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Efficiency of genomic prediction of non-assessed single crosses

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Running title: Genomic prediction of single crosses.

KEYWORDS genomic selection; linkage disequilibrium; general combining ability; specific combining ability; doubled haploids.

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ABSTRACT The objective was to provide additional relevant information on efficiency of prediction of non-assessed single crosses. We derived the genetic model for genomic prediction. The SNP and QTL genotypic data for DH lines, the QTL genotypic data of SCs, and the phenotypic data for DH lines and SCs were simulated assuming 10,000 SNPs, 400 QTLs, two groups of 70 selected DH lines, and 4,900 SCs. The heritabilities for the assessed SCs were 30, 60 and 100%. The scenarios included three sampling processes of DH lines, two sampling processes of SCs for testing, two SNP densities, DH lines from distinct and same populations, DH lines from populations with lower LD, two genetic models, three statistical models, and three statistical approaches. The efficiency of prediction of untested SCs was based on the prediction accuracy and the efficacy of identification of the best 300 (7-9%) non-assessed SCs (coincidence index), computed based on the true genotypic values. Concerning the prediction accuracy and coincidence, our results proved that prediction of untested SCs is very efficient. The accuracies and coincidences ranged from approximately 0.8 and 0.5, respectively, under low heritability, to 0.9 and 0.7, assuming high heritability. Additionally, we highlighted the relevance of the overall LD and evidenced that efficient prediction of untested SCs can be achieved for crops that show no heterotic pattern, for reduced training set size (10%), for SNP density of 1 cM, and for distinct sampling processes of DH lines, based on random choice of the SCs for testing.

INTRODUCTION

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Genomic selection is very commonly used in animal breeding programs, especially for dairy cattle Van Eenennaam et al. (2014). The same cannot yet be said to the same degree concerning crop breeding. The main reasons for the effective application of genomic selection in livestock breeding are: it is efficient, that is, the process has high prediction accuracy, the cost of phenotyping (mainly progeny test) is higher than the cost of genotyping, and the process significantly shortens the selection cycle (Meuwissen et al. 2013). In spite of the many field and simulation-based studies with genomic selection in plant breeding, in general the cost of phenotyping is often still much lower than the cost of genotyping, restricting its application in breeding programs. Jonas and de Koning (2013) consider that genomic selection has the potential to improve existing plant breeding schemes. However, based also on the high diversity and complexity of plant breeding methods, they stated that there are great obstacles to overcome.

An important application of genomic selection in plant breeding is the prediction of untested single crosses (genotypic value prediction) and testcrosses (general combining ability effect prediction) in hybrid breeding (Zhao et al. 2015). Prediction accuracy of barley two- and three-way crosses has been investigated (Philipp et al. 2016). The prediction of untested single crosses was pioneered by Bernardo (1994), based on best linear unbiased prediction (BLUP). Many significant studies on prediction of untested single cross and testcross performance have been published in the last 23 years, focused on the assessment of the prediction accuracy. Most investigations were based on empirical data and estimated the prediction accuracy using a cross-validation procedure. Very few were based on simulated data (Li et al. 2017; Technow et al. 2012a). With no exception, the inference was that prediction of untested single crosses and testcrosses can be an efficient, depending on heritability, training set size, and number of tested inbreds in hybrid combination (both, one, and none parents tested). Remarkably, this conclusion was drawn from studies differing in the type of molecular marker, density of markers, number of inbreds, level of relatedness, diversity, and linkage disequilibrium (LD) between inbreds, heterotic pattern, training set size,

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genetic model, and statistical approach (Zhao et al. 2015). Efficient prediction of barley two- and three-way crosses has been achieved when training and validation sets include the same class of hybrid (Philipp et al. 2016).

Most papers on genomic prediction of maize single cross performance published since 2011 have employed single nucleotide polymorphism (SNP), with the number SNPs filtered ranging from 425 (Zhao et al. 2013a) to 39,627 (Technow et al. 2012a). Based on the physical length of the maize genome (approximately 2,106 megabase pairs (Mb) according to Maize genetics and genomics database), the SNP density ranged from approximately 5 to 0.05 Mb, respectively. For grain yield, the relative prediction accuracies (computed as accuracy/root square of the heritability) in these two papers ranged from 0.27 to 0.62 and from 0.65 to 0.95, respectively. The number of inbreds in each heterotic group was highly variable too, ranging from six and nine (Bernardo 1994) to 75 and 75 (Technow et al. 2012a). The relative accuracy observed by Bernardo (1994) ranged between 0.72 and 0.89. The number of testcrosses ranged between 255 (Windhausen et al. 2012) and 1,894 (Albrecht et al. 2014). The relative accuracies ranged from 0.46 to 0.52 and from 0.33 to 0.65, respectively. The level of relatedness ranged from non-related inbreds in each group (Technow et al. 2012a) to a maximum average value of 0.58 (Bernardo 1995). The relative accuracy obtained by Bernardo (1995) ranged from 0.41 to 0.80. The common heterotic groups were Stiff Stalk and non-Stiff Stalk (Kadam et al. 1916) or Dent and Flint (Technow et al. 2014). The study of Bernardo (1996a) involved nine heterotic groups and the (statistically significant from zero) relative accuracies ranged from 0.43 to 0.88. No study provided clearly greater prediction accuracy of the additive-dominance model relative to the additive model. Finally, only with testcrosses the genomic BLUP (GBLUP) approach outperformed BLUP (Albrecht et al. 2014; Albrecht et al. 2011) concerning prediction accuracy.

Technow et al. (2012a) provided the most comprehensive study on prediction of untested single cross performance. Our assessment on the efficiency of prediction of non-assessed single

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85 crosses provides additional relevant information. Our simulation-based study is the first to provide
86 for breeders a direct measure of efficiency of identification of the best non-assessed single crosses
87 (coincidence index), additionally to the standard prediction accuracy. What is the efficiency of
88 identification of the best 300 untested single crosses if the prediction accuracy is, for example,
89 approximately 0.90? Our results evidence that the efficacy range between 0.60 and 0.70, depending
90 on the doubled haploid (DH) lines derivation process. These measures of efficiency were provided
91 for a large data set (10,000 SNPs, 400 quantitative trait loci (QTLs), 4,900 single crosses) and for
92 low (30%) to high heritability (100%), assuming scenarios not favorable to prediction of non-
93 assessed single cross performance, as low level of relatedness and a not high heterotic pattern. Low
94 heritability has been observed in some CIMMYT's global maize and wheat breeding programs
95 (Crossa et al. 2014). Additionally, we derived the genetic model for genomic prediction, supported
96 by quantitative genetics theory, highlighted the relevance of the overall LD (not only for linked
97 SNPs and QTLs), and evidenced that efficient prediction of untested single crosses can be achieved
98 for crops that show no clear heterotic pattern, as rice, wheat, and barley, for reduced training set
99 size (10%), for SNP density of 1 centiMorgan (cM), and for distinct processes of (DH) lines
100 sampling. Finally, we showed that the choice of the single crosses for testing must be based on a
101 random process, but never by sampling DH or inbreds lines for a diallel. By sampling 76% of the
102 available genotyped DH lines in each group for a diallel (Technow et al. (2012a) sampled 75% of
103 the inbreds), the prediction accuracies and coincidence indexes were 38 to 77% and 39 to 98%
104 lower, respectively, compared with random sampling of 30% of the possible single crosses for
105 testing. Thus, our objective was to provide to breeders additional relevant information on prediction
106 of non-assessed single crosses.

107 ## MATERIALS AND METHODS

108 ### Theory

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Generally, most papers on genomic selection presents only statistical aspects and the genetic models are deduced from gene to SNP effects. Importantly, when there is some quantitative genetics theory, the LD is completely ignored. The theory developed provides, based on quantitative genetics including LD, the genetic model for genomic prediction of single crosses. The model developed offers the genetic background to the models fitted in important previously papers on prediction of untested single crosses and testcrosses (Massman et al. 2013; Technow et al. 2012a; Albrecht et al. 2011). Notice, however, that the derived model has distinct presuppositions.

LD in a group of selected DH or inbred lines

Consider a group of DH or inbred lines selected from a population or heterotic group. Assume also a QTL (alleles B/b) and a SNP (alleles C/c) where B and b are the alleles that increase and decrease the trait expression, respectively. Define the joint genotype probabilities as $P(BBCC) = f_{22}$, $P(BBcc) = f_{20}$, $P(bbCC) = f_{02}$, and $P(bbcc) = f_{00}$, where the subscript indicates the number of copies of the major allele (B and C). The measure of LD between the QTL and the SNP is $\Delta_{bc} = f_{22}f_{00} - f_{20}f_{02}$ (Kempthorne 1954) and the haplotype frequencies are $P(BC) = f_{22} = p_b p_c + \Delta_{bc}$, $P(Bc) = f_{20} = p_b q_c - \Delta_{bc}$, $P(bC) = f_{02} = q_b p_c - \Delta_{bc}$, and $P(bc) = f_{00} = q_b q_c + \Delta_{bc}$, where p is the frequency of the major allele (B or C) and $q = 1 - p$ is the frequency of the minor allele (b or c). Notice that $p_b = f_{22} + f_{20}$ and $p_c = f_{22} + f_{02}$. It is important to highlight the fact that we are not assuming that the QTL and the SNP are linked and in LD in the population or heterotic group, because this is not necessary for genomic prediction. But we are assuming that they are in LD in the group of DH or inbred lines. Furthermore, because selection, genetic drift, and inbreeding (only for inbreds and linked QTLs and SNPs), the gene and genotypic frequencies and the LD values concerning the selected DH or inbred lines cannot be traced to the values in the population or heterotic group.

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SNP genotypic values of DH or inbred lines

The average genotypic value for a group of selected DH or inbred lines is $M_{IL} = m_b + \left(p_b - q_b\right)a_b$, where m_b is the mean of the genotypic values of the homozygotes and a_b is the deviation between the genotypic value of the homozygote of higher expression and m_b. Thus, the average SNP genotypic values for the DH or inbred lines CC and cc are

$$G_{CC} = \frac{1}{f_{.2}}\left[f_{22}\left(m_b + a_b\right) + f_{02}\left(m_b - a_b\right)\right] = M_{IL} + 2q_c\alpha_{SNP} = M_{IL} + A_{CC}$$

$$G_{cc} = \frac{1}{f_{.0}}\left[f_{20}\left(m_b + a_b\right) + f_{00}\left(m_b - a_b\right)\right] = M_{IL} - 2p_c\alpha_{SNP} = M_{IL} + A_{cc}$$

where $\alpha_{SNP} = \left[\frac{\Delta_{bc}}{p_c q_c}\right]a_b = \kappa_{bc}a_b$ is the average effect of a SNP substitution in the group of DH or inbred lines and A is the SNP additive value for a DH or inbred line. Notice that E(A) = 0.

Assuming two QTLs (alleles B and b, and E and e) in LD with the SNP, the average effect of a SNP substitution in the selected DH or inbred lines is $\alpha_{SNP} = \kappa_{bc}a_b + \kappa_{ce}a_e$, where $\kappa_{ce} = \left[\frac{\Delta_{ce}}{p_c q_c}\right]$. Thus, in general, the average effect of a SNP substitution (and the SNP additive value) is proportional to the measure of LD and to the a deviation for each QTL that is in LD with the marker.

SNP genotypic values of single crosses

Aiming to maximize the heterosis, maize breeders commonly assess single crosses originating from selected DH or inbred lines from distinct heterotic groups. Consider n_1 DH or inbred lines from a population or heterotic group and n_2 DH or inbred lines from a distinct population or

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heterotic group. The average genotypic value for the single crosses derived by crossing the DH or inbred lines from group 1 with the DH or inbred lines from group 2 is

$$M_H = m_b + \left(p_{b1}p_{b2} - q_{b1}q_{b2}\right)a_b + \left(p_{b1}q_{b2} + q_{b1}p_{b2}\right)d_b$$

where d_b is the dominance deviation (the deviation between the genotypic value of the heterozygote and m_b).

The average genotypic values for the single crosses derived from DH or inbred lines CC and cc of the group 1 are

$$M_{CC1} = M_H + q_{c1}\kappa_{bc1}\left[a_b + \left(q_{b2} - p_{b2}\right)d_b\right] = M_H + q_{c1}\kappa_{bc1}\alpha_{b2} = M_H + q_{c1}\alpha_{SNP1}$$
$$= M_H + GCA_{CC1}$$

$$M_{cc1} = M_H - p_{c1}\kappa_{bc1}\alpha_{b2} = M_H - p_{c1}\alpha_{SNP1} = M_H + GCA_{cc1}$$

where α_{b2} is the average effect of allelic substitution in the population derived by random crosses between the DH or inbred lines of group 2, α_{SNP1} is the SNP effect of allelic substitution in the hybrid population relative to a SNP derived from group 1, and GCA stands for the general combining ability effect for a SNP locus. Notice that α_{SNP1} depends on the LD in group 1 ($\kappa_{bc1} = \Delta_{bc1}/p_{c1}q_{c1}$) and the average effect of allelic substitution in the population derived by random crosses between the DH or inbred lines of group 2. Further, $E(GCA) = p_{c1}GCA_{CC1} + q_{c1}GCA_{cc1} = 0$. Concerning the single crosses derived from DH or inbred lines CC and cc of the group 2 we have

$$M_{CC2} = M_H + q_{c2}\kappa_{bc2}\left[a_b + \left(q_{b1} - p_{b1}\right)d_b\right] = M_H + q_{c2}\kappa_{bc2}\alpha_{b1} = M_H + q_{c2}\alpha_{SNP2}$$
$$= M_H + GCA_{CC2}$$

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168 $$M_{cc2} = M_H - p_{c2}\kappa_{bc2}\alpha_{bl} = M_H - p_{c2}\alpha_{SNP2} = M_H + GCA_{cc2}$$

169 Notice that E(GCA) = 0 also. The average genotypic values for the single crosses concerning
170 the SNP locus are

171 $$M_{CC1xCC2} = M_H + q_{c1}\alpha_{SNP1} + q_{c2}\alpha_{SNP2} - 2q_{c1}q_{c2}\kappa_{bc1}\kappa_{bc2}d_b$$
$$= M_H + GCA_{CC1} + GCA_{CC2} + SCA_{CC1xCC2}$$

172 $$M_{cc1xcc2} = M_H - p_{c1}\alpha_{SNP1} - p_{c2}\alpha_{SNP2} - 2p_{c1}p_{c2}\kappa_{bc1}\kappa_{bc2}d_b$$
$$= M_H + GCA_{cc1} + GCA_{cc2} + SCA_{cc1xcc2}$$

173 $$M_{CC1xcc2} = M_H + q_{c1}\alpha_{SNP1} - p_{c2}\alpha_{SNP2} + 2q_{c1}p_{c2}\kappa_{bc1}\kappa_{bc2}d_b$$
$$= M_H + GCA_{CC1} + GCA_{cc2} + SCA_{CC1xcc2}$$

174 $$M_{cc1xCC2} = M_H - p_{c1}\alpha_{SNP1} + q_{c2}\alpha_{SNP2} + 2p_{c1}q_{c2}\kappa_{bc1}\kappa_{bc2}d_b$$
$$= M_H + GCA_{cc1} + GCA_{CC2} + SCA_{cc1xCC2}$$

175 where $\kappa_{bc1}\kappa_{bc2}d_b = d_{SNP}$ is the SNP dominance deviation in the hybrid population and SCA
176 stands for the specific combining ability effect for a SNP locus. Notice that $E(SCA) =$
177 $p_{c1}p_{c2}SCA_{CC1xCC2} + p_{c1}q_{c2}SCA_{CC1xcc2} + q_{c1}p_{c2}SCA_{cc1xCC2} + q_{c1}q_{c2}SCA_{cc1xcc2} = 0$ and
178 , for each group, E(SCA|CC) = E(SCA|cc) = 0. That is, the expectation of the SNP SCA effects
179 given a SNP genotype for the common DH or inbred line is also zero. Notice also that the four
180 genotypic values depends on four parameters (M_H, α_{SNP1}, α_{SNP2}, and d_{SNP}).

181 Assuming two QTLs (alleles B and b, and E and e) in LD with the SNP, the SNP dominance
182 deviation is $d_{SNP} = \kappa_{bc1}\kappa_{bc2}d_b + \kappa_{ce1}\kappa_{ce2}d_e$. Thus, generally, the SNP dominance deviation
183 (and the SNP SCA effect) is proportional to the product of the LD values in both groups of DH or
184 inbred lines and to the dominance deviation for each QTL that is in LD with the marker.

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185 The previous model expressed as a function of the GCA and SCA effects is that proposed by
186 Massman et al. (2013), but these authors assumed $GCA_{CC} + GCA_{cc} = 0$ (for each heterotic group
187 and for each SNP) and $SCA_{CC1xCC2} = SCA_{cc1xcc2} = -SCA_{CC1xcc2} = -SCA_{cc1xCC2}$.
188 Technow et al. (2012b) have used a standard extension from QTL to SNP, defining the single cross
189 genotypic value for a SNP as a function of the SNP a and d deviations. That is,
190 $M = M_H + u_1 a_1 + u_2 a_2 + u_3 d$, where u_1 and u_2 equal to 1/2 or −1/2 if the corresponding DH or
191 inbred line is homozygous for distinct SNP alleles (CC or cc), and u_3 equal to 0 if the single cross
192 is homozygous or 1 if heterozygous.

193 ***SNP genotypic values of single crosses from DH or inbred lines derived from the same***
194 ***population or heterotic group***

195 Well defined heterotic groups are known for maize, but not for special maize as popcorn and
196 sweet corn and for other crops as wheat (Zhao et al. 2013b), rice (Xu et al. 2014), and barley
197 (Philipp et al. 2016). Thus, for many breeders, it is interesting to know about the efficiency of
198 genomic prediction of singles crosses when there are no heterotic groups. Assuming n DH or inbred
199 lines derived from the same population or heterotic group, the average genotypic values for the
200 single crosses concerning the SNP locus are

201 $$M_{CCxCC} = M + 2q_c\alpha_{SNP} - 2q_c^2\kappa_{bc}^2 d_b = M + 2GCA_{CC} + SCA_{CCxCC}$$

202 $$M_{ccxcc} = M - 2p_c\alpha_{SNP} - 2p_c^2\kappa_{bc}^2 d_b = M + 2GCA_{cc} + SCA_{ccxcc}$$

203 $$M_{CCxcc} = M + 2(q_c - p_c)\alpha_{SNP} + 2p_c q_c \kappa_{bc}^2 d_b = M + GCA_{CC} + GCA_{cc} + SCA_{CCxcc}$$

204 where $M = m_b + (p_c - q_c)a_b + 2p_c q_c d_b$ is the hybrid population mean,
205 $\alpha_{SNP} = \kappa_{bc}[a_b + (q_b - p_b)d_b] = \kappa_{bc}\alpha_b$ is the average effect of a SNP substitution in the hybrid
206 population, and $d_{SNP} = \kappa_{bc}^2 d_b$ is the SNP dominance deviation. Notice that the SNP GCA effects

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are equal to half the SNP additive value for the single crosses (A), the SNP SCA effects are the SNP dominance deviations for the single crosses (D), and that the three genotypic values depends on three parameters (M, α_{SNP}, and d_{SNP}). Notice also that E(GCA) = E(A) = E(SCA) = E(SCA|CC) = E(SCA|cc) = E(D) = 0.

Accuracy of single cross genomic prediction

Assuming a QTL and a SNP in LD in the two groups of DH or inbred lines, the predictor of the single cross QTL genotypic value is the single cross SNP genotypic value (because they are proportional). Thus, the covariance between the predictor and the genotypic value is

$$\begin{aligned}
\mathrm{Cov}\left(\tilde{G},G\right) &= f_{22}^{1}f_{22}^{2}\left[M_H+GCA_{CC1}+GCA_{CC2}+SCA_{CC1xCC2}\right]\left[M_H+GCA_{BB1}+GCA_{BB2}+SCA_{BB1xBB2}\right]+\\
&+f_{22}^{1}f_{20}^{2}\left[M_H+GCA_{CC1}+GCA_{cc2}+SCA_{CC1xcc2}\right]\left[M_H+GCA_{BB1}+GCA_{BB2}+SCA_{BB1xBB2}\right]+\\
&\cdots\\
&+f_{00}^{1}f_{00}^{2}\left[M_H+GCA_{cc1}+GCA_{cc2}+SCA_{cc1xcc2}\right]\left[M_H+GCA_{bb1}+GCA_{bb2}+SCA_{bb1xbb2}\right]-\left(M_H\right)^2\\
&=p_{c1}q_{c1}\left(\kappa_{bc1}\alpha_{b2}\right)^2+p_{c2}q_{c2}\left(\kappa_{bc2}\alpha_{b1}\right)^2+4p_{c1}q_{c1}p_{c2}q_{c2}\left(\kappa_{bc1}\kappa_{bc2}d_b\right)^2\\
&=p_{c1}q_{c1}\left(\alpha_{SNP1}\right)^2+p_{c2}q_{c2}\left(\alpha_{SNP2}\right)^2+4p_{c1}q_{c1}p_{c2}q_{c2}\left(d_{SNP}\right)^2\\
&=\sigma^{2(1)}_{GCA_{SNP}}+\sigma^{2(2)}_{GCA_{SNP}}+\sigma^{2}_{SCA_{SNP}}=\sigma^{2}_{G(SNP)}
\end{aligned}$$

where the GCA and SCA effects for the QTL are $GCA_{BB1}=q_{b1}\alpha_{b2}$, $GCA_{bb1}=-p_{b1}\alpha_{b2}$, $GCA_{BB2}=q_{b2}\alpha_{b1}$, $GCA_{bb2}=-p_{b2}\alpha_{b1}$, $SCA_{BB1xBB2}=-2q_{b1}q_{b2}d_b$, $SCA_{BB1xbb2}=2q_{b1}p_{b2}d_b$, $SCA_{bb1xBB2}=2p_{b1}q_{b2}d_b$, and $SCA_{bb1xbb2}=-2p_{b1}p_{b2}d_b$, σ^2_{GCA} and σ^2_{SCA} are the GCA and SCA variances for the SNP locus, and σ^2_G is the SNP genotypic variance. The GCA and SCA variances for the QTL are $\sigma^{2(1)}_{GCA}=p_{b1}q_{b1}\left(\alpha_{b2}\right)^2$,

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$\sigma^{2(2)}_{GCA} = p_{b2}q_{b2}\left(\alpha_{b1}\right)^2$, and $\sigma^2_{SCA} = 4p_{b1}q_{b1}p_{b2}q_{b2}\left(d_b\right)^2$. The QTL genotypic variance is $\sigma^2_G = \sigma^{2(1)}_{GCA} + \sigma^{2(2)}_{GCA} + \sigma^2_{SCA}$ Thus, the single cross prediction accuracy is

$$\rho_{\tilde{G},G} = \sqrt{\frac{\sigma^2_{G(SNP)}}{\sigma^2_G}}$$

Assuming s SNPs,

$$\rho_{\tilde{G},G} = \sum_{r=1}^{s} \sigma^2_{G(SNP(r))} / \sqrt{\sigma^2_{\tilde{G}}\sigma^2_G}$$

where $\sigma^2_{\tilde{G}}$ is the variance of the predicted single cross genotypic values and σ^2_G is the single cross genotypic variance. Further,

$\alpha_{SNP(r)1} = \sum_{i=1}^{k'}\left[\frac{\Delta_{ri1}}{p_{r1}q_{r1}}\right]\alpha_{i2} = \sum_{i=1}^{k'}\kappa_{ri1}\alpha_{i2}$, where k' is the number of QTLs in LD with the SNP r) in group 1, and

$d_{SNP(r)} = \sum_{i=1}^{k''}\left[\frac{\Delta_{ri1}}{p_{r1}q_{r1}}\right]\left[\frac{\Delta_{ri2}}{p_{r2}q_{r2}}\right]d_i = \sum_{i=1}^{k''}\kappa_{ri1}\kappa_{ri2}d_i$ where k'' is the number of QTLs in LD with the SNP r in both groups

Notice that because the accuracy of genomic prediction of single crosses depends on the squares of the average effects of SNP substitution and the SNP dominance deviations, it is not affected by the linkage phase (coupling or repulsion), as it does not depend on linkage. But it depends on the magnitude of the LD in each group of DH or inbred lines.

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Assuming single crosses derived from DH or inbred lines of a single population or heterotic group we have $\sigma^2_{G(SNP)} = 2p_c q_c (\alpha_{SNP})^2 + (2p_c q_c d_{SNP})^2$ and $\sigma^2_G = 2p_b q_b (\alpha_b)^2 + (2p_b q_b d_b)^2$.

The statistical model for single cross genomic prediction

Assume n_1 and n_2 (several tens) DH or inbred lines from two populations or heterotic groups genotyped for s (thousands) SNPs and the experimental assessment of h (few hundred) single-crosses (h much lower than $n_1.n_2$) in e (several) environments (a combination of growing seasons, years, and locals). Defining y as the adjusted single cross phenotypic mean, the statistical model for prediction of the average effects of SNP substitution and the SNP dominance deviations is

$$y = M_H + \sum_{r=1}^{s} \left(z1_r \alpha_{SNP1_r} + z2_r \alpha_{SNP2_r} + z3_r d_{SNP_r} \right) + \text{error}$$

where $z1_r = q_{r1}$, $z2_r = q_{r2}$, and $z3_r = -2q_{r1}q_{r2}$ if the SNP genotypes for the DH or inbred lines are CC (group 1) and CC (group 2), $z1_r = -p_{r1}$, $z2_r = -p_{r2}$, and $z3_r = -2p_{r1}p_{r2}$ if the SNP genotypes for the DH or inbred lines are cc (group 1) and cc (group 2), $z1_r = q_{r1}$, $z2_r = -p_{r2}$, and $z3_r = 2q_{r1}p_{r2}$ if the SNP genotypes for the DH or inbred lines are CC (group 1) and cc (group 2), and $z1_r = -p_{r1}$, $z2_r = q_{r2}$, and $z3_r = p_{r1}q_{r2}$ if the SNP genotypes for the DH or inbred lines are cc (group 1) and CC (group 2).

Regarding the single crosses obtained from DH or inbred lines of the same population or heterotic group we have

$$y = M + \sum_{r=1}^{s} \left(z1_r \alpha_{SNP_r} + z2_r d_{SNP_r} \right) + \text{error}$$

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where $z1_r = 2q_r$ and $z2_r = -2q_r^2$ if the SNP genotypes for the DH or inbred lines are CC and CC, $z1_r = -2p_r$ and $z2_r = -2p_r^2$ if the SNP genotypes for the DH or inbred lines are cc and cc, and $z1_r = 2(q_r - p_r)$ and $z2_r = 2p_r q_r$ if the SNP genotypes for the DH or inbred lines are CC and cc.

The statistical problem of genomic prediction when there are a very large number of molecular markers and relatively few observations have been addressed thorough several regularized whole-genome regression and prediction methods (Daetwyler et al. 2013; de Los Campos et al. 2013). Then, the predicted effects of SNP substitution ($\tilde{\alpha}$) and SNP dominance deviations ($\tilde{d}$) must be used to provide genomic prediction of non-assessed single crosses. The predicted genotypic value for a non-assessed single cross of DH or inbred lines from two groups is

$$\tilde{G} = \hat{M}_H + \sum_{r=1}^{s} \left(z1_r \tilde{\alpha}_{SNP1_r} + z2_r \tilde{\alpha}_{SNP2_r} + z3_r \tilde{d}_{SNP_r} \right)$$

For a non-assessed single cross of DH or inbred lines from the same group, the predicted genotypic value is

$$\tilde{G} = \hat{M} + \sum_{r=1}^{s} \left(z1_r \tilde{\alpha}_{SNP_r} + z2_r \tilde{d}_{SNP_r} \right)$$

Simulation

The SNP and QTL genotypic data for DH lines, the QTL genotypic data of single crosses, and the phenotypic data for DH lines and single crosses were simulated using the software *REALbreeding*. The program has been developed by the first author using the software *REALbasic 2009* (Viana et al. 2017a; Viana et al. 2017b; Viana et al. 2016; Azevedo et al. 2015; Viana et al. 2013). Based on our input, the software distributed 10,000 SNPs and 400 QTLs in ten chromosomes (1,000 SNPs and 40 QTLs by chromosome). The average SNP density was 0.1 cM. The QTLs were distributed in the regions covered by the SNPs (approximately 100 cM/chromosome). Initially, *REALbreeding* sampled 700 DH lines from two non-inbred populations

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(heterotic groups) in LD (350 from each population). The populations were composites of two populations in linkage equilibrium. In a composite, there is LD only for linked SNPs and QTLs (Viana et al. 2016). The number of DH lines from each S_0 plant was one (scenario 1) or ranged from 1 to 5 (scenario 2). We also sampled 350 DH lines from each population after three generations of selfing (using the single seed descent process). The number of DH lines from each S_3 plant ranged from 1 to 5 (scenario 3). For each scenario, the software then crossed 70 selected DH lines from each population, using a diallel design. The heritability for the DH lines was 30%.

The genotypic values of the DH lines and of the single crosses were generated assuming a single set of 400 QTLs and two degrees of dominance. To simulate grain yield and expansion volume, a measure of popcorn quality, we defined positive dominance ($0 < (d/a)_i \leq 1.2$, i = 1, ..., 400) and bidirectional dominance ($-1.2 \leq (d/a)_i \leq 1.2$), respectively, where d/a is the degree of dominance. To compute the genotypic values, *REALbreeding* used our input relative to the maximum and minimum genotypic values for homozygotes. For grain yield and expansion volume, we defined 140 and 30 g/plant and 55 and 15 mL/g, respectively. The phenotypic values were obtained from the sum of the population mean, genotypic value, and experimental error. The error variance was computed from the broad sense heritability. To avoid outliers, we defined the maximum and minimum phenotypic values as 160 and 10 g/plant and 65 and 5 mL/g.

The heritabilities for the assessed single crosses were 30, 60, and 100%. Thus, the genotypic value prediction accuracies of the assessed single crosses were 0.55, 0.77, and 1.00, respectively. For each scenario were processed 50 resamplings of 30 and 10% of the single crosses (1,470 and 490 assessed single crosses). That is, we predicted 70 and 90% of the single crosses (3,430 and 4,410 non-assessed single crosses). Additionally, to assess the relevance of the number of DH lines sampled, we fixed the number of DH lines to achieve the same number of assessed single crosses, using a diallel. That is, we sampled 50 times 38 and 22 DH lines in each group for a diallel (scenario 4), generating 1,444 and 484 single crosses for assessment, respectively. We called these

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303 processes as sampling of single crosses (scenarios 1 to 3) and sampling of DH lines (scenario 4).
304 Other additional scenarios were: genomic prediction of single crosses from selected DH lines from
305 same heterotic group (interestingly for wheat, rice, and barley breeders, for example) (scenario 5)
306 and from selected DH lines from populations with lower LD (scenario 6), to emphasize that the
307 prediction accuracy depends on the LD in the groups of DH or inbred lines. A last scenario
308 (seventh) was genomic prediction of single crosses under an average density of one SNP each cM.
309 This lower density was obtained by random sampling of 100 SNPs per chromosome using a
310 *REALbreeding* tool (*sampler*). To investigate the single cross prediction efficiency based on our
311 model and on the models proposed by Massman et al. (2013) and Technow et al. (2012b), we used
312 another *REALbreeding* tool (*Incidence matrix*) to generate the incidence matrices for the three
313 models and for the two DH lines sampling processes. To assess the relevance of the SCA effects
314 prediction on genomic prediction of single cross performance, we also fitted the additive model
315 (including only the GCA effects). For comparison purpose, we also processed single cross
316 prediction based on GBLUP (with the observed additive and dominance relationship matrices) and
317 BLUP (with the expected additive and dominance relationship matrices).

318 **Statistical analysis**

319 The methods used for prediction were ridge regression BLUP (RR-BLUP), GBLUP and
320 BLUP. For the analyses we used the *rrBLUP* package (Endelman 2011). The accuracies of single
321 cross genotypic value prediction were obtained by the correlation between the true values of the
322 non-assessed single crosses computed by *REALbreeding* and the values predicted by RR-BLUP,
323 GBLUP, and BLUP. We also computed the efficiency of identification of the 300 non-assessed
324 single crosses of higher genotypic value (coincidence index). The parametric average coincidence
325 index was computed by ordering the average phenotypic values of the 4,900 single crosses for each
326 heritability and for each DH lines derivation process. Regarding grain yield, for heritability of 30%
327 the coincidence index was 0.2533, 0.2833, and 0.2433 assuming one DH line per S_0 plant, one to

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five DH lines per S_0 plant, and one to five DH lines per S_3 plant, respectively. The corresponding values for heritability of 60% were, respectively, 0.4800, 0.4900, and 0.4567. Concerning expansion volume, the corresponding values for heritabilities of 30 and 60% were, respectively, 0.2600, 0.2833, and 0.2700, and 0.4733, 0.5100, and 0.4533. The assumed average parametric coefficient index was 0.26 and 0.48 for heritabilities of 30 and 60%, respectively, for both traits. For the population structure analysis we employed *Structure* (Falush et al. 2003) and fitted the no admixture model with independent allelic frequencies. The number of SNPs, sample size, burn-in period, and number of MCMC (Markov chain Monte Carlo) replications were 1,000 (sampled at random), 140 (70 DH lines from each population), 10,000, and 40,000, respectively. The number of populations assumed (*K*) ranged from 1 to 4, and the most probable *K* value was determined based on the inferred plateau method (Viana et al. 2013). The LD analyses were performed with *Haploview* (Barrett et al. 2005).

Data availability

REALbreeding is available upon request. The data set is available at https://doi.org/10.6084/m9.figshare.5035130.v1. Data citation:

Viana, José Marcelo Soriano; Pereira, Helcio Duarte; Mundim, Gabriel Borges; Piepho, Hans-Peter; Fonseca e Silva, Fabyano (2017): Efficiency of genomic prediction of non-assessed single crosses. figshare. https://doi.org/10.6084/m9.figshare.5035130.v1

RESULTS

The parametric mean and genotypic variance in the populations 1 and 2 were 108.5 and 87.3 (g/plant) and 4.7680 and 6.2580 $(g/plant)^2$. The DH lines derivation processes (one and one to five per S_0 plant and one to five per S_3 plant) provided, for each population, selected DH lines with similar mean (approximately 97 and 76 g/plant for populations 1 and 2), inbreeding depression (approximately −10 and −13% for populations 1 and 2), and genotypic variance (approximately 6 and 7 $(g/plant)^2$ for populations 1 and 2) and groups of single crosses also similar for mean

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353 (approximately 103 g/plant), heterosis (approximately 19%), and genotypic variance
354 (approximately 4 $(g/plant)^2$). Because we derived one to few DH lines from unrelated S_0 and S_3
355 plants, the average level of relatedness between the selected DH lines was very low (zero and zero,
356 0.0041 and 0.0041, and 0.0054 and 0.0074 assuming one DH line per S_0, one to five DH lines per
357 S_0, and one to five DH lines per S_3, for populations 1 and 2, respectively). Concerning SNP data,
358 the frequency distribution of the minor allele frequency (MAF) and the absolute value of the
359 difference between a SNP allele frequency were also similar for both groups of selected DH lines,
360 regardless of the DH line derivation process (Figure 1a, b, c). The average MAF was 0.33,
361 regardless of the population and DH line derivation process. However, the evidence obtained by the
362 population structure analysis was that the DH lines belong to two distinct subpopulations (suggested
363 *K* equal to 2.4 by the inferred plateau method). The percentages of non-polymorphic SNPs were
364 very low (0.1 to 0.4%). No differences between allelic frequencies were observed for only 1.7 to
365 2.1% of the SNPs. For approximately 70% of the SNPs, the absolute difference between allelic
366 frequencies ranged from 0.1 to 0.6. Regarding LD, for the groups of selected DH lines the evidence
367 based on the analysis of chromosome 1 (no difference between chromosomes is expected) is that
368 LD extents for up to 35 cM, regardless of the DH lines derivation process (Figure 1c, d). Ignoring
369 the non-significant LD values (LOD score lower than 3), for 17 to 20% of the SNP pairs the r^2
370 values ranged from 0.2 to 0.5 (average of 0.16, regardless of the DH lines group and derivation
371 process).

372 Assuming our model, average SNP density of 0.1 cM, training set size of 30%, positive
373 dominance (grain yield), additive-dominance model, and sampling of single crosses, the prediction
374 accuracies of the non-assessed single crosses were greater than the accuracies of the assessed single
375 crosses for low (up to 46% higher) and intermediate (up to 16% higher) heritabilities (Table 1;
376 Figure 2a). As the prediction accuracy of assessed single crosses approaches 1.0, the accuracy of the
377 non-assessed single crosses approaches approximately 0.9 (up to 11% lower). Sampling one to five

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DH lines per S_3 plant was only slightly superior to the other DH lines derivation processes, regardless of the prediction accuracy of the assessed single crosses (up to 5% higher). Fitting the additive model provided essentially the same prediction accuracies since the maximum decrease was approximately 1%. No significant differences between the prediction accuracies of non-assessed single crosses were also observed assuming bidirectional dominance (expansion volume). The differences compared to positive dominance ranged from approximately –5 to 2%. However, a striking difference was observed between the sampling processes of single crosses for testing. Random sampling of single crosses provided much greater prediction accuracies of non-assessed single crosses, compared to sampling DH lines for a diallel. The increases in the accuracies by sampling single crosses ranged from approximately 38 to 77%, proportional to the heritability. Decreasing the average SNP density to 1 cM led to a slight decrease in the prediction accuracy of non-assessed single crosses of approximately –4%). Decreasing the training set size to 10% decreased the prediction accuracy of non-assessed single crosses in approximately –5 to –15%, inversely proportional to the heritability. To evidence that the prediction accuracy of non-assessed single crosses depends on the level of (overall) LD in the groups of selected DH or inbred lines, we derived DH lines from the same base populations after 10 generations of random crosses (to decrease the LD). The accuracies were also high, ranging from 0.83 to 0.95, proportional to the heritability. The prediction accuracies of non-assessed single crosses from DH lines of the same population were equivalent to the accuracies for single crosses derived from DH lines belonging to distinct heterotic groups, ranging from 0.83 to 0.91, also proportional to the heritability. Comparing our statistical model with the models proposed by Massman et al. (2013) and Technow et al. (2012a), we observed no differences for the prediction accuracies of non-assessed single crosses (maximum difference of 1%). Finally, no significant differences between the prediction accuracies for RR-BLUP, GBLUP, and BLUP occurred (maximum of 2%), excepting for one to five DH lines per S_3 plant, where BLUP was 9 to 10% inferior, regardless of the heritability.

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Concerning the coincidence index, in general the inferences are the same established from the prediction accuracy analysis (Table 2; Figure 2b). There were no differences between the coincidence indexes regarding our model and the models proposed by Massman et al. (2013) and Technow et al. (2012a) (maximum difference of 3%), and between the RR-BLUP, GBLUP, and BLUP approaches, except for one to five DH lines per S_3 plant, where BLUP was –19 to –27% inferior, proportional to the heritability. The coincidence indexes were also high for single crosses derived from selected DH lines obtained from the base populations with lower LD (ranging from 0.55 to 0.76, proportional to the heritability) and from selected DH lines of the same population (ranging from 0.61 to 0.76, also proportional to the heritability). Sampling single crosses for assessment also provided much greater coincidence index compared to sampling DH lines for a diallel (39 to 98% higher, proportional to the heritability). Decreasing the SNP density and the training set size decreased the coincidence index from 5 to 10% (proportional to the heritability) and from 17 to 26% (inversely proportional to the heritability), respectively. The maximum difference in the coincidence index by fitting the additive-dominant and the additive models was –3%. Only for one DH line per S_0 plant the coincidence indexes assuming bidirectional dominance were slightly greater than the values assuming positive dominance (9 to 14% greater). This sampling process of DH lines provided the higher values of coincidence index, compared to the other sampling processes (7 to 26% higher, inversely proportional to the heritability). Finally, the coincidence index of the non-assessed single crosses are greater than the parametric values for all assessed single crosses assuming low (up to 117% higher) and intermediate (up to 39% higher) heritabilities (Table 1). However, as the parametric coincidence of assessed single crosses approaches 1.0, the coincidence values of the non-assessed single crosses approach approximately 0.60 to 0.74 (up to 26 to 40% lower), depending on the DH line sampling process.

DISCUSSION

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427 It was twenty-three years ago today, Bernardo (1994) taught the breeders to use BLUP (more
428 precisely, GBLUP) for predicting untested maize single cross performance. BLUP, as well known,
429 is the Henderson's (1974) approach for genetic assessment. Based on the prediction accuracies
430 obtained by Bernardo (1994, 1995, 1996a, 1996b, 1996c), for grain yield and other traits (distinct
431 genetic controls), a breeder should realize that the performance of untested single crosses can be
432 effectively predicted using relationship information from molecular or pedigree data, unbalanced
433 and large data set, and diverse heterotic patterns. This general inference has been confirmed with
434 maize (Zhao et al. 2015) and other important crops, as rice (Xu et al. 2014), wheat (Zhao et al.
435 2013b) and barley (Philipp et al. 2016), along the last 20 years. Why, then, there is no published
436 evidence that prediction of untested single crosses is of general use by breeders of worldwide seed
437 companies? What should be additionally proved to make prediction of untested single crosses as
438 successful as the Jenkins' (1934) method for predicting double crosses performance was? We
439 believe that this paper offers a significant contribution.

440 Our assessment on efficiency of prediction of untested single cross performance keeps some
441 similarities with few earlier studies but sharp differences for most previous investigations. This
442 study is based on simulated data set, as the study of Technow et al. (2012a), assuming 400 QTLs
443 distributed along ten chromosomes. Thus, the prediction accuracies and coincidence indexes (a
444 measure of untested single crosses selection efficiency) are for really non-assessed single crosses
445 since the values were computed based on the true genotypic values of the non-assessed single
446 crosses and not on a cross-validation procedure involving assessed single crosses. This does not
447 mean that we consider simulated data better than field data or have any criticism on the cross-
448 validation procedure. We know that simulated data, because the presuppositions, cannot integrally
449 describe the complexity of populations and genetic determination of traits (Daetwyler et al. 2013).
450 To highlight the relevance of (overall) LD, our study is based on scenarios not favorable to
451 prediction of untested single cross performance: very low level of relationship between the DH

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452 lines, low and intermediate heritabilities for the assessed single crosses, and not higher heterotic
453 pattern. In the studies of Massman et al. (2013) and Bernardo (1994, 1995, 1996a) the relationship
454 among inbreds from the same heterotic group ranged from 0.11 to 0.58. Riedelsheimer et al. (2012)
455 observed high relationship only between the non-Stiff Stalk inbreds. Technow et al. (2012a)
456 assumed non-related inbreds. For most of the investigations on prediction of untested single crosses
457 and testcrosses, the grain yield heritability ranged from 0.72 to 0.88. The common heterotic patterns
458 in these previous studies are Stiff Stalk and non-Stiff Stalk, and Dent and Flint. The MAF in the
459 groups of Dent and Flint inbreds were approximately 0.10 and 0.20, respectively, and
460 approximately 20% of the SNPs showed a difference of allelic frequency of at least 0.6.

461 Concerning the prediction accuracy and the efficiency of identification of the superior 300
462 non-assessed single crosses, our results prove that prediction of untested single crosses is a very
463 efficient procedure (note that we are not saying genomic prediction), specially for low and
464 intermediate heritabilities of the assessed single crosses. The prediction accuracy of the non-
465 assessed single crosses under low (0.55 to 0.71) and intermediate (0.74 to 0.87) accuracies of
466 assessed single crosses achieved 0.85 and 0.89, respectively. It is important to highlight that these
467 are not relative accuracies. Most important, the coincidence of the non-assessed single crosses
468 under low (0.26 to 0.39) and intermediate (0.44 to 0.66) parametric coincidences of assessed single
469 crosses achieved 0.59 and 0.64, respectively. For high heritability (80 to 95%; accuracies from 0.89
470 to 0.97), as observed in most of the studies on prediction of untested single cross performance, we
471 can state (based on values predicted by fitting a quadratic regression model) that the prediction
472 accuracy of non-assessed single crosses is up to only 10% lower (0.87 to 0.92) and, most
473 impressive, the coincidence index can range from 0.61 to 0.71 (parametric coincidences between
474 0.72 to 0.93). Under maximum accuracy of assessed single crosses (1.0), the prediction accuracy
475 and coincidence of non-assessed single crosses achieved 0.93 and 0.76. Thus, assuming high
476 heritability, high density, and training set size of 30%, the accuracy can achieve 0.92 and the

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efficiency of identification of the best 9% of the non-assessed single crosses can achieve 0.71. It is important to highlight that this efficacy can be higher by using more related DH or inbred lines, under high LD. Thus, we strong recommend that maize breeders, as well as rice, wheat, and barley breeders, make widespread use of prediction of non-assessed single crosses, at least for preliminary screening or prior to field testing.

To take advantage of genomic prediction, Kadam et al. (2016) recommend redesigning hybrid breeding programs. However, because breeders are unlikely to rely solely on genomic predictions when selecting superior untested hybrids, Technow et al. (2014) believe that genomic prediction will be combined with field testing of the most promising experimental hybrids. For grain yield, the prediction accuracies observed by Bernardo (1994, 1995, 1996a) ranged from 0.14 to 0.80, proportional to the heritability (in the range 35-74%) and training set size. The non-relative accuracies (relative accuracy x root square of heritability) observed in the studies of Kadam et al. (2016), Technow et al. (2014), Massman et al. (2013), Technow et al. (2012a), and Riedelsheimer et al. (2012) ranged between 0.20 and 0.86, also proportional to the heritability (in the range 53-98%) and training set size.

We hope that readers of this paper have realized the importance of (overall) LD for effective prediction of non-assessed single crosses, as well as genetic variability (see the parametric accuracy of genomic prediction). Breeders have no control over LD and relatedness between the DH or inbred lines. However, selection should always provide high level of overall LD in the groups of selected DH or inbred lines. Comparison of our LD assessment with the LD analyses from other studies is inadequate because we have distances in cM and not in base-pairs. But in general the level of LD was high (r^2 of approximately 0.3) only for SNPs separated by up to 0.5 Mb (Technow et al. 2014; Massman et al. 2013; Technow et al. 2012a; Riedelsheimer et al. 2012). To maximize the prediction accuracy and the efficiency of identification of the best non-assessed single crosses it is necessary to adopt the random sampling of single crosses for testing instead of the random sampling

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of DH or inbred lines for a diallel. This is because sampling 30 or even 10% of the single crosses leads to single crosses for testing derived from all DH or inbred lines from each group. In our case, in every resampling assuming training set size of 30 and 10% we always get groups of assessed single crosses (1,470 and 490 single crosses, respectively) derived from the 70 DH lines of each group. However, sampling DH lines for a diallel provided 1,440 and 484 single crosses for testing derived from 38 and 22 DH lines, respectively. Thus, the sampling of single crosses provides best prediction of the SNP average effects of substitution. Riedelsheimer et al. (2012) emphasized the need for large genetic variability to obtain high prediction accuracies. Further, their results indicated that pairs of closely related lines and population structuring only weakly contributed to the high prediction accuracies. Regarding dominance, because it can be a relevant genetic effect, breeders should always fit the additive-dominance model to maximize the prediction accuracy and the efficiency of identification of the best non-assessed single crosses. Interestingly, in most of the studies on prediction of non-assessed single crosses the prediction accuracy did not significantly increase when modeling SCA in addition to GCA effects (Zhao et al. 2015).

Concerning SNP density and training set size, factors related with the costs of genotyping and phenotyping, breeders should find a balance between efficiency and expenses, since maximizing SNP density and training set size maximizes the efficiency of untested single cross prediction. Based on our results, because the decreases in the prediction accuracy (approximately 4%) and coincidence index (5 to 10%) by decreasing the average SNP density from 0.1 to 1 cM are of reduced magnitude, we consider sufficient to employ custom genotyping to provide an average SNP density of 1 cM. Decreasing the training set size from 30 to 10% of the single crosses does not significantly affect the prediction accuracy under intermediate to high heritability (decrease of up to 9%), but the coincidence index can be reduced in up to 21%. However, considering that the coincidence index will be kept in the range 0.48 to 0.61, proportional to the heritability, and that the maximum values are in the range 0.48 to 0.61, we also consider sufficient to assess at least 10% of

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the possible single crosses. As highlighted by Zhao et al. (2015), marker density only marginally affects the prediction accuracy of untested single crosses. For biparental populations, a plateau for the accuracy is reached with a few hundred markers. Technow et al. (2014) did not find an improvement of prediction accuracies by using higher SNP density. Additionally, the increase in the training set size led to a relative small increase in the prediction accuracy. However, the prediction accuracies obtained by Riedelsheimer et al. (2012) under high density (38,019 SNPs) were substantially greater than those reached with a low-density marker panel (1,152 SNPs). In the study of Technow et al. (2012a), the prediction accuracies increased with SNP density and number of parents tested in hybrid combination.

The DH lines sampling process, the heterotic pattern, and the statistical approach should not be worries for breeders. However, under high heritability notice that sampling more than one DH line per S_0 or S_3 plant provided the higher coincidence values and high prediction accuracy in our study. For rice, wheat, and barley breeders our message is: high prediction accuracy and high efficiency of identification of superior non-assessed single crosses does not depend on heterotic groups but on the (overall) LD in the group or in each group of DH or inbred lines. In other words, the efficiency of prediction of non-assessed single crosses derived from DH or inbred lines from the same population can be as high as the efficiency of prediction of untested single crosses derived from DH or inbred lines from distinct heterotic groups. This is not confirmed comparing the relative prediction accuracies for grain yield of maize untested single crosses (from approximately 0.50 to 0.95, for most studies) with those obtained with rice, wheat, and barley untested hybrids (0.50 to 0.60, approximately) (Philipp et al. 2016; Xu et al. 2014; Zhao et al. 2013b). However, the lower relative prediction accuracies for untested rice, wheat, and barley hybrids should be due to prediction of two- and three-way crosses. Regarding the statistical approach, our model did not provide an increase in the efficiency of non-assessed single cross prediction, compared to the models proposed by Massman et al. (2013) and Technow et al. (2012a). It is important to highlight

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552 that our results showed that these two models are really identical (data no shown). Thus, because
553 the simplified definition of the incidence matrices for these two previous models, it is quite safe to
554 use any of them. Finally, the choice between the statistical approaches RR-BLUP (prediction of
555 genotypic values of non-assessed single crosses based on prediction of SNP average effects of
556 substitution), GBLUP (prediction of genotypic values of non-assessed single crosses based on
557 additive and dominance genomic matrices), and BLUP (prediction of genotypic values of non-
558 assessed single crosses based on additive and dominance matrices from pedigree records) is not a
559 serious worry for breeders too. Our evidence is that there is no significant difference between RR-
560 BLUP and GBLUP regarding prediction accuracy and efficiency of identification of the best
561 untested single crosses. Further, even when the level of relatedness between the DH or inbred lines
562 in each group is low, in general BLUP is as efficient as genomic prediction, excepting when the DH
563 lines are derived from inbred population. Thus, DNA polymorphism is not essential for an efficient
564 prediction of non-assessed single cross performance. In his review on genomic selection in hybrid
565 breeding, Zhao et al. (2015) state that the choice of the biometrical model has no substantial impact
566 on the prediction accuracy of untested single crosses. Technow et al. (2014) observed that
567 prediction methods GBLUP and BayesB resulted in very similar prediction accuracies. In the study
568 of Massman et al. (2013), BLUP and RR-BLUP models did not lead to prediction accuracies that
569 differed significantly. Comparing GBLUP and BayesB, Technow et al. (2012a) concluded that the
570 latter method produced significantly higher accuracies for the additive-dominance model.

571 Our main contributions on the prediction efficiency of non-assessed single cross performance
572 are: 1) the prediction accuracy of untested single crosses ranged from approximately 0.80 to 0.90 as
573 the heritability of tested single crosses ranged from low (30%) to high (100%); however, the
574 efficacy of identification of the best 9% of the untested single crosses ranged from approximately
575 0.50 to 0.70, depending on the DH lines sampling process; 2) the prediction accuracy for crops
576 showing no defined heterotic pattern can be as efficient as with maize, for which there is well

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577 defined heterotic groups; this is because the most important factor affecting the prediction
578 efficiency is the overall LD; 3) to maximize prediction accuracy and coincidence the choice of
579 single crosses for testing should be based on a random process; this procedure maximizes the
580 number of DH lines in hybrid combination and provides better predictions of the SNP average
581 effects of substitution and dominance deviations; 4) because non significant decreases in the
582 prediction accuracy and coincidence, the prediction of untested single crosses can be efficient
583 assuming reduced training set size (10%) and SNP density of 1 cM; 5) RR-BLUP and GBLUP
584 provides equivalent prediction efficiencies of untested single crosses; 6) excepting for DH lines
585 derived from inbred populations, BLUP is as efficient as genomic prediction of untested single
586 crosses; and 7) the theoretical accuracy shows that the prediction accuracy is not affected by the
587 linkage phase.

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666 **Table 1** Average prediction accuracies of non-assessed single crosses and its standard deviation,
667 assuming single crosses from selected DH lines, 30 and 10% of assessed single crosses, two traits
668 (grain yield - GY, g/plant, and expansion volume - EV, mL/g), two sampling processes of single
669 crosses, four statistical models, three DH lines sampling processes, two genetic models, and three
670 accuracies of assessed single crosses

Trait	Samp. proc.	Statistical model	DH lines	Gen. mod.	Accuracy of assessed single crosses		
					0.55	0.77	1.00
GY	SCs	Viana et al.	$1/S_0$	AD	0.7790 ± 0.0124	0.8447 ± 0.0066	0.8859 ± 0.0018
				A	0.7688 ± 0.0132	0.8380 ± 0.0067	0.8821 ± 0.0019
			$1\text{-}5/S_0$	AD	0.7947 ± 0.0125	0.8525 ± 0.0072	0.8896 ± 0.0025
				A	0.7895 ± 0.0126	0.8465 ± 0.0077	0.8858 ± 0.0027
			$1\text{-}5/S_3$	AD	0.8010 ± 0.0145	0.8678 ± 0.0054	0.9276 ± 0.0025
				A	0.7954 ± 0.0145	0.8627 ± 0.0056	0.9238 ± 0.0026
			$1\text{-}5/S_3$	AD[a]	0.7718 ± 0.0161	0.8371 ± 0.0079	0.8888 ± 0.0043
			$1\text{-}5/S_3$	AD[b]	0.6836 ± 0.0277	0.7885 ± 0.0139	0.8817 ± 0.0049
			$1/S_0$	AD[c]	0.8293 ± 0.0131	0.8944 ± 0.0049	0.9479 ± 0.0017
			$1\text{-}5/S_3$	AD[d]	0.8267 ± 0.0082	0.8928 ± 0.0043	0.9083 ± 0.0023
		Massman et. al.[e]	$1/S_0$	AD	0.7874 ± 0.0118	0.8519 ± 0.0053	0.8924 ± 0.0026
			$1\text{-}5/S_0$	AD	0.7982 ± 0.0140	0.8622 ± 0.0055	0.8973 ± 0.0025
			$1\text{-}5/S_3$	AD	0.8074 ± 0.0112	0.8753 ± 0.0056	0.9314 ± 0.0026
		GBLUP	$1/S_0$	AD	0.7841 ± 0.0122	0.8477 ± 0.0064	0.8906 ± 0.0019
			$1\text{-}5/S_0$	AD	0.7973 ± 0.0124	0.8574 ± 0.0070	0.8978 ± 0.0019
			$1\text{-}5/S_3$	AD	0.7911 ± 0.0146	0.8639 ± 0.0056	0.9319 ± 0.0023
		BLUP	$1/S_0$	AD	0.7855 ± 0.0129	0.8541 ± 0.0059	0.8899 ± 0.0019
			$1\text{-}5/S_0$	AD	0.7803 ± 0.0143	0.8435 ± 0.0074	0.8830 ± 0.0024
			$1\text{-}5/S_3$	AD	0.7227 ± 0.0203	0.7915 ± 0.0077	0.8373 ± 0.0048
	DHs	Viana et al.	$1/S_0$	AD	0.5012 ± 0.0416	0.5117 ± 0.0467	0.5343 ± 0.0467
			$1\text{-}5/S_0$	AD	0.4827 ± 0.0423	0.5000 ± 0.0420	0.5036 ± 0.0465
			$1\text{-}5/S_3$	AD	0.5799 ± 0.0437	0.6106 ± 0.0413	0.6357 ± 0.0429
EV	SCs	Viana et al.	$1/S_0$	AD	0.7779 ± 0.0157	0.8458 ± 0.0069	0.8820 ± 0.0024
			$1\text{-}5/S_0$	AD	0.8019 ± 0.0155	0.8656 ± 0.0050	0.9055 ± 0.0020
			$1\text{-}5/S_3$	AD	0.7589 ± 0.0143	0.8424 ± 0.0058	0.9165 ± 0.0027

[a]density of 1 cM; [b]training set of 490 single crosses (10%); [c]after 10 generations of random crosses;[d]single crosses from DH lines of the same population; [e]and Technow et al..

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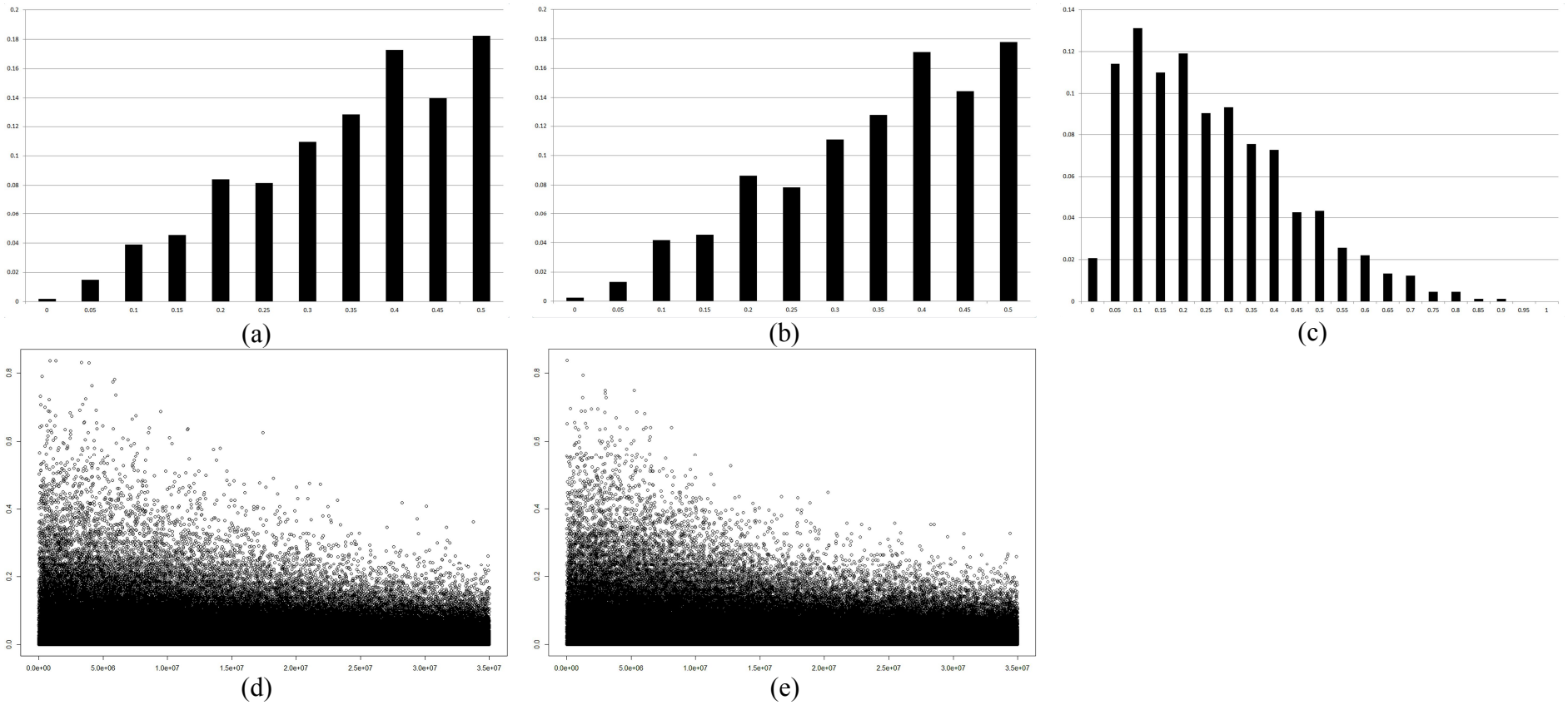
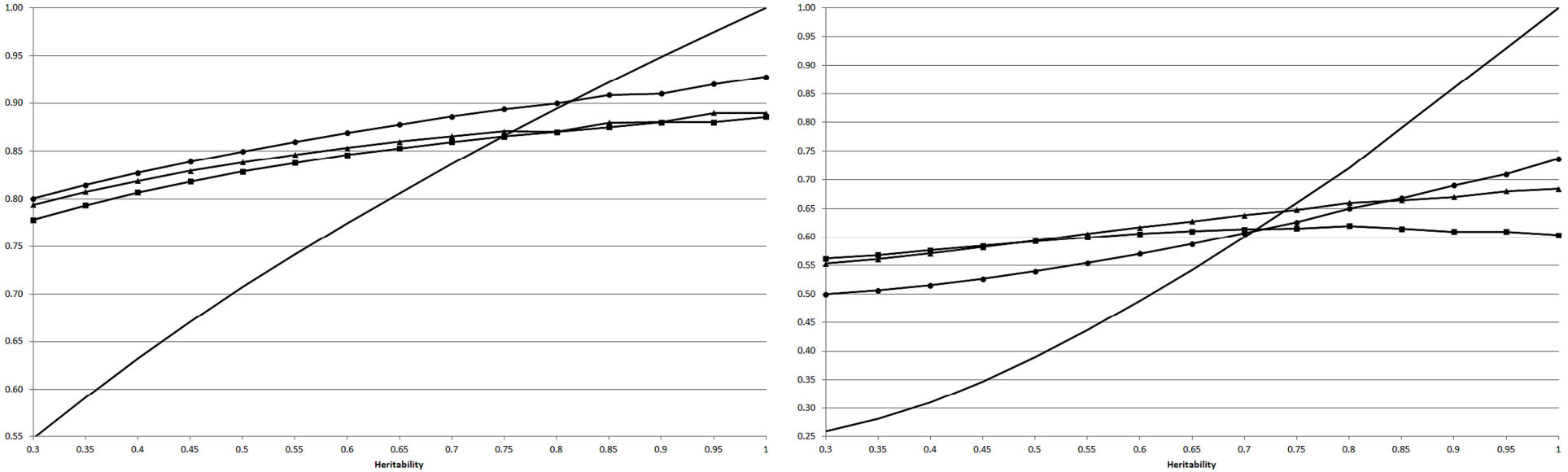
671 **Table 2** Average coincidence of the best 300 predicted single crosses and its standard deviation,
672 assuming single crosses from selected DH lines, 30 and 10% of assessed single crosses, two traits
673 (grain yield - GY, g/plant, and expansion volume - EV, mL/g), two sampling processes of single
674 crosses, four statistical models, three DH lines sampling processes, two genetic models, and three
675 parametric coincidence of assessed single crosses

Trait	Samp. proc.	Statistical model	DH lines	Gen. mod.	Coincidence of assessed single crosses		
					0.26	0.48	1.00
GY	SCs	Viana et al.	$1/S_0$	AD	0.4523 ± 0.0334	0.5525 ± 0.0190	0.6037 ± 0.0170
				A	0.4396 ± 0.0346	0.5449 ± 0.0176	0.5976 ± 0.0172
			$1\text{-}5/S_0$	AD	0.5686 ± 0.0273	0.6369 ± 0.0221	0.6842 ± 0.0140
				A	0.5640 ± 0.0283	0.6299 ± 0.0221	0.6816 ± 0.0152
			$1\text{-}5/S_3$	AD	0.5129 ± 0.0235	0.6044 ± 0.0200	0.7363 ± 0.0183
				A	0.5063 ± 0.0225	0.5993 ± 0.0193	0.7305 ± 0.0190
			$1\text{-}5/S_3$	AD[a]	0.4881 ± 0.0278	0.5691 ± 0.0229	0.6620 ± 0.0215
			$1\text{-}5/S_3$	AD[b]	0.3805 ± 0.0511	0.4797 ± 0.0354	0.6087 ± 0.0233
			$1/S_0$	AD[c]	0.5528 ± 0.0298	0.6489 ± 0.0203	0.7571 ± 0.0162
			$1\text{-}5/S_3$	AD[d]	0.6116 ± 0.0214	0.7156 ± 0.0150	0.7581 ± 0.0166
		Massman et. al.[e]	$1/S_0$	AD	0.4670 ± 0.0346	0.5663 ± 0.0174	0.6157 ± 0.0157
			$1\text{-}5/S_0$	AD	0.5651 ± 0.0310	0.6431 ± 0.0164	0.6955 ± 0.0144
			$1\text{-}5/S_3$	AD	0.5279 ± 0.0291	0.6139 ± 0.0204	0.7423 ± 0.0172
		GBLUP	$1/S_0$	AD	0.4622 ± 0.0308	0.5660 ± 0.0190	0.6092 ± 0.0163
			$1\text{-}5/S_0$	AD	0.5650 ± 0.0280	0.6384 ± 0.0204	0.6849 ± 0.0137
			$1\text{-}5/S_3$	AD	0.5010 ± 0.0245	0.5937 ± 0.0216	0.7294 ± 0.0168
		BLUP	$1/S_0$	AD	0.4641 ± 0.0331	0.5709 ± 0.0176	0.6081 ± 0.0127
			$1\text{-}5/S_0$	AD	0.5531 ± 0.0323	0.6272 ± 0.0194	0.6699 ± 0.0130
			$1\text{-}5/S_3$	AD	0.4172 ± 0.0258	0.4731 ± 0.0211	0.5377 ± 0.0196
	DHs	Viana et al.	$1/S_0$	AD	0.2753 ± 0.0374	0.3056 ± 0.0445	0.3169 ± 0.0401
			$1\text{-}5/S_0$	AD	0.3268 ± 0.0642	0.3400 ± 0.0691	0.3461 ± 0.0728
			$1\text{-}5/S_3$	AD	0.3699 ± 0.0583	0.3931 ± 0.0579	0.4300 ± 0.0633
EV	SCs	Viana et al.	$1/S_0$	AD	0.5156 ± 0.0331	0.6081 ± 0.0159	0.6599 ± 0.0146
			$1\text{-}5/S_0$	AD	0.5506 ± 0.0285	0.6337 ± 0.0203	0.6944 ± 0.0141
			$1\text{-}5/S_3$	AD	0.4746 ± 0.0294	0.5843 ± 0.0174	0.7141 ± 0.0171

[a]density of 1 cM; [b]training set of 490 single crosses (10%); [c]after 10 generations of random crosses;[d]single crosses from DH lines of the same population; [e]and Technow et al..



676 **Figure 1** Frequency distribution of the MAF in the groups of selected DH lines (a and b) and the absolute value of the difference between a SNP allele
677 frequency (c), and LD (r^2) in relation to distance (cM) in the two groups of selected DH lines (d and e), regarding SNPs in chromosome 1 separated by
678 zero to 35 cM, assuming one DH line per S_0 plant.



679 **Figure 2** Predicted accuracies (a) and coincidence indexes (b) for untested single crosses (square: $1/S_0$; triangle: $1\text{-}5/S_0$; circle: $1\text{-}5/S_3$), and parametric
680 accuracies and coincidence indexes for tested single crosses (continuous line), assuming our model, average SNP density of 0.1 cM, training set size of
681 30%, positive dominance (grain yield), additive-dominance model, and sampling of single crosses.