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ORIGINAL PAPER

Prediction of single-cross hybrid performance for grain yield and grain dry matter content in maize using AFLP markers associated with QTL

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Abstract Prediction methods to identify single-cross hybrids with superior yield performance have the potential to greatly improve the efficiency of commercial maize (*Zea mays* L.) hybrid breeding programs. Our objectives were to (1) identify marker loci associated with quantitative trait loci for hybrid performance or specific combining ability (SCA) in maize, (2) compare hybrid performance prediction by genotypic value estimates with that based on general combining ability (GCA) estimates, and (3) investigate a newly proposed combination of the GCA model with SCA predictions from genotypic value estimates. A total of 270 hybrids was evaluated for grain yield and grain dry matter content in four Dent × Flint factorial mating experiments, their parental inbred lines were genotyped with 20 AFLP primer-enzyme combinations. Markers associated significantly with hybrid performance and SCA were identified, genotypic values and SCA effects were estimated, and four hybrid performance prediction approaches were evaluated. For grain yield, between 38 and 98 significant markers were identified for hybrid performance and between zero and five for SCA. Estimates of prediction efficiency (R^2) ranged from 0.46 to 0.86 for grain yield and from 0.59 to 0.96 for grain dry matter content. Models enhancing the GCA approach with SCA estimates resulted in the highest prediction efficiency if the SCA to GCA ratio was high. We conclude that it is advantageous for prediction of single-cross hybrids to enhance a GCA-based model with SCA effects estimated from molecular marker data, if SCA variances are of similar or larger importance as GCA variances.

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Introduction

Identifying single-cross hybrids with superior yield performance is of fundamental importance in commercial maize (*Zea mays* L.) breeding programs. Several hundred single-cross combinations are tested by breeders each year in extensive yield trials. The test procedure is expensive and time-consuming, therefore, only a limited subset of all possible single crosses can be tested. Prediction methods for performance of single crosses have always been a major issue in hybrid breeding owing to the potential to greatly improve the efficiency of commercial breeding programs. As maize germplasm is commonly organised in genetically divergent heterotic groups, predicting the performance of inter-group hybrids is of greatest interest to maize breeders.

Predicting the performance of hybrids from per se performance of their parental inbred lines failed due to masking dominance effects (Smith 1986; Hallauer 1990). Estimates of genetic distances (GD) between the parental lines based on unselected DNA markers alone were not promising for predicting performance

of inter-group hybrids (cf. Melchinger 1999). These findings were in agreement with theoretical results of Charcosset and Essioux (1994), who attributed the low correlation between heterosis and GD to (1) no or only loose linkage of heterosis-affecting QTL to the molecular markers employed in estimating GD and (2) different linkage phases between the QTL and marker alleles in the maternal and paternal gametic arrays, as expected more frequently with inter-group hybrids.

Best linear unbiased prediction (BLUP) was investigated by Bernardo (1994, 1996) to predict performance of untested single crosses using phenotypic information of related single crosses and genetic relationships among their parental inbreds. As an extension based on trait and marker data, TM–BLUP was proposed, inferring the identity by descent of unobservable QTL alleles from molecular marker data and thereby modelling the covariances associated with QTL (Bernardo 1998, 1999). However, TM–BLUP resulted only in marginal improvements for predicting single-cross performance, compared with the ordinary BLUP approach.

Vuylsteke et al. (2000a) determined associations of amplified fragment length polymorphism (AFLP) markers with hybrid performance for grain yield (GY) and with specific combining ability (SCA) across hybrids from a diallel among lines from different heterotic groups. Estimates of genotypic value for all hybrids were obtained from the summed effects of significant markers. They provided the basis for prediction of hybrid performance and SCA by a linear regression approach. The predictions obtained with this approach were encouraging, but comparisons with established procedures for prediction of inter-group hybrids are lacking.

General combining ability (GCA) estimates of the parental lines provide an established and simple approach to predict hybrid performance (Cockerham 1967; Melchinger et al. 1987). Prediction based on GCA alone ignores SCA, which is related to specific heterosis and an important component of hybrid performance (Gardner and Eberhart 1966). Improving the simple GCA model with SCA predictions obtained by the approach of Vuylsteke et al. (2000a) has not been investigated hitherto.

Our objectives were to (1) identify marker loci associated with QTL for hybrid performance or SCA in maize using the approach devised by Vuylsteke et al. (2000a), (2) compare hybrid performance prediction by genotypic value estimates with that based on GCA estimates, and (3) investigate a newly proposed combination of the GCA model with SCA predictions from genotypic value estimates for the performance prediction of inter-group hybrids.

Materials and methods

Plant materials and field trials

In total, 52 maize elite inbred lines developed at the breeding program of the University of Hohenheim were used as parental lines for the factorial crosses under evaluation. The inbreds comprised 38 Dent lines with Iodent or Iowa Stiff Stalk Synthetic background, and 14 Flint lines with European Flint or Flint/Lancaster background. Four Dent × Flint factorial mating experiments (14×7, 11×4, 14×6, 11×4), further referred to as Exp. 1–4, were produced, providing a total of 270 hybrids. Thereby, eight Dent lines and six Flint lines were included in more than one factorial. Each factorial was evaluated in an 1-year experiment, with field trials at four to six locations in Germany under diverse agroecological conditions. The trials were evaluated in two-row plots using adjacent α-designs with two to three replications. The hybrid performance of the crosses was recorded for GY in Mg ha^{-1} adjusted to 155 g kg^{-1} grain moisture and for grain dry matter content (GDMC) in percent.

Biometrical analysis of phenotypic data

Adjusted entry means and effective error mean squares (Cochran and Cox 1957) derived from analysis of variance (ANOVA) of each trial were used to calculate the combined ANOVA for each experiment. Variance components and GCA and SCA effects of the factorial mating designs (Comstock and Robinson 1952) were estimated with the following model:

$$y_{ijk} = \mu + g_i' + g_j'' + s_{ij} + l_k + gl_{ik}' + gl_{jk}'' + sl_{ijk} + e, \quad (1)$$

where y_{ijk} = mean performance of the hybrid between parental inbreds i and j at location k; μ = overall mean; g_i' and g_j'' = GCA effects; s_{ij} = SCA effect; l_k = effect of location k; gl_{ik}', gl_{jk}'', and sl_{ijk} = GCA × location and SCA × location interaction effects of inbreds i and j with location k; $e \sim N(0, \sigma_e^2)$ = residual error of y_{ijk}. All effects were assumed random and determined by BLUP. Broad-sense heritabilities (h^2) were estimated on an entry-mean basis (Fehr 1987), and 95% confidence intervals of heritabilities were calculated according to Knapp and Bridges (1987). BLUP for GCA and SCA effects were calculated with SAMM (Butler et al. 2004), a software package, which uses core FORTRAN routines from the program AS-Reml™ (Gilmour et al. 2002). The remaining analyses of field data were performed with PLABSTAT (Utz 2003).

Analysis of molecular data

The 52 inbred lines were assayed for AFLP markers based on published protocols (Vos et al. 1995). Genotyping was conducted with 20 AFLP primer-enzyme combinations, 10 of which were *Eco*RI/*Mse*I (E33/M47, E33/M50, E33/M51, E33/M61, E33/M62, E35/M50, E38/M47, E38/M51, E39/M47, and E39/M50) and the remaining 10 were *Pst*I/*Mse*I (P12/M47, P12/M49, P12/M50, P12/M59, P12/M61, P12/M62, P13/M49, P18/M49, P18/M59, and P18/M62) (Vuylsteke et al. 1999). Positions of mapped AFLP bands were obtained from an integrated AFLP map (Peleman et al. 2000), a subset of which was chosen to avoid multiple markers at identical mapping positions.

Selection of markers and identification of QTL regions

For each experiment, markers associated significantly with hybrid performance and SCA were identified. Following Vuylsteke et al. (2000a), the genotypic class of each marker-hybrid combination was determined from marker data of the homozygous parental inbreds, generally resulting in three possible classes: homozygous present (*MM*), homozygous absent (*mm*), and heterozygous (*Mm*). Across all hybrids, each marker was tested with the rank sum test of Kruskal–Wallis for effects of marker genotypic class on hybrid performance or SCA. For testing marker associations with hybrid performance, a significance level of $P = 0.001$ was used. In the study of Vuylsteke et al. (2000a), this significance level resulted in the highest efficiency for prediction of hybrid performance, when compared with other scenarios. Following these authors, a significance level of $P = 0.005$ was used for testing marker associations with SCA.

For further analyses, significant markers were retained only if (1) marker genotypes were available for all hybrids and (2) all three genotypic classes were represented by at least one hybrid. These markers are referred to as fully informative. Chromosomal regions harbouring significant QTL across experiments were identified with a moving interval approach. An interval was defined for each selected locus, beginning 3 cM before and ending 3 cM after the locus. Intervals comprising significant loci in at least two experiments were regarded as chromosomal regions with effects across experiments.

Prediction models for hybrid performance and specific combining ability based on genotypic values

Additive (a) and dominance (d) effects for hybrid performance were estimated for each selected marker from the arithmetic means of the genotypic classes *MM*, *mm*, and *Mm* (Vuylsteke et al. 2000a). Marker allele *M* describes the presence of an AFLP fragment and *m* the absence of that fragment. The genotypic value for each hybrid was estimated by the total contribution of the selected markers to hybrid performance ($\mathrm{TCSM_{HP}}$) and was used as predictor for hybrid performance in a linear regression approach.

For prediction of SCA on the basis of genotypic value estimates, two approaches were employed (Vuylsteke et al. 2000a). Analogous to the partitioning of hybrid performance into GCA and SCA, the $\mathrm{TCSM_{HP}}$ was partitioned into general ($\mathrm{GCSM_{HP}}$) and specific ($\mathrm{SCSM_{HP}}$) contributions of the selected markers to hybrid performance, where the TCSM_{ij} of a hybrid between inbreds i and j can be written as:

$$\mathrm{TCSM}_{ij} = \mu + \mathrm{GCSM}_i + \mathrm{GCSM}_j + \mathrm{SCSM}_{ij}. \quad (2)$$

The linear regression of SCA on $\mathrm{SCSM_{HP}}$ across all tested hybrids was chosen as a first model for prediction of SCA. For the second approach, additive and dominance effects for SCA were estimated for each marker from the arithmetic means of the genotypic classes *MM*, *mm*, and *Mm*. Using the markers that were significantly associated with SCA, the total contribution of the selected markers to SCA ($\mathrm{TCSM_{SCA}}$) was calculated for each hybrid. Linear regression of SCA on $\mathrm{TCSM_{SCA}}$ across all hybrids resulted in a second model for prediction of SCA.

Prediction of hybrid performance based on general and specific combining abilities

For untested hybrids, no SCA estimates exist. Therefore, a simple approach for the hybrid performance prediction of untested hybrids was based solely on the GCA estimates:

$$\tilde{y}_{ij} = \hat{\mu} + \hat{g}_i' + \hat{g}_j''. \quad (3)$$

We extended the GCA approach for hybrid performance prediction by using SCA estimates ($\hat{s}_{ij}$) from the two SCA prediction models presented above, so that:

$$\tilde{y}_{ij} = \hat{\mu} + \hat{g}_i^{'} + \hat{g}_j^{''} + \hat{s}_{ij} \text{ with } \hat{s}_{ij} = \begin{cases} a+b & \text{SCSM}_{\text{HP}} \\ a+b & \text{TCSM}_{\text{SCA}} \end{cases} \text{ or } \quad (4)$$

Substituting $\hat{s}_{ij}$ with estimates from the first model for SCA prediction resulted in the $SCSM_{HP}$-based GCA+SCA approach for hybrid performance prediction (referred to as GCA+SCA 1 approach), substituting $\hat{s}_{ij}$ with estimates from the second model for SCA prediction resulted in the $TCSM_{SCA}$-based GCA+SCA approach for hybrid performance prediction (referred to as GCA+SCA 2 approach).

Evaluating the efficiency of prediction models for hybrid performance

The efficiency of the four approaches predicting the performance of untested hybrids was evaluated by a leave-one-out cross-validation similar to the jackknife procedure (Vuylsteke et al. 2000a). Briefly, the full data set of n hybrids was split into two subsets. The estimation set comprised n–1 hybrids for the estimation of model parameters. The remaining one hybrid was not included in the parameter estimation and formed the test set, for which the predictions with each chosen model were done on the basis of parameters derived from the estimation set. This procedure was performed n times, designating each of the n hybrids as test set. To evaluate the prediction efficiency, the proportion of explained variance (R^2) was calculated for the correlation between the n predicted and observed hybrid performance values.

Results

Biometrical analysis of field data

Estimates of GCA variance components (Table 1) for GY ranged from 0.078 to 0.428 with highest values for Exp. 2 and generally higher GCA variances for Dent lines compared with Flint lines. The SCA variance components for GY were highest (0.170) for Exp. 1 and were remarkably higher than for the remaining experiments (0.029–0.072). Broad-sense heritabilities (h^2) for GY ranged from 68.1 to 81.6%, with the lowest value for Exp. 3. When averaging GCA variance over Flint and Dent, the ratio of SCA to GCA variance ranged from 0.19 to 1.12, with highest values for Exp. 1. For GDMC, the ratio of SCA to GCA variance ranged from 0.06 to 0.42, again with highest values for Exp. 1.

Identification of chromosomal regions with significant QTL

The AFLP assays resulted in 1835 dominantly scored AFLP markers (Table 2) of which 910 were uniquely mapped. For GY, the number of markers with significant ($P < 0.001$) genotypic effects for hybrid performance ranged from 38 (Exp. 4) to 98 (Exp. 1), of which 28–67 were fully informative and therefore selected for calculation of $TCSM_{HP}$. The number of markers with significant ($P < 0.005$) genotypic effects for GY SCA ranged from zero (Exp. 2) to five (Exp. 1), with zero to three being fully informative and therefore selected for calculation of $TCSM_{SCA}$. For GDMC, between 24 and 55 fully informative markers were significant for hybrid performance, between 0 and 13 were significant for SCA.

All markers with significant effects for GY hybrid performance and known map positions were shown in a linkage map (Fig. 1), including the results of all four experiments. For every chromosome, regions were identified where QTL with significant GY hybrid performance effects were detected in at least two of the four experiments. Chromosomal regions with significant loci in at least three experiments are located on chromosome 1 (55, 68–73 cM), chromosome 3 (62–64 cM), chromosome 4 (59–63 cM), chromosome 6 (45–48 cM), chromosome 8 (82–83 cM), chromosome 9 (82–84 cM), and chromosome 10 (59–60 cM). All markers with significant effects for GDMC hybrid performance and known map positions were shown in a linkage map (Fig. 2), including the results of all four experiments. For every chromosome, regions were identified where QTL with significant GDMC hybrid performance effects were detected in at least two of the four experiments. Chromosomal regions with significant loci in at least three experiments are located on chromosome 1 (70–73, 85–87 cM), chromosome 2 (51–54, 91–93 cM), chromosome 3 (56–58, 83–85 cM), chromosome 4 (61–64 cM), and chromosome 9 (82–84 cM).

Prediction models for specific combining ability and hybrid performance

For the first model of SCA prediction for GY, the estimates of correlations (r) and their significance levels varied substantially among the four experiments (Table 3). Highly significant ($P < 0.001$) correlations were detected only for Exp. 1, where 26.4% of the observed SCA variation could be explained. For the

Table 1 Means, variance components, and heritabilities of grain yield and grain dry matter content from field experiments with factorial crosses, comprising *n* hybrids and *l* locations

Criterion	Experiment			
	1	2	3	4
Experimental setup				
n	98	44	84	44
l	6	5	4	6
Grain yield (Mg ha^{-1})				
Overall mean	11.72	12.10	11.38	9.82
Variance components				
GCA Dent	0.203 (0.091, 0.765)***	0.428 (0.195, 1.569)***	0.193 (0.087, 0.733)***	0.220 (0.099, 0.825)***
GCA Flint	0.100 (0.032, 1.333)***	0.339 (0.086, 22.730)*	0.117 (0.030, 6.726)	0.078 (0.019, 5.904)*
SCA	0.170 (0.114, 0.278)***	0.072 (0.030, 0.352)*	0.066 (0.034, 0.176)***	0.029 (0.011, 0.172)*
GCA Dent × loc	0.153 (0.100, 0.262)***	0.053 (0.019, 0.447)*	0.129 (0.075, 0.272)***	0.071 (0.038, 0.177)***
GCA Flint × loc	0.135 (0.080, 0.279)***	0.462 (0.227, 1.401)***	0.256 (0.134, 0.675)***	0.120 (0.060, 0.352)***
SCA × loc	0.173 (0.125, 0.254)***	0.169 (0.095, 0.382)***	0.125 (0.079, 0.230)***	0.067 (0.032, 0.215)***
Ratio of variance components				
SCA:GCA	1.12	0.19	0.43	0.19
Broad-sense heritabilities (%)				
h^2	80.3 (72.7, 85.3)	81.6 (69.2, 88.2)	68.1 (53.8, 77.2)	81.6 (69.6, 88.1)
Grain dry matter content (in %)				
Overall mean	67.7	71.4	71.6	68.5
Variance components				
GCA Dent	1.068 (0.534, 3.105)***	2.709 (1.293, 8.825)***	2.886 (1.466, 8.091)***	2.612 (1.248, 8.490)***
GCA Flint	0.404 (0.150, 2.930)***	5.130 (1.585, 87.484)***	2.812 (1.046, 20.088)***	1.332 (0.402, 25.875)***
SCA	0.307 (0.213, 0.482)***	0.224 (0.111, 0.668)***	0.330 (0.215, 0.569)***	0.174 (0.089, 0.487)***
GCA Dent × loc	0.262 (0.176, 0.432)***	0.056 (0.014, 3.209)	0.435 (0.273, 0.802)***	0.163 (0.084, 0.440)***
GCA Flint × loc	0.248 (0.149, 0.491)***	1.056 (0.524, 3.121)***	0.600 (0.318, 1.533)***	0.464 (0.238, 1.270)***
SCA × loc	0.300 (0.236, 0.396)***	0.335 (0.200, 0.671)***	0.175 (0.104, 0.357)***	0.315 (0.207, 0.538)***
Ratio of variance components				
SCA:GCA	0.42	0.06	0.12	0.09
Broad-sense heritabilities (%)				
h^2	91.0 (87.5, 93.3)	95.4 (92.4, 97.1)	93.9 (91.1, 95.6)	95.1 (91.8, 96.8)

Variance components with their significance levels and 95% confidence intervals (in parentheses) were given for general combining ability (GCA), specific combining ability (SCA), and interactions with locations (loc). The GCA variances were averaged over Flint and Dent for calculation of the SCA:GCA variance ratio. Broad-sense heritabilities (h^2) and their 95% confidence intervals (in parentheses) were calculated for the entries

*, ****F*-test for variance components significant at the 0.05 and 0.001 probability levels, respectively

second model of SCA prediction, markers could be selected for Exps. 1 and 4, resulting in highly significant ($P < 0.001$) and significant ($P < 0.01$) correlations, and explaining 25.6 and 21.4% of the observed SCA variation for GY. The estimates of correlation (r) of GY hybrid performance with all four predictors were highly significant ($P < 0.001$). Correlations of hybrid performance with $TCSM_{HP}$ were generally lowest, ranging from 0.79 (Exp. 1) to 0.89 (Exp. 2 and Exp. 4). Differences among the three GCA-based approaches were very small, with the exception of Exp. 1, where both GCA+SCA approaches showed higher correlations (0.89) compared with the GCA approach (0.85).

For the first model of SCA prediction for GDMC, the estimates of correlations (r) and their significance levels varied substantially among the four experiments (Table 3). For the second model of SCA prediction, markers could be selected for Exps. 1–3, resulting in highly significant ($P < 0.001$) correlations, and explaining 31.3–47.3% of the observed SCA variation for GDMC. Correlations of GDMC hybrid performance with $TCSM_{HP}$ were generally lowest, ranging from 0.86 (Exp. 1) to 0.95 (Exp. 2). Differences among the three GCA-based approaches were small, again with the exception of Exp. 1, where both GCA+SCA approaches showed higher correlations (0.94 and 0.96) compared with the GCA approach (0.93).

Table 2 Number of selected AFLP markers

Criterion	Experiment			
	1	2	3	4
Grain yield (Mg ha^{-1})				
Markers selected for hybrid performance				
Total	98 (67)	70 (40)	65 (44)	38 (28)
Mapped	56 (41)	37 (27)	29 (23)	21 (15)
Markers selected for SCA				
Total	5 (3)	0 (0)	1 (0)	1 (1)
Mapped	0 (0)	0 (0)	1 (0)	1 (1)
Grain dry matter content (in %)				
Markers selected for hybrid performance				
Total	64 (40)	92 (55)	73 (39)	34 (24)
Mapped	32 (18)	54 (36)	44 (24)	20 (12)
Markers selected for SCA				
Total	22 (13)	11 (7)	5 (5)	0 (0)
Mapped	9 (6)	5 (3)	1 (1)	0 (0)

Markers with significant genotypic effects for hybrid performance and specific combining ability (SCA) for grain yield and grain dry matter content were selected from 1835 markers, of which 910 were uniquely mapped. Only fully informative markers (numbers given in brackets) were selected for subsequent analyses

Evaluating the efficiency of prediction models for hybrid performance

The prediction efficiency of the four hybrid performance prediction approaches, evaluated by cross-validation and measured by the proportion of explained variance (R^2), ranged from 46.2 to 86.3% for GY (Table 3). Efficiencies were consistently lowest for the $TCSM_{HP}$ approach in all experiments. Among the GCA-based approaches, differences in efficiency were largest for Exp. 1, where R^2 of the GCA+SCA 1 approach was greatest (56.5%). The SCA:GCA ratio for GY in this experiment was higher than in Exps. 2–4, where the efficiencies of the GCA-based approaches hardly differed. In a comparison across all models, the R^2's for Exp. 1 were lowest, followed by Exp. 3. For GDMC, the prediction efficiencies ranged from 58.7 to 95.9% and were consistently lowest for the $TCSM_{HP}$ approach. Among the GCA-based approaches, differences in efficiency were largest for Exp. 1, where R^2 of the GCA+SCA 2 approach was greatest (82.8%).

Discussion

The approaches for hybrid performance prediction were evaluated across four experiments, which mostly differed in the experimental size, set of parental lines, set of trial locations, and year of trial. Accordingly, the four experiments differed in the results from the biometrical analyses of GY field data. For Exps. 2 and 4 the ratios of SCA variance to GCA variance (averaged over Flint and Dent) were low. In Exp. 2, the low SCA:GCA ratio was due to a high GCA variance, whereas in Exp. 4 it was due to a low SCA variance. These results agree with the findings of Melchinger (1999) on the basis of data from Dhillon (1990), describing an SCA:GCA ratio of 0.30 for ear yield of inter-group hybrids. However, for Exp. 1, the SCA:GCA ratio was relatively high (1.12), due to a larger SCA variance. The SCA:GCA ratio is relevant

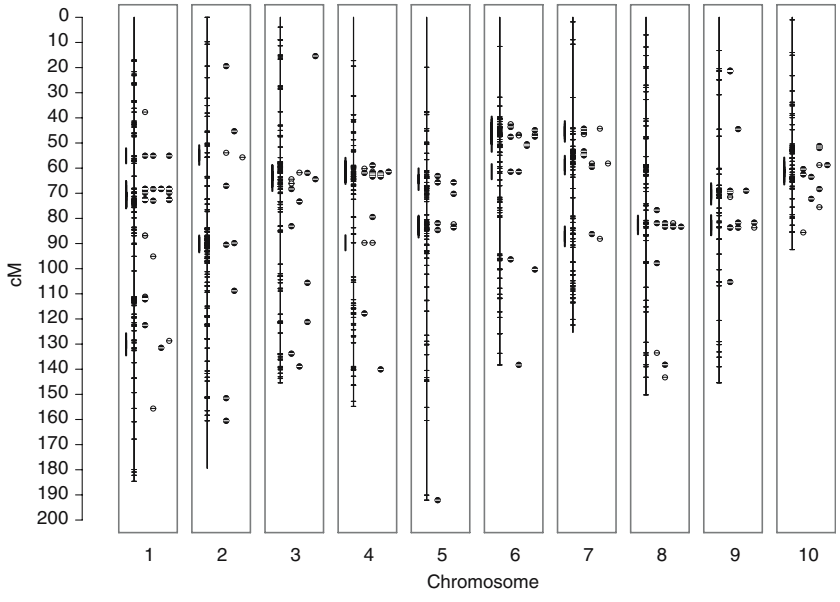

Fig. 1 Map of markers with significant genotypic effects for grain yield hybrid performance. Markers are plotted according to their chromosome number and map position (cM). *Full dots* indicate fully informative markers, whereas *empty dots* indicate non-fully informative markers. For each chromosome, *dots* are arranged in four columns that correspond to Experiments 1–4 (*from left to right*). *Bold lines* along the chromosome indicate regions with significant loci in at least 2 of 4 experiments

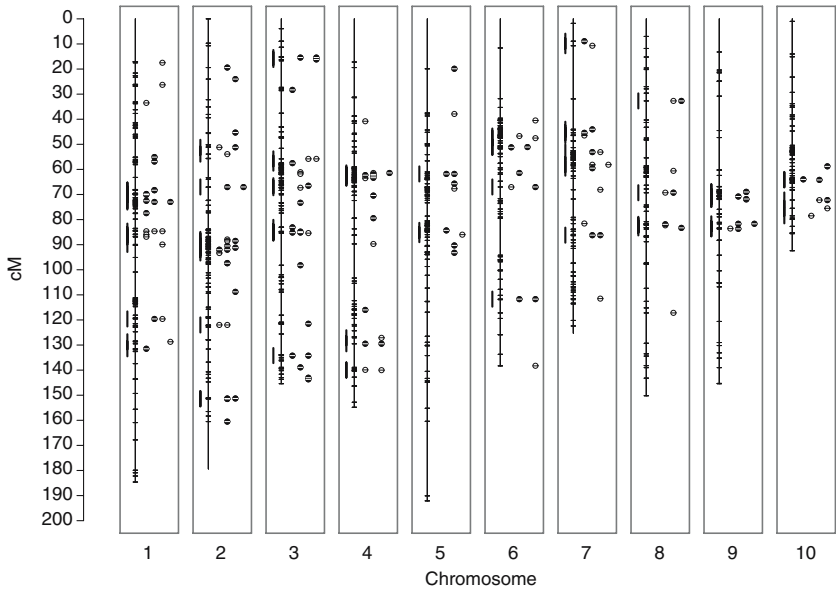

Fig. 2 Map of markers with significant genotypic effects for grain dry matter content hybrid performance. Markers are plotted according to their chromosome number and map position (cM). *Full dots* indicate fully informative markers, whereas *empty dots* indicate non-fully informative markers. For each chromosome, *dots* are arranged in four columns that correspond to Experiments 1–4 (*from left to right*). *Bold lines* along the chromosome indicate regions with significant loci in at least 2 of 4 experiments

for the following discussion on numbers of selected loci and the prediction efficiencies compared across the experiments.

The inbred lines in each experiment can be regarded as a random sample of lines from a base population consisting of all potential parental lines of hybrid varieties between the Flint and Dent germplasm pools in Central Europe. In consequence, the factors in the model for analysis of the mating design were regarded as random. This allows (1) for estimation of variance components and thereby deriving measures such as the SCA to GCA ratio, (2) for estimation of GCA main effects for unbalanced data sets which is the case in cross-validation, and (3) results in BLUPs which are appropriate estimates if the aim of the data analysis is selection of superior genotypes (Smith et al. 2005).

Identification of QTL

The markers that were identified for their significant influence on hybrid performance or SCA were the foundation for the estimation of genotypic values of the hybrids and, consequently, for the prediction of hybrid performance or SCA. The Kruskal–Wallis test is a nonparametric version of the one-way ANOVA (Kruskal and Wallis 1952) and was employed to test the association of markers with hybrid performance or SCA. The sampling distribution of the Kruskal–Wallis statistic is a very close approximation of the chi-square distribution provided that each class comprises at least five observations. Thus, a minimum of five hybrids per class would be recommendable, however, for the present data similar prediction efficiency results were obtained provided that each class comprises at least one observation (data not shown). In order to compare the results with Vuylsteke et al. (2000a), we followed their procedure, including markers only if each of the three genotypic classes was represented by at least one hybrid.

For all experiments, between 28 and 67 fully informative markers were identified for GY hybrid performance at a significance level of $P = 0.001$ and used for hybrid performance prediction. In our study more significant markers were found compared with the work of Vuylsteke et al. (2000a), where 20 markers were identified for hybrid performance at the same significance level. Our experiments comprised between 44 and 98 inter-group hybrids, whereas Vuylsteke et al. (2000a) analysed 53 inter-group hybrids from a 13 by 13 half-diallel experiment, evaluated at three sites in Northern Italy with randomised complete block designs and three replications. Their parental inbred lines were genotyped with 71 primer combinations (Vuylsteke et al. 2000b), resulting in 1385 AFLP markers, which mapped on the B73 × Mo17 Recombinant Inbred high-density AFLP linkage map (Vuylsteke et al. 1999). In our study, genotyping the inbred lines with 20 primer combinations resulted in 1835 AFLP markers, of which 910 uniquely mapped on a nearly identical map (Peleman et al. 2000).

For GY SCA, we detected only few markers at a significance level of $P = 0.005$, whereas Vuylsteke et al. (2000a) identified 25 markers at the same significance level. In their data sets, the SCA and GCA variances were of similar order, indicating a higher relevance of SCA compared to our experiments. The 53 inter-group

Table 3 Correlations of specific combining ability (SCA) for grain yield and grain dry matter content with (1) the specific contribution of selected markers to hybrid performance ($SCSM_{HP}$) and (2) the total contribution of selected markers to SCA ($TCSM_{SCA}$). Correlations of hybrid performance for grain yield and grain dry matter content with (1) the total contribution of selected markers to hybrid performance ($TCSM_{HP}$), (2) predictions from general combining ability (GCA) of parental inbreds, (3) $SCSM_{HP}$-based GCA+SCA 1 predictor, and (4) $TCSM_{SCA}$-based GCA+SCA 2 predictor. Proportions of the explained variance (R_2) of hybrid performance prediction based on the four approaches were determined by a cross-validation procedure

Criterion	Experiment			
	1	2	3	4
Grain yield (Mg ha^{-1})				
Correlation of SCA with:				
$SCSM_{HP}$	0.51***	0.19	0.22*	0.27
$TCSM_{SCA}$	0.51***	–	–	0.46**
Correlation of hybrid performance with:				
$TCSM_{HP}$	0.79***	0.89***	0.85***	0.89***
GCA	0.85***	0.98***	0.95***	0.98***
GCA+SCA 1 ($SCSM_{HP}$)	0.89***	0.98***	0.95***	0.98***
GCA+SCA 2 ($TCSM_{SCA}$)	0.89***	–	–	0.99***
Proportion of explained variance determined by cross-validation:				
$TCSM_{HP}$	46.2%	73.3%	59.0%	65.8%
GCA	53.9%	86.0%	77.9%	86.3%
GCA+SCA 1 ($SCSM_{HP}$)	56.5%	85.5%	76.9%	86.0%
GCA+SCA 2 ($TCSM_{SCA}$)	52.8%	–	–	85.7%
Grain dry matter content (in %)				
Correlation of SCA with:				
$SCSM_{HP}$	0.20	0.47**	0.24*	0.35*
$TCSM_{SCA}$	0.65***	0.69***	0.56***	–
Correlation of hybrid performance with:				
$TCSM_{HP}$	0.86***	0.95***	0.89***	0.87***
GCA	0.93***	0.99***	0.98***	0.99***
GCA+SCA 1 ($SCSM_{HP}$)	0.94***	0.99***	0.98***	0.99***
GCA+SCA 2 ($TCSM_{SCA}$)	0.96***	1.00***	0.99***	–
Proportion of explained variance determined by cross-validation				
$TCSM_{HP}$	58.7%	87.4%	69.8%	62.9%
GCA	78.3%	95.5%	92.9%	93.4%
GCA+SCA 1 ($SCSM_{HP}$)	76.6%	95.9%	93.1%	93.3%
GCA+SCA 2 ($TCSM_{SCA}$)	82.8%	95.9%	92.5%	–

*, **, ***Pearson's product moment correlation coefficient (r) significant at the 0.05, 0.01, and 0.001 probability levels, respectively

hybrids from their half-diallel were produced with parental lines from Iowa Stiff Stalk Synthetic, Lancaster Sure Crop and miscellaneous origin, and therefore stratification effects explain higher SCA variances compared with the situation of hybrids between lines exclusively from two divergent heterotic groups (Reif et al. 2005). The SCA variance is defined as a component of the total genotypic variance for hybrid performance, and in cases where the SCA variance was small, this would explain the lower number of markers, which were significantly associated with SCA compared to the number of markers significantly associated with hybrid performance. This is supported by our results for GY and GDMC, where the number of SCA associated markers was highest for Exp. 1, which showed for both traits the highest SCA:GCA ratio of all experiments. Summarizing, the number of markers identified for GY hybrid performance was comparable to literature findings and the number of markers associated with SCA was very low, in dependence to the SCA:GCA ratio.

Mapping of QTL regions across experiments

For GY hybrid performance, regions with significant loci in at least two of four experiments were found on all chromosomes. Moreover, five regions with significant loci in all experiments were detected. The experiments differed in their set of parental inbreds, factorial size, year of trial, and set of locations, which in

conjunction with genotype by environment interactions (Table 1) may account for those cases, in which significant QTL were identified only in a subset of the experiments. Vuylsteke et al. (2000a) reported 20 loci significantly associated with hybrid performance, of which four were identical or close to the following regions identified in our study: chromosome 1 at 95 cM in one experiment, chromosome 2 at 54–56 cM and chromosome 6 at 61 cM in two experiments, and chromosome 4 at 59–63 cM in all four experiments. For GDMC hybrid performance, regions with significant loci in at least two of four experiments were found on all chromosomes. Many QTL regions for GDMC mapped in the same regions as QTL for GY. Overlaps between both traits for regions with significant QTL in at least three experiments were found on chromosome 1 at 72–73 cM, chromosome 4 at 61–63 cM, and chromosome 9 at 82–84 cM. Summarizing, a considerable number of chromosomal regions was identified, comprising QTL that were significantly associated with hybrid performance of GY or GDMC in multiple experiments.

Prediction of specific combining ability

For GY SCA prediction based on $SCSM_{HP}$ (first model), the highest correlation was found for Exp. 1. This experiment also showed the highest SCA:GCA ratio. For Exps. 2–4, where the correlations were clearly lower, the rank order did loosely correspond to the SCA:GCA ratio. The correlations in Exp. 1 ($r = 0.51$) were similar to the respective results ($r = 0.49$) of Vuylsteke et al. (2000a). For the GY SCA prediction based on $TCSM_{SCA}$ (second model), the correlations were similar or higher compared with the first model. In comparison with the analysis of Vuylsteke et al. (2000a), where 36.8% of the SCA variance was explained with the second model based on 25 selected markers, our results showed lower coefficients of determination (Exp. 1: 25.6%, Exp. 4: 21.4%), however based on a very low number of selected markers (Exp. 1: three markers, Exp. 4: one marker).

Identification of significant QTL is a prerequisite for the determination of a genotypic value and, thus, for obtaining the predictor of SCA. However, it was observed that with a very low number of selected markers (three GY markers in Exp. 1, one GY marker in Exp. 4, five GDMC markers in Exp. 3), the correlation of SCA with genotypic value $TCSM_{SCA}$ can be comparable or even higher than the correlation with $SCSM_{HP}$, which was based on a considerably larger number of selected markers. These results indicate that already with a small number of selected markers, sound predictions with $TCSM_{SCA}$ could be obtained, as long as a minimum number of significant markers was available for determination of genotypic values.

Prediction of hybrid performance

The observed correlations of hybrid performance with all investigated predictors were highly significant for all experiments (Table 3). In the analysis of inter-group crosses from a diallel experiment, Vuylsteke et al. (2000a) obtained the correlation $r = 0.79$ of maize GY hybrid performance with TCSM on basis of 20 selected markers. This result is in the range of our findings for the $TCSM_{HP}$ approach, with r between 0.79 and 0.89 on basis of 28–67 selected markers. Ranking the predictors by their correlation coefficients resulted in the same order for each experiment: correlations of hybrid performance with $TCSM_{HP}$ were notably smallest, followed by GCA effects estimated from the parental inbreds, and the combined GCA+SCA predictors.

All markers were tested separately from each other for their association with hybrid performance or SCA. Thus, it cannot be ruled out that multiple markers, which are closely linked to the same QTL, were selected for prediction. However, as the additive and dominance effects of all selected markers were added into the genotypic value estimates, the true contribution of such a QTL would be overestimated and consequently may increase the prediction error. This issue becomes apparent from results of Vuylsteke et al. (2000a; Tables 2, 3), where identical additive and dominance effects were estimated for closely linked selected markers. In our data, closely linked selected markers were also observed for GY and GDMC hybrid performance (Figs. 1, 2). To investigate the effect of closely linked selected markers, we compared the prediction of hybrid performance on basis of all mapped selected markers with a subset of the mapped selected markers (data not shown). The marker subset was determined by the following rules: if two significant markers had (a) less than 5 cM map distance and no non-significant markers in between or (b) less than 3 cM map distance with non-significant markers in between, the marker with the higher P-value was discarded. Across all experiments and traits, this procedure resulted in a reduction of the number of selected markers by 8–37%, indicating that a substantial proportion of markers is affected by this issue. However, the correlation of hybrid performance with $TCSM_{HP}$ changed by –0.2 to 1.5% and for the GCA+SCA 1 approach by –1.2 to 0.1%. These results indicate that for our data closely linked markers did not have a

substantial influence on the prediction of hybrid performance.

Prediction of untested hybrids was assessed with a cross-validation procedure, measuring the prediction efficiency (Table 3) of the models by the proportion of the explained variance (R^2). Across all models, prediction efficiencies were generally higher for experiments with lower SCA:GCA ratio. For the $TCSM_{HP}$ approach, R^2 estimates for GY ranged from 0.46 to 0.73 for all four experiments and were similar or higher compared with $R^2 = 0.45$, obtained by Vuylsteke et al. (2000a). However, for all experiments analysed in our study, the simple approach based on GCA estimates from the parental inbreds and without using molecular marker data was able to predict hybrid performance for GY of inter-group maize single crosses with higher efficiency than the $TCSM_{HP}$ approach.

The highest prediction efficiency of all models for GY and GDMC hybrid performance in Exp. 1 was obtained with our two proposed models, which enhance the simple GCA approach with SCA estimates predicted on the basis of molecular data. For the other experiments, the prediction efficiencies of the SCA enhanced approaches were similar to the simple GCA approach. The superiority of the proposed SCA enhanced models can be explained by the comparably high SCA:GCA ratio in Exp. 1, emphasizing the advantage of including SCA estimates for hybrid performance prediction. Such an increased relevance of SCA exists in the early phase of establishing a hybrid breeding program and in breeding programs, which rely on more than two divergent heterotic pools. In practical maize breeding programs, the extent of GCA and SCA variances is rarely predictable. Thus, for the prediction of single-cross hybrids, an approach is advantageous if its prediction efficiency is convincingly high under both conditions, low and high SCA:GCA ratios. This was the case with our proposed prediction procedure, enhancing a GCA-based model with SCA effects predicted on the basis of molecular marker data.

The proportions of explained variance from cross-validation (Table 3) were consistently smaller than the squared correlations of hybrid performance with the predictors across the full set of hybrids (Table 3). This difference is due to the error of predicting one hybrid on the basis of the remaining hybrids from a full factorial, as was performed in the cross-validation procedure. Prediction efficiencies obtained in this way can be used to compare the models. However, in practical breeding programs not only one hybrid, but a larger proportion of a factorial experiment has to be predicted. This should be considered in future cross-validation analyses. So far, the approach is based on a biallelic model. Consequently, the model could be extended to allow for the use of multiallelic marker data such as simple sequence repeats (SSR).

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