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Re-evaluation of the prospects of marker-assisted selection for improving insect resistance against *Diatraea* spp. in tropical maize by cross validation and independent validation

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Abstract Cross validation (CV) and validation with an independent sample (IV) are new biometric approaches in QTL analysis to obtain unbiased estimates of QTL effects and the proportion of the genetic variance explained by the detected marker-QTL association (*p*). Our objective with these methods was to obtain a realistic picture on the prospects of marker-assisted selection (MAS) for improving the resistance of maize against the tropical stem borer species *Diatraea grandiosella* (SWCB) and *Diatraea saccharalis* (SCB). Published QTL mapping studies on leaf-damage ratings (LDR) with populations of $F_{2:3}$ lines and recombinant inbred lines (RIL) from crosses CML131×CML67 and Ki3×CML139 of tropical maize inbreds were re-analyzed with CV and IV. With CV, the reduction in *p* for LDR compared to *p* obtained with the whole data set varied between 41.0 and 79.6% in the populations of $F_{2:3}$ lines and between 30.1 and 65.2% in the two populations of RIL. Estimates of *p* for SCB LDR were similar for CV and IV. For SWCB LDR, *p* estimates obtained with IV were larger than those obtained with CV in CML131×CML67. The reverse was observed for Ki3×CML139. Under the assumption of identical selection intensities, and based on the re-estimates of *p*, MAS using only molecular marker information is less-efficient than conventional phenotypic selection (CPS). MAS combining marker and phenotypic data increases the relative efficiency by only 4% in comparison to CPS. In conclusion, MAS for improving SWCB and SCB LDR seems not-promising unless additional QTLs with proven large effects are available or the costs of marker assays are considerably reduced.

Keywords Insect resistance · QTL · Cross validation · Maize · Marker-assisted selection (MAS)

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Introduction

The southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar, and the sugarcane borer (SCB), *Diatraea saccharalis* Fabricius, are serious insect pests in tropical and subtropical areas of Central and Latin America. In the small-scale, low-input farming systems prevailing in these regions, low yield stability is partly due to the highly variable damage caused by SWCB and SCB.

Resistance to SWCB and SCB larvae feeding in tropical maize germplasm was reported to be quantitative with mainly additive gene action for first-generation leaf feeding resistance (Hinderliter 1983; Thome et al. 1992). QTL studies confirmed these results (Bohn et al. 1996, 1997; Groh et al. 1998; Khairallah et al. 1998). QTLs with predominantly additive gene effects were found on all maize chromosomes, except for chromosomes 4 and 6. Moreover, a high genotypic correlation was found between SWCB and SCB leaf-feeding resistance suggesting a common genetic basis for the resistance against both insect species. Bohn et al. (1997) reported seven QTLs with pleiotropic effects on chromosomes 1, 5, 7 and 9 in an F_2 population derived from a cross between a resistant and a highly susceptible tropical inbred line. However, these QTLs explained only half of the genotypic variance for SWCB and SCB leaf-feeding resistance.

A preliminary comparison of QTL consistency across populations and generations based on common chromosomal-bin positions revealed a low consistency of QTL positions across different populations but a moderate agreement between QTLs found in different generations for a given population (Groh et al. 1998). The authors explained the lack of consistency by: (1) different sets of

QTLs segregating in the different populations, (2) dominance effects that are not detectable in populations of RIL, (3) epistasis, and (4) a low power of QTL detection due to small population sizes (n<200).

The primary goal of QTL studies is to provide marker-QTL associations for MAS programs. For a successful application of MAS it is essential to have a detailed knowledge about the putative location and effects of genetic factors influencing the target trait. MAS is more efficient than CPS if: (1) QTL positions are estimated with high precision in order to choose markers showing a minimum of recombination with the QTL, (2) QTL effects are estimated without bias due to genotypic and environmental sampling, and (3) p is sufficiently large for the examined trait (Lande and Thompson 1990).

Computer simulations (Utz and Melchinger 1994; Beavis 1998) demonstrated that with the currently used methods of QTL analysis, such as simple interval mapping (Lander and Botstein 1989) or composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994), the estimates of individual QTL effects and p can be severely inflated. Furthermore, confidence intervals for QTL positions are fairly large for the population sizes commonly used in QTL mapping experiments (van Ooijen 1992; Visscher et al. 1996). Following a proposal by Lande and Thompson (1990), Melchinger et al. (1998) employed independent population samples for estimating QTL effects and confirmed that estimates of the phenotypic and genotypic variance explained by QTLs detected in a calibration sample were substantially inflated. Consequently, the prospects of MAS are generally assessed too optimistically.

The overall goal of this study was to assess the prospects of MAS for improving the level of insect resistance of tropical and subtropical maize germplasm based on reanalyses of published QTL experiments (Bohn et al. 1996, 1997; Groh et al. 1998; Khairallah et al. 1998). Our objectives were: to (1) determine the bias in estimates of QTL effects and p obtained from populations of $F_{2:3}$ lines using cross validation and validation with independent populations of RIL, and (2) to draw realistic conclusions about the prospects of MAS for increasing the level of leaf-feeding resistance against SWCB and SCB.

Materials and methods

QTL studies used for re-analyses

Four maize inbred lines were selected as parents: CML131, a highly susceptible, subtropical white dent line out of CIMMYT's population 42, was crossed to CML67, a highly resistant, tropical red/yellow semi-dent line from Antigua Group 2. Ki3, a tropical yellow flint line from Suwan1 with susceptibility to *Diatraea* spp., was crossed to CML139, a resistant, subtropical yellow semi-flint line selected out of Dominican Republic Group 1 and Antigua Group 2. For the first cross a total of 171 $F_{2:3}$ lines and 187 RILs, subsequently denoted as [CML131×CML67]-F2 and [CML131×CML67]-RIL, respectively, and for the second cross 475 $F_{2:3}$ lines and 158 RILs, subsequently denoted as [Ki3×CML139]-F2 and [Ki3×CML139]-RIL, were used for QTL mapping (see Table 1). The RILs of each cross were derived from another sample of F_2 plants. All field experiments with manual infestations of SWCB or SCB larvae were conducted at CIMMYT's experimental stations at Tlaltizapán and Poza Rica, Mexico, during 1991 to 1995. Each year-location or year-season combination was treated as an environment in the subsequent statistical analyses. For a detailed description of the QTL studies see Bohn et al. (1996 and 1997), Groh et al. (1998) and Khairallah et al. (1998).

For evaluating the level of antibiosis against SWCB and SCB, every plant was manually infested with 30 to 45 neonate SWCB or SCB larvae at the six- to eight-leaf stage (mid-whorl) by mixing freshly hatched larvae with maize-cob grits and applying the mixture into the plant whorl using a mechanical dispenser (Mihm 1983). Leaf feeding damage caused by the insect larvae was assessed on each infested plant 2 to 3 weeks after infestation using a leaf-damage rating scale from 1 (no visible leaf damage) to 10 (dead growing point, all leaves with long lesions) as described by Ortega et al. (1980) and Thome et al. (1992).

For each parental cross, separate linkage maps were constructed for the F_2 and RIL populations. Linkage maps for cross CML131×CML67 were based on 190 F_2 individuals and 98 RFLP marker loci, and 187 RILs and 136 RFLP marker loci. Linkage maps for cross Ki3×CML139 were constructed using 475 F_2 individuals and 128 RFLP marker loci, and 143 RILs and 146 RFLP marker loci. In total, 57% of the RFLP marker loci used in [CML131×CML67]-F2 were also employed for linkage-map construction in [CML131×CML67]-RIL, whereas 72% of RFLP marker loci used in [Ki3×CML139]-F2 were also employed for [Ki3×CML139]-RIL. Software package MAPMAKER (Lander et al. 1987) was employed for linkage-map construction.

Data analyses

QTL re-mapping and re-estimation of their effects for SCB and SWCB LDR for the whole data sets was performed as described by Groh et al. (1998) using means across environments with PLABQTL (Utz and Melchinger 1996), which employs interval mapping by the regression approach (Haley and Knott 1992) in combination with the use of selected markers as cofactors (Jansen and Stam 1994; Zeng 1994). The selection of cofactors by stepwise regression was described by Melchinger et al. (1998). For comparison with most preceding QTL mapping studies, a LOD threshold of 2.5 was chosen for declaring a putative QTL significant, ensuring a comparison-wise type-I error of P_c<0.0092 for the F_2 populations and of P_c<0.0032 for the populations of RILs. Estimates of QTL positions were obtained at the position where the LOD score assumed its maximum in the region under consideration. The proportion of the genotypic variance explained by all detected QTLs (p) was estimated from the ratio

$$\hat{p} = \frac{R^2_{adj}}{\hat{h}^2},$$

where R^2_{adj} is an estimator of the proportion of the phenotypic variance explained by all detected marker-QTL-marker associations (Utz et al. 2000) and $\hat{h}^2$ is the heritability of the respective trait on an entry mean basis (Hallauer and Miranda 1981).

Cross validation

For testing the effect of genotypic sampling, we applied a five-fold cross-validation procedure (CV/G) as described in detail by Utz et al. (2000). The whole data set (DS) containing the entry means across environment 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 the 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 permutating the respective subsets for ES and TS, five different CV/G runs are possible for the five-fold CV. To increase the precision of estimates of p, additional CV/G runs were generated by using 40 dif-

ferent randomizations for assigning genotypes to the respective subsamples, yielding a total of 200 replicated CV/G runs.

Estimates of p obtained from ES and TS were compared to determine the magnitude of bias in p due to genotypic sampling. Following Utz et al. (2000), the proportion of the genotypic variance explained by the detected QTLs 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:

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

Estimates of R^2_{adj} were devided by $\hat{h}^2$ to avoid the attenuation effect in error-in-variables models. Using a LOD threshold of 2.5, each CV/G run yielded different estimates for the number of QTLs, 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 relative bias in estimates of p_{ES} was calculated based on CV/G results as $(1-\tilde{p}_{TS.ES}/\tilde{p}_{ES})$. The average number of QTLs was determined as the mean across replicated CV/G runs. A more-detailed analysis was performed for putative QTLs for SWCB and SCB on chromosome 5. The precision of QTL positioning was determined by the relative frequency of detected QTLs for 2,000 replicated CV/G runs in 1-cM intervals along chromosome 5 from the ES with k=5. 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 ($\hat{a}_{TS}$) was determined by multiple regression based on: (1) the map positions of all QTLs detected in ES, and (2) the marker genotypes of the $F_{2:3}$ or RILs in TS at the respective flanking marker loci according to described procedures (Haley and Knott 1992; Utz and Melchinger 1996). Subsequently, the median $\tilde{a}_{TS}$ was calculated across all CV/G runs for a given position.

Validation with independent samples

The populations of RILs were used for independent validation of QTL effects and p estimates obtained with the populations of $F_{2:3}$ lines. The position of QTLs for SWCB and SCB LDR detected in populations of $F_{2:3}$ lines were transferred to their respective position on the RIL linkage maps based on the relative position of the QTLs in an interval built by the markers common to both populations as described in detail by Groh et al. (1998).

The same ES as in CV/G were used to predict genotypic values $Q_{VS.ES}$ for the validation set (VS). The adjusted squared correlation coefficient between $Q_{VS.ES}$ and the phenotypic entry means (Y_{VS}) from the [CML131×CML67]-RIL and the [Ki3×CML139]-RIL, divided by the heritability of the trait under study, served as an unbiased estimate of the genotypic variance explained by the putative QTLs detected in the ES:

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

Estimates of $p_{VS.ES}$ were calculated as the median $\tilde{p}_{VS.ES}$ of replicated runs. The relative bias in estimates of p_{ES} was calculated based on results obtained by IV as $(1-\tilde{p}_{VS.ES}/\tilde{p}_{ES})$.

Efficiency of MAS

The relative efficiency (*RE*) of MAS over CPS was determined based on the formula derived by Lande and Thompson (1990). It was assumed that molecular-marker scores can be recorded without errors, and selection intensities of MAS and CPS are of equal size. If selection is only performed on marker loci, the efficiency relative to CPS of the same selection intensity was calculated as $RE_p=\sqrt{p/h^2}$. For MAS, which combines phenotypic data and molecular marker information, *RE* was calculated as

$$RE_c = \sqrt{\frac{p}{h^2} + \frac{(1-p)^2}{1-h^2 p}}.$$

Results

Trait means, variances and heritabilities

Quantitative genetic parameters for SWCB and SCB leaf-feeding resistance of $F_{2:3}$ and RIL populations derived from crosses CML131×CML67 and Ki3×CML139 were presented in detail by Bohn et al. (1997), Groh et al. (1998), and Khairallah et al. (1998). The mean LDR across insect species, populations and generations varied between 4.9 and 6.9 (Table 1). For cross CML131×CML67, SWCB larvae feeding caused higher LDR values than SCB larvae feeding especially in the RIL. Genotypic variances ($\hat{\sigma}^2_g$) among $F_{2:3}$ lines and RILs were highly significant ($P<0.01$) for SWCB LDR and SCB LDR in both crosses. For [Ki3×CML139]-RIL, the $\hat{\sigma}^2_g$ of SWCB LDR was substantially smaller than for the other populations. Estimates of the genotype×environment interaction variance ($\hat{\sigma}^2_{ge}$) for LDR were highly significant ($P<0.01$) and of similar size for SWCB and SCB. In the $F_{2:3}$ populations, $\hat{\sigma}^2_{ge}$ was significantly larger than in the RIL populations for SWCB and SCB LDR. This resulted in greater $\hat{h}^2$ values for SWCB and SCB LDR in the RIL

Table 1 Information about the mapping populations used for the re-analysis of QTLs involved in the leaf-damage ratings (LDR) against tropical maize stem-borer species *D. grandiosella* (SWCB) and *D. saccharalis* (SCB) as well as means and variance components for LDR

Cross	Generation	Number of		Insect	Means		Variance components		Reference
		Families	Markers		$\bar{P}$[a]	$\bar{X}$	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_{ge}$	
					1–10 rating scale				
CML131×CML67	$F_{2:3}$	171	98	SWCB	6.6±0.1[b]	6.9±0.1	0.33±0.05**	0.20±0.04**	Bohn et al. 1997
				SCB	6.4±0.1	6.3±0.1	0.29±0.05**	0.22±0.04**	Bohn et al. 1997
	$F_{6:7}$	187	136	SWCB	6.0±0.3	6.3±0.0	0.22±0.03**	0.06±0.02**	Groh et al.1998
				SCB	4.7±0.2	4.9±0.1	0.36±0.05**	0.08±0.03**	Groh et al.1998
Ki3×CML139	$F_{2:3}$	475	128	SWCB	6.4±0.0	6.5±0.0	0.21±0.03**	0.63±0.03	Khairallah et al. 1998
	$F_{7:8}$	158	146	SWCB	6.1±0.2	5.9±0.1	0.11±0.03**	0.09±0.03**	Groh et al.1998

** Variance component was significant at the 0.01 probability level

[a] $\bar{P}$=mean of parents, $\bar{X}$=mean of population

[b] Standard errors are attached

Table 2 Comparison of results obtained from QTL studies performed with populations of $F_{2:3}$ and RILs derived from the crosses CML131×CML67 and Ki3×CML139 analyzed with composite interval mapping, five-fold cross validation, and independent validation (for details see text)

Item	Parameter estimate[b]	CML131×CML67				Ki3×CML139	
		SCB-LDR[a]		SWCB-LDR		SWCB-LDR	
		$F_{2:3}$	RIL	$F_{2:3}$	RIL	$F_{2:3}$	RIL
Data set (DS)	h^2	0.59	0.75	0.64	0.72	0.50	0.62
	No. of QTLs	10	5	5	10	11	11
	$\hat{p}_{DS}$ (%)	84.2	69.8	46.0	77.5	68.4	79.2
Estimation set (ES)	No. of QTLs	8.4	5.8	6.8	8.9	9.3	9.0
		1, 12[c]	1, 13	2, 14	2, 24	5, 14	3, 20
	$\tilde{p}_{ES}$ (%)	81.7	70.3	55.2	73.4	62.7	74.8
		60.3, 96.3[d]	54.6, 81.7	30.3, 78.3	56.2, 85.8	48.5, 75.4	51.5, 95.9
Test set (TS)	$\tilde{p}_{TS.ES}$ (%)	38.5	48.7	11.3	38.1	37.0	26.0
		9.5, 76.2	19.4, 75.1	1.4, 35.8	6.4, 67.5	17.1, 60.2	4.7, 65.0
Validation set (VS)	$\tilde{p}_{VS.ES}$ (%)	35.9		28.8		14.2	
		17.7, 52.1		10.1, 44.4		7.4, 22.8	

[a] LDR=leaf damage rating
[b] Number of QTL in ES calculated as the mean; $\tilde{p}_{ES}$, $\tilde{p}_{TS.ES}$, and $\tilde{p}_{VS.ES}$ denote the median across all cross-validation and validation runs
[c] Range of number of QTLs
[d] Percentiles of 5 and 95%

Table 3 Position of QTLs detected for SWCB and SCB leaf-damage ratings (LDR) and their respective additive effects determined using the whole data set ($\hat{a}_{DS}$), 200 cross-validation runs ($\hat{a}_{TS.ES}$), or an independent sample of RILs ($\hat{a}_{VS.ES}$)

Chr.[b]	Position	$\hat{a}_{DS}$	$\hat{a}_{TS.ES}$[a]				$\hat{a}_{VS.ES}$
			Median	(10, 90)	Percentile	Freq.[c]	
	cM		1-10 rating scale			%	1-10 rating sc.
			CML131×CML67				
SWCB LDR							
1	102	–0.25**	–0.26	–0.42	–0.03	3.0	–0.04
1	158	–0.24*	–0.09	–0.45	0.05	4.0	–0.14**
5	98	–0.26**	–0.20	–0.43	0.06	23.0	–0.10**
7	61	–0.25**	–0.23	–0.40	0.05	3.5	–0.10*
9	70	–0.34**	–0.37	–0.54	–0.16	8.0	–0.24**
SCB LDR							
1	163	–0.23**	–0.24	–0.58	0.23	6.0	–0.10*
2	24	–0.23**	–0.27	–0.87	0.33	5.0	–0.06
2	126	–0.15**	–0.22	–0.66	–0.01	14.5	–0.02
5	73	–0.28**	–0.28	–0.36	–0.07	5.5	–0.11*
5	135	–0.14**	–0.15	–0.33	0.11	20.0	–0.11*
7	51	–0.22**	–0.25	–0.49	–0.08	3.5	–0.15**
8	59	–0.26**	–0.15	–0.32	0.21	6.5	–0.15**
9	58	–0.24**	–0.32	–0.43	–0.08	5.5	–0.40**
9	97	–0.22**	–0.32	–0.53	–0.16	8.0	–0.07
10	84	–0.17**	–0.12	–0.37	0.11	22.5	–0.01
			Ki3×CML139				
SWCB LDR							
2	0	–0.01	0.02	–0.12	0.12	12.5	–0.06
3	124	–0.23**	–0.17	–0.35	–0.03	17.0	–0.08
3	188	–0.18**	–0.18	–0.32	–0.07	20.5	–0.07
4	16	0.19**	0.05	–0.20	0.32	3.0	0.06
5	4	–0.14**	–0.11	–0.17	0.00	6.0	–0.02
5	164	–0.20**	–0.21	–0.31	–0.12	9.0	–0.07
5	198	–0.03	–0.09	–0.28	0.10	13.0	0.05
6	8	0.24**	0.19	0.13	0.28	7.5	0.04
7	96	–0.12**	–0.11	–0.23	0.13	8.0	0.06
8	44	–0.17**	–0.18	–0.30	–0.10	8.5	–0.13*
9	132	–0.21**	–0.12	–0.26	–0.05	8.5	–0.19**

*, ** Significant at the 0.05 and 0.01 probability level, respectively
[a] Median and percentiles were calculated based on 200 CV runs
[b] Chr.=chromosome
[c] Freq.=frequency of QTL detection across 200 CV runs

populations than in the $F_{2:3}$ population of the cross CML131×CML67. The phenotypic correlation coefficient between SWCB LDR and SCB LDR was $\hat{r}_p$=0.62 (P<0.01) in the [CML131×CML67]-F2 and $\hat{r}_p$=0.76 (P<0.01) in the [CML131×CML67]-RIL.

QTL analyses

Using the entire DS, ten QTLs for SCB LDR and five QTLs for SWCB LDR were detected in the [CML131×CML67]-F2 (Table 2). In the [CML131×CML67]-RIL, five QTLs were detected for SCB LDR and ten QTLs for SWCB LDR. For SWCB LDR, 11 QTLs were found in the [Ki3×CML139]-F2 and the [Ki3×CML139]-RIL. Simultaneously, these QTLs explained between 46.0 to 84.2% of ($\hat{\sigma}_g^2$).

In the 200 ES, the mean number of detected QTLs varied from 5.8 to 9.3 for the SCB and SWCB LDR and the median $\tilde{p}_{ES}$ exceeded 55% across all mapping populations. For both traits $\tilde{p}_{ES}$ was smaller than the respective $\hat{p}_{DS}$ value in most populations, except for SCB LDR in the [CML131×CML67]-RIL and SWCB LDR in the [CML131×CML67]-F2. Cross validation resulted in $\tilde{p}_{TS.ES}$ values that were substantially reduced in comparison with $\tilde{p}_{ES}$ values. The reduction ranged from 30.1% for SCB LDR in the [CML131×CML67]-RIL to 79.6% for SWCB LDR in the [CML131×CML67]-F2.

The use of RILs as independent population samples for validating the QTL results obtained in the respective F_2 populations showed no consistent results across insect species and parental crosses. In cross CML131×CML67, estimates of $\tilde{p}_{TS.ES}$ and $\tilde{p}_{VS.ES}$ were of similar size for SCB LDR, whereas $\tilde{p}_{VS.ES}$ was substantially larger than $\tilde{p}_{TS.ES}$ for SWCB LDR. In contrast, $\tilde{p}_{VS.ES}$ was 45% smaller than $\tilde{p}_{TS.ES}$ in the cross Ki3×CML139.

Additive effects calculated in the DS ($\hat{a}_{DS}$) of [CML131×CML67]-F2 for SWCB and SCB LDR ranged from –0.34 to –0.14 (Table 3). Dominance effects were significant only for the second QTL on chromosome 1 for SWCB LDR. For [Ki3×CML139]-F2, the $\hat{a}_{DS}$ ranged from –0.23 to 0.24, with two QTLs on chromosomes 2 and 5 displaying significant (P<0.01) dominance. The $\tilde{a}_{TS.ES}$ values obtained by CV/G at the QTL positions determined with the DS varied between –0.37 and –0.09 for the [CML131×CML67]-F2 and between –0.21 to 0.19 for [Ki3×CML139]-F2. For half of the detected QTLs the range of $\tilde{a}_{TS.ES}$ estimates included negative, but also positive, values. Applying IV, four out of five QTLs for SWCB LDR and six out of ten QTLs for SCB LDR detected in the [CML131×CML67]-F2 yielded significant (P<0.05) $\hat{a}_{VS.ES}$ values. For the cross Ki3×CML139, only two QTLs detected in the F_2 population showed a significant (P<0.05) additive effect also in the independent population of RILs. In CML131×CML67, significant estimates of the absolute value of $a_{VS.ES}$, denoted as $|\hat{a}_{VS.ES}|$, were smaller than $|\hat{a}_{DS}|$ and $|\tilde{a}_{TS.ES}|$ for SWCB LDR QTLs, except for the QTL on chromosome 1. In contrast, $|\hat{a}_{VS.ES}|$ was smaller than $|\hat{a}_{DS}|$ for most of the SCB LDR QTLs but $|\hat{a}_{VS.ES}|$ was larger than $|\tilde{a}_{TS.ES}|$ for half of the SCB LDR QTLs. Significant values of $|\hat{a}_{VS.ES}|$ were smaller than $|\hat{a}_{DS}|$ and $|\tilde{a}_{TS.ES}|$ for SWCB LDR QTLs in Ki3×CML139.

Table 4 Relative efficiencies of marker-assisted selection (MAS) for SCB and SWCB leaf-feeding resistance in two tropical maize populations purely based on marker data or based on phenotypic and molecular data. Relative efficiencies were calculated with estimates of p obtained from composite interval mapping without cross-validation, with cross-validation, and independent validation

Item	Used $\hat{p}$	CML131×CML67		Ki3×CML139
		SCB	SWCB	SWCB
Pure MAS[a]	$\hat{p}_{DS}$[b]	1.15	0.91	1.17
	$\tilde{p}_{ES}$	1.08	1.05	1.12
	$\tilde{p}_{TS.ES}$	0.72	0.50	0.88
	$\tilde{p}_{VS.ES}$	0.75	0.72	0.53
Combined MAS	$\hat{p}_{DS}$	1.17	1.10	1.23
	$\tilde{p}_{ES}$	1.14	1.15	1.21
	$\tilde{p}_{TS.ES}$	1.04	1.03	1.11
	$\tilde{p}_{VS.ES}$	1.05	1.06	1.04

[a] Pure MAS=MAS based on marker data only; combined MAS=MAS based on phenotypic and marker data
[b] DS, data set; ES, estimation set, TS, test set; VS, validation set

Efficiency of MAS

Based on $\tilde{p}_{ES}$, estimates of *RE* ranged from 1.05 to 1.12 for MAS based on molecular-marker data, and from 1.14 to 1.21 for MAS combining phenotypic data with information on molecular markers (Table 4). In comparison to *RE* estimates based on $\tilde{p}_{ES}$, *RE* values of both MAS schemes dropped to a minimum of 0.5, if *RE* was calculated using $\tilde{p}_{TS.ES}$ and $\tilde{p}_{VS.ES}$ for cross the CML131×CML67. For cross Ki3×CML139, the reduction in *RE* based on $\tilde{p}_{VS.ES}$ was larger than that obtained by using $\tilde{p}_{TS.ES}$ across both MAS schemes.

Discussion

The improvement of the host plant resistance of tropical and subtropical maize germplasm against SWCB and SCB is a major breeding objective. Based on the estimated relative small number of genes involved in the leaf-feeding resistance against both insect pests, as well as the preponderance of additive gene action, recurrent selection procedures are useful tools for increasing the level of resistance (Smith et al. 1989). However, recurrent selection and screening techniques for evaluating insect resistance are laborious and time consuming. To overcome these problems, we have previously proposed to use MAS for those genomic regions that significantly improve insect resistance without adversely affecting other important agronomic traits (Bohn et al. 1996). The prospects of MAS to improve both SWCB and SCB resistance simultaneously were regarded to be high be-

cause of: (1) high values for $\hat{p}_{DS}$, (2) QTL×environment interactions of negligible size for most QTLs conferring resistance, (3) the high genotypic correlation between SWCB and SCB resistance, and (4) the advent of marker systems that are more-cost efficient than RFLPs. However, QTL effects and *p* were estimated from the same data as used for QTL detection (Bohn et al. 1997; Groh et al. 1998; Khairallah et al. 1998). Based on the results of recent publications (Melchinger et al. 1998; Utz et al. 2000), we concluded that the QTL effects reported for SWCB and SCB resistance and $\hat{p}_{DS}$ were upwardly biased and a re-analysis of these data with new methods was appropriate.

Two approaches can be applied to obtain less-biased estimates of QTL effects and *p*. Firstly, Utz et al. (2000) employed cross-validation to eliminate the bias due to model selection caused by genotypic and environmental sampling. Secondly, Lande and Thompson (1990) suggested the use of two independent samples derived from the same parental cross, one being used for QTL detection and the other subsequently for estimation of the QTL effects at the respective positions. In this study, both approaches were used to obtain realistic estimates of QTL effects and *p*.

Validation of QTL effects and *p*

Based on the whole data set, we detected 5 to 11 QTLs for insect resistance in populations of $F_{2:3}$ lines and RILs derived from the crosses CML131×CML67 and Ki3×CML139. In contrast, the number of QTLs detected for LDR varied between 1 and 24 across all 200 CV/G runs. Possible explanations for the varying subsets of QTL detected by CV/G are (1) the genotypic sampling performed by CV/G, and (2) the small size of the mapping populations (n<200, with a single exception) resulting in a low power of QTL detection. The latter was confirmed by the considerably smaller range of detected QTLs across all ES for the large [Ki3×CML139]-F2 mapping population (n=475). Our observations are in agreement with previous studies (Beavis 1998), which showed that the probability of identifying the same set of QTL across different samples of the same population is low, if the trait under study is governed by multiple genes with small effects and the mapping population sizes are small. As a consequence, for each ES a different statistical model for estimating the genetic effect of QTLs was selected, resulting in a wide range of p_{ES} estimates.

In most cases, $\tilde{p}_{ES}$ was smaller than the respective value of $\tilde{p}_{DS}$. This decrease was expected, because ES contains only 80% of the genotypes of the whole data set resulting in a reduced power of QTL detection. However, for SWCB LDR on average two additional QTLs were detected using ES, resulting in a $\tilde{p}_{ES}$ value that exceed $\tilde{p}_{DS}$ by 17%. The appearance of extra QTLs for SWCB can be explained by two putative QTLs that were right below the level of significance within the whole data set (on chromosomes 5 and 8, data not shown); with genotypic sampling in ES these QTLs were frequently above the critical LOD threshold of 2.5.

The inflation of $\tilde{p}_{ES}$ compared with $\tilde{p}_{TS.ES}$ was attributable to genotypic sampling (CV/G) and ranged from 30 to 80%. The relative bias found in the cross CML131×CML67 was larger in the $F_{2:3}$ families than in the RIL population for LDR of both insect species. This result can be explained by the larger h^2 estimates for LDR in RILs than for $F_{2:3}$ families. However, for the cross Ki3×CML139 the relative bias of estimation was larger for the RILs than for the population of $F_{2:3}$ lines. It can be hypothesized that this result was mainly caused by the contrasting number of lines evaluated for each generation ($F_{2:3}$=475, RILs=158). Similar to our study, Utz et al. (2000) evaluated the bias of *p* in testcrosses of 344 F_3 maize lines applying a five-fold CV/G. They found that $\tilde{p}_{TS.ES}$ was substantially reduced as compared to $\tilde{p}_{ES}$ and substantiated their findings by re-analyzing barley and maize QTL studies from the literature. Using the CV/G scheme, the bias ranged from 30 to 70%. However, the largest reduction in $\tilde{p}_{TS.ES}$ was observed by CV with simultaneous genotypic and environmental sampling (CV/GE). Therefore, Utz et al. (2000) recommended the CV/GE scheme to get unbiased estimates of *p*. However, in the case of small G×E interactions, they suggested that the use of CV/G is sufficient to assess the prospects of MAS. In our studies, SWCB and SCB resistance were evaluated in a small number of environments (U≤3) and QTL×E interactions were not significant for most LDR QTLs. Therefore, CV was restricted to CV/G. In addition, the results of Utz et al. (2000) showed that the difference between $\tilde{p}_{TS.ES}$ values based on CV/G and CV/GE were in most cases small, hence, a large part of the reduction found with CV/GE was attributable to genotypic sampling. Based on these findings, it can be concluded that in our study a significant proportion of the unknown bias of *p* for SWCB and SCB LDR was removed by CV/G.

Inconsistent results were observed if the QTL effects and *p* obtained for populations of $F_{2:3}$ lines were validated with an independently derived set of RILs from the same cross. For the population [CML131×CML67]-F2, the median $\tilde{p}_{VS.ES}$ for SCB LDR was of similar size as $\tilde{p}_{TS.ES}$ but about twice as large for SWCB LDR. In contrast, $\tilde{p}_{VS.ES}$ was markedly smaller than $\tilde{p}_{TS.ES}$ for SWCB LDR in the [Ki3×CML139]-F2. Several reasons may explain the observation that $\tilde{p}_{TS.ES}$ was larger than $\tilde{p}_{VS.ES}$. (1) Some QTLs detected in the F_2 populations were false positives. (2) The most-likely QTL position determined in the F_2 population was not the most-likely QTL position in the RIL, even though the QTL support intervals overlapped. (3) Linkage disequilibrium between marker loci and QTLs are reduced in higher selfing generations. (4) Dominance effects were included in the model for estimating QTL effects in the F_2 populations but not in the RIL populations. Even though the resistance against SWCB and SCB was mainly determined by additive effects, dominance may not be negligible in the inheritance of these traits (see Table 3, Bohn et al. 1996; Khairallah

et al. 1998). (5) F_2 and RIL populations were evaluated for their level of insect resistance in different environments and, therefore, environmental effects may contribute to the finding that $\tilde{p}_{TS.ES}$ was larger than $\tilde{p}_{VS.ES}$. However, using $F_{2:3}$ lines and RILs evaluated in different environments, we expect to obtain *p* estimates that are corrected for genotypic and environmental sampling. A possible reason why $\tilde{p}_{TS.ES}$ was smaller than $\tilde{p}_{VS.ES}$ is that for independent validation the same validation sample (RIL) was utilized throughout. Therefore, the observed results with independent validation can be considerably influenced by the specific RIL sample used for the VS.

Although it was possible to obtain more reliable $\hat{p}_E$ and R^2_{adj} values in this study by avoiding bias causing model selection, both estimates are still biased. The *p* and R^2_{adj} estimators are the ratios of two estimators, and it is a well established fact that ratios can be biased even if the nominator and denominator estimators are not biased. In contrast to Charcosset and Gallais (1996), who postulated that R^2_{adj} values are unbiased, calculations using Equation 27.93 for R^2 derived by Kendall and Stuart (1961) showed that R^2_{adj} values are still inflated. However, this bias can be considered to be small (Utz, unpublished data). In addition, the regression method used for QTL mapping underestimates the true R^2_{adj} value (Xu 1995). Therefore, it can be assumed that both biases do partly cancel each other out.

Applying 200 CV/G runs, the median additive effect was determined for each putative QTL position computed with DS. The $\hat{a}_{TS.ES}$ values obtained for each QTL position varied within a wide range. However, comparing a_{DS} estimates with $\tilde{a}_{TS.ES}$ no clear trend towards reduced additive effects was observed. In general, additive effects determined by IV were considerably smaller and showed a different ranking than the respective effects obtained with DS and CV/G. This reduction can be explained by genotypic and environmental sampling effects and QTL×environment interactions. In addition, all reasons leading to a reduction in $\hat{a}_{VS.ES}$ compared with $\tilde{a}_{TS.ES}$ apply here, because the additive effect of a QTL is associated with the partial R^2 value and, consequently, with *p* (Melchinger et al. 1998).

QTL position

Confidence intervals for QTL positions are large if small population sizes are used for QTL detection (van Ooijen 1992; Visscher et al. 1996). In order to gain information about the position of a QTL in a different ES, we adopted the concept of QTL intensity distributions proposed by Sillanpää and Arjas (1998) and Utz et al. (2000). Based on 2,000 CV/G runs, we produced QTL frequency distributions for SWCB and SCB leaf-damage ratings to determine the most-likely QTL position. The QTL frequency distributions for SWCB and SCB LDR followed approximately the LOD curves obtained with CIM for the whole data set. For leaf-damage ratings, two well-defined QTL frequency peaks were detected for SWCB and SCB on chromosome 5 (Fig. 1). The first QTLs for SWCB and SCB LDR are located in adjacent marker intervals and both QTL frequency distributions overlap. This result has two major effects on applying MAS. First, if the QTL position is not localized with high precision in the ES, it is unlikely to obtain unbiased estimates of the true genetic QTL effects in TS. Second, if the QTL for SWCB and the first QTL for SCB LDR have to be combined in one genotype by MAS, a large marker bracket has to be used (Bracket: Markers 3 and 8; interval length about 100 cM). However, if the marker bracket is large, the probability that one of the QTLs is lost due to recombination during successive generations of MAS is high.

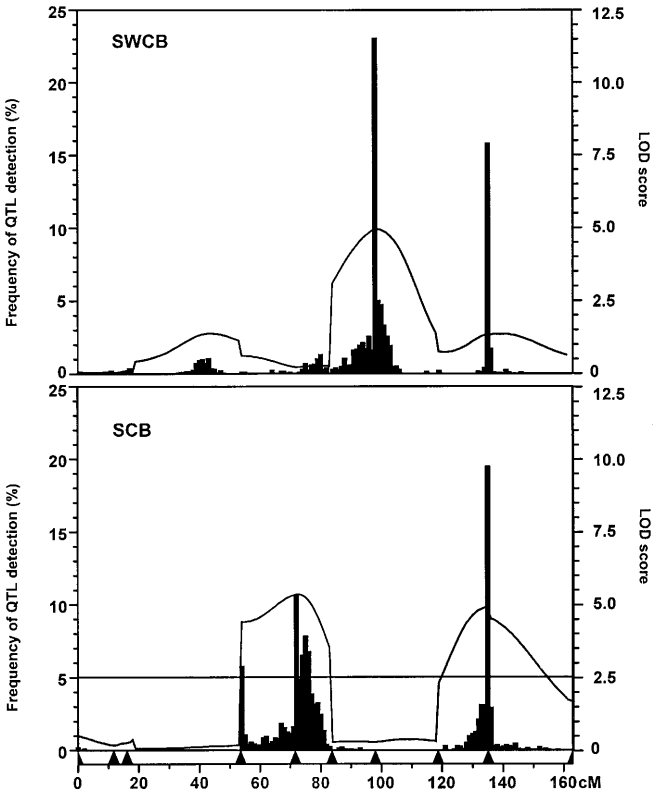

Fig. 1 QTL frequency distribution for SWCB and SCB leaf feeding resistance at 1cM intervals on chromosome 5 obtained from 2000 cross validation runs for the F_2 population derived from cross CML131×CML67. The solid line indicates the LOD curves determined from the entire data set using composite interval mapping. Marker positions are denoted by triangles

For both insect species a second frequency peak was detected at the same chromosomal position, even though no QTL was detected at this position for SWCB LDR with CIM using the DS (Fig. 1). For each random sample of genotypes used for calibration, a new set of cofactors was determined for CIM. In most instances, only one of the putative QTL positions for SWCB was significant, depending upon which marker was selected as a cofactor on chromosome 5.

Prospects of MAS

The relative efficiency of MAS was determined based on the new estimates of *p* obtained with CV/G and IV, and the assumptions that: (1) the selection intensity is the same for MAS solely based on molecular markers and MAS combining information on markers with data on phenotypic variation, implying equal costs for genotyping and phenotyping, and (2) marker data points are recorded without error. Values of *RE* were notably below 1 for $p_{TS.ES}$ and $p_{VS.ES}$ estimates, when MAS was solely based on molecular markers. If both molecular-marker information and phenotypic data were combined for MAS, *RE* values approached 1.0. This indicated that the efficiency was only slightly improved, when marker and phenotypic data were combined for MAS to increase the level of SWCB or SCB resistance. These results are in agreement with empirical studies from the literature. Early studies based on isozyme markers showed that MAS was as effective as CPS (Stuber et al. 1982; Frei et al. 1986; Stuber and Edwards 1986). Stromberg et al. (1994) found no significant differences between MAS and CPS for yield improvement. However, neither MAS nor CPS improved yield significantly. Eathington et al. (1997) studied the possibility to predict the testcross performance of $S_{4:5}$ lines by S_1 testcross data, net molecular score based on S_1 genotypes, and combined indices. They found S_1 phenotypes to be better for predicting $S_{4:5}$ testcross data than molecular markers.

In contrast to the *RE* estimate proposed by Lande and Thompson (1990), Knapp (1998) introduced an alternative efficiency measure for MAS. This measure predicts the effect of MAS based on the number of genotypes necessary to select at least one superior genotype. Applying simulations, Knapp (1998) showed that the efficiency of MAS was increased by reducing the number of progeny to be tested, if MAS was solely based on those markers with highly significant effects on the target trait. Therefore, Knapp (1998) proposed to base MAS on QTLs for which the most-accurate information is available. In order to obtain "bona fide" QTLs, he proposed to use high LOD thresholds for QTL detection. However, increasing the LOD threshold did not result in less-biased *p* estimates for SWCB and SCB LDR in our study (data not shown). Similar results were found by Utz et al. (2000). As an alternative to the "bona fide" QTL approach, we recommend to employ cross validation to select marker-QTL associations for MAS with the largest effect on the respective trait.

The key parameter for assessing the prospects of MAS is $p_{TS.ES}$. In order to obtain reliable and high $p_{TS.ES}$ values for quantitative traits, large population sizes (n>500) and a high number of test environments have to be employed. However, mandatory large-scale experiments are not an option for most breeders due to financial and logistic restrictions.

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