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Association mapping in an elite maize breeding population

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Abstract Association mapping (AM) is a powerful approach to dissect the genetic architecture of quantitative traits. The main goal of our study was to empirically compare several statistical methods of AM using data of an elite maize breeding program with respect to QTL detection power and possibility to correct for population stratification. These models were based on the inclusion of cofactors (Model A), cofactors and population effect (Model B), and SNP effects nested within populations (Model C). A total of 930 testcross progenies of an elite maize breeding population were field-evaluated for grain yield and grain moisture in multi-location trials and fingerprinted with 425 SNP markers. For grain yield, population stratification was effectively controlled by Model A. For grain moisture with a high ratio of variance among *versus* within populations, Model B should be applied in order to avoid potential false positives. Model C revealed large differences among allele substitution effects for trait-associated SNPs across multiple plant breeding populations. This heterogeneous SNP allele substitution effects have a severe impact for genomic selection studies, where SNP effects are often assumed to be independent of the genetic background.

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Introduction

Dissection of quantitative traits with association mapping (AM) is a promising strategy (Yu et al. 2008; Lu et al. 2010). AM in plant genetics is commonly based on several segregating bi-parental populations, which are analyzed together (e.g., Wu et al. 2002; Stich et al. 2008; Kover et al. 2009; Reif et al. 2010). The controlled crosses result in more balanced allele frequencies compared with association mapping in a diverse panel of lines by enhancing QTL detection power and decreasing confounding effects of population structure (McMullen et al. 2009).

The resolution of AM in multiple segregating populations and the required marker density for a genome-wide scan for QTLs are defined by the extent of linkage disequilibrium (LD) present in the population of the parental genotypes (Myles et al. 2009). In natural populations LD is a function of mutation, recombination, selection, migration, and mating pattern (Flint-Garcia et al. 2003). Therefore, it has to be examined empirically for the germplasm under study to determine the number of required markers for a genome-wide scan for QTLs (Rafalski 2002). In heterotic groups of European elite maize breeding programs a high extent of LD was observed based on a limited number of (approximately 100) SSR markers (Reif et al. 2005; Stich et al. 2005). This suggests that genome-wide AM in breeding populations is feasible even with a

medium marker density. In contrast, Van Inghelandt et al. (2011) observed a fast decay of LD in four European heterotic groups and suggested that between 4,000 and 65,000 SNP markers are needed for genome-wide association mapping in elite maize breeding populations. Consequently, further research is needed to investigate the required marker density for genome-wide AM in elite maize breeding populations.

Several statistical approaches based on linear (e.g., Yu et al. 2008) or linear mixed models (e.g., Stich et al. 2008) have been proposed for AM in multiple segregating populations. Although a balanced experimental design underlies AM in multiple segregating populations, biometric models should correct for population stratification if population structure is associated with the trait under consideration (Reif et al. 2010). Based on a simulation appraisal, Yu et al. (2008) compared the use of (i) cofactors to correct for population stratification with a (ii) biometrical model including a general population effect and found a lower detection power of QTL for the latter model. Reif et al. (2010) evaluated empirically the two above-described models and observed that both the approaches yielded huge variation with respect to QTL detection. Moreover, the substantial differences were observed between mapped QTLs depending on the genetic background of maize (e.g. Mihaljevic et al. 2004) which can be interpreted as AM in multiple segregating populations should be conducted by applying biometrical models with marker effects nested within segregating populations. Nevertheless, to the best of our knowledge a model comparison considering also nested models has not yet been performed.

Here, 930 maize test cross progenies from 11 segregating populations data were used to (1) estimate the extent of linkage disequilibrium in the population of the parental lines, (2) compare empirically different statistical models for AM in multiple segregating populations, and (3) evaluate variation of SNP allele substitution effects in different populations.

Materials and methods

Plant materials

Nine elite inbred lines originating from the stiff stalk heterotic group were used as parents and crossed in an incomplete diallel design (Supplementary Table S1). In total, 11 segregating populations were generated by single seed descent until the F_3 generation and in-vivo doubled haploid (DH) induction. The 930 genotypes (292 F_3 and 638 DHs) were crossed to a tester. The tester was an elite inbred line from the opposite European heterotic pools. All plant materials used in this study are proprietary to Syngenta Seeds.

Field experiments

The 930 test cross progenies were evaluated in unreplicated trials in six environments in 2007. The environments were Campagnola (Italy, 59 m above sea level (asl)), Codogno (Italy, 63 m asl), S. Martino di V. (Italy, 4 m asl), S. Dona (Italy, 3 m asl), Vegas bajas (Spain, 192 m asl), and Almodovar (Spain, 64 m asl). Two-row plots (8.2 m^2) were machine planted (5.5–7.0 plants m^{-2}). Data were recorded for (1) grain yield in Mg ha^{-1}, adjusted to 155 g kg^{-1} of grain moisture and (2) grain moisture content in g kg^{-1} at harvest stage.

Phenotypic data analysis

All quantitative genetic parameters were estimated on the basis of the 930 testcross progenies. Combined analysis across locations was performed using the following statistical model:

$$y_{ip} = \mu + g_i + E_p + d_{ip},$$

where y_{ip} is the phenotypic value for the ith maize line in the pth environment, μ is an intercept term, g_i is the genetic effect of the ith genotype, E_p is the effect of the pth environment, and d_{ip} is the residual, which is the sum of genotype × environment interaction effects and single environmental residuals (Searle 1971). Variance components were determined by the restricted maximum likelihood (REML) method assuming a random model. Heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_e/E)$, where E refers to the number of environments and σ^2_e refers to residuals variance (confounded with σ^2_{GxE}) (Melchinger et al. 1998). Heritability on a plot basis (h^2_{plot}) was estimated as $\sigma^2_G/(\sigma^2_G + \sigma^2_e)$. In addition, Best linear unbiased estimators (BLUEs) across the six locations for 930 testcross progenies were calculated for both traits by assuming fixed genotypic effects.

Molecular data analyses

The DNA was extracted by following a modified SDS-potassium-acetate protocol (Dellaporta et al. 1983). SNP detection was performed using Taqman technology (Applied Biosystems). Within each segregating population, observed genotypic frequency at each marker locus was checked for deviations from Mendelian segregation ratios and allele frequency of 0.5 using a χ^2 test, and Bonferroni–Holm procedure was applied to correct for multiple tests (Holm 1979). The resulting 425 polymorphic high-quality SNP loci were used to construct an integrated linkage map based on the principle described by Stam (1993) by using the JoinMap Ver. 3.0 program (Van Ooijen and Voorrips

2001) (Supplementary Fig. S1). The integrated map is based on results of the eleven populations using Kosambi mapping function (Kosambi 1944). Genetic structure among the nine parents and their 930 genotypes were analyzed by applying principal coordinate analysis (PCoA) (Gower 1966) based on the modified Rogers' distances of the individuals (Wright 1978). Extent of LD between all pairs of loci was determined by estimating r^2 as described by Hill and Robertson (1968) using the data of the nine parents. The PCoA and LD analysis were performed by using the software Plabsoft (Maurer et al. 2008).

Association mapping in multiple segregating populations

For the AM analyses, an additive genetic model was chosen for the testcross progenies as described by Utz et al. (2000). We applied a two-step procedure for QTL detection. In a first step, stepwise multiple linear regression was used to select a set of cofactors based on the Schwarz (1978) Bayesian Criterion (SBC). Cofactor selection was performed using PROC GLMSELECT implemented in the statistical software SAS (SAS Institute 2008). In the second step, we calculated a P value for the association of each marker with the phenotypic value for the F test with a full model (with marker effect) against a reduced model (without marker effect) (for details see Reif et al. 2010). Genome-wide scans for QTLs were conducted using statistical software R (R Development Core Team 2010).

The three different models used in the present study were as follows

$$\mathbf{Y} = \mathbf{l}\mu + \mathbf{X_q}b_q + \sum_{c \neq q} \mathbf{X_c}b_c + \varepsilon, \qquad \text{(ModelA)}$$

where $\mathbf{Y}$ is a $N \times 1$ column vector of the BLUE values of phenotypic data of N testcross progenies ($N = 930$ in our case) coming from P populations ($P = 11$); $\mathbf{l}$ is a $N \times 1$ column vector containing constant 1; μ is the intercept; $\mathbf{X_q}(\mathbf{X_c})$ is a $N \times 1$ column vector containing those marker-types (delegated by 0-1-2) of each individual at marker q (cofactor c); b_q (b_c) is the expected substitution effect of marker q (cofactor c); and ε is the vector of the residuals of the model.

Model B includes population as additional effect to correct for populations stratification:

$$\mathbf{Y} = \mathbf{l}\mu + \mathbf{X_p}\mathbf{M_p} + \mathbf{X_q}b_q + \sum_{c \neq q} \mathbf{X_c}b_c + \varepsilon, \qquad \text{(ModelB)}$$

where $\mathbf{Y}$, $\mathbf{l}$, μ, $\mathbf{X_q}$ ($\mathbf{X_c}$), b_q (b_c) and ε are as described in Model A; $\mathbf{X_p}$ is a $N \times P$ matrix whose elements were 0 or 1 according to whether a progeny i belonged to population p and $\mathbf{M_p}$ is a $P \times 1$ vector of population effects.

Model C is a nested model where both cofactors and assumed QTLs effects were nested within populations:

$$\mathbf{Y} = \mathbf{l}\mu + \mathbf{X_p}\mathbf{M_p} + \mathbf{W_q}\mathbf{E_q} + \sum_{c \neq q} \mathbf{W_c}\mathbf{E_c} + \varepsilon, \qquad \text{(ModelC)}$$

where $\mathbf{Y}$, $\mathbf{l}$, μ, $\mathbf{X_p}$, $\mathbf{M_p}$ and ε are as described in Model B; $\mathbf{W_q}(\mathbf{W_c})$ is a $N \times P$ matrix containing marker-types (delegated by 0-1-2) of each individual in population p at marker q (cofactor c); $\mathbf{E}_q(\mathbf{E}_c)$ is a $P \times 1$ vector of the expected substitution effects of marker q (cofactor c) in population p.

The Bonferroni–Holm procedure (Holm 1979) was used to detect markers with significant ($P < 0.05$) main effects. The proportion of the phenotypic variance explained by QTL was determined by the estimator R^2_{adj} as described by Utz et al. (2000). In a final fit of all detected QTLs, the total proportion of explained genotypic variation was estimated as $p = R^2_{adj}/h^2$ following Utz et al. (2000). This measure standardizes the proportion of phenotypic variance explained by the QTL with the heritability, which facilitates evaluation of MAS as independent of phenotypic data (Utz et al. 2000). The proportion of the genotypic variance explained by individual QTL was calculated as the ratio of $p = R^2/h^2$. The prediction accuracy of grain moisture for each individual population and across populations was calculated as a square of the correlation between QTLs effects with observed BLUEs.

Results

The ANOVA across environments revealed significant ($P < 0.01$) variances for genotypes in all individual populations for both traits except for PopDxB and PopExB for grain yield and PopDxH for grain moisture (Table 1). For grain yield, genotypic variance among populations ($\sigma^2_{\text{G-Among}} = 0.13$) was of the same magnitude as that of within populations ($\sigma^2_{\text{G-Within}} = 0.19$). In contrast, for grain moisture, genotypic variance among populations ($\sigma^2_{\text{G-Among}} = 68.0$) was substantially higher than within populations ($\sigma^2_{\text{G-Within}} = 23.6$). Heritability was higher for grain moisture (0.72) compared with grain yield (0.51). Grain yield ranged from 10.32 to 15.49 Mg ha^{-1} with a mean of 13.45 Mg ha^{-1}. Grain moisture averaged 231.6 g kg^{-1}. We observed a weak yet significant correlation between grain yield and grain moisture ($r = 0.11$, $P < 0.01$).

The first two principal coordinates explained 28.8% of the total variation (Fig. 1). The PCoA revealed a population structure of the nine parents with five clusters: (1) A, (2) E and F, (3) B and C, (4) D and I, and (5) G and H. Moreover, progenies were placed with respect to at least one PC in between their parents. The average genetic map

Table 1 Mean, range, genotypic variance (σ^2_G), error variance (σ^2_e), and heritability (h^2) for grain yield (Mg ha^{-1}) and grain moisture (g kg^{-1}) in maize based on 930 test cross progenies of 11 segregating populations

Population	PopAxD	PopAxE	PopAxF	PopAxG	PopCxE	PopDxB	PopDxH	PopDxI	PopExB	PopExH	PopGxI	Total
Grain yield												
Mean	13.31	13.46	13.85	13.45	13.33	13.74	13.03	12.87	13.82	13.11	12.81	13.45
Min	11.54	11.26	11.69	10.32	11.63	12.25	11.20	11.42	12.46	11.80	11.19	10.32
Max	15.15	15.12	15.49	14.88	14.78	15.15	13.99	14.34	14.84	14.92	14.32	15.49
σ^2_G	0.25***	0.22***	0.31***	0.41***	0.32***	0.08	0.19**	0.30***	0.04	0.23**	0.23***	0.29***
σ^2_e	1.69	1.50	1.73	1.23	1.33	1.74	1.08	1.08	1.31	1.28	0.85	1.67
h^2_{Plot}	0.13	0.13	0.15	0.25	0.20	0.05	0.15	0.22	0.03	0.15	0.21	0.15
h^2_{lines}	0.47	0.47	0.52	0.67	0.59	0.22	0.51	0.63	0.15	0.52	0.62	0.51
Grain moisture												
Mean	234.6	220.4	222.7	244.7	223.4	239.1	235.2	228.6	243.7	233.8	228.5	231.6
Min	211.9	199.3	195.4	223.4	202.7	215.2	219.0	212.8	224.5	220.6	215.0	195.4
Max	255.4	244.1	239.4	261.6	240.7	259.3	248.5	242.1	261.1	249.2	245.3	261.6
σ^2_G	66.90***	35.28***	38.97***	39.13***	74.95***	32.54***	18.84	29.88***	15.83**	22.17**	17.71**	101.3***
σ^2_e	172.76	172.43	164.67	123.42	187.35	177.09	167.75	128.05	168.72	165.34	147.03	242.1
h^2_{Plot}	0.28	0.17	0.19	0.24	0.29	0.16	0.10	0.19	0.09	0.12	0.11	0.30
h^2_{lines}	0.70	0.55	0.59	0.66	0.71	0.52	0.40	0.58	0.36	0.45	0.42	0.72

, * Significant at the 0.01 and 0.001 level of probability, respectively

h^2_{plot} and h^2_{lines} refers to the heritability of plot and lines mean basis, respectively. For details see "Materials and methods" section

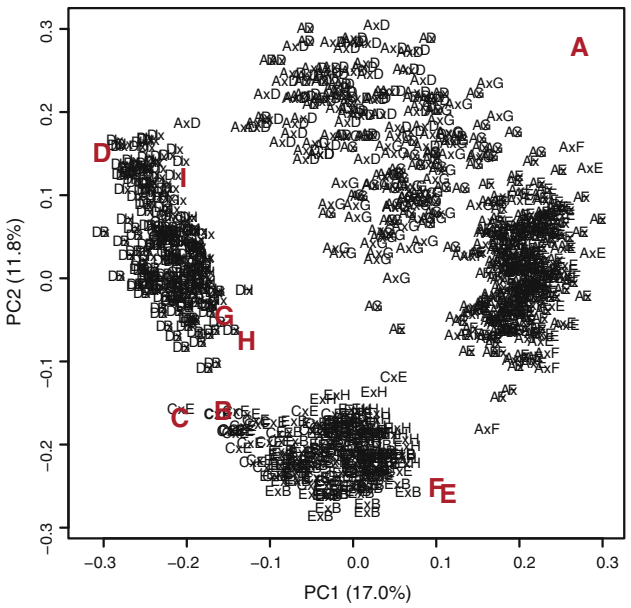

Fig. 1 Principal coordinate analysis of the 9 parents and their 930 progenies based on modified Rogers' distance estimates. Percentages in parentheses refer to the proportion of variance explained by the principal coordinate

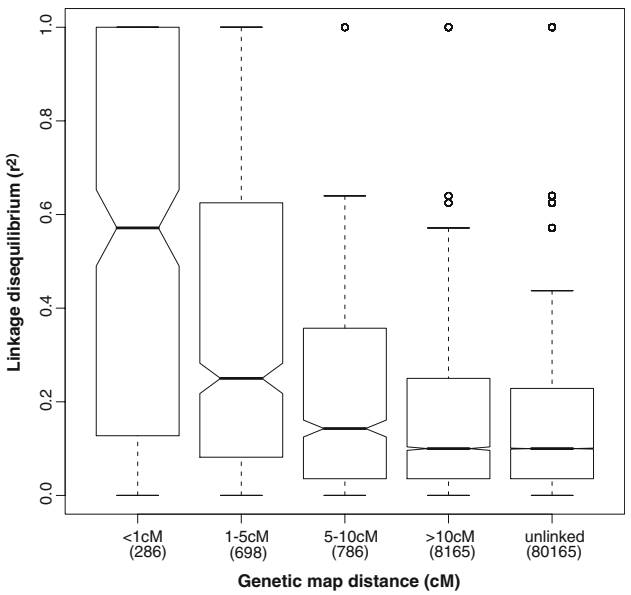

Fig. 2 Box plots of linkage disequilibrium (r^2) between SNP markers as a function of genetic map distance determined in the population of nine parental lines. Number of marker pairs belongs to each group was presented in *brackets*

distance between adjacent markers in our study was 2.9 cM (Supplementary Fig. S1). Estimates of r^2 among adjacent loci pairs averaged 0.49. We observed high LD when the markers were placed up to 1 cM distance. LD decreased to a base level of $r^2 = 0.1$ within 10 cM (Fig. 2).

Genome-wide scans identified main effect QTLs distributed throughout the whole genome for grain yield and grain moisture (Figs. 3, 4). Overlap of QTLs across all three models was low, but overlap across Model A and B was high for grain yield (Table 2). For grain yield, less QTL were detected for Model C (4) compared with Model A (6) and Model B (6). The proportion of genotypic variance explained by the detected QTLs was highest for Model B (44.1%) followed by Model A (40.8%), and Model C (17.9%). For grain moisture, most QTL were detected by Model A (17) followed by Model B (10), and Model C (6). Model A (71.9%) explained a higher proportion of the genotypic variance than Model B (27.4%) and Model C (8.1%). Prediction accuracy (R^2) of grain moisture content based on total QTLs detected by Model A was varied from 0.12 to 0.50 for individual populations and 0.52 for across populations (Fig. 5).

We estimated allele substitution effects for all trait-associated SNPs within each population by applying Model C (Table 3). The analyses revealed huge differences among allele substitution effects for trait-associated SNPs: For grain yield, 56% of the detected QTLs showed SNP allele substitution effects which changed even in sign while comparing the 11 segregating populations. Moreover, for grain moisture, 72% of the QTLs displayed allele substitution effects changing in sign among the 11 segregating populations.

Discussion

In plant breeding programs segregating populations with varying size are routinely evaluated in intensive field trials. During the past decade, costs of genotyping decreased steadily and, therefore, segregating breeding populations are often also fingerprinted with medium- to high-density marker systems (Bernardo and Yu 2007). Genotypic and phenotypic data of several segregating populations enable AM of QTLs underlying important agronomic traits. Previous experimental findings indicated that QTL detection power of AM depends on the choice of an appropriate statistical model (Yu et al. 2008; Reif et al. 2010). This stimulated us to empirically compare several methods of AM in multiple segregating populations using data of an elite maize breeding program with respect to QTL detection power and the possibility to correct for population stratification.

Mapping resolution for genome-wide AM

The resolution and required marker density of genome-wide AM depends on the extent of LD in the population of the parents (Yu and Buckler 2006; Myles et al. 2009). LD for closely linked markers with a genetic map distance smaller than 1 cM was high with average r^2 values of 0.6 (Fig. 2). LD decreased rapidly within a window of 10 cM up to a base level of $r^2 = 0.1$. This result is in contrast with

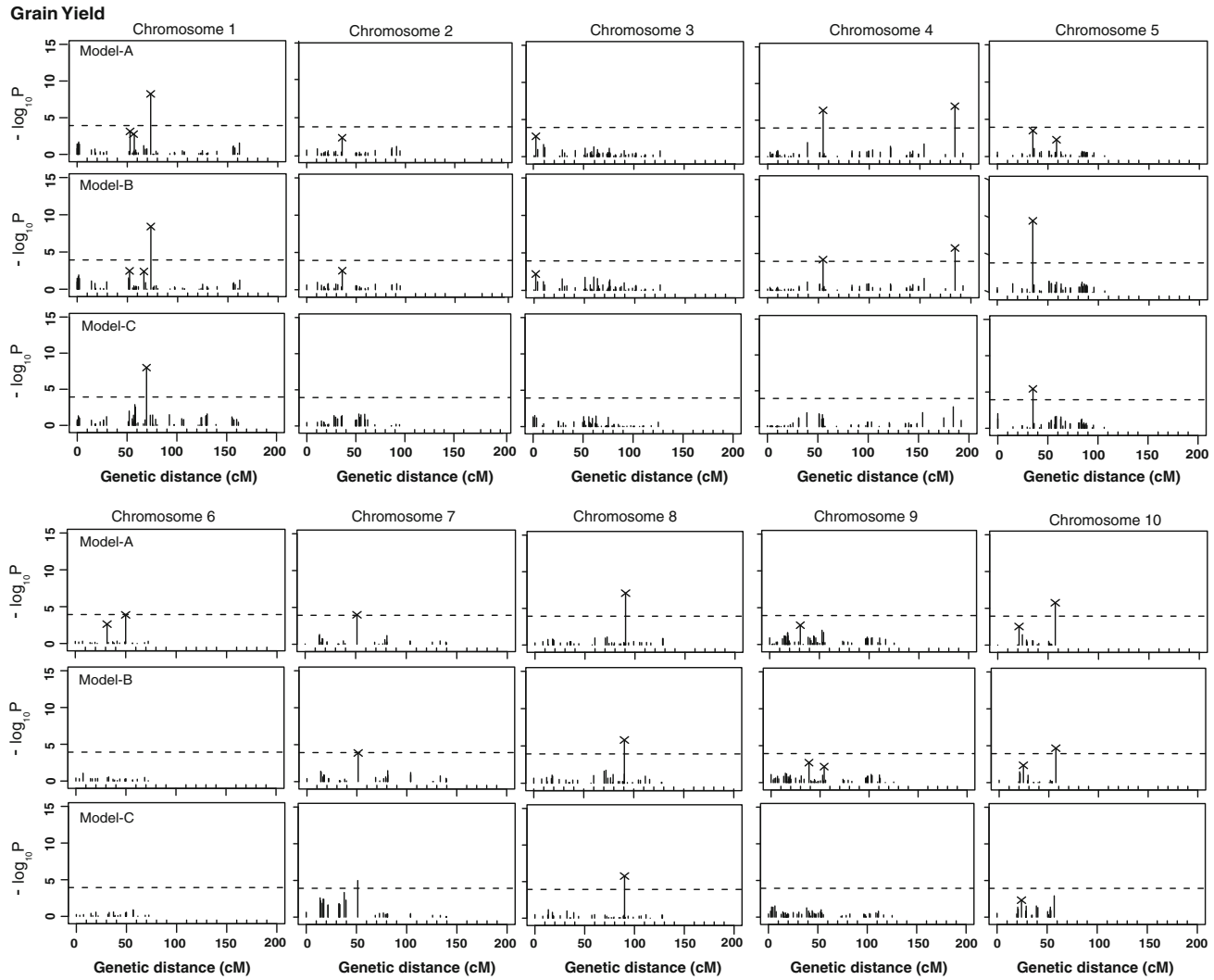

Fig. 3 SNP markers associated with grain yield in maize based on three statistical models. The *horizontal line* refers to a threshold of $P < 0.05$ applying a Bonferroni–Holm correction for multiple tests. *Multiplication symbol* indicates the selected cofactors

the findings in broad-based germplasm of maize where LD decreased faster (Yan et al. 2009). Moreover, the extent of LD was also higher in our survey compared with a study based on European elite maize breeding populations (Van Inghelandt et al. 2011). This can be explained not only by differences in the population history but also by the possibility to obtain slightly upward biased r^2 values in small size populations (Yan et al. 2009). Even though we might have slightly overestimated r^2 values, the observed high average LD ($r^2 = 0.49$) between adjacent markers in our study clearly indicates that at least medium to large effect QTLs should be detected with the applied marker density.

Quality of phenotypic data

Power of QTL detection in AM is determined by several factors including the number of genotypes as well as the genetic architecture and heritability of the trait under evaluation (Yu et al. 2008). The present study is based on a vast elite maize breeding population with 930 individuals, which were intensively phenotyped for grain yield and grain moisture. Heritability on a plot basis was slightly higher for grain yield and similar for grain moisture than previous findings in maize (Schön et al. 2004; Smalley et al. 2004) resulting in genotypic values estimated with high accuracy. The precision of the phenotypic data is also reflected by significant genetic variation observed for almost all single segregating populations (Table 1). Consequently, quality of phenotypic data should facilitate QTL detection with substantial power. Nevertheless, the observed high ratio of genetic variance among *versus* within population for grain moisture suggests that population stratification has to be considered in the biometrical model for AM in multiple segregating populations.

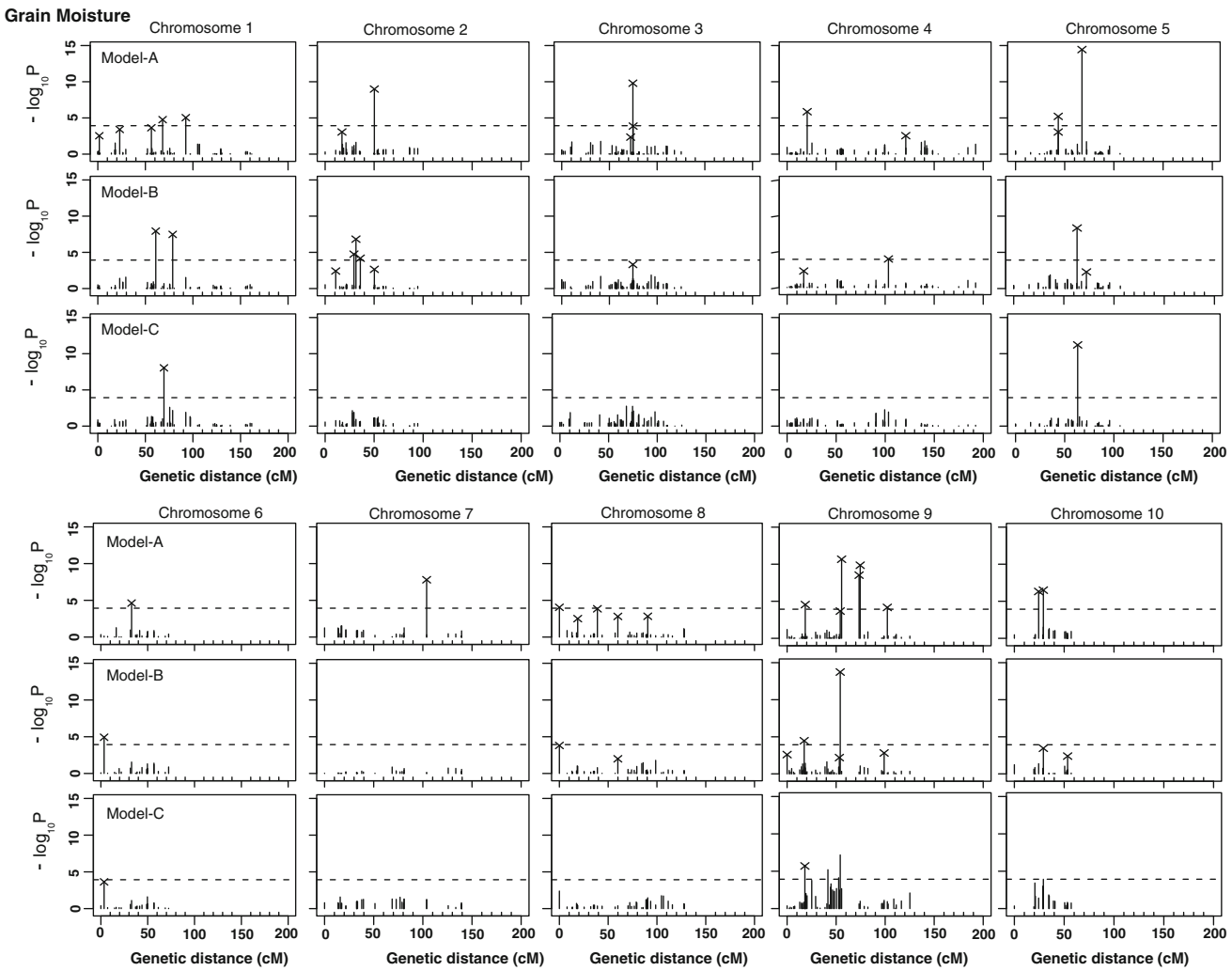

Fig. 4 SNP markers associated with grain moisture in maize based on three statistical models. The *horizontal line* refers to a threshold of $P < 0.05$ applying a Bonferroni–Holm correction for multiple tests. *Multiplication symbol* indicates the selected cofactors

Comparison of biometrical models for AM in multiple segregating populations

Linear mixed models have been used recently for AM in multiple segregating populations (e.g., Stich et al. 2008) for correcting population stratification within and among segregating populations using a kinship matrix. This approach faces the problem, however, of reduction in QTL detection power due to overcorrection of population stratification. Furthermore, large mapping populations cause severe problems for solving mixed model equations due to high computational load (Zhang et al. 2010). Consequently, we have not considered linear mixed models in our study. Instead, we used linear regression models correcting for population stratification with cofactors (Model A) or with an additional fixed population effect (Model B) (Yu et al. 2008). These models are similar to composite interval mapping approaches suggested for bi-parental populations (Jansen and Stam 1994; Zeng 1994), exploiting cofactors to correct for population stratification and controlling for genetic background noise within segregating populations leading to an increased QTL detection power.

For grain yield, overlap of QTLs detected with Model A and B was high with a comparable explained genotypic variance in the final fit based on all QTLs detected with the respective biometrical model (Table 2). This clearly indicated that for this trait modeling a population effect does not appear to be required and population stratification is effectively controlled by using solely cofactors. This is supported by similar R^2 values explained by cofactors of Model A ($R^2 = 0.21$) compared with cofactors plus

Table 2 Analysis of trait-associated markers, allele substitution (α) effects, the explained proportion of the genotypic variance p_G (%), and the total phenotypic variance (R^2) of the association mapping in multiple segregating populations based on three different statistical models

Chromosome	QTL position	Model-A		Model-B		Model-C	
		α-effect	p_G (%)	α-effect	p_G (%)	α-effect	p_G (%)
Grain yield (Mg ha^{-1})							
1	69.5	–	–	–	–	0.20	6.1
1	73.3	0.16	6.3	0.21	10.6	–	–
4	54.6	−0.15	5.7	−0.12	3.7	–	–
4	184.4	−0.07	1.2	−0.13	3.3	–	–
5	35.1	–	–	−0.17	5.7	−0.17	5.3
7	51.3	−0.10	2.2	–	–	−0.11	5.1
8	90.0	–	–	–	–	0.17	5.5
8	90.6	0.21	7.3	0.18	5.7	–	–
10	57.1	0.31	7.7	0.27	6.1	–	–
Total p_G (%)			40.8		44.1		17.9
R^2 (%)			20.8		22.5		9.1
Grain moisture (g kg^{-1})							
1	60.9	–	–	1.5	1.7	–	–
1	68.1	1.3	0.7	–	–	–	–
1	69.5	–	–	–	–	2.3	2.5
1	78.7	–	–	−2.0	3.4	–	–
1	92.5	−1.7	2.1	–	–	–	–
2	29.4	–	–	−2.5	2.0	–	–
2	31.5	–	–	1.3	0.4	–	–
2	36.0	–	–	−0.7	0.1	–	–
2	50.3	−1.4	1.1	–	–	–	–
3	74.6	1.9	1.5	–	–	–	–
4	20.5	2.4	2.8	–	–	–	–
4	103.4	–	–	−2.4	3.2	–	–
5	43.4	−4.2	3.1	–	–	–	–
5	63.2	–	–	3.7	8.1	2.6	3.2
5	67.5	−2.6	3.8	–	–	–	–
6	3.4	–	–	4.5	2.8	–	–
6	32.8	1.5	1.4	–	–	–	–
7	103.9	3.0	4.1	–	–	–	–
8	0.0	1.0	0.7	–	–	–	–
9	17.4	–	–	0.4	0.1	–	–
9	18.1	–	–	–	–	−1.1	1.0
9	18.5	−1.6	1.5	–	–	–	–
9	41.6	–	–	–	–	0.9	0.6
9	52.6	–	–	–	–	0.1	0.4
9	54.2	–	–	1.2	1.1	1.2	1.1
9	55.6	5.3	7.7	–	–	–	–
9	73.4	2.5	2.1	–	–	–	–
9	74.5	−2.3	2.2	–	–	–	–
9	102.2	1.0	0.6	–	–	–	–
10	24.4	−4.5	2.0	–	–	–	–
10	29.1	−1.8	1.7	–	–	–	–
Total p_G (%)			71.9		27.4		8.1
R^2 (%)			51.8		19.7		5.8

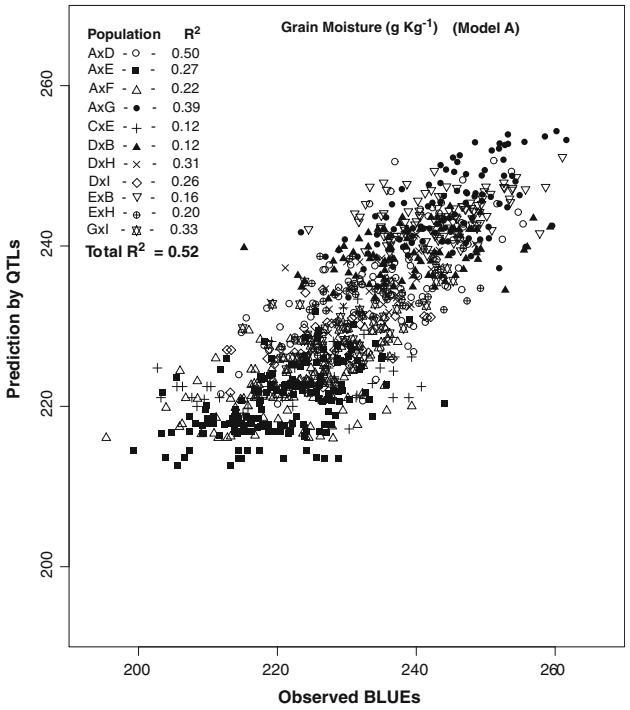

Fig. 5 Prediction of grain moisture content by QTLs detected based on Model A for 11 individual populations

population effect in Model B ($R^2 = 0.23$). Consequently, for AM of grain yield, use of Model A and B seems to be appropriate in our study.

For grain moisture, overlap of QTL was low and Model A detected more QTLs explaining nearly three times higher genotypic variance in the final fit of all detected QTLs compared with Model B (Table 2). The higher number of QTLs detected with Model A than with Model B for grain moisture can be explained by an inflated number of false positive QTLs due to an insufficient control of population stratification. This is supported by substantially lower R^2 values explained by cofactors of Model A ($R^2 = 0.59$) compared with cofactors plus population effect in Model B ($R^2 = 0.70$). Nevertheless, the higher number of QTLs detected for grain moisture with Model A than with Model B can also be interpreted as an indicator that Model A exploits the genotypic variance among populations more efficiently leading to an increased QTL detection power (Yu et al. 2008; Reif et al. 2010). Based on the detected QTLs in Model A, grain moisture was predicted with quite high prediction accuracy (Fig. 5). Nevertheless, the prediction accuracy (R^2) of Model A has to be interpreted carefully, because although predictive power of QTL is high across populations, R^2 for individual populations was low with an average of 0.26 (Fig. 5). As prediction of differences among populations can be done very efficiently based on mean parental performance (e.g., Melchinger et al. 1998), marker-based prediction of performance within single segregating populations is of particular interest, but the prediction accuracy for individual populations in the present study was quite low (Fig. 5). Consequently, for AM in multiple segregating populations for traits with a high amount of genetic variance among populations, use of Model A is not appropriate and Model B should be applied.

The number of QTLs detected with Model C is substantially lower compared with Model A and B (Table 2). This can be explained by the lower number of degrees of freedom for AM in multiple segregating populations based on nested models compared with Model A and B. Therefore, application of Model C for QTL detection can be only recommended in case of large segregating populations.

Model C revealed a large variation in SNP allele substitution effects (Table 3) with QTL effects changing even in sign for both traits, grain yield and grain moisture (Table 3). This variation in allele substitution effects is as expected on the basis of previous studies in maize (e.g., Melchinger et al. 1998; Frascaroli et al. 2009; Schön et al. 2010) and can be explained by differences in allele frequency (Falconer and Mackay 1996), epistasis (Blanc et al. 2006), and multiple alleles (Calus et al. 2008). The large variation in SNP allele substitution effects has an impact on the accuracy of genomic selection, where SNP effects are often assumed to be independent of the genetic background (e.g., Meuwissen et al. 2001; Gianola et al. 2006; Habier et al. 2007; de los Campos et al. 2009; Luan et al. 2009). Problems of heterogeneous SNP allele substitution effects across multiple populations might be reduced accommodating epistasis (Habier et al. 2010) and considering multiple alleles (Calus et al. 2008). Nevertheless, reliability of SNP allele substitution effects across multiple plant breeding populations should be investigated in more detail.

Conclusions

The empirical comparison of the three biometrical models for AM in multiple segregating populations suggested that the choice of appropriate model is depends on the genetic variance among populations. For traits with a low ratio of variance among *versus* within populations, Model A or B can be recommended. Nevertheless, for traits with pronounced genetic variance among populations, Model B should be applied in order to avoid potential false positives. Model C is well suited to estimate the predictive power of the QTL detected with Model A and B within populations. This enables a robust judgment of the potential of marker-assisted selection in the context of plant breeding.

Table 3 Allele substitution (α) effects of the trait-associated markers estimated within the eleven segregating mapping populations

Chromosome	Position (cM)	α-effect										
		PopAxD	PopAxE	PopAxF	PopAxG	PopCxE	PopDxB	PopDxH	PopDxI	PopExB	PopExH	PopGxI
Grain yield (Mg ha^{-1})												
1	69.5	–	−0.01	0.13	–	–	–	–	–	0.15	0.10	–
1	73.3	–	0.20	0.16	–	–	0.06	–	–	–	0.14	–
4	54.6	–	−0.13	−0.11	−0.10	–	−0.15	0.14	–	–	–	−0.01
4	184.4	–	–	–	–	−0.27	−0.17	−0.14	−0.16	−0.12	0.00	−0.14
5	35.1	−0.17	−0.21	−0.13	−0.21	–	–	–	–	–	–	–
7	51.3	−0.01	−0.20	−0.14	−0.07	–	–	–	0.04	–	–	−0.25
8	89.9	0.32	–	–	–	–	0.00	−0.07	–	–	–	0.20
8	90.6	–	–	–	–	–	0.10	0.07	–	–	–	–
10	57.1	–	–	–	–	–	–	−0.11	0.28	–	0.21	0.18
Grain moisture (g kg^{-1})												
1	60.9	–	−1.20	−0.58	–	–	–	3.14	−2.41	−2.89	–	−3.02
1	68.1	0.18	–	–	−1.25	–	0.70	−3.24	0.23	–	–	1.60
1	69.5	–	0.49	2.95	–	–	–	–	–	−1.82	2.26	–
1	78.6	−1.75	−0.98	0.09	−3.55	–	−1.84	2.48	−2.46	−1.01	2.55	0.07
1	92.5	−0.59	−0.50	0.47	0.44	–	–	−2.71	−1.13	−1.13	−2.64	−1.44
2	29.4	–	−1.67	–	–	1.62	–	–	−2.00	−1.26	−2.77	−1.20
2	31.4	−3.44	–	4.28	2.28	5.29	0.30	–	–	–	3.61	–
2	35.9	4.32	−3.02	−4.60	−1.09	–	−0.61	–	–	1.10	–	–
2	50.2	–	−1.56	−1.57	–	−1.45	–	1.55	−1.44	−0.47	–	−1.56
3	74.6	0.89	–	–	1.91	–	–	–	–	1.20	2.56	–
4	20.5	–	–	–	1.05	–	−0.83	–	–	1.39	–	−0.07
4	103.3	–	–	–	–	−3.10	−0.63	−1.07	−0.59	−0.97	−0.90	−1.54
5	43.4	–	–	–	–	–	–	–	0.47	–	–	−1.52
5	63.1	−0.52	−0.37	1.31	1.52	–	–	–	–	–	–	–
5	67.4	−3.78	−2.73	−1.24	−0.08	–	–	–	–	–	–	–
6	3.4	–	–	–	–	−5.09	–	–	–	–	–	–
6	32.8	0.92	–	0.21	–	–	0.92	–	–	–	0.31	1.74
7	103.8	–	–	–	2.19	–	–	−1.27	−3.13	0.17	−0.57	–
8	0.0	1.56	1.98	–	–	0.92	1.47	1.95	−0.19	0.67	2.14	–
9	17.4	9.59	−2.43	−7.43	–	−3.67	0.36	0.39	0.52	−0.87	0.07	–
9	18.1	−12.72	−2.69	5.44	−0.26	–	–	–	–	–	–	–
9	18.5	–	4.66	–	–	–	–	–	–	–	–	–
9	41.6	−0.35	1.14	0.63	–	2.14	0.76	−1.40	1.96	−0.43	0.56	–
9	52.6	0.95	–	–	–	–	1.63	–	–	–	−3.91	0.93
9	54.1	2.75	1.87	1.01	2.19	1.57	–	−2.08	0.08	–	3.12	–
9	55.5	–	–	–	–	0.68	–	7.05	–	–	−1.14	–
9	73.3	−0.34	–	–	0.06	0.58	–	–	0.81	−0.48	−0.74	1.43
9	74.5	–	−2.95	−0.84	–	–	–	–	–	–	–	–
9	102.2	0.04	–	–	0.80	3.04	1.44	−0.31	0.59	–	–	−1.33
10	24.4	–	–	–	–	−1.75	–	–	–	–	–	–
10	29.1	−0.42	−0.68	−0.84	−2.14	–	–	–	–	–	–	–

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