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Use of genomic and phenotypic selection in lines for prediction of testcrosses in maize II: grain yield and plant traits

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Abstract Plant breeders have been trying to predict the performance of hybrids based on their parental performance. One application of molecular markers is its use in selection. The objectives were to map quantitative trait loci (QTL) and verify its congruence in maize lines and in their testcrosses and verify the possibility to select testcrosses from the predicted means of the lines by using information from markers. Two-hundred and fifty six lines and the testcrosses of these lines with two testers were evaluated in six environments, considering grain yield, plant lodging, days to anthesis and silking, anthesis-silking interval, plant and ear height and ear placement. QTL were mapped in the lines and in testcrosses and the predicted means of the lines were computed based on QTL effects and in all markers of the genome. The congruence of QTL detected in the lines and testcrosses were small for all traits. The correlations between the predicted means of the lines and the phenotypic means of the testcrosses ranged from low for grain yield to moderate for cycle and stature traits. The highest coincidences of the lines and selected testcrosses were observed for cycle and stature traits and the lowest for grain yield. Even by using molecular markers information, it is only possible to predict the testcrosses performance from the lines information to less complex traits and with reduced dominance effect. For complex traits and with pronounced dominance effect, information of markers must be obtained directly in the testcrosses, so they can be used for selection.

Keywords Correlation · Endogamy · Hybrids · QTL · Marker assisted selection · Tropical maize

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Introduction

Since the conception of the idea to use hybrids obtained through the crossing of endogamic lines in the breeding of maize (East 1908; Shull 1910; Jones 1918), this technique has been widely applied in breeding programs. With the discovery of the advantages afforded by the use of hybrid maize, innumerable studies have been and continue to be developed in attempts to find an effective way to predict the performance of a hybrid from the performance of its

parental lines. Successful prediction will make the process of obtaining and, principally, of selecting lines much faster and cheaper (Mihaljevic et al. 2005). This has been the motivation for many studies seeking relations between lines and hybrids, for the most diverse range of traits, the results of these studies have proven to be very variable (Hallauer and Miranda Filho 1988).

Quantitative trait loci (QTL) mapping in lines and in their hybrids can provide useful information through which to gain a better understanding at the molecular level of the quantitative aspects of the inheritance of traits, as well as the correlation between trait expression in lines and in the hybrids produced from these lines. This body of information could also assist in the choice of parental lines to be used in the creation of hybrids, through molecular marker assisted selection. This assisted selection could significantly decrease the number of crosses to be evaluated and the time required to obtain superior hybrids, making the hybrid development process faster and cheaper (Stuber and Sisco 1992; Berke and Rocheford 1995; Hospital et al. 1997; Bouchez et al. 2002; Môro et al. 2012).

With the development of highly abundant molecular markers in the genome and costs that continue to decrease, an alternative approach for the use of molecular markers in assisted selection, known as genome-wide selection or genomic selection (GS), has been proposed by Meuwissen et al. (2001). Some results have shown that this strategy is efficient, principally in animal breeding (Kolbehdari et al. 2007; Goddard and Hayes 2007; Long et al. 2007; Legarra and Misztal 2008). Despite this, genomic selection has been little utilized in plant breeding, with most of the studies evaluating genomic selection through simulations (Bernardo and Yu 2007; Bernardo 2008, 2009; Liu et al. 2008; Heffner et al. 2009; Massman et al. 2013; Jacobson et al. 2014) but, in maize, some results have shown promising accuracy estimates using GS (Mendes and Souza Júnior 2016).

Some studies have been performed with the objective of looking for congruence between the mapped QTL in lines and in hybrids and/or testcrosses obtained from the lines, focussing on different traits and types of population (Beavis et al. 1994; Groh et al. 1998; Austin et al. 2000; Mihaljevic et al. 2005; Peng et al. 2013). In general, these studies have found few coincident QTL for any of the traits investigated.

However, for the majority of these studies the parental generations and the crosses were evaluated in different environments, typically the generations were evaluated in the same area but in different years. It is then possible that the low QTL congruence may have occurred as a consequence of high QTL × environment interactions.

A comparison of QTL coincidences between lines and their testcrosses, when the two generations are evaluated in the same environment, eliminates QTL × environment interactions from the analyses, allowing a more precise examination of QTL congruence between the two generations, lines and their testcrosses. Thus, the objectives of this study were to map the QTL for several traits in S_1 lines of maize and in testcrosses of these lines with two testers, and to test the congruence of the QTL of the chosen traits. Further objectives were to examine the use of information derived from the S_1 lines to predict the behaviour of the testcrosses and to select the superior testcrosses. Trait prediction was pursued by examining the correlations for the chosen traits between the phenotypic means of the testcrosses and each of the following means for the S_1 lines: phenotypic mean, mean predicted using the QTL map of the S_1 lines, and mean predicted from all the molecular markers of the S_1 lines. The effects of information source on superior testcross selection were investigated by identifying coincidences between superior testcrosses selected according to testcross phenotypic means and S_1 lines selected on the basis of three sources of S_1 information: phenotypic mean, QTL map, and all the molecular markers.

Materials and methods

Genetic material

Parental inbred lines L-08-05F and L-14-04B were used to develop a reference F_2 population. Both inbreds were developed by the Maize Breeding Program of the Department of Genetics, at the "Luiz de Queiroz" School of Agriculture, University of São Paulo, Brazil (ESALQ/USP). Inbred L-08-05F, with orange flint kernels, was developed from the population IG-1, and inbred L-14-04B, with yellow dent kernels, was developed from the population BR-106. These populations and their respective inbreds are in

different heterotic groups and they are genetically divergent for several traits, and both populations were derived from tropical germplasm (Sibov et al. 2003; Sabadin et al. 2008; Moreira et al. 2009; Môro et al. 2012). The parental inbreds were crossed and three F_1 plants, previously tested against the parental inbreds to check their genetic identity with microsatellite markers, were selfed to develop the F_2 population. Two-hundred and fifty-six plants were randomly taken and selfed to develop $F_{2:3}$ progenies, which corresponded to the S_1 lines used in this work. These were crossed with two tester lines L-04-05F and L-02-03D, also belonging to the Maize Breeding Program of the Department of Genetics of ESALQ/USP. Inbred line L-04-05F was derived from the IG-1 population, while inbred line L-02-03D was obtained from the IG-2 population. These tester lines belong to different heterotic groups, and are divergent for various traits. In order to obtain the testcrosses, the S_1 lines were used as the female parent (through detasseling) and crossed with each tester line in isolation plots, thereby producing two testcrosses for each of the S_1 lines, totalling 256 testcrosses with L-04-05F (TC1) and 256 testcrosses with L-02-03D (TC2). This study thus used 256 S_1 lines, together with their respective testcrosses.

Experimental procedure

The evaluation of the S_1 lines and the testcrosses was performed in six environments, each combination of location and year being considered as a distinct environment. The 256 lines and their respective testcrosses were evaluated on two experimental stations, Department of Genetics (ESALQ/USP) and Anhembi. The experiments were conducted over three farming years, with two replications per environment; the trials with the S_1 lines and the testcrosses were set up in adjacent areas and were, therefore, in the same environment. For both the trial with the S_1 lines and in the two trials with the testcrosses, the experimental design adopted was a simple 16×16 lattice. Each plot comprised a row of length 4 m sewed with 50 seeds, after thinning 20 plants remained in each plot. The final spacing between the plants was 0.20 m, while the spacing between the rows was 0.80 m, corresponding to a population of approximately 62,500 plants ha^{-1}. Evaluations were made of the following traits: grain yield (GY, in t ha^{-1}) adjusted to a moisture content of 15% and corrected to a mean

stand through analysis of the covariance; plant height and ear height (PH and EH, respectively, in cm), obtained as means over five competitive selected plants of the plot; ear placement (EP = EH/PH); days to anthesis and days to silking (DA and DS, respectively, in days), obtained as the number of days after planting when 50% of the plants in a plot were in anthesis (DA) and when 50% showed silking (DS); anthesis-silking interval (ASI = DS−DA); and plant lodging (PL, as % of plants in the plot), obtained from the number of plants found to have been flattened and broken in the plot at the time of harvesting, corrected to a mean stand through analysis of the covariance.

Analyses of variance and covariance

All of the analyses of variance and covariance were performed using the PROC GLM procedures of the SAS software package (SAS Institute Inc. 2001). For each environment conducted with the lines and testcrosses individual analyses of variance were performed, according to the mathematical model for lattice-type experiments (Cochran and Cox 1966). Starting from the adjusted means obtained from these individual analyses, analyses of combined variance of the environment were performed, using a random model. For the testcrosses, in addition to the combined analyses, analyses of grouped combined variance were performed, in which the trials conducted with the two testcrosses were grouped together. Analyses of covariance between the S_1 lines and the testcrosses of each tester were also conducted, using the same procedures as for the analyses of variance, obtained the coefficients of genetic and phenotypic correlation of the evaluated traits in the lines and in the testcrosses.

Genetic map

The genetic map used, and the procedures used to develop it, was previously described by Sibov et al. (2003) and Môro et al. (2012). The F_2 plants that gave rise to the $F_{2:3}$ progenies (S_1 lines) were genotyped with microsatellite markers and the genetic map was developed using MAPMAKER/EXP version 3.0b (Lincoln et al. 1992). The map present a total of 177 markers distributed along the 10 linkage groups. The genetic map spanned 2052 cm in length with an average interval of 11.60 cm between adjacent markers.

QTL mapping

The program QTL Cartographer version 1.17 (Basten et al. 2003) was used to perform the QTL mapping, "*window size*" was set to 10 cM and "*walking speed*" to 1 cM, and module *Jzmapqtl* was selected. *Jzmapqtl* implements composite interval mapping (CIM) (Zeng 1994) expanded to analysis across multiple environments (Jiang and Zeng 1995). For the mapping in the S_1 lines the model used was $y_{jm} = b_{0m} + b_m^* x_j^* + d_m^* z_j^* + \sum_l^t (b_{lm} x_{jl} + d_{lm} z_{jl}) + e_{jm}$ and, in testcrosses, the model was similar but the dominance effects are absent, were y_{jm} is the phenotypic value of the j-th genotype evaluated in the m-th environment; b_{0m} is the mean effect for environment m; b_m^* corresponds the additive effect of the probable QTL applicable to environment m for the lines and the allelic substitution effect of the probable QTL applicable to environment m for the testcrosses; x_j^* is the identifying variable of the genotype of the probable QTL which takes the values 0, 1 and 2 for the genotypes *qq*, *Qq* and *QQ*, respectively, according to probabilities that depend on the recombination fraction between marker i and the QTL, conditional on the genotypes of the flanking markers i and $i + 1$; d_m^* is the dominance effect of the probable QTL applicable to environment m for the lines; z_j^* is the identifying variable of the genotype of the probable QTL, which assumes values of 0 for the homozygote genotypes (*qq* or *QQ*) and 1 of a heterozygote genotype (*Qq*), according to probabilities that depend on the recombination fraction between marker i and the QTL, conditional on the genotypes of the flanking markers i and $i + 1$; b_{lm} is the partial regression coefficient between the phenotypic values and the values attributed to x_{jl}; x_{jl} are the identifying variables associated with cofactor l and t is the number of markers selected as cofactors; d_{lm} is the partial regression coefficient between the phenotypic values and the values attributed to z_{jl} for the lines; z_{jl} are the identifying variables associated with cofactor l and t is the number of markers selected as cofactors for the lines; and e_{jm} is the residual effect associated with the j-th genotype in the m-th environment.

The cofactors considered in the analyses were selected per environment, using a "*stepwise*" (forward/backward) regression procedure, with $\alpha = 0.05$ as the critical value for the inclusion or exclusion of a marker in the model. After this first round of cofactor selection, a second selection was applied to the selected cofactors, to leave at most the five most informative cofactors for each environment. This was performed to prevent overparameterization of the model, which can introduce biases in the estimates obtained (Basten et al. 2003).

The critical values for the tests to establish the presence of a QTL and a QTL × environment interaction was given by the number of independent tests, according Vieira et al. (2000). For the present study, these critical values were 25.3 and 21.4, which correspond to LOD scores of 5.5 and 4.7, respectively, larger than those used in most studies in maize (Mihaljevic et al. 2005; Lima et al. 2006).

Congruence of mapped QTL in lines and testcrosses

In order to identify positional congruence between the QTL detected in the S_1 lines and those in their testcrosses, estimates were made of the confidence intervals for each mapped QTL, expressed as a "*one-LOD support interval*" (Lander and Botstein 1989). The confidence intervals of the detected QTL in congruent regions in the lines and in the testcrosses were compared, when overlap of these intervals occurred it was considered probable that a common QTL had been identified.

Prediction of line means

Means were predicted for the S_1 lines on the basis of the effects of the QTL. Neglecting epistatic effects between the QTL, these means ($\bar{Y}_{QS_1}$) were obtained from the equation $\hat{y} = \hat{\mu}\mathbf{1} + \mathbf{X}\hat{\beta}$, in which $\hat{y}$ is the vector holding the predicted means for the S_1 lines; $\hat{\mu}$ is the overall mean of the S_1 lines; $\mathbf{1}$ is a vector of ones; $\mathbf{X}$ is the matrix of the genetic predictors of the additive and dominance effects of the mapped QTL, with dimensions $N_{S1} \times (2 \times N_{QTL})$, where $N_{S1} = 256$ and N_{QTL} is the number of mapped QTL; and $\hat{\beta}$ is the vector of the genetic values of the mapped QTL, that is, the additive and dominance effects of the mapped QTL. The predictors of the additive effects (a) were obtained from the difference the conditional probabilities of the QTL would present for the genotypes QQ and qq, given the genotypes of the flanking markers of this QTL, while the predictors of

the dominance effects (d) were obtained from the conditional probabilities the QTL would display for the genotype Qq, given the genotypes of the flanking markers, that is Predictor (a) = P(QQ|Mi_Mj_) − P(qq|Mi_Mj_) and Predictor (d) = P(Qq|Mi_Mj_). The probabilities were obtained using the R/QTL package of the software R.

The genotypic values (best linear unbiased predictor—BLUPs) of the molecular markers were obtained using the mixed model $\mathbf{y} = \boldsymbol{\mu}\mathbf{1} + \mathbf{Xg} + \mathbf{e}$, in which: $\mathbf{y}$ is the vector of dimension $N_{S_1} \times 1$ ($N_{S_1} = 256$) holding the phenotypic means of the S_1 lines; $\boldsymbol{\mu}$ is the overall phenotypic mean of the S_1 lines; $\mathbf{1}$ is the vector of ones of dimension $N_{S_1} \times 1$ which relates the mean to the vector $\mathbf{y}$; $\mathbf{X}$ is the matrix of dimensions $N_{S_1} \times N_m$ ($N_m = 177$), whose elements are defined as follows: 1 if the S_1 plant is homozygote for the marker originated from the L14-04B line, −1 if homozygote for the L08-05F originated marker, and 0 if heterozygote; $\mathbf{g}$ is the $N_M \times 1$ vector of the BLUPs of the genetic values of the markers (GVM/BLUP), treated as random effects; and $\mathbf{e}$ is the $N_M \times 1$ vector of the residuals.

The genetic values of the markers were estimated using a mixed-models methodology (Henderson 1984). The BLUPs of the genetic values were obtained, assuming that the variance from each marker is equal to σ_G^2/N_m, where σ_G^2 is the genetic variance of the S_1 lines and N_m the number of markers (Meuwissen et al. 2001). Starting from the genotypes of the markers of the S_1 lines and the estimates of their genetic values, predictions were obtained of the means for the lines based upon all the markers of the genome ($\bar{Y}_{GS_1}$), through the equation $\hat{\mathbf{y}} = \hat{\mu}\mathbf{1} + \mathbf{X}\hat{\mathbf{g}}$. In this equation, the parameters correspond to those previously described, and have the same dimensions, but with the following differences: the vector $\hat{\mathbf{y}}$ holds the estimates of the predicted means of the S_1 lines; $\hat{\mu}$ is the estimate of the overall mean of the S_1 lines; and $\hat{\mathbf{g}}$ is the vector holding the estimates of the BLUPs of the genetic values of the markers.

Correlations and coincidences for selected superior testcrosses

Correlation coefficients between the phenotypic and predicted means of the lines and the phenotypic means of the testcrosses for the two testers were estimated.

Lists of selected genotypes were then generated for the S_1 lines, based in turn on the phenotypic means, the means predicted from the mapped QTL ($\bar{Y}_{QS_1}$), and the means predicted from the genetic values ($\bar{Y}_{GS_1}$). Selection lists were also developed for the two testcrosses, based on their phenotypic means. Selection was then applied to these lists with selection intensities of 10% (best 26 genotypes) and 20% (best 52 genotypes). The number of superior genotypes coincidences for the lines and for the testcrosses in these selections were then counted.

Results

Analyses of variance and covariance

Highly significant differences ($p \leq 0.01$) were detected between the lines and the lines × environments interaction was also significant for all the analysed traits. Highly significant differences were also detected for the testcrosses, however the testcrosses × environments interaction was not significant for any of the traits for both the testcrosses (data not presented). The means of the lines differed from the means of both testcrosses for GY, DA and PH. For DS, the mean of the lines only differed from the mean of the TC2 testcrosses. For the other traits, there were no significant differences between the means. The experimental coefficients of variation of the lines were greater than for the testcrosses, probably due to higher mean trait values presented by testcrosses. The estimates of the variance components were all positive and significant ($p \leq 0.05$), with the exception of the interaction testcrosses × environment which was not significant for both testers, for all the traits. The only occurrence of a significant difference ($p \leq 0.05$) between the two testcrosses was for the genetic variances of GY, DA and DS, for these traits, the TC2 genetic variance presented the largest estimate in relation to TC1. For all the traits, for both the lines and testcrosses, the heritability coefficients evaluated based on means were significantly different from zero ($p \leq 0.05$) (Table 1).

The significant coefficients of genetic correlation between the lines and the testcrosses varied from low for GY (0.35) to high for the ASI (0.90), both with TC2. For the significant phenotypic correlations, the

Table 1 Means values, range of variation (R), experimental coefficients of variation CV (%), genetic variances ($\hat{\sigma}_G^2$), interaction genotype × environment variances ($\hat{\sigma}_{G\times E}^2$), heritability coefficients ($\hat{h}_{\bar{x}}^2$), with respective confidence intervals, for the traits evaluated in S_1 lines and testcrosses (TC)

Generation[a]	Parameters	Trait[b]							
		GY	PL	DA	DS	ASI	PH	EH	EP[c]
S_1	Mean (R)	5.27 (2.01;10.67)	7.20 (0.00;43.95)	68.70 (62.35;74.05)	69.85 (61.49;76.53)	1.15 (−2.21;3.81)	192.00 (161.15;229.92)	101.86 (79.66;123.46)	0.53 (0.45;0.59)
	CV (%)	19.91	50.32	2.57	2.66	111.65	5.27	7.34	4.68
	$\hat{\sigma}_G^2$	1.73 [1.44;2.12]	0.54 [0.42;0.72]	4.02 [3.34;4.95]	5.85 [4.88;7.16]	0.64 [0.51;0.84]	80.47 [66.09;100.98]	43.79 [35.91;54.86]	0.54 [0.44;0.67]
	$\hat{\sigma}_{G\times E}^2$	0.69 [0.61;0.79]	0.72 [0.58;0.91]	1.33 [1.09;1.67]	1.62 [1.35;1.98]	0.50 [0.37;0.71]	49.58 [41.78;60.26]	26.68 [22.37;32.47]	0.29 [0.24;0.35]
	$\hat{h}_{\bar{x}}^2$	0.90 [0.88;0.92]	0.65 [0.58;0.71]	0.89 [0.86;0.91]	0.91 [0.89;0.93]	0.70 [0.64;0.75]	0.83 [0.79;0.86]	0.82 [0.79;0.86]	0.84 [0.81;0.87]
TC1	Mean (R)	9.81 (8.44;11.31)	3.40 (0.16;12.90)	61.23 (58.81;63.43)	63.94 (61.68;66.50)	2.71 (0.49;4.42)	237.39 (222.37;253.03)	131.61 (118.07;147.25)	0.55 [0.51;0.59]
	$\hat{\sigma}_G^2$	0.10 [0.08;0.13]	0.04 [0.03;0.07]	0.20 [0.16;0.26]	0.33 [0.27;0.41]	0.10 [0.08;0.14]	10.17 [8.23;13.01]	11.34 [9.30;14.31]	0.10 [0.08;0.13]
	$\hat{h}_{\bar{x}}^2$	0.68 [0.61;0.74]	0.37 [0.24;0.48]	0.73 [0.68;0.78]	0.83 [0.80;0.86]	0.58 [0.50;0.66]	0.77 [0.72;0.81]	0.81 [0.78;0.85]	0.81 [0.78;0.85]
TC2	Mean (R)	10.53 (7.33;12.55)	5.74 (0.38;17.20)	62.41 (59.93;66.03)	63.52 (60.34;67.19)	1.10 (−0.34;2.84)	243.54 (224.56;259.15)	129.34 (112.97;143.06)	0.53 (0.49;0.57)
	$\hat{\sigma}_G^2$	0.18 [0.15;0.24]	0.06 [0.04;0.10]	0.36 [0.30;0.46]	0.56 [0.47;0.69]	0.08 [0.06;0.11]	11.89 [9.64;15.14]	10.24 [8.29;12.97]	0.10 [0.08;0.13]
	$\hat{h}_{\bar{x}}^2$	0.72 [0.66;0.77]	0.37 [0.24;0.48]	0.83 [0.79;0.86]	0.88 [0.86;0.90]	0.57 [0.48;0.65]	0.78 [0.73;0.82]	0.78 [0.74;0.82]	0.80 [0.77;0.84]
TC	CV (%)	9.93	58.81	2.12	1.94	60.51	3.52	5.92	4.25

Confidence intervals at the 0.95 probability level

[a] S_1, TC1, TC2 and TC refer to S_1 lines, testcrosses with the L-04-05F line tester, testcrosses with the L-02-03D line tester and to the average between testcrosses, respectively

[b] GY grain yield (tonnes per hectare), PL plant lodging (percentage), DA days to anthesis (days), DS days to silking (days), ASI anthesis-silking interval (days), PH plant height (cm), EH ear height (cm), EP ear placement

[c] For EP the genetic and genotype × environment variances were multiplied by 1000

Table 2 Phenotypic ($\hat{r}_{\bar{P}}$) and genetic ($\hat{r}_G$) correlations between S_1 lines and their testcrosses (TC) for several traits

Trait	Tester L-0405F (TC1)		Tester L-0203D (TC2)	
	$\hat{r}_{\bar{P}}$	$\hat{r}_G$	$\hat{r}_{\bar{P}}$	$\hat{r}_G$
GY	0.11[ns]	0.12[ns]	0.29**	0.35**
PL	0.35**	0.68**	0.37**	0.72**
DA	0.54**	0.68**	0.65**	0.74**
DS	0.67**	0.76**	0.73**	0.81**
ASI	0.53**	0.82**	0.58**	0.90**
PH	0.44**	0.58**	0.40**	0.51**
EH	0.57**	0.71**	0.55**	0.69**
EP	0.66**	0.81**	0.65**	0.78**

GY grain yield (tonnes per hectare), *PL* plant lodging (percentage), *DA* days to anthesis (days), *DS* days to silking (days), *ASI* anthesis-silking interval (days), *PH* plant height (cm), *EH* ear height (cm), *EP* ear placement

[ns] , ** Non-significant e significantly different from zero at the 0.01 probability level, respectively

values were found to vary from low for GY (0.29) to high for DS (0.73), again both with TC2 (Table 2). Except for grain yield for both the phenotypic and genetic correlations, there were no differences in the magnitudes of the estimates of the coefficients as a function of the tester used, that is, the estimates of the coefficients were close for the two testcrosses. With the exception of PL, there were no marked differences between the genetic and phenotypic correlations for either of the testcrosses.

QTL mapping and congruence of mapped QTL

The number of mapped QTL for GY in the S_1 lines was 16, of which 88% displayed significant interaction with the environment. For the other traits, the number of mapped QTL varied from 9 for DA to 21 for PL and PH, the smallest percentage of QTL showing a significant interaction with the environment was observed for DA (56%) and the largest was with ASI (83%). For GY of the TC1 and TC2 16 and 17 QTL were mapped, of which 50 and 65% presented significant interaction with the environment. For the other traits, in the TC1, the number of mapped QTL ranged from 7 for DS to 19 for EP, the smallest percentage of QTL showing a significant interaction with the environment was observed for DS (43%) and the largest with PL (82%). In the TC2, the number of

mapped QTL varied from 9 for DA to 19 for DS, the smallest percentage of QTL displaying significant interaction with the environment was 50%, observed for PH, while the largest percentage was 73%, which occurred for EH (Table 3).

Only one GY QTL was coincident between the lines and the TC1 and the TC2. For the other evaluated traits, the numbers of QTL coincidences between the lines and the testcrosses were also very low. Examining the TC1, the number of QTL coincident with the lines varied from zero for PL, DA, PH and EH to 3 for EP while, for the TC2, this number varied from zero for ASI and PH to 2 for DS and EP. There were no QTL coincidences between the lines and the testcrosses which were simultaneously coincident with both the testcrosses, that is, no mapped QTL in the lines was detected in the same region in both the testcrosses TC1 and TC2 (Table 3). The numbers of coincident QTL between the two testcrosses were also very low, ranging from zero coincident QTL for DS to 4 for GY, EH and ASI (data not presented).

Correlations between line and testcross means, and coincidences in selection of superior testcrosses

The correlation coefficients between the means predicted for the lines on the basis of the effects of the QTL of the lines ($\bar{Y}_{QS_1}$) and the phenotypic means of the testcrosses for GY were 0.13 and 0.23 for the TC1 and TC2, respectively. For the other traits the coefficient estimates varied from 0.23 for ASI in the TC2 to 0.44 for EP in the TC1. Examining the correlations between the means predicted for the lines based on all the markers ($\bar{Y}_{GS_1}$) and the testcross phenotypic means, the correlation coefficients for GY were 0.13 and 0.26 for the TC1 and TC2, respectively. For the other traits, the smallest estimated coefficient occurred for PL in the TC1 (0.27), while the largest value was found for DS in both the testcrosses (0.62) (Table 4). Thus, as was observed with the phenotypic means of the S_1 lines ($\bar{Y}_{PS_1}$) when molecular marker information is used, the weakest correlation coefficients were obtained for GY and PL, and the strongest for the traits related to the cycle and stature of the plants. Although the testers used were of different origin, this was not seen to have an effect on the correlation coefficients for either $\bar{Y}_{QS_1}$ or $\bar{Y}_{GS_1}$.

Table 3 Number of mapped QTL and QTL with significant interaction with environment, number and percentage of coincident QTL between S_1 lines and testcrosses (TC)

Generation[a]	Number		Coincident	
	QTL	QTL × environment	Number[b]	Percentage[b]
Grain yield (t ha^{-1})				
S_1	16	14	–	–
TC1	16	8	1	6.25
TC2	17	11	1	6.25
Plant lodging (%)				
S_1	21	13	–	–
TC1	11	9	0	0.00
TC2	14	11	1	4.76
Days to anthesis (days)				
S_1	9	5	–	–
TC1	11	7	0	0.00
TC2	9	6	1	11.11
Days to silking (days)				
S_1	16	13	–	–
TC1	7	3	1	6.25
TC2	19	11	2	12.50
Anthesis-silking interval (days)				
S_1	12	10	–	–
TC1	16	10	1	8.33
TC2	1	7	0	0.00
Plant height (cm)				
S_1	21	16	–	–
TC1	11	6	0	0.00
TC2	12	6	0	0.00
Ear height (cm)				
S_1	13	9	–	–
TC1	16	8	0	0.00
TC2	15	11	1	7.69
Ear placement				
S_1	19	14	–	–
TC1	19	9	3	15.79
TC2	10	7	2	10.53

[a] S_1, TC1 and TC2 refer to S_1 lines, testcrosses with the L-04-05F line tester and testcrosses with L-02-03D line tester, respectively

[b] Number and percentage of QTL were mapped in the lines and that have been mapped in congruent regions in testcrosses (when overlap of these intervals occurred)

With a selection intensity of 10%, applied on GY, the number of coincidences between the TC1 selected according to the testcross phenotypic means and the S_1 lines selected according to the means $\bar{Y}_{PS_1}$, $\bar{Y}_{QS_1}$ and $\bar{Y}_{GS_1}$ were 2, 2 and 3 testcrosses, respectively, while for the TC2 there were 6, 5 and 6 coincidences, respectively. For the other traits, the number of coincidences found varied from 2 for PL in the TC1 for lines selected according to $\bar{Y}_{QS_1}$, to 16 for DS in the TC2 for lines selected according to $\bar{Y}_{PS_1}$. With a selection intensity of 20% applied on GY the number of coincidences found in the phenotypically selected TC1 and the S_1 lines selected according to the means $\bar{Y}_{PS_1}$, $\bar{Y}_{QS_1}$ and $\bar{Y}_{GS_1}$ were 12, 13 and 12 testcrosses, respectively, while for the TC2 there were 15, 12 and 16 testcross coincidences, respectively. For the other traits the number of coincidences varied from 12 for PL in the TC2 for S_1 lines selected according to $\bar{Y}_{QS_1}$, to 35 for the TC2 with S_1 line selection according to DA and DS based on $\bar{Y}_{PS_1}$ (Table 5).

Table 4 Correlations coefficients between phenotypic means ($\bar{Y}_{PS_1}$), means predicted based on the effects of the QTL ($\bar{Y}_{QS_1}$) and means predicted based on all the markers ($\bar{Y}_{GS_1}$) of the S_1 lines with phenotypic means of the testcrosses (TC) for several traits

Testcross[a]	Trait	$\bar{Y}_{QS_1}$	$\bar{Y}_{GS_1}$	$\bar{Y}_{PS_1}$
TC1	GY	0.13*	0.13*	0.11[ns]
	PL	0.32**	0.27**	0.35**
	DA	0.28**	0.49**	0.54**
	DS	0.38**	0.62**	0.67**
	ASI	0.32**	0.49**	0.53**
	PH	0.25**	0.37**	0.44**
	EH	0.28**	0.46**	0.57**
	EP	0.44**	0.57**	0.66**
TC2	GY	0.23**	0.26**	0.29**
	PL	0.26**	0.35**	0.37**
	DA	0.30**	0.53**	0.65**
	DS	0.36**	0.62**	0.73**
	ASI	0.23**	0.52**	0.58**
	PH	0.31**	0.36**	0.40**
	EH	0.24**	0.44**	0.55**
	EP	0.41**	0.56**	0.65**

GY grain yield (tonnes per hectare), *PL* plant lodging (percentage), *DA* days to anthesis (days), *DS* days to silking (days), *ASI* anthesis-silking interval (days), *PH* plant height (cm), *EH* ear height (cm), *EP* ear placement

[ns] ,*,** Non-significant, significantly different from zero at the 0.05 probability level and significantly different from zero at the 0.01 probability level, respectively

[a] TC1 and TC2 refer to to testcrosses with the L-04-05F line tester and testcrosses with L-02-03D line tester, respectively

Discussion

The analyses of variance showed the existence of genetic variability for the lines and the testcrosses for all of the traits, as well as performance differentials of the lines across the different environments. Although the experimental coefficients of variation for some traits were a little high, the values are similar to those found in the literature for all the traits considered, in both the lines and in the testcrosses, indicating that the data were obtained with good experimental precision (Hallauer and Miranda Filho 1988; Mihaljevic et al. 2005; Lima et al. 2006; Sabadin et al. 2008).

The magnitudes of the estimates of the genetic variances for the lines were larger than for the testcrosses, which was expected, since the lines displayed the larger amplitude of variation for the means of all the traits. In the case of testcrosses, the genetic variance is a function of the allelic substitution effect of the tester on the loci which control the trait, which in turn depends on their degree of dominance (Bernardo 2002). Inspecting the means ranges of variation of the two testcrosses, it can be seen that the variation for the TC2 was larger for all of the traits, resulting in a greater variability. Furthermore, the inbred L-04-05F, used as tester to give the TC1, has a common origin (IG-1) with one of the parents (L-08-05F) of the S_1 population, this accounts for the lower divergence found for this tester with the S_1 population when compared against the other tester (L-02-03D), which gave the TC2. The heritability coefficients found for the lines were, in general, greater than those of the testcrosses, which may have occurred as a consequence of the greater genetic variance of the S_1 generation in relation to the testcrosses. Overall, taking into consideration the traits analysed and the number of evaluation environments, the genetic components values are in accord with those reported by Hallauer and Miranda Filho (1988). The estimates of the genetic and phenotypic correlation coefficients showed that, as can be observed from the majority of the results in the literature, they are strongest for the least complex traits. These traits have higher heritability coefficients and are the traits for which additive effects are predominant (Smith 1986; Hallauer and Miranda Filho 1988; Groh et al. 1998; Mihaljevic et al. 2005; Peng et al. 2013).

The QTL mapping results showed that large numbers of QTL were mapped for all of the traits and that a substantial number of these QTL displayed significant interaction with the environment, confirming both the quantitative inheritance and the complexity of all the traits considered. Similar results have been obtained in other studies of QTL mapping in maize (Groh et al. 1998; Austin et al. 2000; Mihaljevic et al. 2005; Lima et al. 2006; Sabadin et al. 2008; Peng et al. 2013). For testcrosses, although no significant interaction with the environment was detected by analysis of variance, there was interaction of QTL with the environment, probably because QTL with contrary signal (direction) can have their effects cancelled and in the average of all QTL, interaction it is not detected. Examination of the coincidences in the mapped QTL between the lines and the testcrosses suggests that the QTL mapping is directly influenced by the genetic background of the population. Further,

Table 5 Coincidence of superior S_1 lines and testcross selected, in percentage and number (in parenthesis), considering selection based on phenotypic means ($\bar{Y}_{PS_1}$), means predicted based on the effects of the QTL ($\bar{Y}_{QS_1}$) and predicted based on all the markers ($\bar{Y}_{GS_1}$) of the S_1 lines and phenotypic means of the testcrosses (TC), for 10% (26 genotypes) and 20% (52 genotypes) selection intensity (SI) for several traits

Testcross[a]	Trait	SI = 10% (26)			SI = 20% (52)		
		$\bar{Y}_{PS_1}$	$\bar{Y}_{QS_1}$	$\bar{Y}_{GS_1}$	$\bar{Y}_{PS_1}$	$\bar{Y}_{QS_1}$	$\bar{Y}_{GS_1}$
TC1	GY	7.69 (2)	7.69 (2)	11.54 (3)	23.08 (12)	25.00 (13)	23.08 (12)
	PL	15.38 (4)	7.69 (2)	15.38 (4)	38.46 (20)	26.92 (14)	28.85 (15)
	DA	19.23 (5)	15.38 (4)	26.92 (7)	46.15 (24)	34.62 (18)	46.15 (24)
	DS	42.31 (11)	30.77 (8)	34.62 (9)	59.62 (31)	42.31 (22)	57.69 (30)
	ASI	42.31 (11)	19.23 (5)	30.77 (8)	46.15 (24)	38.46 (20)	51.92 (27)
	PH	30.77 (8)	26.92 (7)	23.08 (6)	42.31 (22)	32.69 (17)	34.62 (18)
	EH	34.62 (9)	34.62 (9)	30.77 (8)	46.15 (24)	38.46 (20)	42.31 (22)
	EP	46.15 (12)	30.77 (8)	50.00 (13)	51.92 (27)	42.31 (22)	48.08 (25)
TC2	GY	23.08 (6)	19.23 (5)	23.08 (6)	28.85 (15)	23.08 (12)	30.77 (16)
	PL	19.23 (5)	19.23 (5)	23.08 (6)	30.77 (16)	23.08 (12)	25.00 (13)
	DA	34.62 (9)	30.77 (8)	34.62 (9)	67.31 (35)	38.46 (20)	57.69 (30)
	DS	61.54 (16)	38.46 (10)	50.00 (13)	67.31 (35)	40.38 (21)	65.38 (34)
	ASI	26.92 (7)	23.08 (6)	34.62 (9)	50.00 (26)	34.62 (18)	55.77 (29)
	PH	26.92 (7)	23.08 (6)	11.54 (3)	36.54 (19)	40.38 (21)	34.62 (18)
	EH	19.23 (5)	11.54 (3)	7.69 (2)	44.23 (23)	32.69 (17)	44.23 (23)
	EP	53.85 (14)	34.62 (9)	46.15 (12)	51.92 (27)	40.38 (21)	48.08 (25)

[a] TC1 and TC2 refer to testcrosses with the L-04-05F line tester and testcrosses with L-02-03D line tester, respectively

GY grain yield (tonnes per hectare), *PL* plant lodging (percentage), *DA* days to anthesis (days), *DS* days to silking (days), *ASI* anthesis-silking interval (days), *PH* plant height (cm), *EH* ear height (cm), *EP* ear placement

there is evidence that the tester used influences the mapping analyses, indicating the occurrence of QTL × tester interactions. These interactions are probably due to the effects of the specific alleles of the tester being used, and to the dominance or epistatic effects of the QTL (Beavis et al. 1994; Groh et al. 1998; Austin et al. 2000; Lu et al. 2003; Mihaljevic et al. 2005; Peng et al. 2013). Another possible explanation for the low rate of QTL coincidences is the homozygote condition of the lines, while is not "normal" for maize. The phenotype of a line could be "masked" by one or a few pairs of loci with deleterious genes in homozygosis. The crossing of these homozygote lines produces testcrosses in which the heterozygosis of these loci is restored and so also the natural condition for the species, eliminating the expression of the majority of the deleterious genes which were in homozygosis (Smith 1986). The results obtained in this work are consistent with reports in the literature (Beavis et al. 1994; Groh et al. 1998; Austin et al. 2000; Mihaljevic

et al. 2005; Peng et al. 2013), because, although the correlation coefficients varied from low to high, few of the QTL were coincident between the lines and the testcrosses. In these published studies, however, the parent generations and the crosses were evaluated generally in the same area but in different years, corresponding therefore to different environments, and, for this reason, the QTL × environment interaction interfered in the number of coincident QTL. The low congruence found in the present study, may occur as the result of the dominance or epistatic effects of the QTL and/or through the effects of the alleles of the tester. Therefore, in the previously reported studies, the lack of congruence cannot be explained by the QTL × environment interaction alone.

The correlations between the phenotypic means of the testcrosses and the predicted means of the lines based on the effects of the QTL ($\bar{Y}_{QS_1}$) displayed lower estimated correlation coefficients than the correlations with means for the lines predicted on the basis of all

the markers ($\bar{Y}_{GS_1}$), indicating that possibly many loci that affect the traits were not detected in the QTL mapping. These results are in agreement with previous reports (Meuwissen et al. 2001; Bernardo and Yu 2007; Lorenzana and Bernardo 2009; Massman et al. 2013), which have shown that the $\bar{Y}_{GS_1}$ are more accurate than the $\bar{Y}_{QS_1}$, in the sense that the $\bar{Y}_{GS_1}$ provide values which are closer to the phenotypic means ($\bar{Y}_{PS_1}$). More detailed examination of the results obtained shows that, even applying the molecular marker information, it is only possible to predict the performance of the testcrosses from data collected for the lines for the cycle and plant stature traits. For those traits with accentuated dominance effects and the more complex traits, such as grain yield and plant lodging, the selection of the superior lines must be performed starting from the evaluation of these lines in crosses. Accordingly, for grain yield and plant lodging improvement, it will not be possible to use marker assisted selection or genomic selection in the lines to predict the means of their testcrosses.

The very low number of coincidences found between the superior testcrosses selected on the basis of their phenotypic means and the S_1 lines selected according to the means $\bar{Y}_{PS_1}$, $\bar{Y}_{QS_1}$ and $\bar{Y}_{GS_1}$ predicted from the data collected on the S_1 lines suggests that selection based on predicted means will be inefficient, particularly for grain yield and plant lodging. Just as was observed for the correlation coefficients, the tester did not produce an obvious effect on the number of coincidences in the testcrosses selected, that is, the coincidence rate was low for both testcrosses. For both the selection intensities used, the highest numbers of coincidences in the testcrosses selected generally occurred for the cycle and plant stature traits, that is, for the least complex traits and the traits for which additive effects are predominant. For the more complex traits and traits for which dominance effects are predominant, such as grain yield, the selected testcross coincidences were much fewer, independent of the selection intensity adopted, indicating that for complex traits that the coincidences are probably random in their origin. These results concerning the influence of the type of trait on the number of coincidences in the selected testcrosses are consistent with the information gathered from the estimated correlation coefficients. Thus, independent of how the S_1 means were obtained, from phenotypic information or from the molecular markers of the lines, the strongest correlations were obtained for the cycle and plant stature traits.

Comparing the three types of selection, it was found that, for the two selection intensities applied and for both the testcrosses, the number of coincident superior testcrosses did not show marked differences according to the source of the S_1 means: phenotypic data, QTL, and all the markers of the lines. When differences did occur, the highest coincidence percentages were observed for selection based on the phenotypic means of the lines and based on predictions of the S_1 means obtained by considering all the markers of the lines. Therefore, genomic selection could be successfully performed on the lines, for the cycle and plant stature traits. Introducing genomic selection will increase the efficiency of breeding programs, since it will be possible to perform the selection through the genotyping of seeds or seedlings. This acceleration of the time necessary to complete a selection cycle (Johnson 2004; Lorenzana and Bernardo 2009; Môro et al. 2012; Mendes and Souza Júnior 2016) will in turn permit increases in the size of the samples and the focussing of the available resources on the best genotypes (Eathington et al. 2007). The application of genomic selection to traits such as disease tolerance, which generally show less complex inheritance, is likely to become a fundamental tool for breeding programs. Genomic selection permits the selection of tolerant and resistant genotypes in the initial phases of their cultivation and can be performed without the presence of the pathogen in the testing area.

Concluding remarks

The results show that the correlations between the traits in the lines and in their testcrosses are low for traits with a predominance of dominance effects and medium for traits for which additive effects are predominant. Even for the traits which displayed medium levels of correlation between the lines and testcrosses, the number of mapped QTL coincidences between the lines and testcrosses is very low. From this observation, it appears that the great majority of the QTL responsible for the expression of a trait in the lines are not expressing this trait in the testcrosses obtained from these lines, or that the expression is very different in the testcrosses.

Building upon this understanding, it was found that even using marker information to select superior testcrosses from data gathered from the lines, the results are similar to those obtained by considering the phenotypic information of the lines, independent of the tester used. Therefore, by using genomic selection, a "soft" selection for the least complex traits and those with reduced dominance effects could be started during the line creation phase. For the more complex traits and those showing marked dominance effects, the selection would necessarily have to be performed through the evaluation of the lines in crosses and, therefore, for this group of traits, the marker information to be used in the selection will have to be obtained from and used directly in the population of testcrosses.

Although for some traits it was not possible to use markers to predict the behaviour of the testcrosses from data gathered on the lines, for other traits useful testcross predictions could be obtained. For these genomically predictable traits, marker information could be used as a tool to assist the selection of superior genotypes in breeding programs thereby increasing the efficiency of the selection process, by diminishing the time necessary to complete each selection cycle. This leads to increases in the gains obtained per year relative to those offered by phenotypic selection, the advantages are especially evident when the evaluation of the phenotypes is difficult, expensive, very time consuming, requires specialised labour, is subjective, or when highly specific environments are needed for cultivar raising. Genomic selection does not depend on the environment, and can also be performed outside the ideal timeframe for cultivation or may even be performed directly on seeds. The choice for the cultivar breeder of whether or not to use molecular markers as a tool in breeding programs will depend on the cost/benefit relationship for the application of genomic selection, making the decision over which selection strategy to adopt a distinctive feature of each program.

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