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ORIGINAL PAPER

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QTL mapping for European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm

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Abstract In hybrid breeding the performance of lines in hybrid combinations is more important than their performance per se. Little information is available on the correlation between individual line and testcross (TC) performances for the resistance to European corn borer (ECB, *Ostrinia nubilalis* Hb.) in maize (*Zea mays* L.). Marker assisted selection (MAS) will be successful only if quantitative trait loci (QTL) found in F_2 derived lines for ECB resistance are still expressed in hybrid combinations. The objectives of our study were: (1) to identify and characterize QTL for ECB resistance as well as agronomic and forage quality traits in a population of testcrossed $F_{2:3}$ families; (2) to evaluate the consistency of QTL for per se and TC performances; and (3) to determine the association between per se and TC performances of $F_{2:3}$ lines for these traits. Two hundred and four $F_{2:3}$ lines were derived from the cross between maize lines D06 (resistant) and D408 (susceptible). These lines were crossed to D171 and the TC progenies were evaluated for ECB resistance and agronomic performance in two locations in 2000 and 2001. Using these TC progenies, six QTL for stalk damage rating (SDR) were found. These QTL explained 27.4% of the genotypic variance in a simultaneous fit. Three QTL for SDR were detected consistently for per se and TC performance. Phenotypic and genotypic correlations were low for per se and TC performance for SDR. Correlations between SDR and quality traits were not significant. Based on these results, we conclude that MAS will not be an efficient method for improving SDR. However, new molecular tools might provide the opportunity to use QTL data as a first step to identify genes involved in ECB resistance. Efficient MAS procedures might then be based on markers designed to trace and to combine specific genes and their alleles in elite maize breeding germplasm.

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Introduction

The European corn borer (ECB, *Ostrinia nubilalis* Hb.) is a pest of maize (*Zea mays* L.) with growing importance in European maize production. In contrast to the U.S. corn belt, where the ECB has up to four generations, only one generation is observed in Central Europe. Leaf feeding and stem tunneling by ECB larvae reduce plant growth and cause stalk lodging and ear dropping, resulting in severe yield losses of up to 30% (Bohn et al. 2000).

Chromosomal regions affecting ECB resistance in the resistant U.S. inbred B52 were first identified using translocation stocks (Onukogu et al. 1978). B52 and the resistant inbreds DE811 and Mo47 were also investigated in QTL studies in crosses with susceptible inbreds B73 and Mo17 (Lee 1993; Schön et al. 1993; Jampatong et al. 2002). In agreement with the translocation study, the QTLs with the largest effects on ECB resistance were detected on chromosomes 1 and 2. In early maturing European dent maize, QTL found for tunnel length and stalk damage ratings explained approximately one half of the genotypic variance. However, agreement of QTL results across the different mapping populations and the resistance traits measured was low. This low consistency was explained by the partly different genetic basis of the

different resistance traits, as indicated by the low to moderate correlation between these traits, and the low power of QTL detection (Melchinger et al. 1998). In addition, the populations used might differ for the set of segregating resistance gene alleles and their epistatic interactions (Stuber 1995).

Even though the consistency across these studies was low, simulation experiments showed that most QTL detected in F_2 populations are not likely to be false positives (Beavis 1994) and might be used for marker assisted selection. However, after 10 years of genetic studies using molecular markers to identify a large set of putative QTL, the question of whether these can be used for marker assisted selection to improve ECB resistance in maize is unanswered. A few studies concluded that MAS might be promising (Jampatong et al. 2002; Flint-Garcia et al. 2003), whereas other studies pointed out that the use of MAS would not result in increased selection gains based on relative efficiency calculations (Bohn et al. 2000; Papst et al. 2001). However, in order to finally judge the prospects of MAS to improve ECB resistance in maize, information on the correlation between per se and testcross (TC) performance for ECB resistance is needed.

In hybrid breeding programs the performance of maize lines in hybrid combinations is more important than their performance per se. Little information is available on the correlation between line per se and hybrid performance for ECB resistance in temperate maize germplasm. Correlations between per se and TC performance were high for stalk damage ratings, but low for tunnel length and yield reduction caused by ECB larvae feeding (Kreps et al. 1998). In tropical maize the association between per se and TC performance was low for corn borer resistance (Thome et al. 1992; Groh et al. 1998) and the consistency of QTL mapped for per se and TC performance was poor. These results suggest that MAS can only be successfully employed to improve ECB resistance in maize if QTL identified in lines per se are expressed in hybrid combinations. Therefore, the objectives of our study were: (1) to identify and characterize QTL for ECB resistance, as well as agronomic and forage quality traits, in a population of testcrossed $F_{2:3}$ families derived from a cross between two early-maturing European dent lines; (2) to evaluate the consistency of QTL for per se and TC performances; and (3) to determine the association between per se and TC performances of $F_{2:3}$ lines for these traits.

Materials and methods

Plant materials

Dent lines D06 (ECB resistant) and D408 (ECB susceptible) were crossed to produce 230 $F_{2:3}$ families; these families were evaluated for per se performance and used for QTL mapping as described in detail by Bohn et al. (2000). Out of this set of $F_{2:3}$ families, 204 families were testcrossed by crossing ten randomly chosen plants per family with D171, a susceptible line from the flint pool. Seeds were harvested from all ten plants and bulked to evaluate TC progenies.

Field trials

Experiments with manual infestation of ECB larvae and those under protection (insecticide application without infestation) were conducted in Pulling and Frankendorf in the summer seasons of 2000 and 2001. Forage quality traits were evaluated in Frankendorf and Straubing in 2001. All experimental sites are located in southeastern Germany and are characterized by increasing ECB population densities over the past 5 years (Zellner, Landesanstalt für Landwirtschaft Freising, personal communication). Each year-location combination was treated as an environment in the subsequent statistical analyses. A total of 210 TC entries were evaluated, including the 204 $F_{2:3}$ families and duplicate entries of the parental lines, and the F_1 hybrid as duplicate entries. The experimental design was a $21\times10\alpha$-design with two replications at all locations. The experimental unit was a two-row plot with 50 plants, 4 m long, and a row spacing of 0.75 m. Trials were over-planted and later thinned to a final plant density of 8 plants/m^2. The first and the last two plants of each row were eliminated before grain harvest. These plants were used for forage quality analyses in 2001.

ECB treatment

An average number of 20 neonate ECB larvae were applied three times at weekly intervals for a total of about 60 larvae per plant. Freshly hatched larvae were mixed with maize-cob grits and placed into the whorl or leaf collar of maize plants with special dispensers (Mihm 1983). The manual infestation was synchronized with the natural occurrence of ECB moths between the end of June and mid-July. The plant development stages varied at infestation time from mid-whorl stage to tasseling or silking. Egg masses for manual infestation were provided by Dr. P. Aupinel, INRA, Le Mangeraud, France. The insecticide-protected whole plots were treated with FASTAC SC applied twice starting at the end of June in 10- to 14-day intervals.

Evaluation of resistance and agronomic traits

Resistance against ECB larvae feeding was determined using stalk damage ratings (SDR) based on a 1–9 rating scale (1 for intact plants, 9 for dropped ears or breakage below the ear) as described by Hudon and Chiang (1991). Grain yield, in tonnes per hectare, under protection (GYP) and manual infestation (GYI) was adjusted to 15.5% grain moisture, and the relative grain yield reduction (RGY) was calculated as (GYI/GYP)×100. The date of anthesis (ANT), in days after sowing, plant height (PHT), in centimeters, and dry matter concentration (DMC), as a percentage, were recorded for plants from the insecticide-protected plots. The four plants, not harvested for grain yield in the insecticide protected plots, were hand-harvested without ears at the end of September to determine dry matter concentration of stover (DMCS), in per cent, cellulose-digestible organic matter (CDOM) (De Boever et al. 1986), concentration of crude protein (CP) (Kjeldhal 1883), concentration of crude fiber (CF) (Naumann and Bassler 1998), water-soluble carbohydrates (WSC) (Luff and Schoorl 1929), in vitro digestible organic matter (IVDOM) (Tilley and Terry 1963), and digestibility of neutral detergent fiber (DNDF) (Dolstra and Medema 1990), in g kg^{-1} 10^{-1}. All forage quality traits were analyzed by near-infrared reflectance spectroscopy using calibrations provided by KWS Saat AG, Germany.

Marker and linkage analyses

Details on the marker analyses and the linkage map development were presented by Bohn et al. (2000). Briefly, a total of 230 F_2 plants were genotyped for 93 RFLP and two SSR marker loci. The linkage map was constructed using MAPMAKER3.0b (Lander et al. 1987) software. Linkage between two markers was declared significant in two-point analyses if the LOD score ($\log_{10}$ of the

likelihood odds ratio) exceeded a threshold of 3.0. After determining linkage groups and the correct linear arrangement of marker loci along the linkage groups, recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgans (cM) by Haldane's mapping function (Haldane 1919).

Statistical analyses

Analyses of variance were performed on field data from each experiment within each environment. Adjusted entry means and effective error mean squares were used to compute the combined analyses of variance and covariance across environments for experiments with and without ECB infestation. The sums of squares for entries (210 *df*) were subdivided into the variation among TC progenies of the $F_{2:3}$ families (203 *df*) and the orthogonal contrasts among the means TC progenies of the $F_{2:3}$ families versus the midparental value and P1 versus P2. Components of variance for the TC progenies were computed considering all effects in the statistical model as random. Estimates of the genotypic variance (σ_g^2), genotype×environment interaction variance (σ_{ge}^2), error variance (σ^2), and phenotypic variance (σ_p^2) and their standard errors were calculated as described by Searle (1971). Heritabilities (h^2) for TC progenies were computed on an entry-mean basis and confidence intervals on $\hat{h}^2$ were estimated according to Knapp et al. (1985). Phenotypic ($\hat{r}_p$) and genetic ($\hat{r}_g$) correlation coefficients were calculated among resistance and agronomic traits by applying standard methods (Mode and Robinson 1959). PLABSTAT (Utz 2001) and SAS (SAS Institute 1996) software packages were used for all calculations.

QTL analyses

QTL analyses were performed on the subset of 201 TC progenies for which both molecular and phenotypic data were available. Composite interval mapping (CIM) was employed for QTL detection and estimation of QTL effects. A LOD threshold of 2.5 was chosen for declaring a putative QTL significant, ensuring a comparison-wise error rate of $P<0.0036$ and an experiment-wise error rate of $P<0.30$. Estimates of QTL positions were obtained at the position, where the LOD score reached its maximum in the region under consideration. All putative QTL were examined for presence of digenic epistatic effects and QTL × environment interactions. The proportion of the phenotypic variance explained by all QTL was determined by the adjusted coefficient of determination of regression (R_{adj}^2) fitting a model including all detected QTL. The proportion of the genotypic variance explained by all QTL for one trait (p) was calculated as $p = R_{adj}^2/h^2$.

Five-fold cross validation (CV/G) was performed for the $F_{2:3}$ lines per se and their TC progenies following procedures described by Utz et al. (2000) and Bohn et al. (2001). The whole data set (DS) containing the entry means across environments for each mapping population was randomly split into k=5 disjoint subsets. Four subsets were combined to form the estimation set (ES) for QTL detection and estimation of genetic effects. The remaining subset formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data. By permuting the subsets, five different CV/G runs are possible for a five-fold CV/G. Subsets were formed randomly 200 times, yielding a total of 1,000 replicated CV/G runs. Following Utz et al. (2000), the proportion of the genotypic variance explained by the detected QTL in TS ($\hat{p}_{TS.ES}$) was calculated from the adjusted squared correlation coefficient between the phenotypic entry means observed in TS (Y_{TS}) and the predicted genotypic values ($Q_{TS.ES}$) on the basis of results derived from ES, divided by the heritability of the trait under study:

$$\hat{p}_{TS.ES} = \frac{R_{adj}^2(Y_{TS}, Q_{TS.ES})}{\hat{h}^2}.$$

Using a LOD threshold of 2.5, each CV/G run yielded different estimates for the number of QTL, their location, and genetic effects in the ES. Estimates of p in ES and TS were calculated as the median $\tilde{p}$ over all replicated CV/G runs. The average number of QTL was determined as the mean across replicated CV/G runs. The median additive genetic effect $\tilde{a}_{ES}$ was calculated for each scanned chromosomal position. For each $\hat{a}_{ES}$, the corresponding additive effect from TS ($\hat{a}_{TS.ES}$) was determined by multiple regression based on the map positions of all QTL detected in ES and the marker genotypes of the $F_{2:3}$ family in TS at the respective flanking marker loci, according to described procedures (Haley and Knott 1992; Utz and Melchinger 1996). Subsequently, the median $\tilde{a}_{TS.ES}$ was calculated across all CV/G runs for a given position.

Relative efficiency of MAS

The relative efficiency (RE) of MAS over conventional phenotypic selection was calculated according to the formula of Lande and Thompson (1990). Assumed molecular-marker scores were recorded without errors, and selection intensities of MAS and CPS were equal. If selection is only performed on marker loci, the efficiency was calculated as $RE = \sqrt{p/h^2}$. If phenotypic and molecular data were combined, the *RE* was calculated by using the formula $RE_c = \sqrt{p/h^2 + (1-p)^2/(1-ph^2)}$.

Correlation between TC progenies and $F_{2:3}$ families

Associations between per se and TC performance of $F_{2:3}$ families were determined using the following three methods. Firstly, phenotypic correlations ($\hat{r}_p$) were estimated on entry-mean basis for per se and TC performance of the 204 $F_{2:3}$ families. Because per se and TC performance was not evaluated in the same locations, genotypic correlation ($\hat{r}_g$) was calculated as $\hat{r}_g = MP/E\sqrt{\hat{\sigma}_{gT}^2 \times \hat{\sigma}_{gL}^2}$, where MP is the mean product of entry means for per se and TC performance, E is the number of environments, $\hat{\sigma}_{gT}^2$ is the estimated genotypic variance for TC performance, and $\hat{\sigma}_{gL}^2$ is the estimated genotypic variance for the per se performance. PLABSTAT (Utz 2001) was used to perform the necessary calculations. Secondly, the correlation coefficient between LOD profiles (r_{LOD}) determined in the mapping experiments for per se and TC performance was calculated. Significance of this correlation was determined by employing a permutation test (Keightley and Knott 1999). The data were permuted 1,000 times by randomizing the order of chromosomes independently for each mapping experiment. The reordered chromosomes were lined up and the correlation between profiles was calculated under the null hypothesis that LOD profiles were not correlated. All computations were performed using the software program CORRESP (Utz 2002). Thirdly, multiple regression was used to determine the combined effect of the QTL positions identified for per se perfor- mance in TC progenies. The pro-por-tion of σ_g^2 explained by these chromosomal regions for TC performance was calculated as $p_{TL} = R_{adj}^2\left(Y_{TC}, Q_{TC.per\ se}\right)/h^2$, where $R_{adj}^2\left(Y_{TC}, Q_{TC.per\ se}\right)$ is the adjusted squared correlation coefficient between the phenotypic entry means observed in TC progenies (Y_{TC}) and the predicted genotypic values ($Q_{TC.per\ se}$) on the basis of QTL results derived from the per se performance and h^2 is the heritability for TC performance of the trait under study. All calculations were performed with PLABQTL (Utz and Melchinger 1996) software.

Results

Phenotypic data

TC performance of $F_{2:3}$ families

TC progeny means of parental lines D06 and D408, their F_1 hybrid, and the population TC mean were not significantly different for all traits (Table 1). Genotypic variances were highly significant ($P<0.01$) for all traits and σ^2_{ge} estimates were significant ($P<0.01$) only for SDR, GYP, DMC, and quality trait DNDF (data not shown for DNDF). Heritabilities were low for RGY, GYP and quality traits DMCS, CP, and DNDF ($\hat{h}^2 \leqslant 0.45$) (data for quality traits not shown) but of moderate size for all other traits ($0.50 < \hat{h}^2 < 0.71$).

QTL for per se performance

Here, we report QTL for ECB resistance and agronomic traits obtained by using five-fold cross validation. Corresponding results found without cross validation were presented in a previous paper (Bohn et al. 2000).

Resistance traits

For SDR two QTL on chromosome1 and one each on chromosomes 6 and 8 were detected. These four QTL explained 27.6% of $\hat{\sigma}^2_g$ (Table 2). QTL detection frequencies ranged from 19.4% to 100%. For tunnel length two QTL were found on chromosome 5 and one each on chromosomes 1, 2, 3, 7, 8, and 10. These QTL explained in a simultaneous fit 10.6% of $\hat{\sigma}^2_g$. The QTL detection frequencies varied between 19.6% and 79.5%.

Agronomic traits

A total of 32 QTL were found for agronomic traits GYP (3 QTL), GYI (2 QTL), PHT (7 QTL), ANT (10 QTL), and DMC (10 QTL) (data not shown). Most of them showed additive gene action. The proportion of $\hat{\sigma}^2_g$ explained by the QTL detected for each trait ranged from 17.3% to 27.0 %. Quantitative trait loci found for grain yield under insecticide protection and ECB infestation explained less than 3% of $\hat{\sigma}^2_g$. Five out of 32 QTL were detected in more than 97% of all cross validation runs for each trait. The remaining QTL were found with frequencies between 13.0% and 94.6 %.

Table 1 Testcross means of parental lines D06 and D408, their F_1 generation, and 204 $F_{2:3}$ families, as well as estimates of variance components and heritabilities for resistance, agronomic, and quality traits evaluated at two locations in 2000 and 2001

Generation	Entries (no.)	Resistance traits		Agronomic traits				Quality traits	
		SDR (1–9 scale)[a]	RGY (%)	GYP (t ha^{-1})	PHT (cm)	ANT (days)	DMC (%)	CF (g kg^{-1}10^{-1})	IVDOM (g kg^{-1}10^{-1})
Testcross means[b]									
D06	2	3.39±0.36	94.4±0.8	7.73±0.17	274.9±3.6	83.4±0.8	69.3±0.02	33.48±0.08	66.6±1.16
D408	2	4.30±0.04	87.3±4.4	8.36±0.18	270.3±0.7	83.8±0.9	67.1±0.22	30.41±1.01	72.44±2.37
P[c]	4	3.84±0.57	90.8±4.9	8.05±0.39	272.6±3.4	83.6±0.7	68.2±1.29	31.94±1.87	69.52±3.70
F_1	2	3.97±0.06	88.3±5.1	8.18±0.01	271.5±2.6	81.9±1.0	68.0±0.35	31.94±0.13	68.98±0.38
$F_{2:3}$	204	3.34±0.77	89.1±5.1	8.11±0.42	273.4±7.7	83.1±1.2	68.0±0.85	32.00±0.89	69.19±1.74
Variance components									
σ^2_g		0.08±0.17**	8.93±3.01**	0.76±0.19**	41.53±5.90**	0.94±0.14**	0.47±0.08**	0.43±0.09**	1.74±0.33**
σ^2_{ge}		0.04±0.17**	1.77±4.16	0.55±0.21**	2.93±4.02	0.11±0.10	0.22±0.06**	0.07±0.09	0.33±0.33
σ^2_e		0.68±0.56**	5.60±4.70**	0.36±0.30**	67.64 ±55.36**	13.71±11.20**	0.16±0.13**	4.11±3.36**	24.32±19.87**
Heritability									
$\hat{h}^2$		0.50	0.35	0.45	0.71	0.69	0.65	0.53	0.55
90% C.I. on $\hat{h}^2$		(0.34; 0.63)	(0.14; 0.59)	(0.28; 0.59)	(0.62; 0.78)	(0.59; 0.77)	(0.53; 0.73)	(0.39; 0.64)	(0.42; 0.66)

** Variance component was significant at the 0.01 probability level.

[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, PHT = plant height, ANT = days to anthesis, DMC = dry matter content, CF = content of crude fiber, IVDOM = in vitro digestible organic matter.

[b] Standard errors are attached.

[c] P = mean of parental testcross hybrids.

Table 2 Position of detected QTL and their respective additive effects for stalk damage ratings (*SDR*) and tunnel length (*TL*) determined using the whole data set ($\hat{a}$) or 200 five-fold cross validation runs ($\hat{a}_{TS.ES}$). Parameters were estimated from phenotypic per se data of 210 $F_{2:3}$ families derived from the cross D06×D408 evaluated at two locations in 1995

Bin[b]	Position (cM)	$\hat{a}$[a]	$\hat{a}_{TS.ES}$			$\hat{p}$[d]
			Median	(10; 90) Percentile	Frequency (%)[c]	
Stalk damage ratings		1–9 scale				
1.02	46	–0.21	–0.08	(–0.11; –0.07)	19.4	
1.06	166	0.27	0.24	(0.23; 0.24)	88.6	
6.07	144	0.27	0.25	(0.23; 0.25)	84.0	
8.05	58	–0.26	–0.27	(–0.28; –0.26)	100.0	27.6
Tunnel length		cm				
1.07	202	0.54	0.36	(0.35; 0.40)	79.2	
2.04	110	–0.70	0.36	(–0.40 ;–0.25)	19.6	
3.09	304	0.32	0.28	(0.27; 0.31)	79.5	
5.03	80	–0.74	–0.21	(–0.27; –0.20)	36.9	
5.04	102	0.80	0.26	(0.23; 0.33)	27.3	
7.05	102	0.32	–0.01	(–0.04; 0.03)	27.5	
8.05	58	–0.55	–0.23	(–0.23; –0.17)	50.3	
10.06	148	0.86	0.46	(0.42; 0.54)	29.1	10.6

[a] Median and percentiles were calculated based on 200 five fold CV/G runs.
[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (Gardiner et al. 1993).
[c] Frequency of QTL detection across 200 five fold CV/G runs.
[d] $\hat{p}$ = proportion of genotypic variance explained by detected QTL calculated as R^2_{adj} / heritability in 200 cross validation runs (for heritability and results without cross validation see Bohn et al. 2000).

Table 3 Position of detected QTL and their respective additive effects for stalk damage ratings (*SDR*), content of crude fiber (*CF*), in vitro digestibility of organic matter (*IVDOM*) determined using the whole data set ($\hat{a}$) or 200 five-fold cross validation runs ($\hat{a}_{TS.ES}$). Parameters were estimated from phenotypic data of TC progenies of 204 $F_{2:3}$ families derived form the cross D06×D408 evaluated at two locations in the years 2000 and 2001; bold letters indicate common QTL positions across per se and TC evaluations

Bin[b]	Position (cM)	$\hat{a}$[a]	$\hat{a}_{TS.ES}$			$\hat{p}$[d]
			Median	(10; 90) Percentile	Frequency (%)[c]	
Stalk damage ratings		1–9 scale				
1.01	**25**	**–0.31**	–0.26	(-0.26; - 0.24)	**91.7**	
3.09	315	0.20	0.14	(0.13; 0.15)	62.1	
6.06	**110**	**0.59**	0.53	(0.52; 0.54)	**86.0**	
7.04	180	0.25	0.06	(0.04; 0.08)	41.0	
8.04	**30**	**–0.29**	**–0.17**	(–0.18; –0.16)	**66.7**	
10.04	160	0.37	0.11	(0.10; 0.13)	54.5	**27.4**
Crude fiber		g kg^{-1} 10^{-1}				
3.01	20	0.35	0.45	(0.37; 0.46)	24.1	
6.05	80	–0.42	–0.48	(–0.54; –0.48)	63.7	
9.02	28	0.29	0.28	(0.25; 0.32)	14.6	**4.7**
In vitro digestibility of organic matter		g kg^{-1} 10^{-1}				
8.06	76	0.65	0.55	(0.52; 0.56)	69.8	
9.01	26	–0.47	–0.60	(–0.66; –0.53)	47.9	**3.3**

[a] Median and percentiles were calculated based on 200 five fold CV/G runs.
[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (Gardiner et al. 1993).
[c] Frequency of QTL detection across 200 five fold CV/G runs.
[d] $\hat{p}$ = proportion of genotypic variance explained by detected QTL calculated as R^2_{adj} / heritability in 200 cross validation runs.

QTL for TC performance

Resistance traits

Six QTL for SDR on chromosomes 1, 3, 6, 7, 8, and 10 were found, explaining between 6.7% and 13.4% of $\hat{\sigma}^2_p$ (Table 3). In the 1,000 CV/G runs, the mean number of detected QTL was 4.4 for SDR, which explained 27.4% of $\hat{\sigma}^2_g$ in a simultaneous fit. The frequencies of QTL detection in cross validation varied from 41.0% to 91.7%. Except for QTL in bins 1.01 and 8.04, the resistance allele originated from the resistant parent D06. No QTL for RGY was detected.

Agronomic traits

A total of 16 QTL were found for GYP (4 QTL), GYI (3 QTL), PHT (4 QTL), ANT (3 QTL), and DMC (2 QTL) (data not shown). One QTL for PHT accounted for 17.7% of $\hat{\sigma}^2_p$, whereas the other QTL explained between 3.8% and 13.6% of $\hat{\sigma}^2_p$. Averaged across cross validation runs,

Table 4 Phenotypic ($\hat{r}_p$) and genotypic ($\hat{r}_g$, below the diagonal) correlation coefficients among resistance and agronomic traits calculated in a population of 204 testcrossed $F_{2:3}$ families derived from the cross D06×D408

	Resistance traits		Agronomic traits				
	SDR	RGY	GYP	GYI	PHT	ANT	DMC
SDR		–0.44**	–0.03	–0.41**	–0.29**	–0.32**	0.18**
RGY	–0.84^{++}		–0.32**	0.63**	0.16*	0.13	–0.08
GYP	–0.03	0.01		0.52**	0.27**	0.25**	0.01
GYI	–0.58^{++}	0.69^{++}	0.46^{++}		0.38**	0.31**	–0.07
PHT	–0.44^{++}	0.36^{++}	0.27^{++}	0.46^{++}		0.43**	–0.14*
ANT	–0.50^{++}	0.23^{+}	0.54^{++}	0.53^{++}	0.58^{++}		–0.37**
DMC	0.30^{++}	–0.09	–0.07	–0.13^{+}	–0.18^{+}	–0.45^{++}	

*,** Phenotypic correlation was significant at the 0.05 and 0.01 probability levels, respectively.
$^{+}$, $^{++}$Genotypic correlation exceeded once or twice its standard error, respectively.
[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, GYI = grain yield under infestation, PHT = plant height, ANT = date of anthesis, DMC = dry matter content.

QTL explained between 3.3% (DMC) and 18.1% (PHT) of $\hat{\sigma}_g^2$ in a simultaneous fit. One QTL each for GYP (bin 5.07), GYI (bin 3.08), and PHT (bin 9.02) and two for ANT (bins 3.03 and 8.03) were detected in more than 97% of the 1,000 cross validation runs. QTL×environment interactions were significant (P<0.05) for ANT, DMC, and GYP.

Quality traits

Twenty-two QTL were detected for all evaluated forage quality traits (2 QTL for DNDF, IVDOM; 3 QTL for CF, DMCS; 4 QTL for CDOM, CP, and WSC; data not shown, for CF and IVDOM see Table 3). Each QTL explained between 5.7% and 10.7% of $\hat{\sigma}_p^2$ and between 3.3% and 8.0% of $\hat{\sigma}_g^2$ in a simultaneous fit. The frequency of QTL detection varied between 14.6% (CF, bin 9.02) and 89.6% (WSC, bin 10.06) of the cross validation runs. QTL×environment interactions were significant (P<0.05) for DNDF.

Correlations between resistance and agronomic traits

The genotypic and phenotypic correlation coefficients between RGY and SDR were highly significant (P<0.01) and negative (Table 4). Associations between agronomic and resistance traits were moderate to low. Stalk damage ratings were negatively associated with GYI, PHT, and ANT but positively correlated with DMC. Significant but low genotypic correlations (r_g >-0.27) were found between SDR and quality traits CDOM, DNDF, IVDOM, and WSC (data not shown).

Correlations between per se and TC performance

Phenotypic correlations between per se and TC performance of $F_{2:3}$ families were highly significant (P<0.01) and of moderate size for SDR, PHT, ANT, and DMC (Table 5). Corresponding genotypic correlations were moderate to high for all traits except RGY. The correlation between LOD profiles (r_{LOD}) for per se and TC performance showed significant (P<0.01) values only for SDR, PHT, and ANT. QTL identified for per se performance explained between 23% and 27% of σ_g^2 for TC performance for SDR, PHT, and ANT. Estimates of p_{TL} were practically zero for all other traits.

Table 5 Phenotypic ($\hat{r}_p$) and genotypic ($\hat{r}_g$) correlation coefficients between per se and TC performance of 204 $F_{2:3}$ families of cross D06×D408 as well as correlation coefficients between LOD profiles (r_{LOD}) and the proportion of the genotypic variance in TC performance explained by QTL detected for per se performance (p_{TL})

Trait[a]	$\hat{r}_p$	$\hat{r}_g$	r_{LOD}[b]	p_{TL}
SDR	0.33**	0.62^{++}	0.27*	23.1
RGY	0.09	0.30^{+}	0.10	–[c]
GYP	0.16*	0.30^{++}	–0.16	2.0
GYI	0.17*	0.27^{++}	0.12	2.1
ANT	0.56**	0.74^{++}	0.34*	26.7
PHT	0.69**	1.00^{++}	0.44**	27.0
DMC	0.47**	0.63^{++}	0.06	1.9

*,** Phenotypic correlation was significant at the 0.05 and 0.01 probability levels, respectively.
$^{+}$, $^{++}$Genotypic correlation exceeded once or twice its standard error, respectively.
[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, GYI = grain yield under infestation, PHT = plant height, ANT = days to anthesis, DMC = dry matter content.
[b] Correlation coefficients of LOD profiles for resistance and agronomic traits across testcross progenies and $P_{2:3}$ families.
[c] No QTL was detected for RGY.

Discussion

Consistency of QTL for per se and TC performance

The prime goal of maize breeding is to identify new lines with superior performance in hybrid combinations. Selection of potential parents is based on their general combining ability (GCA) to increase the probability of finding superior hybrid combinations. This is necessary, because performance of lines per se does not provide an adequate measure of their value in hybrid combinations for most traits of agronomic importance (Hallauer 1990).

The predictive value of GCA depends on the relative importance of GCA and the specific combining ability (SCA) for the trait under study. Based on these relationships, it seems logical to use QTL detected for TC performance or QTL found for per se performance with consistent expression in TC for identifying potential hybrid parents using MAS. However, QTL for TC performance might be tester-specific, resulting in the need to develop several QTL populations with different testers. In addition, the use of QTL for per se performance in MAS is attractive, because this information is available at least two generations earlier than is TC performance.

Therefore, the detection of QTL contributing to the GCA of a line for a specific trait and the consistency of QTL across line per se and TC evaluations, as well as between testers, are of central importance for developing a successful MAS program. The level of consistency depends on the power of QTL detection in per se and TC evaluations, the type of gene action displayed by the genes involved in the inheritance of the trait under study, and the specific allelic effects of the chosen tester. The power of detecting QTL for per se and TC performance is a function of the size of the QTL effects, the heritability of the trait under study, and the size of the mapping populations. In addition, the power of detecting the same QTL in per se and in TC evaluations is the product of the power of QTL detection in the separate studies. Therefore, a QTL will only be consistently detected in per se and TC evaluations, if the power of QTL detection is high in both studies.

In per se evaluations of $F_{2:3}$ families most likely QTL with additive effects will be found, because only a quarter of the dominance effects present in the F_1 generation can be detected in a population of $F_{2:3}$ families. In contrast, the average effect of a gene substitution is determined in TC evaluations. The average effect of a gene substitution is a function of additive and dominance effects as well as allele frequencies in the tester. The latter shows the influence of the tester allele. If the tester allele is dominant or partially dominant over the alleles of the two parental lines, it may mask the effect of the QTL allele segregating in the mapping population and hence this QTL is not detected for TC performance.

For TC performance, we detected six QTL for SDR explaining an average of one quarter of the genotypic variance across cross-validation runs. Three of these were in adjacent chromosomal bins (on chromosomes 1, 6, and 8) to QTL for SDR per se performance. In agreement with the above outlined expectations, QTL for per se performance displaying overdominance were not detected for TC performance, if their additive effect was not of considerable size, such as for the QTL for SDR in chromosomal bin 1.06. In addition, QTL alleles of the tester may be dominant over the alleles of the parental lines. These effects and the decreased genotypic variance displayed by testcrossed $F_{2:3}$ families reduced the power of QTL detection using TC progenies and resulted in a low consistency of QTL across per se and TC evaluations. Despite these disadvantages of TC progenies in QTL detection, three QTL were found for TC performance that remained undetected for per se performance. One possible explanation for this result could be the use of a highly susceptible tester. This tester was selected based on its high level of susceptibility to ECB larvae feeding combined with otherwise good agronomic performance (Schulz et al. 1997). In the case of a susceptible tester, specific interactions of the tester and segregating alleles facilitate the detection of new QTL (Kreps et al. 1998). In addition, this finding might be due to the low power of QTL detection in the per se and TC evaluations as a result of the relatively small population size (N<210), the low to moderate heritabilities for the evaluated ECB resistance traits, and the fact that per se and TC evaluations were performed in different environments.

Similar results were reported by Groh et al. (1998), who evaluated two tropical maize populations of recombinant inbred lines for their resistance against tropical stem borer species (*Diatraea* spp.) in per se and TC evaluations. Based on the mostly additive gene action found in early generations of both RIL populations (Bohn et al. 1996), it was expected to find several common resistance genes between per se and TC evaluations. But the reported consistency was low. The authors explained their findings by the low power of QTL detection in the TC progenies and the evaluation of both progeny types in different environments.

In a set of 16 European flint and 24 dent lines, correlations between per se and TC performance were high for stalk damage ratings, but low for tunnel length and yield reduction caused by larvae feeding (Kreps et al. 1998). In tropical maize the association between per se and TC performance was low for corn borer resistance (Thome et al. 1992). In accordance with these studies, we found low but significant phenotypic and genotypic correlation coefficients between per se and TC performance. However, based on the size of these correlations, it was not surprising that only half of the QTL detected in $F_{2:3}$ lines per se were rediscovered using their TC pro-genies.

In most ECB QTL studies, the majority of QTL associated with resistance showed additive effects and only to a minor extent dominance (Bohn et al. 2000; Papst et al. 2001; Krakowsky et al. 2002). Therefore, tight correlations between per se and TC evaluations were expected. However, as already state above, QTL with additive effects will be preferentially detected in populations of $F_{2:3}$ families, resulting in an underestimation of dominance involved in the inheritance of ECB resistance and in an overestimation of the association between per se and TC performance. In addition, predictions were based on QTL that often explained less than 50% of the genetic variance for the evaluated ECB resistance trait.

Clustering of QTL for insect resistance

The QTL regions for SDR detected only for TC performance in our study were located in adjacent intervals known to carry QTL for tunnel length observed for per se

performance (chromosomes 3, 7, and 10). All QTL found for TC performance were located adjacent to QTL regions detected for stem borer resistance in other temperate and tropical maize populations (Schön et al. 1993; Groh et al. 1998; Khairallah et al. 1998; Bohn et al. 2000; Papst et al. 2001; Jampatong et al. 2002; Krakowsky et al. 2002). A compilation of all known QTL positions based on their bin location showed that QTL for stem borer resistance were not randomly distributed across the maize genome but occur in clusters on chromosome 1, 5, and 9. This information is a possible starting point to determine candidate genes involved in the inheritance of stem borer resistance. Previous findings suggested cell wall fortification caused by increased lignin content as one putative resistance mechanism (Buendgen et al. 1990; Bergvinson et al. 1996). Known genes of the lignin biosynthesis pathway are located in the stem borer resistance gene clusters. It might be possible to substantiate the hypothesis that lignin is an important factor in stem borer resistance by applying new molecular tools to determine the association between allelic variation at candidate gene loci and a specific phenotype (McMullen et al. 1998; Buckler and Thornsberry 2002).

Correlations between traits

We determined tissue digestibility characteristics to test the hypothesis that cell wall fortification is one possible resistance mechanism. In our study no significant association was found between digestibility traits (CDOM, IVDOM, DNDF) and SDR. This is in good agreement with Kreps et al. (1998), who evaluated a set of 41 inbred lines for their per se and TC performance and reported no significant phenotypic correlation between in vitro digestibility of organic matter (IVDOM) and ECB resistance. Two possible reasons might account for the lack of association between digestibility traits and ECB resistance. First, the physical properties of a lignin polymer largely depend on its monolignol subunit composition. In maize, genes are known that directly influence lignin content in cell walls and its subunit structure. Specific alleles at these loci may cause the production of lignin with a subunit composition in cell walls that result in an increased IVDOM without compromising cell wall strength. These genes might also improve digestibility traits without increased SDR. Next to cell wall fortification and reduced forage quality, high concentrations of foliar phenolic acids are assumed to increase resistance to insect herbivores by causing oxidative stress in the midgut of insects (McMullen et al. 1998). Phenols are oxidized to quinones, which bind amino acids and proteins reducing their nutrional value and/or bioavailability and thus inhibiting larval development (Felton et al. 1989; Duffey and Felton 1991; McMullen et al. 1998). However, tobacco plants overexpressing a key gene involved in phenolic production did not exhibit higher levels of resistance against *Heiothis virescens* (Johnson and Felton 2001).

We found a negative association between SDR and ANT and a positive association between SDR and DMC in testcrosses in accordance with previous studies (Groh et al. 1998; Bohn et al. 2000; Magg et al. 2001). Bohn et al. (2000) found $F_{2:3}$ family genotypes that combined early flowering with a high level of ECB resistance. They conjectured, by examining graphical genotypes, that the correlation between ANT and ECB resistance was mainly caused by tight linkage instead of pleiotropy. The $F_{2:3}$ families, which combined early flowering with a high level of ECB resistance for per se performance, also showed this trait combination for TC performance.

Prospects of MAS for ECB resistance

The main goal of QTL mapping is the identification of chromosomal regions involved in the inheritance of economically important quantitative traits as a starting point for MAS. In the case of improving ECB resistance, costs for mass rearing of larvae and manual infestation are high and h^2 of ECB resistance traits are low. Therefore, marker-based technologies could offer a more efficient way to develop new genotypes with improved ECB resistance. In our companion study, the relative efficiency of MAS over conventional phenotypic selection was 0.87 for SDR indicating that conventional phenotypic selection is more efficient than MAS (Bohn et al. 2001). Even with low relative efficiencies (RE<1) MAS may be competitive over conventional phenotypic selection, if cost-effective PCR-based marker systems are available and costs of artificial infestation are high. However, the effectiveness of MAS strongly depends on the accuracy of QTL mapping results. Here, we reanalyzed data reported by Bohn et al. (2001) using CV. QTL for SDR explained 27.6% of σ_g^2 resulting in a low relative efficiency of MAS over conventional phenotypic selection (RE=0.47). If MAS and conventional phenotypic selection were combined, values of RE approached 1.05. This result suggested only a small gain in selection response employing MAS in selection programs for improving ECB resistance.

What are possible alternative approaches to utilize QTL information for stem borer resistance gathered in multiple studies over the last 10 years? A first step to utilize this wealth of information would be the performance of a meta-analysis (Goffinet et al. 2000) to confirm the hypothesis that QTL for stem borer resistance occur in clusters. These clusters might be large and will contain hundreds of genes, but based on knowledge about putative resistance mechanisms it might be possible to identify the underlying biochemical pathways and respective candidate genes. Their effect on stem borer resistance might be tested with association studies. Association studies might also provide plant breeders with information about the allelic variation that can be exploited for each resistance gene. In this case, MAS will be based on gene sequences

that allow tracing and combining candidate genes and their specific alleles directly.

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