\}$$

where N(i, j) is the total number of bands common to lines i and j, and N(i) and N(j) are the total number of bands present in i and j respectively, considering all probe-enzyme or primer combinations. This distance measure is equal to one minus the genetic similarity coefficients originally devised by Dice (1945) and first used for RFLP data by Nei and Li (1979). Values of GD may range from 0 (identical profiles for all markers in the two inbreds) to 1 (no bands in common). For pure-breeding lines, as in our study, GD corresponds to the Rogers' distance (Rogers 1972) used in earlier RFLP studies (Melchinger et al. 1990 a, b, 1991; Livini et al. 1992) for the case when only probe-enzyme combinations that yield a single-band RFLP pattern are employed.

Analysis of variance of data from individual experiments and combined across locations were conducted following Cochran and

**Table 1** Maize inbreds and their parentage

| Lines | Source/pedigree[a] | Lines | Source/pedigree |
|---|---|---|---|
| Iowa Stiff Stalk (BSSS) | | Lancaster Sure Crop (LSC) | |
| B14A | (Cuzco × $B14^8$) rust res. sel. | C103 | Lancaster Sure Crop |
| B37 | BSSSC0 | C123 | (C102 × C103) Sel. |
| B73 | BSSSC5 | Lo881 | Syn. C103 |
| B84 | BSSSC7 | Mo17 | CI 187–2 × C103 |
| Lo950 | Pioneer 3183 self | Va59 | (C103 × T8) × (K4 × $C103^2$) |
| Lo951 | Pioneer 3183 self | | |
| Miscellaneous | | | |
| H55 | $Hy^2$ × Mo21A | | |
| Pa91 | [(Wf9 × Oh40B)$S_4$ × (Ind28-$11^2$ × L317)$S_4$] | | |

[a] Livini et al. (1992)

Cox (1957). Diallel analyses were performed with the $F_1$ data to estimate general combining ability (GCA) and specific combining abiliy (SCA) effects using Griffing's Model 1 of Method 4 (1956). Similarly, the GD values associated with 78 $F_1$ hybrids were partitioned into general (GGD) and specific genetic distances (SGD) as described by Melchinger et al. (1990 a). Simple correlations were calculated for various combinations of yield means, SCA, GD and SGD values. Associations among the 13 inbreds were determined from cluster analysis based on GD estimates. The UPGMA clustering method (or "group average" or "average linkage" cluster analysis) was used for hierarchical clustering, and the necessary computations were performed using a NTSYS-pc program (Rohlf 1989).

## Results

### RFLP vs AFLP

A total of 508 RFLP bands were detected when considering the 13 inbreds tested with all probe-enzyme combinations. Of the 149 probe-enzyme combinations used in this study, 135 (91%) revealed polymorphism across the 13 inbreds assayed. The majority (72%) of the polymorphic probe-enzyme combinations gave single-banded RFLP patterns. The remaining yielded multiple-banded RFLP patterns, suggesting the presence of repeated binding sequences in the genome for the corresponding DNA clones. The number of RFLP variants per probe-enzyme combination ranged from 2 to 5 in the former case and from 2 to 9 in the latter case, with an average of 3.31 and 4.97, respectively.

For AFLP analysis, a total of six primer combinations was used to assay the 13 inbreds. These permitted the production of approximately 500 selectively amplified DNA fragments ranging in size from 60 to 600 nucleotides and the identification of 209 polymorphic markers. On average 30–120 distinguishable bands were observed after selective amplification with each primer combination, and an average of 34.8 of these AFLP bands were found to be polymorphic among lines with a range from 19 to 52.

### Genetic distances among inbreds

A summary of the GDs between lines from BSSS, LSC and miscellaneous heterotic groups is presented in Table 2. RFLP and AFLP markers gave almost identical mean GD values (46.2 vs 45.9). Moreover, GDs based on AFLP data had a similar range (19.5–63.7) to the range of GDs calculated from RFLP data (24.1–59.9). For both molecular markers the subset means for GDs were, as expected, significantly greater for combinations of lines of different origin (51.9 and 50.7) than for the BSSS × BSSS (34.7 and 36.4) and LSC × LSC (31.1 and 37.2).

The correlation coefficient between RFLP- and AFLP-GDs was 0.65, indicating that the two classes of markers may assay different parts of the genome. All RFLP markers are located on the genomic map and were chosen to be evenly distributed throughout the maize genome. Thirty eight AFLP markers (18%) used in this study appear randomly distributed in the genome while the others have an unknown genomic localization (our unpublished data).

### Cluster analysis of RFLP and AFLP data

The dendrograms from UPGMA cluster analyses of GDs based on either RFLP and AFLP data are presented in Fig. 1. Both have a high co-phenetic coefficient ($r = 0.91$ and 0.90 respectively for RFLPs and AFLPs) and therefore show a good fit with GD values.

Clustering based on RFLP data resulted in two major groups (Fig. 1 a). One cluster was comprised of lines derived from, or related to, BSSS along with H55, while the other was composed of five LSC-related inbreds along with Pa91. Within the BSSS group, B73 and B84, derived from advanced cycles of recurrent selection programs of the BSSS population, formed a separate subgroup from Lo950 and Lo951, developed from Pioneer 3183, a commercial hybrid. Inbreds B14A and B37 were loosely aggregated with the cluster of B73- and Lo950-related lines. Within the Lancaster group, C103 and C123 were highly clustered and most distantly merged with Lo881, Mo17, and Va59; Pa91, which has approximately half of its germplasm from LSC, merged in this subgroup.

The AFLP-based dendrogram assigned the 13 inbreds to three major groups (Fig. 1 b): (1) the BSSS-related lines; (2) H55 along with Pa91; and (3) the

**Table 2** Genetic distances [Mean, minimum (Min), maximum (Max) and standard deviation (SD) × 100] calculated from RFLP and AFLP data for a combination of all lines and for different subsets of combinations

| Combination of lines | (*n*) | RFLP markers GD × 100 | | | | AFLP markers GD × 100 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Min | Max | SD | Mean | Min | Max | SD |
| Among all | (78) | 46.2 | 24.1 | 59.9 | 8.1 | 45.9 | 19.5 | 63.7 | 10.7 |
| BSSS × BSSS | (15) | 36.4 | 24.7 | 43.1 | 6.2 | 34.7 | 19.5 | 44.8 | 7.6 |
| LSC × LSC | (10) | 37.2 | 24.1 | 47.3 | 7.8 | 31.1 | 20.5 | 40.1 | 6.4 |
| Different origin[a] | (53) | 50.7 | 43.9 | 59.9 | 3.6 | 51.9 | 41.7 | 63.7 | 5.7 |

[a] This group comprises all combinations excluding the BSSS × BSSS and LSC × LSC subsets

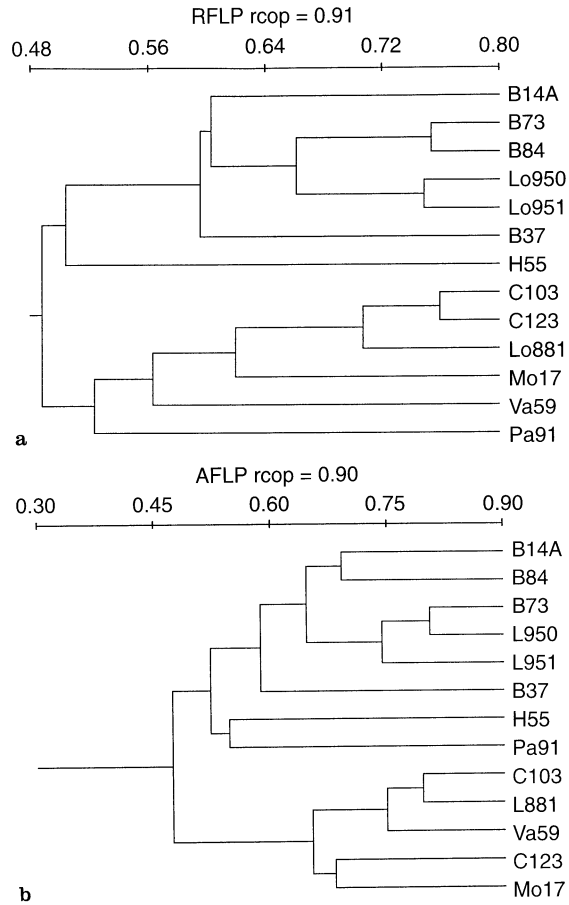


**Fig. 1a,b** Association among lines revealed by UPGMA cluster analysis of Dice genetic similarity (GS) coefficients calculated from RFLP data **a** and AFLP data **b**

Lancaster lines. In addition, when compared to the RFLP-based dendrogram, discrepancies in forming subgroups within the major groups were noted. Within the BSSS group B73 and Lo950 were clustered together in a major subgroup with Lo951. B14A clustered together with B84, while B37 was loosely aggregated with the other BSSS-related lines. The H55 and Pa91 inbreds were in a separate group that merged with the group of the BSSS-related lines. Three of the LSC lines (C103, Lo881, and Va59) were clustered together in one major subgroup, while C123 and Mo17 were in a separate subgroup.

## Hybrid performance

The mean, standard error, and range of variation for the grain yield of all entries tested in the three environments are given in Table 3. The magnitude of variation was appreciably large in each environment. Grain yield was maximum at Bergamo; the experimental mean in this location was 12.49 t $ha^{-1}$ and one single-cross hybrid yielded approximately 16 t $ha^{-1}$. The coefficient

**Table 3** Means, standard errors (SE) and ranges for grain yield averaged over 78 maize hybrids evaluated at three locations in 1996

| Environment | Grain yield | | |
|---|---|---|---|
| | Mean $\pm$ SE (t/ha) | Range (t $ha^{-1}$) | CV (%) |
| Bergamo | 12.49 $\pm$ 0.21 | 7.11–16.03 | 8.4 |
| Luignano | 9.98 $\pm$ 0.27 | 4.33–15.30 | 14.0 |
| Turano | 8.83 $\pm$ 0.20 | 3.55–12.10 | 15.8 |
| Overall mean | 10.43 $\pm$ 0.17 | 3.55–16.03 | 10.8 |

of variation ranged from 8.4 to 15.8% with an overall mean of 10.8% which is an acceptable value for average yields superior to 8.0 t $ha^{-1}$.

Genotypic differences among $F_1$ crosses for grain yield were highly significant ($P \leqslant 0.01$) in all environments as well as in the combined analysis of variance across environments (data not shown). Similarly, the cross $\times$ environment interaction was significant, but the sums of squares for the interaction were less than for the main effects of crosses. Also, GCA and SCA variances were highly significant ($P \leqslant 0.01$) and of a similar order of magnitude, while the GCA $\times$ environment and SCA $\times$ environment interaction variances were not significant.

The highest performance for grain yield was obtained from crosses between Lo881 and three BSSS-related lines (B73, B84, Lo950) (Table 4). Crosses H55 $\times$ Lo881 and Lo951 $\times$ Pa91 also produced a high yield. Estimates of positive SCA effects were greatest for H55 $\times$ C103, B14A $\times$ Mo17, and Lo950 $\times$ Lo881. Crosses among C103-related inbreds had in several instances a relatively low yield and significant negative SCA estimates. Crosses among BSSS-derived inbreds were also frequently lower in grain yield and in estimates of SCA, although crosses involving Lo951 and other BSSS-derived inbreds (B73, B37, and B84) had a relatively high grain yield.

## Relationship of genetic distance to hybrid performance

The estimates of simple correlations ($r$) of GDs with $F_1$ performance for grain yield (F1P) and SGD with SCA effects are presented in Table 5.

The correlation coefficients of GDs calculated for RFLP and AFLP data with grain yields for the entire set of 78 hybrids were highly significant ($P \leqslant 0.01$) but only of moderate size. The $r$ value was 0.36 for the GD based on RFLPs and 0.51 for the GD based on AFLPs. By contrast, for both classes of molecular markers a lack of relationship were noted between these two variables in the three subsets of crosses. It must be emphasized, however, that the results obtained from the BSSS $\times$ BSSS and LSC $\times$ LSC group of crosses should be interpreted with caution because they are based on a small number of crosses. Estimates of

**Table 4** Grain yield means (t $ha^{-1}$) and estimates of specific combining ability (in brackets) for 78 single-cross maize hybrids averaged over three locations

| Inbreds | B37 | B73 | B84 | Lo950 | Lo951 | H55 | C103 | C123 | Lo881 | Mo17 | Va59 | Pa91 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B14A | 9.7[a] (− 0.17) | 9.4 (− 1.43**) | 10.7 (− 0.50) | 10.1 (− 0.51) | 12.4 (0.23) | 10.3 (− 1.11**) | 8.8 (− 0.53) | 10.0 (1.37**) | 9.4 (− 1.55**) | 12.6 (2.58**) | 10.1 (0.78*) | 11.7 (0.85*) |
| B37 | | 10.4 (0.04) | 10.0 (0.72*) | 10.3 (0.16) | 12.2 (0.50) | 10.7 (− 0.24) | 8.4 (− 0.46) | 8.1 (− 0.06) | 11.0 (0.52) | 10.4 (0.85*) | 8.5 (− 0.35) | 10.3 (− 0.08) |
| B73 | | | 10.0 (− 1.69*) | 8.2 (2.90**) | 11.8 (− 0.86*) | 11.8 (− 0.10) | 11.5 (1.68**) | 9.4 (0.28) | 13.9 (2.46**) | 11.45 (0.89**) | 11.1 (1.29**) | 11.7 (0.36) |
| B84 | | | | 11.2 (− 0.27) | 12.4 (− 0.62) | 10.7 (− 1.57**) | 11.0 (1.01**) | 10.2 (0.71) | 13.7 (1.90) | 11.7 (0.82*) | 11.2 (1.02**) | 11.6 (− 0.10) |
| Lo950 | | | | | 10.0 (− 2.44**) | 11.5 (− 0.19) | 10.2 (0.60) | 9.9 (1.00**) | 13.8 (2.58**) | 11.2 (0.90**) | 10.1 (0.50) | 11.7 (0.58) |
| Lo951 | | | | | | 12.9 (− 0.34) | 12.0 (0.84*) | 11.6 (1.14**) | 12.9 (0.12) | 11.4 (− 0.45) | 12.1 (0.95**) | 13.6 (0.92**) |
| H55 | | | | | | | 13.4 (3.00**) | 10.1 (0.40) | 13.7 (1.68**) | 10.6 (− 0.50) | 10.3 (− 010) | 11.0 (− 0.92**) |
| C103 | | | | | | | | 7.4 (− 0.22) | 5.4 (− 4.54**) | 7.9 (− 1.11**) | 7.1 (− 1.21**) | 10.8 (0.96**) |
| C123 | | | | | | | | | 8.8 (− 0.44) | 6.8 (− 1.51**) | 6.6 (− 1.01**) | 7.5 (− 1.64**) |
| Lo881 | | | | | | | | | | 8.5 (− 2.13**) | 9.1 (− 0.83**) | 10.7 (0.24) |
| Mo17 | | | | | | | | | | | 8.9 (− 0.10) | 10.3 (− 0.23) |
| Va59 | | | | | | | | | | | | 8.9 (− 0.93**) |

[a] LSD (0.05) for $F_1$ means = 1.0

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

*r* values between SGDs and SCA effects were, with both class of markers, for all crosses and individual subsets of crosses positive and in general significant. In particular, a high correlation between the two variables was obtained for the entire set of 78 crosses (0.65 and 0.72), and in the BSSS × BSSS subset for both classes of molecular markers (0.72 and 0.81), whereas in the LSC × LSC subset a relatively high correlation (0.66) was reported only for AFLPs. In addition, significant correlations, although of moderate size, were found also in the subset of unrelated lines (0.38 and 0.47) for RFLP and AFLP, respectively. Finally, it was worth noting that the correlations between GD and SGD calculated from AFLP data with $F_1P$ and SCA effects were higher than those based on RFLPs.

**Table 5** Simple correlations of genetic distance (GD) and specific genetic distance (SGD) based on RFLP and AFLP data respectively, with $F_1$ performance ($F_1P$) and specific combining ability (SCA) of grain yield for all crosses and in different subsets of maize crosses

| Variables | Crosses (*n*) | | | |
|---|---|---|---|---|
| | All (78) | BSSS × BSSS (15) | LSC × LSC (10) | Unrelated lines (53) |
| | | | $F_1P$ | |
| GD-RFLP | 0.36** | 0.31 | 0.28 | − 0.08 |
| GD-AFLP | 0.51** | 0.47 | 0.30 | 0.23 |
| | | | SCA | |
| SGD-RFLP | 0.65** | 0.72** | 0.27 | 0.38** |
| SGD-AFLP | 0.72** | 0.81** | 0.66* | 0.47** |

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

In the plots of grain yield versus GDs, 9 of 10 crosses and 6 of 8 crosses, respectively, for RFLP- and AFLP-based distances in quadrant II involved the inbreds Lo951 and Lo950 in the same cross combinations (Fig. 2 a). For both RFLP- and AFLP-based distances, crosses in quadrant IV had below-average grain yield and above-average GD values. The remaining crosses were located in quadrants I and III, implying a relation of increases in yield to increases in genetic distances for these crosses; this trend was more evident for the AFLP-based distances in comparison to RFLP-based distances. Further examination showed that most of the data points in the upper right quadrant refer to line combinations of the type BSSS × LSC and combinations involving BSSS- and LSC- related lines crossed with the inbred lines H55 and Pa91.

Plots of SGD versus SCA showed that the associations among these variables from both RFLP and AFLP data are largely due to group effects (Fig. 2 b). In particular, for the AFLP-based distances the majority

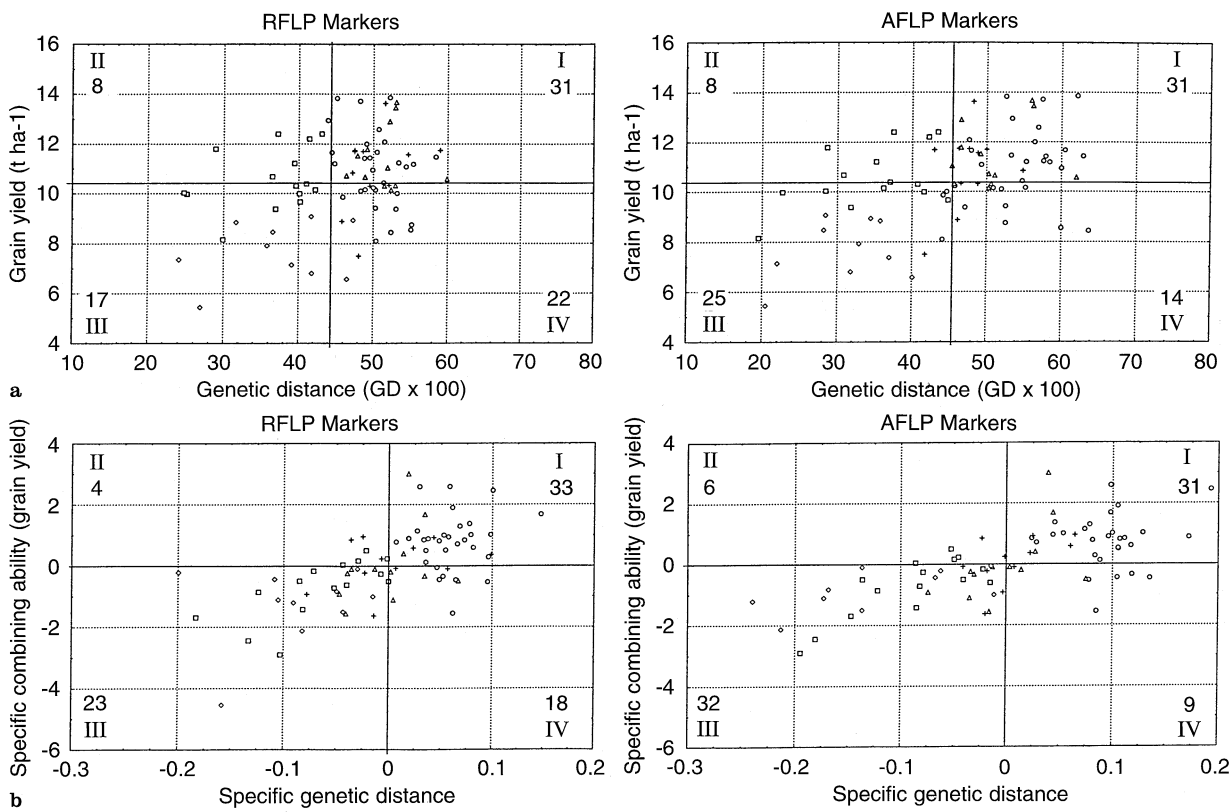


**Fig. 2a, b** Genetic distances (*GD*) versus $F_1$ performances and specific genetic distances versus specific combining ability for grain yield in a diallel of 13 maize inbreds. Quadrants are divided along mean values for the respective axes with *numbers* showing the number of crosses located in the respective quadrant. ○ = BSSS × LSC crosses; □ = BSSS × BSSS crosses; ◇ = LSC × LSCcrosses; △ = unrelated × H55 crosses; + = unrelated × Pa91 crosses

of the crosses between unrelated inbred lines, that is crosses between BSSS × LSC related lines, occupied the upper right quadrant, whereas most of the crosses of the type BSSS × BSSS and LSC × LSC and from closely related lines were positioned in the lower left quadrant.

## Discussion

In the research reported here, we have surveyed genetic divergence among 13 inbred lines of maize with RFLP and AFLP markers and assessed the relationship between molecular polymorphism and hybrid performance in the diallel set of crosses between these 13 parents. The degree of polymorphism revealed by RFLP analysis is in close agreement with the results of comparable studies (Smith et al. 1990; Livini et al. 1992; Mumm and Dudley 1994).

We demonstrate that considerable variation at the inbred level is also detectable with AFLP markers and that the number of polymorphic products obtained with one primer combination can be several fold higher than that obtained by RFLP markers. A higher number of polymorphic variants per assay was identified by AFLP technology compared to RFLPs. In fact, whereas RFLP markers detect at most a few genetic loci in a single hybridization experiments, 100–200 AFLP loci can be surveyed in a single experiment. These results suggest that AFLPs are able to detect a larger number of polymorphisms in a more efficient way in comparison to RFLPs, due to the much higher number of loci assayed in a single multiplex PCR reaction.

The GD values between the inbreds demonstrated that lines from different heterotic groups are genetically more dissimilar than those originating from the same heterotic groups. This observation holds true irrespective of the type of marker used. The relative increase in the mean GD (28.4 and 25.5%, for RFLP and AFLP data, respectively) observed for between-pool as opposed to within-pool combinations was substantially greater than reported in other RFLP studies with maize inbreds (Livini et al. 1992). In the dendrograms obtained from cluster analysis, all lines with defined affiliation to one of the heterotic groups were assigned to the respective main clusters. Thus, RFLP and AFLP data clearly separated lines from the BSSS and LSC heterotic groups and detected pedigree relationship among inbreds. This is in agreement with other RFLP assays in maize (Lee et al. 1989; Melchinger et al. 1991; Ajmone Marsan et al. 1992; Messmer et al. 1992; Mumm and Dudley 1994) and confirm that RFLPs and AFLPs are suitable to define heterotic groups and to identify genetically diverse germplasm sources.

A particular use of genetic markers is the prediction of hybrid performance. Although genetic distances between parents were significantly related with hybrid performance, the estimates of GD did not consistently identify the best crosses. This is similar to results already published (Frei et al. 1986; Lee et al. 1989; Godshalk et al. 1990; Melchinger et al. 1990 a). There are many potential reasons for the finding of poor correlations between genetic distance and hybrid performance. One is that linkage should exist between genes controlling the trait measured and the markers used to estimate genetic distance in order for high correlations to occur (Melchinger et al. 1990 a; Bernardo 1992). In addition, some of the marked chromosome regions could be more important than others in their contribution to $F_1$ yield performance and heterosis. From this point of view, current investigations designed to map QTLs affecting grain yield and related traits confirm that the magnitude of genetic effects for any single QTL contributing to these traits varied considerably, ranging from 5 to 25% of the phenotypic variance (Stuber et al. 1992; Ajmone Marsan et al. 1995). Inadequate genome coverage and different levels of dominance are other reasons suggested for the low correlation between genetic distance and hybrid performance (Melchinger et al. 1990 b).

Although it seems questionable whether such linkages can be identified with reasonable experimental expenditures and whether the identified associations between markers and QTLs are consistent across a wide range of germplasms (Abler et al. 1991), a significant improvement in the correlations between genetic distance and hybrid performance was noted in this study by using AFLP markers in comparison with RFLP markers. It has been reported that, generally, precision improves as more probes or marker loci are employed in the analysis (Tivang et al. 1994). AFLPs may provide a more detailed coverage throughout the genome, which in turn permit one to study genetic diversity as related to hybrid performance. In this respect, recent reports in different plant species have shown that the AFLP technique allows the analysis of thousands of markers in a relatively short time (Meskem et al. 1995; Cervera et al. 1996). In our study the higher number of loci assayed with AFLPs (209) in comparision to RFLPs (135) was not the cause of the superior correlation observed with the former technology. In fact, the correlation calculated combining both sets of data gave a value smaller than those obtained with AFLPs alone (i.e. $r = 0.71$ for SCA and SGD in the data set), in spite of the higher number of loci assayed. Possibly the high resolution of the polyacrylamide gels used for AFLP analysis allows a more precise identification of similar and dissimilar AFLP alleles and, therefore, a more reliable estimation of the genetic distance between genotypes. This may lead to a better prediction of hybrid performance. Additionally, an alternative for obtaining a better correlation between marker heterozygosity and hybrid performance would be the pre-selection of specific markers linked to loci that affect a quantitative trait (Melchinger et al. 1990 a, b; Bernardo, 1992).

A further point worth noting is that the correlations of SGDs with SCA effects were always positive and mostly significant; in particular those based on AFLP data, both for the entire set of 78 crosses and the subset of ten BSSS × BSSS crosses, reached a magnitude suggesting their direct use in predicting heterosis. These results are consistent with the experimental results of previous studies and with quantitative genetic expectations (Melchinger et al. 1992).

In summary, results from this study suggest that molecular marker-based analysis, and in particular AFLP technology, offers a reliable and effective means of assessing genetic variation and for studying relationships among currently and historically important maize inbred lines. This may provide an alternative means for predicting the performance and heterosis of maize hybrids. In particular, correlations between AFLP markers and SCA estimates may have a practical utility in predicting hybrid performance.

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