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Genetic diversity for RFLPs in European maize inbreds:

II. Relation to performance of hybrids within versus between heterotic groups for forage traits

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Summary. Restriction fragment length polymorphisms (RFLPs) have been proposed for the prediction of the yield potential of hybrids and the assignment of inbreds to heterotic groups. Such use was investigated in 66 diallel crosses among 6 flint and 6 dent inbreds from European maize (*Zea mays* L.) germ plasm. Inbreds and hybrids were evaluated for seven forage traits in four environments in the Federal Republic of Germany. Midparent heterosis (MPH) and specific combining ability (SCA) were calculated. Genetic distances (GD) between lines were calculated from RFLP data of 194 clone-enzyme combinations. GDs were greater for flint × dent than for flint × flint and dent × dent line combinations. Cluster analysis based on GDs showed separate groupings of flint and dent lines and agreed with pedigree information, except for 1 inbred. GDs of all line combinations in the diallel were partitioned into general (GGD) and specific (SGD) genetic distances; GGD explained approximately 20% of the variation among GD values. For the 62 diallel crosses (excluding 4 crosses of highly related lines), correlations of GD with F_1 performance, MPH, and SCA for dry matter yield (DMY) of stover, ear, and forage were positive but mostly of moderate size ($0.09 \leq r \leq 0.60$) compared with the higher correlations ($0.39 \leq r \leq 0.77$) of SGD with these traits. When separate calculations were performed for various subsets, correlations of GD and SGD with DMY traits were generally small ($r < 0.47$) for the 36 flint × dent crosses, significantly positive ($r < 0.53$) for the 14 flint × flint crosses, and inconclusive for the 12 dent × dent crosses because of the lack of significant genotypic variation. Results indicated that RFLPs can be used for assigning inbreds to heterotic groups. RFLP-based genetic distance measures seem to be useful for predicting forage yield of (1) crosses between lines from the same germ plasm group or (2) crosses including line combinations from the same as well as different heterotic groups. However, they are not indicative of the hybrid forage yield of crosses between unrelated lines from genetically divergent heterotic groups.

Key words: RFLPs – Heterosis – Genetic distances – Prediction – *Zea mays* L.

Introduction

The identification of lines having superior hybrid performance is the most costly and time-consuming task in breeding programs for hybrid maize (*Zea mays* L.). According to a survey among U.S. maize breeders, a major part of the breeding resources is devoted to evaluating lines in testcrosses and hybrid combinations (Bauman 1981). The efficiency of hybrid breeding programs could be increased if superior crosses could be predicted before field evaluation based on a screening of parental inbreds.

Heterosis accounts for about half of the grain yield of modern maize hybrids (Duvick 1984). Parental genetic divergence has been found to increase the potential for heterosis in crosses among inbreds and, within limits, for interracial crosses of maize (for review, see Hallauer et al. 1988). This suggested the use of indirect measures of genetic diversity as possible predictors for the heterotic response of hybrids.

During the past two decades, experiments have been published relating isozyme dissimilarity between maize inbreds with hybrid performance (for review, see Stuber 1989). In most studies allelic differences at enzyme loci were positively correlated with hybrid grain yield, but the correlations were generally too low to be of predictive

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value. Frei et al. (1986) reported that the correlation differed depending upon whether crosses were produced between lines with similar pedigree background or between lines from different populations. They found a significant correlation between isozyme dissimilarity and higher yield only for the former but not the latter type of crosses.

RFLPs have been suggested as an alternative class of genetic markers for estimating genetic diversity and selecting for increased heterozygosity of hybrids to optimize heterotic response (Burr et al. 1983). In maize, a large number (>500) of mapped DNA clones, well-distributed over the genome, have been developed by public and private research groups (Helentjaris et al. 1988; Burr et al. 1988; Coe et al. 1988) revealing a high level of polymorphism in elite germ plasm (Godshalk et al. 1990; Smith et al. 1990; Melchinger et al. 1991; Boppenmaier et al. 1992). Compared with isozymes, RFLPs allow a more complete and uniform sampling of the maize genome and, thus, should permit a more precise estimation of genetic distances between genotypes. Recent studies in maize (Lee et al. 1989; Smith et al. 1990; Melchinger et al. 1991) suggested that RFLPs can be used to investigate pedigree relationships among inbreds and to assign inbreds to heterotic groups. Lee et al. (1989) and Smith et al. (1990) reported a close association of hybrid performance or heterosis of single crosses to RFLP-based genetic distances of their parental inbreds. In contrast, other researchers (Godshalk et al. 1990; Melchinger et al. 1990a, b; Dudley et al. 1991; Boppenmaier et al. 1992) concluded from their results that RFLP-based distance measures are of limited value in predicting hybrid performance of single crosses between unrelated lines.

All but one of the above-cited studies using RFLPs were undertaken with dent maize inbreds from the U.S. Corn Belt, and the investigated traits were grain yield and yield components. Only Boppenmaier et al. (1992) assayed European maize inbreds and examined forage traits. In contrast to the pure dent × dent crosses commonly employed in U.S. maize hybrids, most of the commercial hybrids grown in Central and Western Europe are crosses between flint and dent inbred lines. Moreover, in many parts of the world, including Europe, maize is cultivated primarily for silage production. To maximize selection advance for forage performance, the breeder has to consider both yield and quality traits of stover and grain simultaneously (Dhillon et al. 1990b; Geiger et al. 1992).

The objectives of the study presented here were to (1) determine RFLP-based genetic distances and hybrid performance as well as heterosis of relevant forage traits for diallel crosses among 6 flint and 6 dent inbred lines adapted to Central Europe and (2) evaluate the usefulness of RFLP-based distance measures for predicting forage yield and quality traits of maize hybrids. For both objectives a comparison was made between single crosses within heterotic groups and those between heterotic groups.

Materials and methods

Genetic materials evaluated

The field data analyzed in this study were taken from an experiment previously described by Dhillon et al. (1990a, b). Their study included 6 flint (D102, DK105, D107, D118, D140, D503) and 6 dent (CO125, W401, D44, D01, D403, D406) maize inbreds. All lines, except CO125 and W401, were developed by W. G. Pollmer at the University of Hohenheim and have been used extensively in the production of commercial hybrids grown in the FRG and France. The genetic background of the lines is given in Table 1. The flint inbreds were derived from Western and Central European flint populations: 2 (D107 and D503) originated directly from synthetic populations; the other 4 represent second-cycle inbreds developed from crosses of elite first-cycle inbreds. The 6 dent lines were derived from recycling lines of Wisconsin and Canadian origin. Two dent lines (D403 and D406) were related by a common high-protein source (Illinois High Protein (IHP)) and had undergone selection for increased grain protein content and protein yield in addition to selection for grain yield and other agronomic traits. The lines were all

Table 1. Genetic background of the 12 maize inbred lines used in this study

Inbred line[a]	Genetic background[b,c,d]
Flint	
D102	75% F2, 12.5% 403, 12.5% EA125/405
DK105	50% GB101, 50% GB35/B
D107	Synthetic of GB18, GB31, GB101, Z583, F2, EP1, and DS7
D118	50% F7, 25% DS7, 12.5% 403, 12.5% EA125/405
D140	75% DK105, 25% JF30sd
D503	50% Syn. PF75, 25% D102, 12.5% DK105, 12.5% F6B.Scag
Dent	
CO125	Unknown
W401	50% W67C, 25% Wis. No. 25, 25% W33, (Anonymous 1989)
D44	50% CO125, 25% W153, 25% 417sd
D01	50% CO125, 25% CO158, 12.5% NE1A, 6.3% W41A, 6.3% W59E
D403	75% CO125, 25% IHP
D406	75% Syn. PD, 9.4% Prid, 6.3% IHP, 4.7% W41A, 4.7% W59E

[a] Lines with initials D or DK were developed by W. G. Pollmer and are proprietary to the University of Hohenheim, W-7000 Stuttgart 70, FRG

[b] W. G. Pollmer (personal communication) unless otherwise stated

[c] Lines with initials GB originated from the German population Gelber Badischer Landmais

[d] Percentage values refer to expected genomic contribution of respective line or synthetic population

highly inbred and had been maintained by self-pollination from seeds of individual ears and rogueing for off-type plants for more than 10 generations. Phenotypic appearance in field tests and RFLP patterns of the inbreds (obtained with single-copy clones yielding only one band per line) gave no evidence of remnant heterozygosity or seed-stock contamination.

The 12 inbred lines were crossed following a diallel mating design to produce all possible 66 F_1 crosses (reciprocals bulked).

Field experiments

Both groups of materials (66 F_1 crosses, 12 inbred lines) were evaluated in 1984 and 1985 at Eckartsweier (University of Hohenheim, Maize Research Station) and at Braunschweig-Voelkenrode (Institute of Grassland and Fodder Research, Federal Agricultural Research Station). The two test sites are located in the south and north of Germany, respectively, and represent diverse agroecological and climatic conditions. The inbred lines and hybrids were grown in separate but adjoining randomized complete-block designs with two replications each. Plots consisted of single rows, 5 m long and spaced at 0.75 m. The experiments at Eckartsweier and Braunschweig-Voelkenrode were machine- and handplanted, respectively, with 66 seeds per row and later thinned to approximately 33 plants (8.5 plants m^{-2}). The harvesting of individual entries at the ensiling stage was staggered on the basis of days to silk.

Data were analyzed for the following traits: dry matter yield (DMY) of stover (stem, leaves, shank, and husks), ear, and forage (stover + ear); net energy content (NEC), expressed in starch equivalents per kilogram dry matter (SE kg^{-1}), of stover and forage; and net energy yield (NEY), expressed in starch equivalents per hectare (SE ha^{-1}), of stover and forage. NEC of stover was determined from acid detergent fiber (ADF) and *in vitro* digestible organic matter (IVDOM), assayed by the laboratory methods of Goering and Van Soest (1970) and Tilley and Terry (1963), respectively, according to the following equation (Zimmer et al. 1980):

$$\mathrm{NEC} = 507.388 - 9.292\ \mathrm{ADF} + 4.477\ \mathrm{IVDOM}\,,$$

with a coefficient of determination (R^2) of 0.92 to NEC determined *in vivo*. NEC of forage was calculated from NEC and DMY of stover and ear by the rule of proportion and assuming a constant NEC of ear dry matter of 768 SE kg^{-1} (Gross 1980).

RFLP analyses

The 12 inbreds were assayed for their respective RFLP patterns. RFLP assays were conducted according to the procedures described by Boppenmaier et al. (1992). We used two restriction enzymes (*Eco*RI and *Hin*dIII) and 101 genomic DNA clones. All maize chromosomes were marked by at least seven DNA probes. Altogether, we analyzed RFLP data from the 194 clone-enzyme combinatios used and listed by Boppenmaier et al. (1992); 8 clone-enzyme combinations were discarded because of problems with unambiguous scoring of autoradiograms.

RFLP profiles on autoradiograms for each clone-enzyme combination were visually scored. A number was assigned to each band according to its migration distance determined by comparison with adjacent lanes of a ladder with restriction fragments of lambda loaded at every eighth lane in the gel. Only full intensity bands were taken into consideration, and two bands were scored as different when they were clearly separated from each other across all lanes in which they appeared. Data were binary coded for subsequent numerical analyses, i.e., presence or absence of a band in a line was coded by 1 or 0, respectively.

Statistical analyses

Analyses of variance (ANOVAs) of F_1 data from individual experiments and combined across environments were conducted following Cochran and Cox (1957). Midparent heterosis (MPH) of each cross was computed by subtracting the respective midparent mean $((P_1 + P_2)/2)$ from the F_1 mean in each environment and subsequently performing combined ANOVAs. In addition to the full set of 66 F_1 crosses, combined ANOVAs of F_1 and heterosis data were performed with a reduced set of 62 F_1 crosses between lines with coancestry $f \leq 0.375$. The 4 crosses excluded were those between highly related lines DK105 × D140, CO125 × D01, CO125 × D403, and D44 × CO125 with estimated coancestry $f = 0.75$, 0.50, 0.75, and 0.50, respectively. Coancestry (f) between related lines was calculated according to the rules and assumptions described by Falconer (1988) and Melchinger et al. (1991), respectively.

Diallel analyses were performed with the F_1 data to estimate general combining ability (GCA) and specific combining ability (SCA) effects. In addition to the reduced set of 62 F_1 crosses, separate combining-ability analyses were performed with the 3 diallel subsets composed of the 14 flint × flint, 12 dent × dent, and 36 flint × dent crosses. Calculations were made according to Griffing's (1956) model 1 of method 4, except in the latter case, where a factorial analysis (Comstock and Robinson 1952) was used. The 4 crosses excluded from the complete diallel and its 3 subsets were treated as missing observations, and PROC GLM of SAS (SAS Institute 1988) was employed for computing least-squares estimates of GCA and SCA effects. For all statistical analyses, a mixed effects linear model was used with the assumptions that genotypes were fixed and replications and environments were random. Accordingly, genetic ratios (GR) analogous to broad-sense heritability were calculated for (1) the reduced set of 62 F_1 crosses and (2) its above-mentioned three subsets as $GR = \phi_c^2\, e\, r/MS_c$, where ϕ_c^2 is the genotypic variance among crosses, MS_c is the mean square due to crosses, r is the number of replications, and e is the number of environments. Dhillon et al. (1990b) may be consulted for further details.

Genetic distance (GD) between pairs of inbreds was estimated as one minus the genetic similarity calculated according to the method of Nei and Li (1979). Properties of this genetic distance measure and its relationship to other measures used in literature have been discussed by Boppenmaier et al. 1992. Analogous to the GCA-SCA subdivision of agronomic F_1 data, GD values associated with the diallel crosses were partitioned into general genetic distances (GGD) and specific genetic distances (SGD) according to the proposal of Melchinger et al. (1990b).

Simple correlations were calculated for GD and SGD with F_1 performance (F1P), SCA, and MPH across environments determined from the combined ANOVAs for the reduced set of 62 F_1 crosses and its three subsets of 14 flint × flint, 12 dent × dent, and 36 flint × dent crosses.

Associations among the 12 lines 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 the PROC CLUSTER program of SAS, subroutine AVERAGE (SAS Institute 1988).

Results and discussion

Genetic variation for RFLPs

Of the 194 clone-enzyme combinations used in this study, 185 (95%) revealed polymorphism across the 12 inbreds assayed. The majority (78%) of the clone-enzyme combinations gave single-banded RFLP patterns (exactly one band for each line). The remaining 42 clone-enzyme combinations yielded multiple-banded RFLP patterns with up to six bands per line, suggesting the presence of repeated binding sequences in the genome for the respective DNA clones (Helentjaris et al. 1988) because in general multiple-banded RFLP patterns occurred simultaneously with both restriction enzymes.

Most (75%) clone-enzyme combinations detected between 2 and 5 RFLP variants across all 12 lines. The average number of RFLP variants per clone-enzyme combination was 4.0 and the maximum number was 11. Only minor differences in the level of polymorphism existed among the 10 chromosomes. Restriction enzymes *Eco*RI and *Hin*dIII each detected on average 4.0 RFLP variants per clone. The level of polymorphism found in our RFLP assay of 12 European maize inbreds was in close agreement with comparable studies of inbreds from the U.S. Corn Belt (Melchinger et al. 1990a; Messmer et al. 1991).

Genetic distances among inbreds

GDs between the parental lines of all diallel crosses ranged from 0.14 for DK105 × D140 to 0.73 for D503 × D44 with an overall mean of 0.54 (Table 2). The 36 flint × dent (F × D) crosses had greater GDs than the 15 flint × flint (F × F) and the 15 dent × dent (D × D) crosses, the subset means being 0.64, 0.51, and 0.51, respectively. However, the range of GD values was narrower in F × D crosses (0.56–0.73) than in F × F (0.14–0.66) and D × D crosses (0.23–0.62), primarily as a consequence of the small GDs between highly related ($f=0.75$) pairs of lines (DK105 × D140, CO125 × D403) in the latter two groups. For each inbred except D01, the maximum GD was observed in combination with D44.

The coefficient of determination (R^2) for the partition of GD into GGD and SGD indicated that 23% of the variation among GD values was explained by GGD. This was about half as much as reported in recent RFLP studies with inbreds from the U.S. Corn Belt (Melchinger et al. 1990a, b). D44 and D118 had large positive GGD, indicating that they represent fairly unique germ plasm within the 6 dent and 6 flint inbreds, respectively. The predominant importance of SGD in the present study was attributable to two causes. First, SGD estimates were mostly positive for F × D crosses and negative for F × F and D × D crosses, indicating the presence of group effects. Second, in harmony with theoretical expectations (Melchinger et al. 1990b), closely related pairs of inbreds (D102 × D503, DK105 × D140, CO125 × D01, CO125 × D403) had large negative SGD estimates. An exception was cross CO125 × D44 with $f=0.50$, but SGD (−0.01) close to zero.

Cluster analysis of RFLP data

The dendrogram obtained from UPGMA cluster analysis of GDs resulted in two major groupings composed of the 6 flint and 6 dent inbreds (Fig. 1). Within the flint heterotic group, two subclusters of related lines were formed: (1) DK105 and its backcross derivative D140 ($f=0.75$) and (2) D503 and its progenitor D102 ($f\approx 0.34$), which in addition are related through the French inbred F2 used in the development of line D102 as well as syn-

Table 2. Genetic distance[a] (GD, above diagonal), general genetic distance (GGD), and specific genetic distance (SGD, below diagonal) calculated from RFLP data of 194 clone-enzyme combinations among 6 flint and 6 dent maize inbred lines

Inbred line	Flint						Dent						General genetic distance
	D102	DK105	D107	D118	D140	D503	CO125	W401	D44	D01	D403	D406	
Flint													
D102		0.61	0.56	0.56	0.60	0.37	0.62	0.60	0.71	0.59	0.63	0.63	0.00
DK105	0.04		0.47	0.58	0.14	0.46	0.66	0.68	0.72	0.64	0.61	0.59	−0.02
D107	−0.04	−0.01		0.66	0.49	0.58	0.66	0.61	0.67	0.60	0.65	0.58	0.01
D118	−0.08	−0.02	0.01		0.58	0.56	0.63	0.67	0.70	0.66	0.64	0.63	0.04
D140	0.04	−0.38	−0.06	−0.01		0.46	0.62	0.62	0.72	0.63	0.59	0.56	−0.03
D503	−0.21	−0.09	−0.00	−0.06	−0.07		0.66	0.62	0.73	0.65	0.62	0.60	0.00
Dent													
CO125	0.07	0.13	−0.09	0.03	0.10	0.12		0.59	0.62	0.32	0.23	0.43	−0.03
W401	0.00	0.10	0.00	0.01	0.05	0.02	0.02		0.62	0.61	0.54	0.43	0.02
D44	0.02	0.07	−0.01	−0.01	0.08	0.06	−0.01	−0.07		0.61	0.61	0.59	0.09
D01	0.01	0.09	0.02	0.04	0.09	0.09	−0.20	0.02	−0.05		0.45	0.49	−0.01
D403	0.07	0.09	0.08	0.04	0.08	0.08	−0.28	−0.02	−0.02	−0.08		0.47	−0.03
D406	0.07	0.07	0.02	0.03	0.05	0.06	−0.07	−0.13	−0.04	−0.04	−0.03		−0.03

[a] Standard errors for genetic distances (GDs) calculated by the jackknife method (Miller 1974) ranged between 0.031 and 0.035

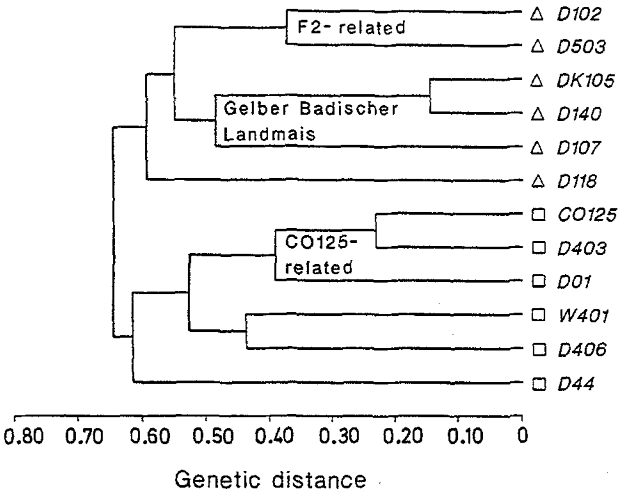

Fig. 1. Associations of 6 flint and 6 dent maize inbred lines revealed by average linkage (UPGMA) cluster analysis of genetic distances (GD) calculated from RFLP data. △ Flint lines, □ dent lines

thetic PF75. Inbred D107 was loosely aggregated with the cluster of DK105-related lines, whereas D118 was clearly separated from the other flint lines.

Within the dent heterotic group, two subgroups were apparent: (1) CO125 and its two derivatives D403 and D01 with $f=0.75$ and 0.50, respectively, and (2) W401 and D406. W401 was a progenitor of synthetic PD involved in the development of D406, but the coancestry of these two lines is unknown because of incomplete pedigree records. Line D44 was most distantly merged with the other dent lines despite of its close relatedness with CO125 ($f=0.50$). D44 differs considerably from its parent CO125 in plant type and early vigor under cool weather conditions. In addition, their cross showed average yield for ear and forage without any evidence of inbreeding depression. We have two alternative explanations for these findings: (1) D44 inherited a much smaller proportion of its genome from CO125 than expected from its pedigree due to selection and/or genetic drift during line development or (2) the pedigree of D44 is wrong. The latter hypothesis could be tested by comparing the RFLP profiles of D44 with those of its three progenitor lines; if the pedigree is incorrect due to pollen or seed contamination, D44 would be expected to display a greater number of bands not present in any of the progenitors.

Clustering of inbreds based on RFLP data clearly separated the lines from the flint and dent heterotic groups. Within heterotic groups, the clustering of inbreds was generally consistent with the expectations based on pedigree information, except for line D44. The first result corroborates the conclusion drawn from recent RFLP studies with U.S. maize inbreds (Lee et al. 1989; Melchinger et al. 1990a, b; Melchinger et al. 1991) in that RFLPs are useful for assigning inbreds to established heterotic groups and for establishing new heterotic groups. The deviating behavior of D44 suggests that further studies with a greater number of lines should be conducted to investigate in detail the association between the coancestry of related lines and the corresponding RFLP-based GD estimates. Such research is needed to examine the potential usefulness of RFLPs for revealing pedigree relationships among inbreds and for determining the true degree of relatedness between lines, as proposed by Melchinger et al. (1991).

Hybrid performance for forage traits: relation to genetic distances

Combined ANOVAs and estimates of GCA and SCA variances and heritability for DMY traits obtained from the complete set of 66 diallel crosses have been given by Dhillon et al. (1990a) and, therefore, are not presented here. Genotypic differences among the reduced set of 62 crosses (4 crosses between highly related lines excluded) were highly significant ($P<0.01$) for all traits (Table 3). This was also apparent from the wide range of F1P for the DMY and NEY traits. Estimates of GR were greater than 0.80 except for NEY of forage. The high GR estimates indicated a predominant role of genotypic variation in comparison with genotype × environment interaction and experimental error for all traits in the materials examined.

Midparent heterosis (MPH), expressed as percentage of the F1P, accounted for 47% of F1P for DMY and NEY of forage and showed a wide range among crosses (Table 3). MPH was smaller for DMY of stover (41%) than for DMY of ear (53%). MPH for NEC of forage and stover was small and negative (−1% and −9%, respectively).

Simple correlations (r) of GD with F1P and MPH, and of SGD with F1P, MPH, and SCA of the 62 crosses were highly significant ($P<0.01$) for DMY of forage and ear and for NEY of forage (Table 4). Estimates of r were consistently smaller for GD (0.33–0.60) than SGD (0.59–0.77). For DMY and NEY of stover, only the correlations involving SGD were significant ($P<0.05$) and of medium size. For NEC traits, GD and SGD were either not significantly correlated or were negatively correlated with F1P, MPH, and SCA.

The correlations generally improved from F1P to MPH and further to SCA in accordance with quantitative-genetic expectations (Melchinger et al. 1990b). The greater correlations of DMY and NEY traits with SGD in comparison with GD are consistent with expectations for the materials under study. For the complete diallel, Dhillon et al. (1990a) reported SCA to contribute more than GCA to the genotypic variation in DMY traits, and this applied also to the reduced set of 62 crosses. A

Table 3. Significance of *F* test for genotypic differences, genetic ratio (GR), and mean, minimum, and maximum of F_1 performance (F1P) and mid-parent heterosis (MPH) in a diallel set of 62[a] maize crosses for forage traits

Trait[b]		*F* test[c]	GR[d]	F_1 performance			Mid-parent heterosis		
				Mean	Minimum	Maximum	Mean	Minimum	Maximum
DMY	Forage	**	0.87	13.90	8.77	18.03	6.57	2.32	10.23
($Mg\ ha^{-1}$)	Stover	**	0.88	6.78	4.30	9.43	2.75	1.04	5.13
	Ear	**	0.81	7.13	4.47	9.13	3.80	1.28	5.56
NEY	Forage	**	0.69	862	565	1,102	403	163	618
($SE\ m^{-2}$)	Stover	**	0.81	315	222	436	111	−4	208
NEC	Forage	**	0.83	621	583	645	−5	−32	29
($SE\ kg^{-1}$)	Stover	**	0.80	465	399	531	−40	−124	33

** Significant at the 0.01 probability level

[a] Four crosses out of 66 (DK105 × D140, CO125 × D403, CO125 × D44, CO125 × D01) involving highly related inbred parents (see Table 1) were excluded from the analyses

[b] DMY = Dry matter yield; NEY = net energy yield; NEC = net energy content

[c] *F* test = genotypic mean squares versus genotype-environment interaction mean squares

[d] GR = genotypic ratio analogous to broad-sense heritability

Table 4. Simple correlations of genetic distance (GD) and specific genetic distance (SGD) with F_1 performance (F1P), mid-parent heterosis (MPH), and specific combining ability (SCA) in a diallel set of 62[a] maize crosses for forage traits

Trait[b]		Variable combination				
		GD versus F1P	GD versus MPH	SGD versus F1P	SGD versus MPH	SGD versus SCA
DMY	Forage	0.33**	0.44**	0.59**	0.68**	0.75**
	Stover	0.09	0.13	0.39**	0.51**	0.61**
	Ear	0.53**	0.60**	0.68**	0.66**	0.77**
NEY	Forage	0.35**	0.47**	0.63**	0.72**	0.77**
	Stover	−0.07	−0.02	0.28	0.40**	0.56**
NEC	Forage	−0.07	0.12	−0.03	0.17	0.00
	Stover	−0.40**	−0.32*	−0.27	−0.03	0.00

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

[a] Four out of 66 crosses (DK105 × D140, CO125 × D403, CO125 × D44, CO125 × D01) involving highly related inbred parents (see Table 1) were excluded from the analyses

[b] DMY = Dry matter yield; NEY = net energy yield; NEC = net energy content

similar inheritance pattern may be expected for NEY traits because variation in NEY traits depends mainly on DMY traits rather than on NEC traits (Geiger et al. 1992).

Plots of GD versus F1P and SGD versus SCA for DMY of forage, stover, and ear are shown in Fig. 2 for all 66 crosses. Obviously, inclusion of the 4 crosses between related lines would result in an upward bias of the correlations because they were all positioned in the lower left quadrant apart from CO125 × D44 for F1P. The plots demonstrate that the elimination of line combinations with negative SGD would substantially increase the frequency of crosses with positive SCA for DMY of forage, stover, and ear. Further examination showed that most of the data points found in the upper right quadrant refer to crosses of type F × D. This result is consistent with the common practice in maize breeding of preferably evaluating crosses between heterotic groups because they are expected to perform generally better than crosses within heterotic groups.

To compare the usefulness of RFLPs in predicting the performance of intergroup versus intragroup hybrids, a subdivision of the 62 crosses into 36 F × D, 14 F × F, and 12 D × D crosses is of interest. In these subsets, we considered only DMY traits for the following reasons: (1) DMY traits are the traits of primary interest, (2) NEY traits gave results similar to those of DMY traits, and (3) NEC traits showed, in three out of four cases, nonsignificant genotypic variation within the F × F and D × D subsets of crosses.

ANOVAs revealed significant ($P < 0.01$) genotypic variation among F × D and F × F crosses for all three DMY traits and among D × D crosses only for DMY of stover (Table 5). GR estimates of the three subsets agreed well with those of the 62 crosses apart from the reduced estimates for DMY of forage and ear in D × D crosses. F × D and D × D crosses had similar but significantly greater overall means for F1P than F × F crosses. As expected, intergroup (F × D) crosses showed appreciably greater MPH than intragroup (F × F, D × D) crosses. For all DMY traits, F × D crosses had the greatest maximum and a broader range of F1P than F × F and D × D crosses. With respect to RFLP-based GD estimates, however, the former had a smaller range and coefficient of variation (CV) than the latter (CV = 0.0006, 0.0014, and 0.0014 for F × D, F × F, and D × D crosses, respectively).

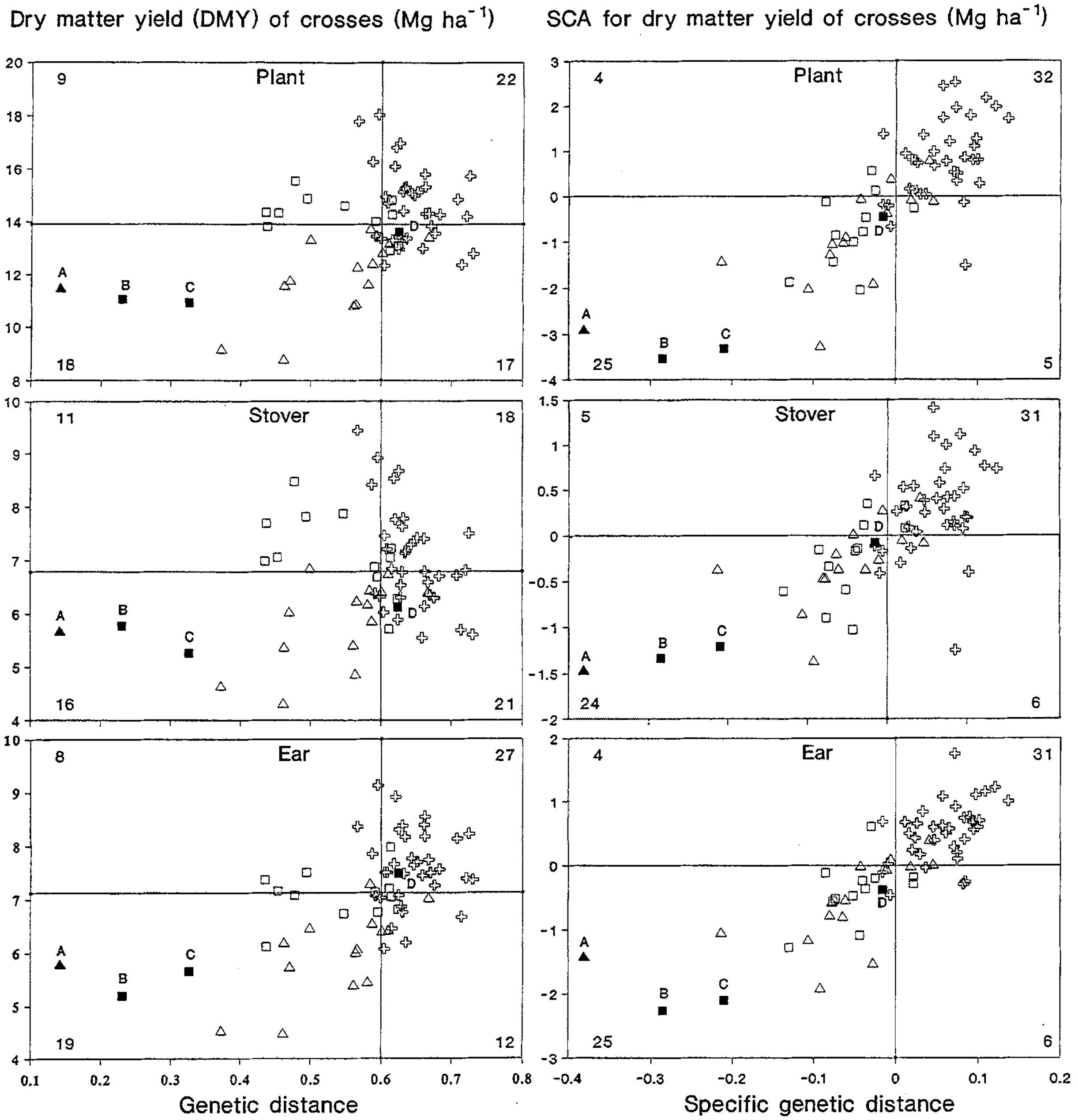

Fig. 2. Genetic distance (GD) versus F_1 performance and specific genetic distance (SGD) versus specific combining ability (SCA) for dry matter yield of forage, stover, and ear in a diallel of 66 maize crosses. Quadrants are divided along mean values for the respective axes with numbers showing the number of crosses located in the respective quadrant. ✥ Flint × dent crosses, △ flint × flint crosses, □ dent × dent crosses. Solid symbols indicate crosses between highly related lines: A DK105 × D140, B CO125 × D403, C CO125 × D01, D CO125 × D44

For the subset of F × D crosses, correlations of SGD with F1P, MPH, and SCA of DMY traits were always positive and mostly significant (Table 6), but their magnitude (<0.44) was too small to be of predictive value. Correlations involving GD were either nonsignificant or negative. The significantly negative correlations of GD with F1P and MPH for DMY of stover were largely attributable to crosses of dent inbred D406 with DK105-related flint lines (DK105, D107, D140), which had comparatively small GD estimates (Table 2) but extremely large F1P and MPH for DMY of stover. For F × F crosses, correlations of both GD and SGD with the other variables were all significantly positive and varied from 0.53 to 0.76. For D × D crosses, the *r* values were not significant except for SGD with SCA for DMY of stover. Concerning the F × F and D × D crosses, the limited numbers of degrees of freedom associated with these correlations should be kept in mind.

Table 5. Significance of *F* test for genotypic differences, genetic ratio (GR), and mean, minimum, and maximum of F_1 performance (F1P) and mid-parent heterosis (MPH) for dry matter yield (DMY) traits and mean, minimum, and maximum of genetic distance (GD) in flint × dent, flint × flint, and dent × dent subsets of maize crosses

Trait[a]		*F*-test[b]	GR[c]	F_1 performance			Mid-parent heterosis		
				Mean	Minimum	Maximum	Mean	Minimum	Maximum
Flint × dent crosses (n = 36)									
DMY ($\mathrm{Mg\ ha^{-1}}$)	Forage	**	0.81	14.62	12.33	18.03	7.28	5.45	10.24
	Stover	**	0.87	7.03	5.54	9.43	3.00	1.43	5.13
	Ear	**	0.67	7.58	6.07	9.13	4.25	3.14	5.56
GD				0.64	0.56	0.73			
Flint × flint crosses (n = 14)									
DMY ($\mathrm{Mg\ ha^{-1}}$)	Forage	**	0.91	11.81	8.77	13.70	5.01	2.32	7.07
	Stover	**	0.87	5.82	4.30	6.84	2.31	1.04	3.30
	Ear	**	0.89	5.99	4.47	7.27	2.70	1.28	3.77
GD				0.54	0.37	0.67			
Dent × dent crosses (n = 12)									
DMY ($\mathrm{Mg\ ha^{-1}}$)	Forage	^{ns}	0.25	14.16	12.90	15.54	6.23	4.87	7.34
	Stover	**	0.80	7.14	5.70	8.47	2.52	1.20	3.47
	Ear	^{ns}	0.20	7.07	6.12	7.98	3.73	2.95	5.12
GD				0.54	0.44	0.62			

**, ^{ns} Significant at the 0.01 and non-significant at the 0.05 probability levels, respectively

[a] DMY = Dry matter yield; GD = RFLP-based genetic distance

[b] *F* test = genotypic mean squares versus genotype-environment interaction mean squares

[c] Genotypic ratio analogous to broad-sense heritability

Table 6. Simple correlations (*r*) of genetic distance (GD) and specific genetic distance (SGD) with F_1 performance (F1P), mid-parent heterosis (MPH), and specific combining ability (SCA) in flint × dent, flint × flint, and dent × dent subsets of maize crosses for dry matter yield (DMY) traits

		Variable combination				
		GD versus F1P	GD versus MPH	SGD versus F1P	SGD versus MPH	SGD versus SCA
		r				
Flint × dent crosses (n = 36)						
DMY	Forage	−0.32	−0.17	0.30	0.38*	0.36*
	Stover	−0.47**	−0.44**	0.18	0.33*	0.24
	Ear	0.01	0.22	0.38*	0.31	0.44**
Flint × flint crosses (n = 14)						
DMY	Forage	0.70**	0.60*	0.76**	0.71**	0.64*
	Stover	0.61*	0.53*	0.68**	0.63**	0.58*
	Ear	0.70**	0.59*	0.74**	0.69**	0.65*
Dent × dent crosses (n = 12)						
DMY[a]	Stover	−0.57	−0.46	0.02	0.22	0.61*

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

[a] Correlation coefficients for DMY of forage and ear were not calculated because the *F* test of genotypic differences was not significant

In summary, our study demonstrated that the associations of RFLP-based genetic distance measures with hybrid performance and heterosis of single crosses depend upon the type of crosses examined. We found substantial differences in the associations for (1) crosses between lines from different heterotic groups, (2) crosses between lines from the same heterotic group, and (3) a mixture of both, as is commonly the case in diallel crosses among lines from different heterotic groups. The correlations of GD and SGD with F1P, MPH, and SCA were appreciably smaller for F × D crosses in comparison with F × F crosses and the entire set of 62 crosses from the diallel (Tables 4 and 6). According to the plots in Fig. 2, the higher correlations for the 62 crosses were attributable to group effects: F × D crosses had mostly large GDs combined with good performance for DMY traits, whereas D × D crosses and F × F crosses showed mostly smaller GDs combined with poorer yields. The limited variation of GDs in F × D crosses, in addition to the various quantitative-genetic reasons discussed in detail by Boppenmaier et al. 1992 may have contributed to the small correlations observed for this group.

Our results are largely in agreement with those from published experiments and may explain the discrepancies reported in literature. Boppenmaier et al. 1992 also investigated correlations between GD and forage traits in a study with 66 F × D crosses between a different set of European flint and dent inbreds and found none of them to be significantly different from zero. Likewise in an experiment with elite U.S. maize inbreds, Godshalk et al. (1990) reported no association between the modified Rogers' distance calculated from RFLP data and grain yield of F_1 hybrids, most of which were intergroup crosses. In experiments with diallel crosses, correlations of RFLP-based distance measures with F1P or SCA for grain yield ranged from low (Dudley et al. 1991) to medium (Melchinger et al. 1990a, b), but were fairly high (Lee et al. 1989) when the parents involved sets of inbreds from different heterotic groups. Smith et al. (1990) reported extremely high correlations of GD, based on 257 RFLP loci, with heterosis ($r=0.87$) and F1P ($r=0.93$) for grain yield in a study that included crosses of elite inbreds from the same as well as different heterotic groups, including crosses between related lines.

The greater correlations obtained for the F × F crosses in comparison with the F × D crosses (Table 6) are consistent with the results reported by Frei et al. (1986). These authors found a close association between GD based on isozymes with grain yield of F_1 crosses only for lines with a similar pedigree background ($r=0.52$) but not for lines with a dissimilar pedigree background ($r=-0.26$). It is worth mentioning that the correlations for the F × F crosses in our study were only slightly reduced when both crosses of highly related lines and crosses between more distantly ($f>0.1$) lines were excluded from the analyses.

Our study suggests the possibility of employing RFLP-based genetic distance measures for predicting hybrid performance, heterosis, and SCA for DMY of forage and ear provided (1) hybrids are to be produced from parents with a similar as well as a dissimilar pedigree background (i.e., intragroup and intergroup crosses) or (2) hybrids are exclusively produced between lines from the same germ plasm group. Under these circumstances, prediction of hybrid performance or SCA may allow the breeder to reject poorer hybrids and to enhance the probability of identifying better hybrids. It may be added that the first situation arises if new inbreds of unknown heterotic pattern or new lines developed from crosses between parents from different heterotic groups (e.g., commercial hybrids) are to be tested in hybrid combinations. The second situation is less frequent in maize breeding, but is relevant in some other crops as, for example, in rape seed (*Brassica napus* L.), where hybrid breeding is feasible, but development of genetically divergent heterotic groups is still in its infancy (H. C. Becker, personal communication).

With respect to crosses between lines from different heterotic groups, our results support the conclusion of Boppenmaier et al. 1992 that genetic distance measures based on an unselected set of RFLP markers are not sufficiently associated with hybrid performance for forage traits to be of any predictive value. In view of the large number (194) of RFLP markers employed in this study, it seems rather unlikely that a further increase in the number of markers would appreciably improve the correlations. Instead, Dudley et al. (1991) recommended the identification of specific marker genotypes for those chromosomal segments that have a significant effect on the expression of the traits of interest. It remains to be investigated whether (1) such markers can be found with reasonable experimental expenditures, (2) the identified linkage relationships exist across a wide range of germ plasm within heterotic groups, and (3) they explain a sufficiently large proportion of the genotypic variation among intergroup hybrids. Research efforts to answer these questions are currently in progress at several private and public institutions.

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