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Genomic prediction of the performance of hybrids and the combining abilities for line by tester trials in maize

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ABSTRACT

The two most important activities in maize breeding are the development of inbred lines with high values of general combining ability (GCA) and specific combining ability (SCA), and the identification of hybrids with high yield potentials. Genomic selection (GS) is a promising genomic tool to perform selection on the untested breeding material based on the genomic estimated breeding values estimated from the genomic prediction (GP). In this study, GP analyses were carried out to estimate the performance of hybrids, GCA, and SCA for grain yield (GY) in three maize line-by-tester trials, where all the material was phenotyped in 10 to 11 multiple-location trials and genotyped with a mid-density molecular marker platform. Results showed that the prediction abilities for the performance of hybrids ranged from 0.59 to 0.81 across all trials in the model including the additive effect of lines and testers. In the model including both additive and non-additive effects, the prediction abilities for the performance of hybrids were improved and ranged from 0.64 to 0.86 across all trials. The prediction abilities of the GCA for GY were low, ranging between − 0.14 and 0.13 across all trials in the model including only inbred lines; the prediction abilities of the GCA for GY were improved and ranged from 0.49 to 0.55 across all trials in the model including both inbred lines and testers, while the prediction abilities of the SCA for GY were negative across all trials. The prediction abilities for GY between testers varied from − 0.66 to 0.82; the performance of hybrids between testers is difficult to predict. GS offers the opportunity to predict the performance of new hybrids and the GCA of new inbred lines based on the molecular marker information, the total breeding cost could be reduced dramatically by phenotyping fewer multiple-location trials.

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops for food, animal feed, and energy in the world [1]. The development and deployment of maize varieties with high yield potentials through breeding is one of the most effective and economical approaches to the increase of maize production and to ensure food security globally. In maize breeding, the two most important activities are the development of inbred lines with high values of the general combining ability (GCA) and specific combining ability (SCA) for grain yield and other agronomic traits, as well as identification of hybrids with high yield potentials [2].

The concepts of GCA and SCA were initially proposed in maize breeding by Sprague and Tatum [3]. The GCA of an inbred or parent is defined as the average performance of the genotype in all hybrid combinations compared with the mean value of all hybrids involved. In contrast, the SCA of a pair of genotypes is defined as the deviation of the realized hybrid performance from the expectation based on the GCAs of the two genotypes and the population mean. The GCA is largely due to additive genetic effects, whereas the SCA is largely attributed to dominance and non-additive epistatic effects [2,3]. In most breeding programs, the most important target trait for

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selection is usually grain yield [4]. At the inbred line development stage, the GCA is the main criterion to advance the newly developed inbred lines to the next cycle as parental lines, while the SCA is an important parameter to identify the best hybrid combinations for grain yield. With the dramatic decrease in cost of the production of doubled haploid (DH) lines, thousands of lines can be generated in a maize breeding program annually [5]. The DH technology leads to a paradigm shift in the maize breeding programs from the generation of the homozygous inbred lines to the estimation of the breeding values of the DH lines [6,7]. In conventional breeding, the breeding values of the inbred lines, i.e. the performance of inbred lines in hybrid combinations, are always evaluated and measured in multiple-location trials, and the GCA and SCA estimations also have to be implemented in multiple-location trials with several specific mating designs, such as the diallel cross, sparse partial diallel cross, and line-by-tester [2,8]. GCA was generally estimated by the ordinary least squares method using the phenotypic data [6,9]. However, multiple-location trials are time-consuming and resource-intensive. Moreover, only a small number of inbred lines can be evaluated using mating designs in multiple-location trials due to practical limitations. In the preliminary stages of testing, the line-by-tester mating design is always used on dozens of inbred lines to evaluate their GCA values. Afterwards, the diallel cross mating design between heterotic groups is used on a limited number of selected inbred lines with good GCA and other agronomic traits in which both GCA and SCA could be accurately estimated [2,4].

Genomic selection (GS) is proposed to perform selection on the untested breeding material based on their genomic data, it can help the breeding programs to reduce phenotyping costs and save time [10,11]. In GS, a training set, for which phenotypic and genotypic data was generated, is used to estimate the effect of genetic markers. The marker effects estimated from the training set are then used to predict the genomic estimated breeding value (GEBV) of individuals in the prediction set, which have been genotyped but not phenotyped [12,13]. In maize, GS has been implemented in many studies, some of them were conducted to predict and select the target traits in inbred lines [14,15], whereas other studies were conducted to predict the performance of hybrids [16,17]. Various statistical models have been developed and applied on GS for grain yield and key agronomic traits, the two most widely used being the genomic best linear unbiased prediction (GBLUP) and ridge regression best linear unbiased prediction (RR-BLUP) [18,19]. Modeling complex genetic effects, such as dominant or epistatic effects, or genotype-by-environment interaction, has the potential for improving the prediction ability for GS, which has been proven to be effective in maize breeding to accelerate genetic gain per unit time and cost [20,21]. However, only a few studies were conducted to predict the GCA and SCA values [22,23], and further studies are needed to improve the ability for predicting the values of GCA and SCA, as well as the performance of hybrids.

The objectives of the present study were to: (1) predict the performance of hybrids for three line-by-tester trials, and estimate the prediction ability on grain yield using several models incorporating different kinds of genotypic data and genetic effects; (2) assess the prediction ability on the GCA and SCA values in three line-by-tester trials; and (3) evaluate the tester effect on the prediction of the performance of hybrids, and estimate the genomic prediction abilitiesof grain yield between testers.

2. Materials and methods

2.1. Plant materials, field experiments, phenotypic data analysis

The phenotypic data of grain yield (GY) collected from two multiple-location trials were used in the present study to conduct genomic prediction analyses. The material and the mating designs in each trial were illustrated in Fig. 1. The first trial, designated as "Trial A", consists of 24 hybrids formed by crossing each line to three testers. The eight inbred lines belong to heterotic group "A" or Tuxpeno, and the three testers belong to heterotic group "B" or non-Tuxpeno. At CIMMYT (International Maize and Wheat Improvement Center), the dent maize kernel type was classified as the heterotic group "A", and the flint maize kernel type was classified as the heterotic group "B". Eight inbred lines were crossed with each tester, respectively. The first tester was an inbred line, and the other two testers were F_1 hybrids formed between two inbred lines belonging to the same heterotic group. "Tester 1" (T1) was one of the parental lines of "Tester 3" (T3), and "Tester 2" (T2) shared a common parental inbred line with T3. The second trial, designated as "Trial B", consists of 72 hybrids. Among these 72 hybrids, 42 hybrids in Trial B were formed between fourteen inbred lines from the heterotic group "B" and three testers. These three testers were F_1 hybrids and designated as Tester 4 (T4), Tester 5 (T5), and Tester 6 (T6), respectively. Each tester was formed between two inbred lines from the heterotic group "A". T5 and T6 shared a common parental line. The other 30 hybrids were formed between ten inbred lines from the heterotic group "A" and three testers. The three testers were F_1 hybrids, and designated as Tester 7 (T7), Tester 8 (T8), and Tester 9 (T9), respectively. Each tester was formed between two inbred lines from the heterotic

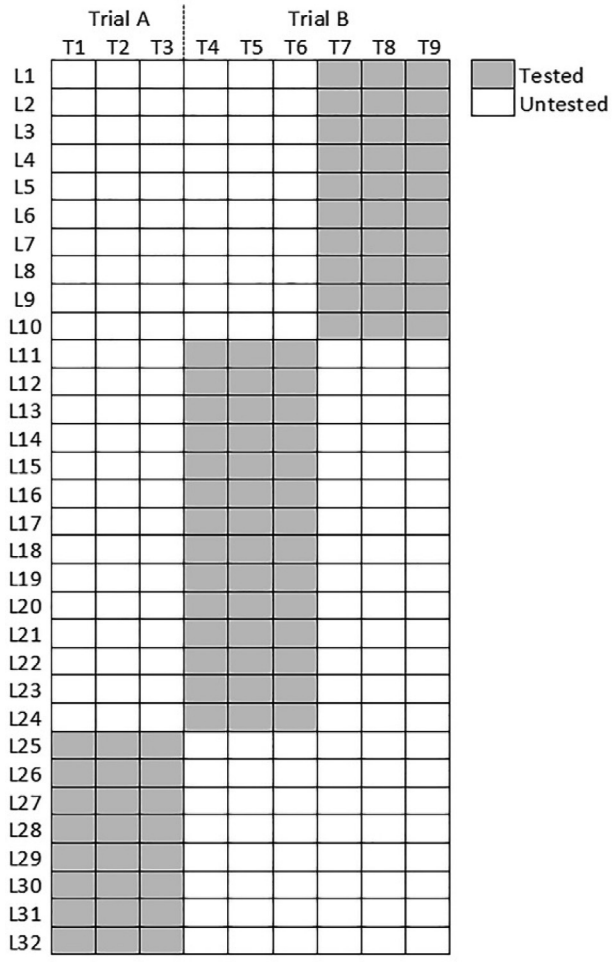

Fig. 1. The breeding material and the mating designs used in Trials A and B. In total, 32 lines and 9 testers were used to form the hybrids for evaluation in Trials A and B. The letter L and T represents the line and tester used to carry out the line-by tester-trial. The dark-colored rectangles indicate the tested hybrids and the white rectangles indicate the untested hybrids.

group "B". T8 and T9 shared a common parental line. Trial A and Trial B were also combined for further analysis, which was defined as "Trial A & B". In tropical maize breeding, three-way cross hybrids are still the main final products, due to the lower seed cost advantage. In three-way cross hybrids, the more commonly used tester is single-cross hybrid.

In each trial, lines and testers from the opposite heterotic groups were crossed in all the possible combinations using the line-by-tester mating design [2,8]. The main purpose of the line-by-tester mating design in maize is to evaluate the GCA of the newly developed inbred lines, and the tester lines representing the genetic diversity of the opposite heterotic group always could classify inbred lines into appropriate heterotic groups and rank inbred lines correctly for performance in hybrid combinations. Trials A and B were evaluated in Mexico in ten and eleven locations, respectively. The total number of observations was 240 in Trial A, and 720 in Trial B. An alpha lattice design was used for both trials with two replications per location. Number of blocks per replication in "Trial A" and "Trial B" was 7 and 20, respectively. The plot size in all the experiments was a single row 5 m long, with 0.75 m between rows, and 0.20 m between plants in each row. Number of blocks per replication in "Trial A" and "Trial B" was 7 and 20, respectively. Phenotypic data were collected at all the locations for the main agronomic traits including grain yield (GY, t ha^{-1}).

2.1.1. Phenotype model based on the performance of hybrids

For each trial, the best linear unbiased estimate (BLUE) values and broad-sense heritability (H^2) of GY were calculated within and across locations using the META-R software version 6.04 [24] (http://hdl.handle.net/11529/10201). The linear mixed models used in META-R are implemented in the LME4 R-package, functions of *lmer()* and REML were used to estimate the variance components [24]. In the equations that follow, within location analysis would not have a genotype-by-environment interaction term.

$$Y_{ijkl} = \mu + g_i + e_j + ge_{ij} + r_k e_j + b_l e_j r_k + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijkl} is the trait of interest, μ is the overall mean, g_i, e_j, and ge_{ij} are the effects of the i-th genotype, j-th environment, and i-th genotype by j-th environment interaction, respectively. $r_k e_j$is the effect of the k-th replication within the j-th environment, and $b_l e_j r_k$ is the effect of the l-th incomplete block within the j-th environment and the k-th replication. ε_{ijkl} is the residual effect of the i-th genotype, j-th environment, k-th replication, and l-th block. n_g represents the number of genotypes in the trial, n_e represents the number of environments, n_r represents the number of replications, and n_b represents the number of blocks, where $i = 1$ to n_g, $j = 1$ to n_e, $k = 1$ to n_r, and $l = 1$ to n_b. Genotype is considered as the fixed effect, whereas all other terms are declared as the random effects. Locations with heritability below 0.05 were excluded from the across location analysis.

Broad-sense heritability (H^2) based on the entry means within trials was estimated as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{n_e} + \frac{\sigma^2}{n_e n_r}} \quad (2)$$

where σ_g^2, σ^2, and σ_{ge}^2 are the genotypic variance, error variance, and genotype-by-environment interaction variance, respectively, and n_r and n_e are the numbers of replications and environments, respectively [2].

2.1.2. Line by tester phenotype model

The estimates of the GCA of the line (GCA_L), the GCA of the tester (GCA_T), and the SCA between the line and tester were estimated with the AGD-R software version 4.1 (https://hdl.handle.net/11529/10203). These values were estimated using the phenotypic data as follows:

$$y_{ijdkm} = \mu + L_i + T_j + L_i T_j + e_d + L_i e_d + T_j e_d + L_i T_j e_d + R_k e_d + b_m r_k + \varepsilon_{ijdkm} \quad (3)$$

where, y_{ijdkm} is the observed value, μ is the general mean, L_i is the effect of i-th line, T_j is the effect of j-th tester, $L_i T_j$ is the effect of i-th line by j-th tester interaction, e_d is the effect of d-th environment, $L_i e_d$ is the effect of i-th line by d-th environment interaction, $T_j e_d$ is the effect of j-th tester by d-th environment interaction, $L_i T_j e_d$ is the effect of the interaction of i-th line, j-th tester, and d-th environment. $r_k e_d$ is the effect of k-th replication nested in d-th environment, $b_m r_k$ is the effect of m-th block nested in k-th replication, and ε_{ijdkm} is the residual. n_l represents the number of lines, n_t represents the number of testers, n_e represents the number of environments, n_r represents the number of replications, and n_b represents the number of blocks, where $i = 1$ to n_l, $j = 1$ to n_t, $d = 1$ to n_e, $k = 1$ to n_r, and $m = 1$ to n_b. Both line and tester are considered as the fixed effects, whereas all other terms are declared as the random effects.

2.2. Genotyping and genotypic data analysis

The parental lines, including lines and testers, were genotyped using the DArT-seq platform (https://www.diversityarrays.com/), and sequencing work was performed at the Genetic Analysis Service for Agriculture (SAGA) laboratory of CIMMYT. SNP calling was conducted as described in the previous study [25]. The physical position of the SNP markers was obtained by aligning the flanking sequences to the reference genome of B73_RefGen_v4.

Initially, 39,659 SNPs with known physical positions were identified for each of the genotyped materials. In TASSEL version 5.0 [26] the SNP marker dataset was filtered with the minor allele frequency (MAF) higher than 0.15, the missing rate below 20%, and the heterozygosity rate below 10%. After filtering, 3212 SNPs were selected to perform further genetic analyses, and all the missing loci were imputed using the maximum likelihood estimation method in R. The marker data of the hybrids crossed between lines and testers were obtained in silico. The heat map of SNP density was created using the *ideogram()* function in the RIdeogram package [27] of the R statistical software [28]. Within each trial, the genetic relationships between the genotyped materials were illustrated using the first two principal components. A principal components analysis (PCA) was conducted and visualized using the functions of *prcomp()* and *plot()* in the R statistical software.

2.3. Genomic prediction of the performance of hybrids

Two models were applied to predict the performance of the hybrids based on the genotypic data of the parental lines, which has been described previously [29]. The first model, i.e. M1, only includes the additive effect. The second model, i.e. M2, includes both the additive and non-additive effects.

The linear model of M1 used to predict the performance of the hybrid is given as follows:

$$y = \mu 1 + Z_L g_L + Z_T g_T + \varepsilon \quad (4)$$

where y is the response vector containing the BLUEs of the hybrids described in 2.1.1; $\mu 1$ is the mean across all environments, and g_L and g_T are the vectors of random effects due to the GCA for lines and testers, respectively. Incidence matrices Z_L and Z_T relate y to g_L, g_T with $g_L \sim N(0, \sigma_L^2 G_L)$ and $g_T \sim N(0, \sigma_T^2 G_T)$, where σ_L^2 and σ_T^2 are variance components associated with GCA_L and GCA_T, and G_L and G_T are genomic relationship matrices for the lines and the testers, respectively. Finally, the residual is $\varepsilon \sim N(0, \sigma_\varepsilon^2 I)$, where σ_ε^2 is the variance associated with the residuals, and the I

is the identity matrix. Relationship matrices G_L and G_T were computed using markers, that is, $G_m = W_m W_m^{'}/p$, where W_m, is the matrix of centered and standardized markers, $m = \{L, T\}$ and p is the number of markers [30]. The diagonal values of G_m are distributed around 1 and therefore, the associated variance components are on the same scale as σ_ε^2.

Model 2 (M2) extended model M1 to include both additive GCA for lines and testers and non-additive effect of the hybrids. This model is given as follows [31]:

$$y = \mu 1 + Z_L g_L + Z_T g_T + Z_H h + \varepsilon \quad (5)$$

where Z_H is an incidence matrix that relates y to h with $h \sim N(0, \sigma_H^2 H)$, where, σ_H^2 is a variance component associated with SCA, and H is a relationship matrix for hybrids; the elements of matrix H can be obtained directly from matrices G_L and G_T described in M1, i.e., H is the matrix product of G_L and G_T. The terms Z_L, Z_T, g_L, g_T and $\boldsymbol{\varepsilon}$ are the same as those in M1.

Model M1, excluding the term $Z_T g_T$, was defined as the "Mck" model in the present study. In the Mck model, the hybrid performance of the inbred lines could be predicted using only the genotypic data of the lines [20] where the mean of the BLUE values of the line crossed with all the testers was used as the phenotypic data.

In both Trials A and B, the genomic prediction abilities between testers were also estimated. In each trial, the testcrosses made between all the inbred lines with one tester were used as the training set, and the testcross made between all the inbred lines with the other different tester were used as the testing set; the prediction abilities between testers were estimated in the testing tests.

Models M1, M2, Mck, as well as the predictions between testers were implemented in BGLR's R library [32]. Inferences were based on 30,000 Gibbs sampler iterations, and the first 15,000 were discarded. In models M1, M2, and Mck, a two-fold cross-validation scheme was implemented and repeated 100 times. In each trial, 50% material was selected as the training set to predict the remaining 50% material as the testing set. The sizes of the training set in M1 and M2 were bigger than that in Mck. Pearson's correlation between the observed and predicted values was estimated in the testing set, and the average Pearson's correlation across 100 replications was defined as prediction ability.

2.4. Genomic prediction of the GCA and SCA values

The GCA values were predicted using the SNP data with the RR-BLUP mixed model. The RR-BLUP model, i.e. M3 in the present study, is described [18] as follows:

$$y = WGu + \varepsilon \quad (6)$$

where $u \sim N(0, I\sigma_u^2)$ is a vector of marker effects, G is the genotype matrix code as {−1, 0, 1} for biallelic SNPs, and W is the design matrix relating lines to the observations (y). Similarly, the SCA values were also predicted with the RR-BLUP mixed model using the in-silico SNP data of the hybrids.

The GCA and SCA values were treated as the target traits to be predicted. The GCA prediction was implemented in two scenarios, i.e. GCA_L and GCA_{LT}. In GCA_L, 50% lines in each trial were selected as the training set to predict the GCA of the remaining 50% lines. In GCA_{LT}, 50% lines and testers were selected as the training set to predict the remaining 50% lines and testers. For SCA prediction, 50% combinations formed between lines and testers were selected as the training set to predict the remaining 50% combinations. The above two-fold cross-validation scheme was repeated 100 times. Within each trial, the average GEBV of GCA or SCA for all the material was calculated across 100 predictions, and the prediction ability was estimated as the correlation between the average GEBVs and the observed phenotypic values across all the material.

3. Results

3.1. Phenotypic analysis results

The information on the summaries of the grand mean, genotype variance, genotype by location variance, residual variance, and heritabilities of GY in all trials are shown in Table 1. In the analysis across locations, the grand means of GY in Trials A, B, and A & B were 5.84, 5.91, and 5.88 t ha^{-1}, respectively. The heritabilities of GY in Trials A, B and A & B were 0.89, 0.91, and 0.89, respectively. In all trials, the genotypic variance values were greater than the genotype-by-location variance values, yet lower than the residual variance values. These results implied that the phenotypic data estimated from the analyses across locations are reliable for further genomic prediction research in the present study, although GY is a complex trait, and it is highly influenced by several factors including genotype-by-location interaction.

In both Trial A and Trial B, the GY values had a normal distribution in most of the individual locations (Fig. S1a, b). The phenotypic correlations of the BLUE values of GY between locations were positively correlated, and most of the phenotypic correlation values were significant at $P < 0.01$. The correlation coefficients in Trial A ranged from 0.024 to 0.77, with a mean of 0.45. In Trial B, they ranged from 0.15 to 0.74, with a mean of 0.45. A few correlation coefficients were low, due to occasional extreme and suboptimal weather conditions that happened in some locations.

The distributions of GCA and SCA values in Trials A, B, A & B are shown in Fig. 2. The GCA values ranged from − 0.61 to 0.46 in Trial

Table 1
Descriptive statistics and analysis of variance (ANOVA) for the target trait of grain yield (GY, t ha^{-1}) in Trials A, B, and A & B.

Descriptive statistics	Trial A	Trial B	Trial A&B
Maximum	10.42	12.93	12.93
Minimum	0.46	1.10	0.46
Grand mean	5.84	5.91	5.88
Median	5.57	5.69	5.67
Genotypic variance	0.34	0.38	0.36
Genotype-by-location variance	0.17	0.15	0.23
Residual variance	0.51	0.50	0.52
Kurtosis	−0.28	−0.11	−0.04
Skewness	−0.18	0.43	0.26
Heritability	0.89	0.91	0.89

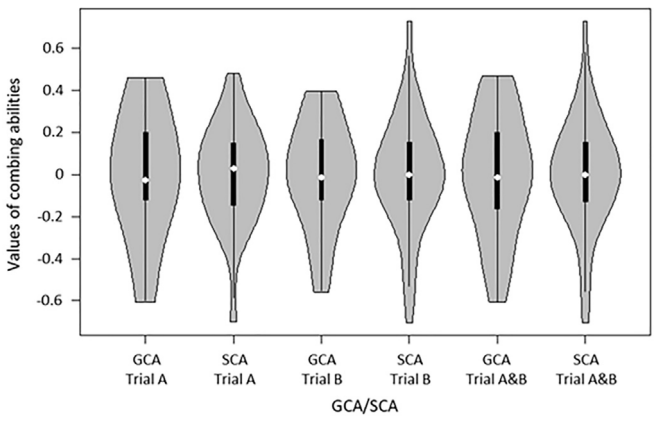

Fig. 2. Violin plots of the values of GCA (general combining ability, t ha^{-1}) and SCA (specific combining ability, t ha^{-1}) for Trials A, B, and A & B.

A, from − 0.56 to 0.40 in Trial B, and from − 0.60 to 0.47 in Trial A & B. The SCA values ranged from − 0.70 to 0.48 in Trial A, from − 0.71 to 0.73 in Trial B, and from − 0.70 to 0.73 in Trial A & B. Sufficient variations were observed for both the GCA and the SCA in all trials.

3.2. Marker data and genetic relationships

The heatmap of the SNP density in each chromosome is shown in Fig. S2. In total, 3212 SNPs distributed in ten chromosomes were used for the further genetic analysis and the number of SNPs per chromosome ranged from 221 on chromosome 10 to 566 on chromosome 1. In all the ten chromosomes in maize, a lower SNP density was observed in the centromeric region. The mean MAF after filtering across all SNPs were 0.24 and 0.25 in Trials A and B, respectively. The mean missing rates after filtering across all SNPs were 0.03 and 0.05 in Trials A and B, respectively.

The genetic relationships between the genotyped materials are illustrated with the PCA plots (Fig. 3). In Trial A, the values of the first two principal components were 51% and 18%, respectively. The testers of T1, T2, and T3, were clustered with each other in one group. The inbred lines were scattered as the second group. In Trial B, the values of the first two principal components were 52% and 14%, respectively. Two groups appeared in the PCA plot of Trial B, the lines and testers from the same heterotic group were clustered in one group, it implied that the testers well represented the genetic diversity of its heterotic group adequately. The testers sharing a common parental line have a closer relationship. The results of PCA plots is consistent with the pedigree information.

3.3. Prediction ability of the GY of hybrids

Prediction abilities of the GY of hybrids were estimated using M1, M2, and Mck models in all trials, and the results are shown in Fig. 4. In all trials, the prediction abilities of the GY of hybrids were high in both M1 and M2 models, i.e. greater than 0.59. In the same trial, the prediction ability of the GY of hybrids estimated using the M2 model were higher than those estimated using the M1 model. The prediction ability of the GY of hybrids estimated using the M1 model was 0.81, 0.68, and 0.59 in Trials A, B, and A & B, respectively. The prediction ability of the GY of hybrids estimated using the M2 model was 0.86, 0.74, and 0.64 in Trials A, B, and A & B, respectively. In all trials, the prediction abilities of the GY estimated using both M1 and M2 models were slightly lower than the heritabilities of the GY estimated using the multiple-location trials. These results imply that the additive effect plays a major role in the prediction of the performance of hybrids in line-by-tester trials, and the prediction model incorporating the non-additive effects could further improve the prediction ability of the performance of the hybrids.

In addition to estimate the prediction ability of the performance of hybrids, the prediction ability of the hybrid performance of the inbred line across all the testers was also estimated, where the Mck model including only the term of $Z_L g_L$ was fitted, and the genotypic data of the inbred line and the phenotypic data of the GY mean of the inbred line crossed with all the testers was used for prediction. The prediction ability of the hybrid performance of the inbred line across all the testers was 0.24, 0.06, and − 0.01 in Trials A, B, and A & B, respectively. The prediction abilities estimated using the Mck model were much lower than the prediction abilities estimated using the M1 and M2 models, indicating that the genotypic data of the testers is very important to improve the prediction ability of the performance of the hybrids. The size of the training set is smaller in the Mck model that is also a potential reason for the lower prediction abilities.

3.4. Prediction abilities of GCA and SCA in line-by-tester trials

The prediction abilities of GCA and SCA were estimated using the M3 model in all trials and the results are shown in Fig. 5. In GCA_L, the training set size of Trials A, B, and A & B was 4, 12, and 16, respectively. The average prediction ability of the GCA of the lines was 0.13, −0.04, and − 0.14 in Trials A, B, and A & B, respectively. In GCA_{LT}, the training set size of Trials A, B, and A & B was 6, 15, and 22, respectively. The prediction ability of the GCA increased, the average prediction ability of the GCA of all material, including both lines and testers, was 0.55, 0.49, and 0.49 in Trials A, B, and A & B, respectively. In the SCA model, the training set size of Trials A, B, and A & B was 12, 36, and 48, respectively. The average prediction ability of the SCA calculated from the 100 replications was − 0.41, −0.26, and − 0.27 in Trials A, B, and A & B, respectively. These results show that the prediction ability of GCA for the line-by-tester trial was moderate by incorporating the information of both lines and testers into the prediction. The SCA for the line-by-tester trial was difficult to predict.

3.5. Genomic prediction abilities of GY between testers

The estimated prediction abilities of the GY between testers are shown in Tables 2 and S1. The prediction abilities between testers in Trial A ranged from 0.07 to 0.41 (Table 2), the prediction abilities

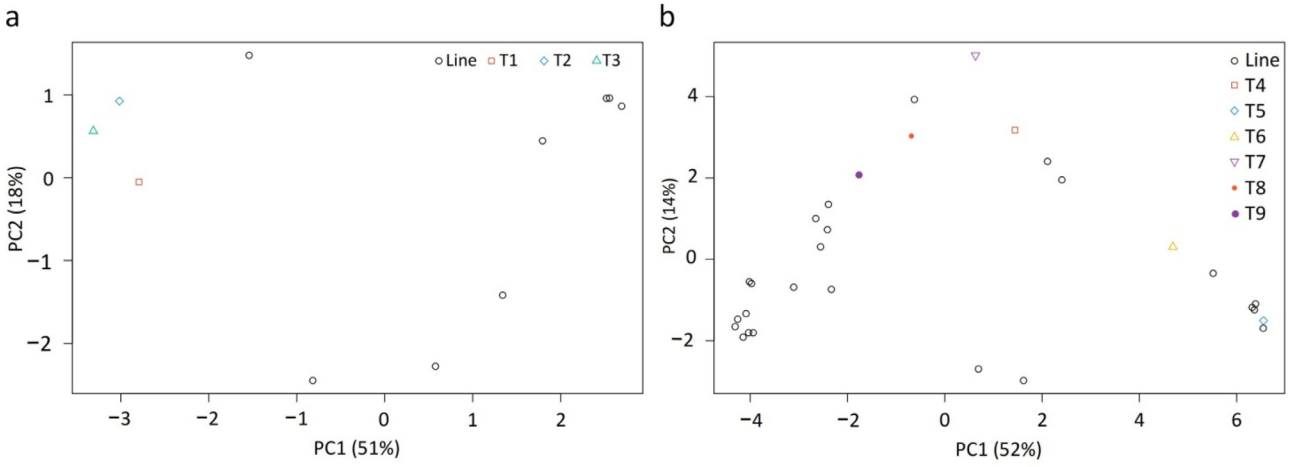

Fig. 3. Genetic relationships illustrated with PCA (principal components analysis) plots. (a) PCA plot of Trial A; (b) PCA plot of Trial B. Nine testers used in Trials A and B are abbreviated as T1 to T9.

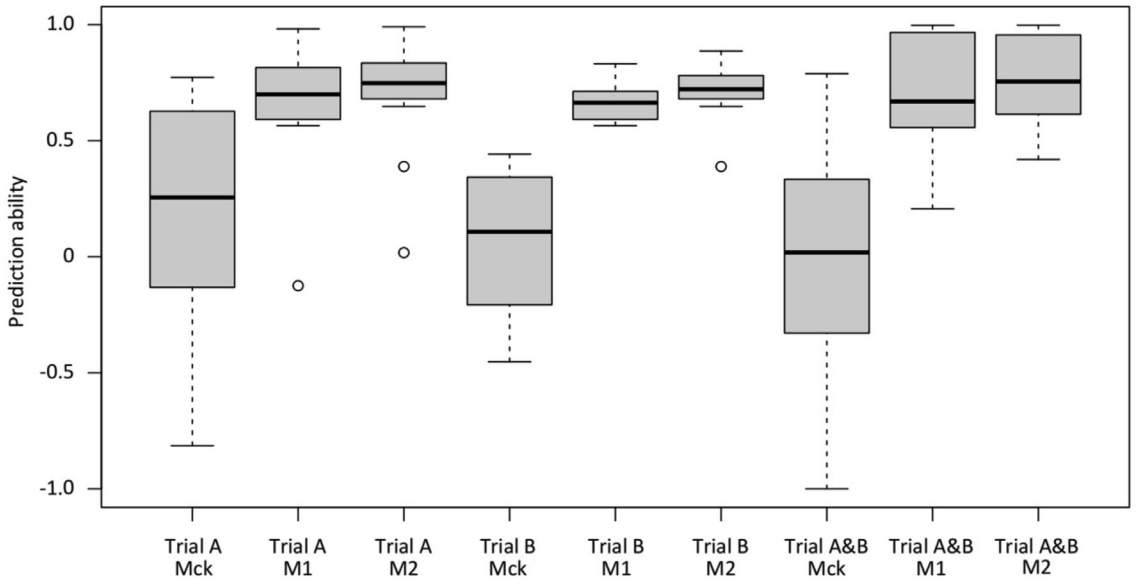

Fig. 4. Prediction abilities of grain yield of the hybrids estimated with the models of M1, M2, and Mck in Trial A, Trial B, and Trial A & B. Mck represents the prediction model using the genotypic data from only lines and only including the additive effect. M1 represents the prediction model using the genotypic data from both lines and testers, and only including the additive effect. M2 represents the prediction model using the genotypic data from both lines and testers, and including both the additive and non-additive effects.

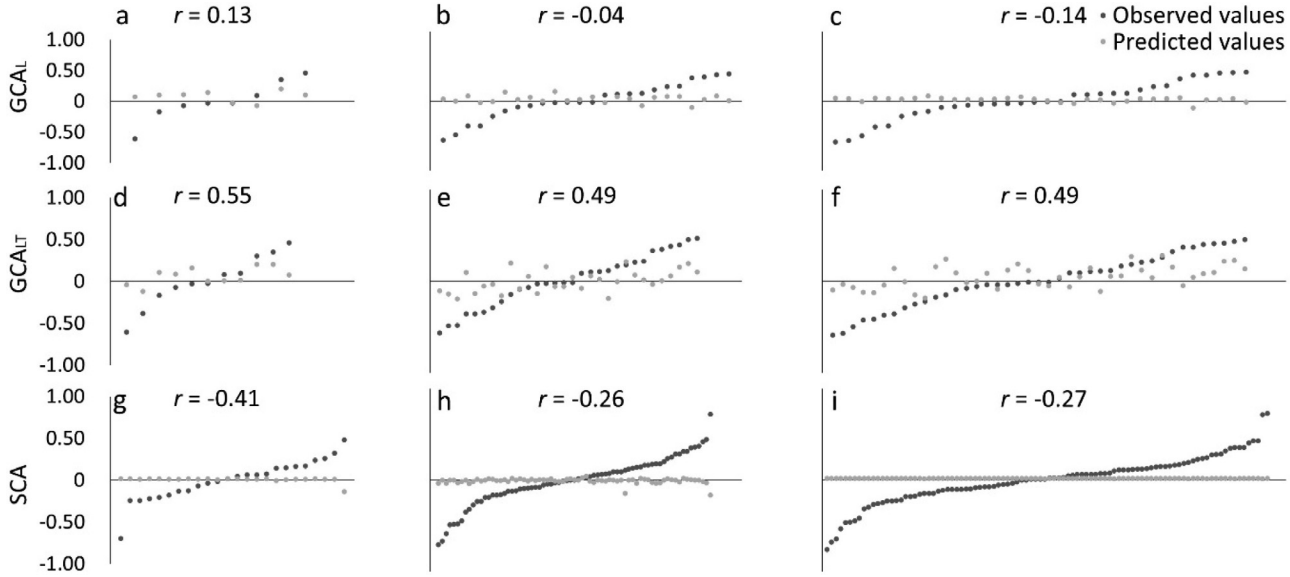

Fig. 5. Prediction abilities (r) of the GCA (General combining ability) and the SCA (Specific combining ability) estimated in Trials A, B, and A & B. a, b, and c are the estimated prediction ability of GCA of the lines (GCA_L) in Trials A, B, and A & B, respectively. d, e, and f are the estimated prediction ability of GCA of the lines and the testers (GCA_{LT}) in Trials A, B, and A & B, respectively. g, h, and i are the estimated prediction ability of SCA in Trials A, B, and A & B, respectively. x-axis stands for the number of genotypes in an ascending order of the observed GCA or SCA values, and y-axis stands for the GCA or SCA values.

of the GY between T1 and T3 were moderate, i.e. 0.38 and 0.39. The highest prediction ability of 0.41 was observed, when the testcrosses made with T2 were used as the training set to predict the testcrosses made with T3. The lowest prediction ability of 0.07 was observed when the testcrosses made with T2 were used as the training set to predict the testcrosses made with T1. These results are in line with the results of genetic relationships, where T1 and T2 are more distantly related; in turn they are closely related between T1 and T3, and between T2 and T3. In general, higher prediction abilities were observed, as the testers were found to be more closely related. However, similar trends were not reflected in Table S1 for Trial B.

Table 2
Prediction abilities of grain yield (GY) between testers in Trial A.

	Tester 1	Tester 2	Tester 3
Tester 1	–	0.34	0.38
Tester 2	0.07	–	0.41
Tester 3	0.39	0.14	–

4. Discussion

GS is a promising genomic tool to predict the phenotypic performance of genotyped materials, without phenotyping. Genomic prediction ability is used to evaluate the effectiveness of GS and must be

moderate to high for GS to be time and cost-effective [33]. In the present study, the prediction abilities of the performance of hybrids were moderate to high across all three trials, the prediction abilities of the GY of hybrids ranged from 0.59 to 0.81 in the M1 model, and from 0.64 to 0.86 in the M2 model. The prediction abilities estimated using both the M1 and M2 models in the present study are higher than those in several earlier GS studies in maize [7,20], in which the prediction abilities were estimated using the genotypic data of inbred lines and the phenotypic data of hybrids formed between inbred lines and testers. These results indicated that incorporating the genotypic data of the testers into prediction helps improve the prediction ability of the performance of the hybrids. The lower prediction abilities estimated from the Mck model also confirmed the importance of the genotypic data of testers for improving prediction ability It implied that GS offers the opportunity to replace the expensive line-by-tester trials phenotyped in multiple-location, where the GEBVs of hybrids can be predicted based on the genotypic data of inbred lines and testers. In addition, the total breeding cost is dramatically reduced by genotyping a few inbred lines and testers with a low-cost per sample genotyping platform, such as DArT-seq, rAmpSeq, and genotyping-by-sequencing [34,35].

Currently, a large number of DH lines can be generated in a maize breeding program every year at an affordable cost. At the line development stage, the major challenge becomes the evaluation of breeding values and combing abilities of newly developed inbred lines, rather than to generate a sufficient number of homozygous inbred lines. For a small number of inbred lines, their breeding values and combining abilities can be easily estimated using the phenotypic data from multiple location trials with special mating designs. However, it is difficult to evaluate the combining abilities for a larger number of inbred lines using phenotypic data, since the number of potential crosses formed between inbred lines grows rapidly, and phenotyping these crosses is resource-intensive. Several GS studies have been conducted to predict the GCA and SCA using molecular marker information, where moderate to high prediction abilities were obtained for the prediction of the GCA. These studies concluded that GS is an effective and efficient approach towards the prediction of the GCA [6,23]. The prediction abilities on the estimation of GCA in the present study ranged from 0.49 to 0.55, which is consistent with the conclusions of previous studies, since it shows that the GCA of inbred lines can be predicted based on the molecular marker information [6,23]. Moreover, the present study also implied that incorporating the GCA factor into the model further improved the prediction of the performance of hybrids, since the prediction abilities of the performance of hybrids ranged from 0.59 to 0.81 in the M1 model. However, the SCA was not well predicted in the present study. The prediction abilities on estimating SCA were negative across all trials, and incorporating the SCA factor into the model only slightly improved the prediction of the performance of hybrids. Negative values of prediction ability in GS mean poor predictions, the prediction results do not have any values for further breeding selection. In Fig. 5, all the predicted SCA values were close to zero, and the variance of the predicted SCA values was very small, which indicated that the SCA values were very difficult to be predicted, there was no linear correlation between the predicted and the observed SCA values. This is mainly due to the fact that the phenotypic data used in the present study was taken from line-by-tester trials, which are mainly designed to accurately estimate the GCA, not the SCA. It also implies that the prediction model needs to be extended in the future studies to include the non-additive effect.

The tester effect plays an important role in the prediction of combining abilities and the performance of hybrids. Several studies have previously implied that modeling the tester effect into the prediction model could improve the prediction abilities of GCA and the performance of hybrids [17,31]. In the present study, the prediction abilities of the performance of hybrids estimated in the M1 and M2 models were much higher than those estimated in the Mck model, indicating that modeling the tester effect into the prediction model could improve the prediction abilities of the performance of hybrids, even though the training set size in the Mck model was a little bit smaller than those in models M1 and M2. The results of the present study also confirmed that modeling the tester effect into the prediction model can improve the prediction abilities of the GCA. The prediction abilities estimated in the GCA_{LT} model were much higher than those estimated in the GCA_{L} model across all trials, even though the size of the training set in GCA_{LT} is slightly bigger than that in GCA_{L}. Furthermore, the genomic prediction abilities of GY between testers were estimated, large variations were observed, moderate prediction abilities presented as the testers are more closely genetically related, and very low prediction abilities presented as the testers are more distantly related. This result showed that the performance of hybrids between testers is difficult to predict, the performance of hybrids should be predicted more accurately by considering the molecular marker information of both lines and testers.

Population size is one of the most important factors affecting the estimation of prediction ability in GS studies. The phenotypic data of the present study is from the lowland tropical maize breeding program of CIMMYT in Mexico, all the trials and crosses were made by the maize breeder, according to the genetic backgrounds of the breeding material, and the appropriate number of tested hybrids included in each breeding trial. Unlike in the genetic studies, the population size of the two trials used in the present study is not so big, because of the feature of breeding trials. To control the phenotyping error in the field, the population size of the breeding trials is always not too big, and only dozens of inbred lines can be included in the line-by-tester trial to evaluate their GCA and SCA values, and each inbred lines is crossed with several testers, the population size per tester is even smaller. The current study focuses more on the breeding application and tries to answer the scientific question of how to predict the performance of hybrids and the combining abilities using the molecular markers and the phenotypic data from the breeding trials, breeding data is more suitable to answer the questions raised by the present study. The results of the present study show that the prediction abilities in Trial A & B are moderate to high, which implies that the population size of the training set can be increased by merging multiple line-by-tester trials, and it can be used to predict the performance of new hybrids and the combing abilities of new inbred lines that have not been evaluated.

5. CrediT authorship contribution statement

Ao Zhang: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Paulino Pérez-Rodríguez:** Data curation, Methodology, Software, Writing - review & editing. **Felix San Vicente:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing. **Natalia Palacios-Rojas:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing. **Thanda Dhliwayo:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing. **Yubo Liu:** Data curation, Formal analysis, Writing - review & editing. **Zhenhai Cui:** Data curation, Formal analysis, Writing - review & editing. **Yuan Guan:** Data curation, Formal analysis, Writing - review & editing. **Hui Wang:** Data curation, Formal analysis, Writing - review & editing. **Hongjian Zheng:** Data curation, Formal analysis, Writing - review & editing. **Michael Olsen:** Funding acquisition, Project administration, Supervision, Writing - review & editing. **Boddupalli M. Prasanna:** Funding acquisition, Project

administration, Supervision, Writing - review & editing. **Yanye Ruan:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Jose Crossa:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Xuecai Zhang:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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