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Identification of QTLs for grain yield and grain-related traits of maize (*Zea mays* L.) using an AFLP map, different testers, and cofactor analysis

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Abstract We exploited the AFLP®[1] technique to map and characterise quantitative trait loci (QTLs) for grain yield and two grain-related traits of a maize segregating population. Two maize elite inbred lines were crossed to produce 229 F_2 individuals which were genotyped with 66 RFLP and 246 AFLP marker loci. By selfing the F_2 plants 229 F_3 lines were produced and subsequently crossed to two inbred testers (T1 and T2). Each series of testcrosses was evaluated in field trials for grain yield, dry matter concentration, and test weight. The efficiency of generating AFLP markers was substantially higher relative to RFLP markers in the same population, and the speed at which they were generated showed a great potential for application in marker-assisted selection. AFLP markers covered linkage group regions left uncovered by RFLPs; in particular at telomeric regions, previously almost devoided of markers. This increase of genome coverage afforded by the inclusion of the AFLPs revealed new QTL locations for all the traits investigated and allowed us to map telomeric QTLs with higher precision. The present study has also provided an opportunity to compare simple (SIM) and composite interval mapping (CIM) for QTL analysis. Our results indicated that the method of CIM employed in this study has greater power in the detection of QTLs, and provided more precise and accurate estimates of QTL positions and effects than SIM. For all traits and both testers we detected a total of 36 QTLs, of which only two were in common between testers. This suggested that the choice of a tester for identifying QTL alleles for use in improving an inbred is critical and that the expression of QTL alleles identified may be tester-specific.

Keywords AFLP markers · QTL mapping · Testcross performance · Composite interval mapping · *Zea mays* L.

Introduction

In the last decade the advent of molecular markers has greatly facilitated the systematic dissection of quantitatively inherited traits into their underlying Mendelian factors (for a review see Lee 1995). This has provided the tools to accelerate plant improvement for a variety of criteria, including yield, by the generation of fine-scale molecular genetic maps to undertake marker-assisted selection (MAS) and positional cloning (for reviews see Tanksley 1993; Lee 1995).

In maize, extensive genome mapping based on DNA restriction fragment length polymorphism (RFLP) markers has been accomplished (Coe et al. 1995; Davis et al. 1999, and references therein). These maps and their associated technology have been used successfully for a number of applications in genetic research and breeding, including quantitative trait locus (QTL) detection (Lee 1995; Khavkin and Coe 1997). However, the use of RFLPs in QTL analysis is an expensive and time-consuming process.

The development of the polymerase chain reaction (PCR) (Saiki et al. 1988) has expanded the repertoire and efficiency of available DNA marker systems, including the AFLP method (Vos et al. 1995). This procedure combines the restriction-site variation similar to that sampled in RFLPs with the exponential amplification of PCR-based marker systems. The major advantages of the AFLP technique over other DNA marker techniques in-

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[1] AFLP® is a registered trademark of Keygene, N.V.

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clude the detection of a large number of polymorphisms from a single PCR reaction without prior sequence information. This is possible within a very short period of time using small amounts of DNA, thus reducing expenses and expediting the construction of high-density linkage maps.

AFLP technology is considered an efficient approach for generating markers due to the high multiplex ratio obtained (Powell et al. 1996; Pejic et al. 1998). The potential of the AFLP method to rapidly create linkage maps has been recently documented in a wide range of plant species (Maheswaran et al. 1997; Alonso-Blanco et al. 1998; Castiglioni et al. 1998; Lu et al. 1998), including maize (Castiglioni et al. 1999; Vuylsteke et al. 1999), as well as in the enrichment of DNA markers near loci of interest for the map-based cloning of plant pathogen-resistance genes (Meksem et al. 1995; Thomas et al. 1995; Rouppe van der Voort et al. 1997).

The objectives of the present study were to investigate the use of the AFLP technique to: (1) determine the genomic location of QTLs affecting the genetic variation of grain yield and two grain-related traits in testcrosses of maize by using simple interval mapping and composite interval mapping approaches; (2) estimate the size of their genetic effects; (3) investigate the consistency of QTLs across testers and (4) explore the possibility of MAS for grain yield.

Materials and methods

Plant materials

The experimental materials were developed by crossing two elite maize inbred lines B73 and A7. These inbreds belong, respectively, to the “Stiff Stalk Synthetic” (BSSS) and “Lancaster Sure Crop” (LSC) heterotic groups. They show a high degree of DNA polymorphism (Livini et al. 1992) and their cross produces a highly heterotic hybrid. From this cross, 229 F_3 families were developed through two selfing generations with each F_3 line tracing back to a different F_2 plant. Each F_3 line was testcrossed to two tester lines, A1 and Mo17, which represent, respectively, the BSSS and LSC germplasms.

Field trials

Basic experimental procedures were as described earlier (Ajmone-Marsan et al. 1995). Briefly, the field trials were carried out according to randomized complete block designs with two replications at two locations in Northern Italy. Cultural conditions were kept as close as possible to the optimal growth conditions in order to achieve high yield levels. The two series of testcross (TC) progenies were evaluated for grain yield (t/ha at 15.5% grain moisture), dry matter concentration (% grain dry matter at harvest), and test weight (kg/hl measured at harvest). Test-weight data were collected at one location only. All data were subjected to standard analysis of variance. Components of variance for genotypes (σ^2_g), genotype-location interactions (σ^2_{gl}), and errors (σ^2_e), were obtained from expectations of mean squares. The standard errors of variance components were computed according to the method of Comstock and Moll (1963). Heritabilities (h^2) for F_3 lines were computed on an entry mean basis and confidence intervals on h^2 were obtained according to Knapp et al. (1985). Phenotypic (r_p) and genotypic (r_g) correlations between the performance of the two TC series were estimated using standard procedures (Mode and Robinson 1959).

DNA extraction

For DNA extraction, the seedlings of each F_3 family were grown in a growth chamber at 25°C with a 16-h photoperiod for 2 weeks. Genomic DNA was extracted from pools of 15–20 shoots of each F_3 family using the CTAB method (Shagai-Maroof et al. 1984).

AFLP analysis

The protocol adopted for the generation of AFLP markers was previously described (Vos et al. 1995). Briefly either *Eco*RI/*Mse*I or *Pst*I/*Mse*I restriction enzyme combinations were used. Eleven *Eco*RI/*Mse*I and nine *Pst*I/*Mse*I AFLP primer pairs were assayed. Adapters and primers used were reported previously (Castiglioni et al. 1999). Following the run, the gel was fixed, dried and exposed to Fujix phosphorimager screens for 16 h. Screens were scanned and visualised using a Fujix BAS-2000 Phosphorimager analysis system.

Scoring AFLP markers

For the analysis of complex AFLP fingerprinting patterns, we have used proprietary scoring software developed specifically for the analysis of AFLPs (Keygene N.V., Wageningen, The Netherlands). The software allows the identification and measurement of specific AFLP bands in a pixel image as produced by X-Ray scans or phosphoimager scans. Bands were scored codominantly and named as previously described (Castiglioni et al. 1999).

Linkage map construction

A linkage map for B73 × A7 was assembled by MAPMAKER (Lander et al. 1987) as previously reported (Castiglioni et al. 1999). Among the 312 RFLP and AFLP markers located on the B73 × A7 map, 195 evenly spaced markers belonging to the framework map, and corresponding approximately to a marker every 10 cM, were used for QTL analysis. Five gaps larger than 30 cM were still present on Chromosomes 1, 3, 4, 5, and 8. QTLs were assigned to centromeric and telomeric regions according to their position compared to region delimiting markers, as described in Castiglioni et al. (1999).

QTL analyses

QTL analyses were performed on mean values of each trait across environments for each experiment involving the two series of TC progenies and using the linkage information given in Fig. 1. For

Fig. 1 Linkage map of the ten maize chromosomes based on the F_2 mapping population derived from the cross of inbred lines B73 and A7. To define chromosome regions, RFLP probes which localise centromeric regions on the reference map by Davis et al. (1998), and in common with our map, have been selected (*underlined* markers). Map distances, on the left side of the bars, are in centimorgans (cM) calculated using the Haldane function. The position of QLTs identified with simple interval mapping (SIM) and composite interval mapping (CIM) are indicated in *white and black boxes*, respectively; *small and capital letters* reported within boxes refer to QTLs identified with testers T1 and T2, respectively. The presence of different QTLs on the same chromosome are indicated with the letters *a, b, c and A, B, C* for SIM and CIM analysis, respectively. *GY*: grain yield; *DW*: dry matter; *TW*: test weight ▶

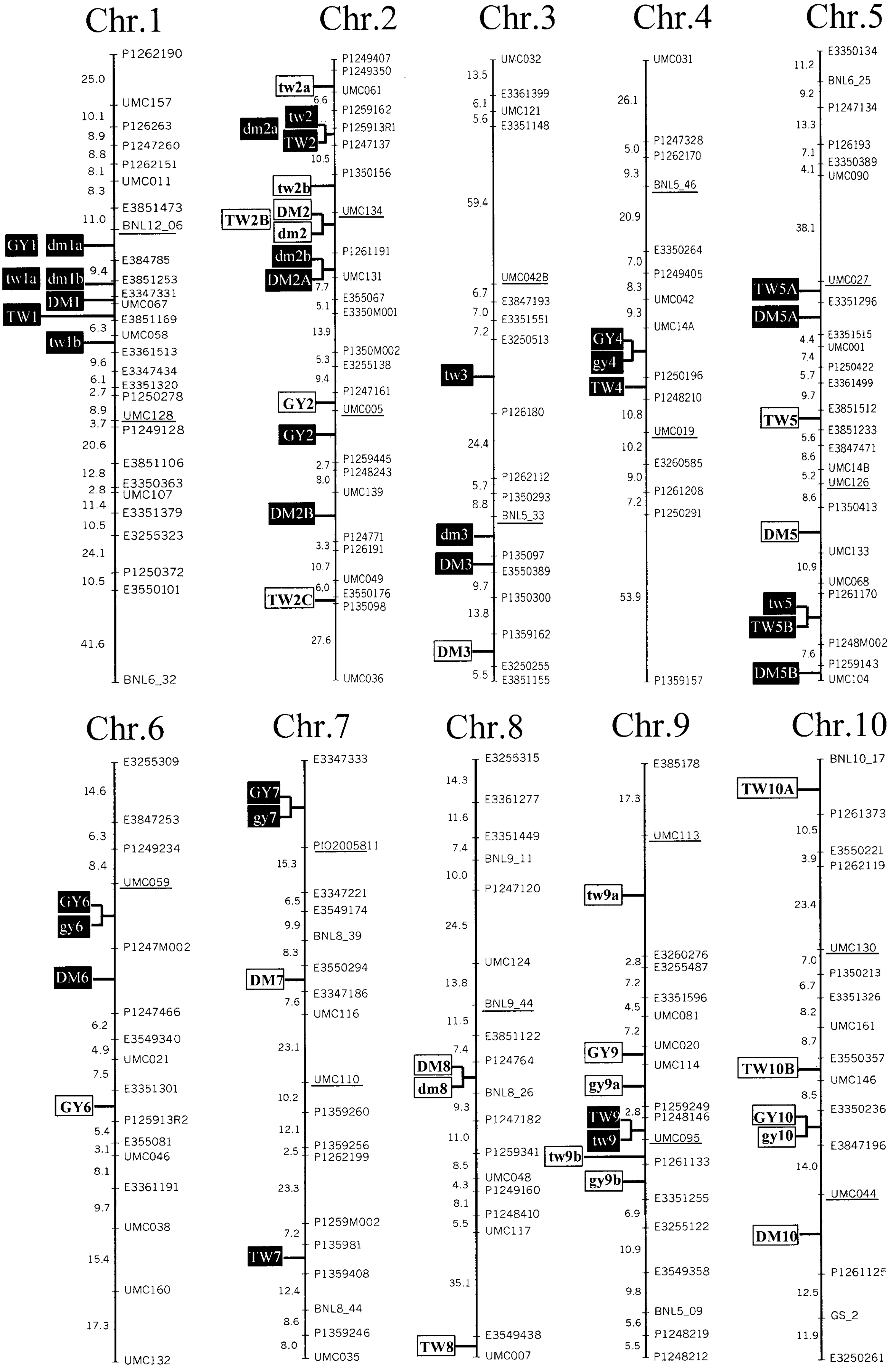

Fig. 1 Legend see page 231

the analysis of linkage between QTLs and molecular markers the simple interval mapping (SIM), proposed by Lander and Botstein (1989), and the composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) statistical methods were used. Following Cowen (1988), an additive genetic model is appropriate for the analysis of TC progenies, because TC progenies from F_2 plants heterozygous at the *i*th marker locus represent a 1:1 mixture of plants carrying alleles from parent P1 and P2.

For interval mapping (both simple and composite) the PLABQTL (program Ver. 1.1, Utz and Melchinger 1996) was employed. For SIM, the model used was $Y_i=b_o+b\ X_i^*+e_i$, where Y_i represents the mean phenotypic value of the i-th family in the TC, b_o is the mean value of the TC carrying the allele from parent P_1 at the marker under consideration; b is the average effect of substituting two alleles of parent P_2 for two alleles of parent P_1 at the marker under consideration; Xi* is an indicator variable taking values 0 or 1 depending on whether the line was homozygous for alleles from parent 1 or 2 and with probability depending on the genotypes of two flanking markers and the position being tested for presence of a QTL (Zeng 1994; Schön et al. 1994; Utz and Melchinger 1995; Bohn et al. 1996; Lübberstedt et al. 1997); e_i are residuals.

For CIM the model used was $Y_i=b_o+b\ X_i^*+\Sigma_k\ b_k\ X_{ik}+e_i$, where Y_i, b_o, b, X_i^*, and e_i are the same as in the SIM model; b_k is a partial regression coefficient of the phenotype Y on the k-th marker; X_{ik} has values 0 or 1 depending on the genotype at the k-th marker present in the i-th family (Zeng 1994; Utz and Melchinger 1995; Bohn et al. 1996). This procedure is a multiple regression procedure adjusting for background effects of markers other than those in the interval being tested. Following the suggestion of Utz and Melchinger (1995) we used as cofactors those markers which were associated with other important QTLs, and not tightly linked with the QTLs beings studied.

QTL effects (α_l) were considered fixed. In PLABQTL (Utz and Melchinger 1995), the phenotypic variance (σ^2_p) explained by a single QTL is calculated as the square of the partial correlation coefficient. This value is the coefficient of determination of the specified QTL and the phenotypic observations, keeping all other QTL effects fixed. The total phenotypic variance (σ^2_p) explained by all QTLs (R^2) was calculated by simultaneously fitting all QTLs detected for a specific trait by a multiple regression model.

For both SIM and CIM, intervals of 2 centiMorgans (cM) between markers and putative QTLs were tested. In these experiments, a LOD score of 3.0 was chosen for declaring a putative QTL. According to simulation studies (Jansen 1994; Zeng 1994) on sample sizes larger than the currently present population, we utilised in our analysis of TC progenies a χ^2 approximation with 2 *df* (1 *df* for the α-effect and 1 *df* for the position of the QTL) to compute a comparison-wise Type-I error probability. With a LOD threshold of 3.0 and 185 marker intervals, the comparison-wise Type-I error probability is <0.027. Estimates of QTL positions were obtained at the point where the LOD score assumed its maximum in the region under consideration. Under CIM, the computation of confidence intervals for the QTL position is still an unsolved problem (Visscher et al. 1996). Therefore, QTLs detected with different testers were regarded as common if their estimated map position was within a 20-cM distance and the estimated α-effects had an identical sign.

Results

Means and heritabilities

Tester Mo17 possessed a significantly ($P<0.01$) higher grain yield than tester A1 in the testcross means of F_3 lines across environments, but the reverse applied for dry matter concentration and test weight (Table 1). For all

Table 1 Estimates of means, variance components, heritabilities, and correlation coefficients of maize TC progenies from parent lines (P1 and P2) and 229 F_3 lines derived from cross P1×P2 and crossed to Mo17 (Exp. 1) and A1 (Exp. 2) for grain yield and grain related-traits

Experiment	Parameter	Grain yield (t/ha)	Dry matter concentration (%)	Test weight[a] (kg/hl)
1	Testcross means			
	P1 (3)[b]	9.18±0.21[c]	73.8±0.18	68.8±0.47
	P2 (3)	10.04±0.21	74.2±0.18	67.5±0.45
	P (6)[d]	9.61±0.15	74.0±0.12	68.2±0.32
	F_3 (229)	9.59±0.03	73.6±0.03	68.0±0.06
	Range of F_3 lines	7.80–12.04	71.9–75.6	66.6–70.3
	Variance components			
	σ^2_g[e]	0.54±0.08**	0.40±0.06**	1.86±0.21**
	σ^2_{gl}	0.26±0.07**	0.19±0.05**	–[f]
	σ^2_e	1.09±0.06	0.71±0.04	1.20±0.09
	Heritability			
	h^2	0.58	0.60	0.76
	90% C.I.[g] on h^2	0.48–0.65	0.51–0.67	0.66–0.83
2	Testcross means			
	P1 (3)	9.10±0.08[c]	75.5±0.17	69.6±0.43
	P2 (3)	9.65±0.08	75.0±0.17	69.6±0.43
	P (6)	9.38±0.06	75.2±0.12	69.9±0.30
	F_3 (229)	9.39±0.03	75.1±0.04	69.0±0.05
	Range of F_3 lines	7.74–11.39	72.8–77.8	–
	Variance components			
	σ^2_g	0.39±0.06**	0.82±0.11**	1.53±0.18**
	σ^2_{gl}	0.06±0.04	0.18±0.08*	–
	σ^2_e	0.86±0.05	1.54±0.09	1.11±0.09
	Heritability			
	h^2	0.62	0.63	0.73
	90% C.I. on h^2	0.54–0.69	0.55–0.70	0.63–0.81
	Correlation coefficients[h]			
	r_p	0.20**	0.44**	0.46**
	r_g	0.28±0.11	0.69±0.09	–

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively

[a] Measured at one location only

[b] Number of entries

[c] Standard errors are attached

[d] P: average of P1 and P2

[e] σ^2_g, σ^2_{gl}, σ^2_e: variance components for genotype, genotype-location interactions, and errors, respectively

[f] No estimate

[g] C.I.: confidence interval

[h] Phenotypic (r_p) and genotypic (r_g) correlation coefficients between TC progenies with T1 and T2

Table 2 Parameters associated with putative QTLs significantly affecting grain yield and grain-related traits estimated from TC progenies of 229 F_3 lines of maize with inbred tester Mo17

Chromosome	Marker		LOD score		σ^2_p exp[c]		Substitution effect (α)[d]	
	SIM[a]	CIM[b]	SIM	CIM	SIM	CIM	SIM	CIM
Grain yield					%		t/ha	
1	–	BNL12.06–E384785	–	3.9	–	7.8	–	0.64
2	–	UMC005–P1259445	–	4.3	–	8.6	–	–0.58
4	UMC14A–P1250196	UMC14A–P1250196	3.9	3.5	7.7	7.1	–0.58	–0.57
6	UMC059–P1247M002	UMC059–P1247M002	5.5	6.1	10.8	11.9	0.77	0.74
7	E3347333–Pio2005811	E3347333–Pio2005811	3.2	3.5	7.5	8.1	–0.64	–0.61
Total[e]					*21.1*	*30.1*	*1.99*	*3.14*
Dry matter concentration							%	
1	BNL12.06–E384785	–	3.9	–	7.7	–	–0.26	–
1	E385153–E3347331	E3347331–UMC067	4.2	7.5	8.2	14.3	–0.48	–0.62
2	P125913R–P1247137	–	4.6	–	9.0	–	–0.31	–
2	P1261191–UMC131	P1261191–UMC131	4.1	5.7	8.0	11.0	–0.50	–0.79
2	–	UMC139–P124771	–	3.3	–	6.6	–	0.53
3	BNL5.33–P135097	P135097–E3550389	4.4	4.8	8.8	9.7	0.74	0.69
5	–	E3351296–E3351515	–	8.3	–	15.7	–	0.66
5	–	P1259143–UMC104	–	4.3	–	8.4	–	–0.31
6	–	P1247M002–P1247466	–	4.0	–	7.8	–	–0.59
Total[e]					*26.1*	*40.1*	*2.29*	*4.19*
Test weight							kg/hl	
1	E385153–E3347331	–	4.7	–	9.3	–	–0.86	–
1	–	UMC067–E3851169	–	4.8	–	9.4	–	–0.98
1	UMC058–E3361513	–	3.1	–	6.2	–	–0.3	–
2	P125913R–P1247137	P125913R–P1247137	5.0	4.7	9.7	9.1	–1.1	–1.39
3	E3250513–P126180	–	3.3	–	6.4	–	0.99	–
4	–	P1250196–P1248210	–	5.0	–	9.7	–	1.00
5	–	UMC027–E3351296	–	4.1	–	8.1	–	1.05
5	P1261170–P1248M002	P1261170–P1248M002	5.1	4.4	9.9	8.7	1.18	1.08
7	–	P125981-P1359408	–	4.7	–	9.3	–	0.82
9	P1248146–UMC095	P1248146–UMC095	5.2	4.6	10.1	9.0	–1.15	–1.19
Total[e]					*35.1*	*41.4*	*5.58*	*7.51*

[a] Simple interval mapping

[b] Composite interval mapping

[c] Percent phenotypic variance explained by QTLs

[d] Average effect of substituting allele B73 by allele A7 in the TC progenies

[e] Estimates of total LOD scores and total variance explained from simultaneous fit of all putative QTLs for the respective traits, sum of effects obtained from absolute values

traits, the range in TC performance of F_3 lines significantly transgressed the TC means of the parental lines.

The TC performance of parents P1 and P2 in Experiment 1 differed significantly for grain yield ($P<0.01$) and test weight ($P<0.05$). In Experiment 2, the TC means of the two parental lines were significantly different for all traits except test weight ($P<0.01$ and 0.05, respectively). The comparison between the average TC performance of F_3 lines and parents in Experiment 1 yielded significant differences for dry matter concentration ($P<0.01$), the parents' mean being highest.

For all traits, genotypic (σ^2_g) differences among F_3 lines were highly significant for both testers. In Experiment 1, the genotype by location interaction (σ^2_{gl}) was consistently greater than zero ($P<0.01$) for the two traits that were evaluated at both locations, while in Exp. 2, σ^2_{gl} was significant ($P<0.05$) only for dry matter concentration. In all cases, estimates of σ^2_{gl} were considerably smaller than σ^2_g. Heritabilities (h^2) were moderately high for all traits in both experiments. For the traits evaluated at different locations, estimates ranged from 0.58 (grain yield) to 0.76 (test weight) in Experiment 1 and from 0.62 (grain yield) to 0.73 (test weight) in Experiment 2. Genotypic correlations (r_g) between the TC performance of F_3 lines for both experiments were moderately high for dry matter concentration while, for grain yield, correlations were only weak and exceeded corresponding estimates of r_p in all cases (Table 1).

QTL analyses

The search for molecular markers associated with the traits was performed by the two algorithms described earlier: SIM and CIM analyses. QTL analyses were carried out with genotypic data for the 195 AFLP marker loci and the linkage information published by Castiglioni et al. (1999). A detailed presentation of our QTL analys-

Table 3 Parameters associated with putative QTLs significantly affecting grain yield and grain-related traits estimates from TC progenies of 229 F_3 lines of maize with inbred tester A1

Chromosome	Marker		LOD score		σ^2_p exp[c]		Substitution effect (α)[d]	
	SIM[a]	CIM[b]	SIM	CIM	SIM	CIM	SIM	CIM
Grain yield					%		t/ha	
2	–	P1247161–UMC005	–	4.0	–	7.9	–	–0.66
6	–	E3351501–P125913Y	–	3.7	–	7.2	–	0.51
9	UMC114–P1259249	UMC020–UMC114	5.2	3.3	9.3	6.5	0.54	0.80
9	P1261133–E3351255	–	3.8	–	7.0	–	0.30	–
10	E3350236–E3847196	E3350236–E3847196	3.2	5.1	6.0	9.8	0.66	0.63
Total[e]					*18.0*	*27.2*	*1.50*	*2.60*
Dry matter concentration							%	
2	UMC134–P1261191	UMC134–P1261191	7.5	9.9	14.1	18.2	–1.31	–1.32
3	–	P1359162–E3250255	–	3.3	–	7.3	–	0.67
5	–	P1350413–UMC133	–	3.1	–	6.1	–	–0.62
7	–	E3550294–E3347186	–	3.6	–	7.1	–	0.57
8	P124764–NBL8.26	P124764–BNL8.26	3.6	9.6	7.0	17.8	0.94	1.03
10	–	UMC044–P1261125	–	3.5	–	7.3	–	0.68
Total[e]					*21.8*	*36.7*	*2.25*	*4.89*
Test weight							kg/hl	
2	P1249350–UMC061	P1249407–P1249350	4.5	3.3	9.6	8.7	–0.74	–0.71
2	P1350156–UMC134	UMC134–P1261191	6.9	4.7	13.1	9.1	–0.97	–1.31
2	–	E3550176–P135098	–	3.1	–	6.2	–	–0.70
5	–	E3851512–E3851233	–	4.5	–	8.8	–	0.88
8	–	E3549438–UMC007	–	4.0	–	7.8	–	–0.57
9	UMC113–E3260276	–	3.9	–	7.8	–	–0.49	–
9	UMC095–P1261133	–	4.5	–	8.8	–	–0.81	–
10	–	BNL10.17–P1261373	–	3.1	–	6.1	–	–0.54
10	–	E3550357–UMC146	–	3.7	–	7.3	–	0.93
Total[e]					*22.8*	*33.6*	*3.01*	*5.64*

[a] Simple interval mapping

[b] Composite interval mapping

[c] Percent phenotypic variance explained by QTLs

[d] Average effect of substituting allele B73 by allele A7 in the TC progenies

[e] Estimates of total LOD scores and total variance explained from simultanous fit of all putative QTLs for the respective traits, sum of effects obtained from absolute values

es in reference to their marker linkage map is given for means across environments because: (1) those QTLs are most important for breeding purposes, and (2) the results of individual environments are consistent with results from the combined data.

The results of QTL mapping for the three traits produced by the two analyses are summarised in Tables 2 and 3, respectively, for the two series of TC progenies, and in Figs. 2, 3 and 4, respectively, for grain yield, dry matter concentration and test weight. A complete list of the number and designation of the selected cofactors applied for each trait and tester can be obtained upon request from the authors.

Grain yield

With tester Mo17, three putative QTLs were identified for grain yield on Chromosome 4 (centromeric region), 6L and 7S, by SIM analysis (Table 2). LOD scores ranged from 3.2 (Chromosome 7) to 5.5 (Chromosome 6). Similarly in the TC with A1 three putative QTLs were identified by SIM analysis on Chromosome 9L (two QTLs), and 10 (centromeric region) (Table 3). In TC with A1, a LOD peak not reaching the significance threshold was noted on Chromosome 6 (Fig. 2); however, this suggestive QTL is apparently mapping to a different position when compared to the significant one identified in the Mo17 TC. The proportion of phenotypic variation explained (σ^2_p) by individual QTLs ranged from 7.5% to 10.8% in the Mo17 TC and from 6.0% to 9.3% in the A1 TC. A comparable proportion of σ^2_p was accounted for by the markers included in the models derived by the two TCs (21.1% in Mo17 and 18.0% in A1). Allele substitution effects ranged from 0.30 t/ha (telomeric QTL on Chromosome 9 in the A1 TC) to 0.77 t/ha (QTL on Chromosome 6 in the Mo17 TC), and the sum of absolute α-effects amounted to 1.99 and 1.50 t/ha in the Mo17 and A1 TCs, respectively.

Fig. 2 Results of the analysis performed for grain yield by simple interval mapping (*SIM*) and composite interval mapping (*CIM*) with tester T1 (Mo17) and tester T2 (A1). Markers are indicated by their progressive numbers on each chromosome. The *solid triangles*, ▲ and ⮝, denote the markers used as cofactors by CIM with testers T1 and T2, respectively

▶

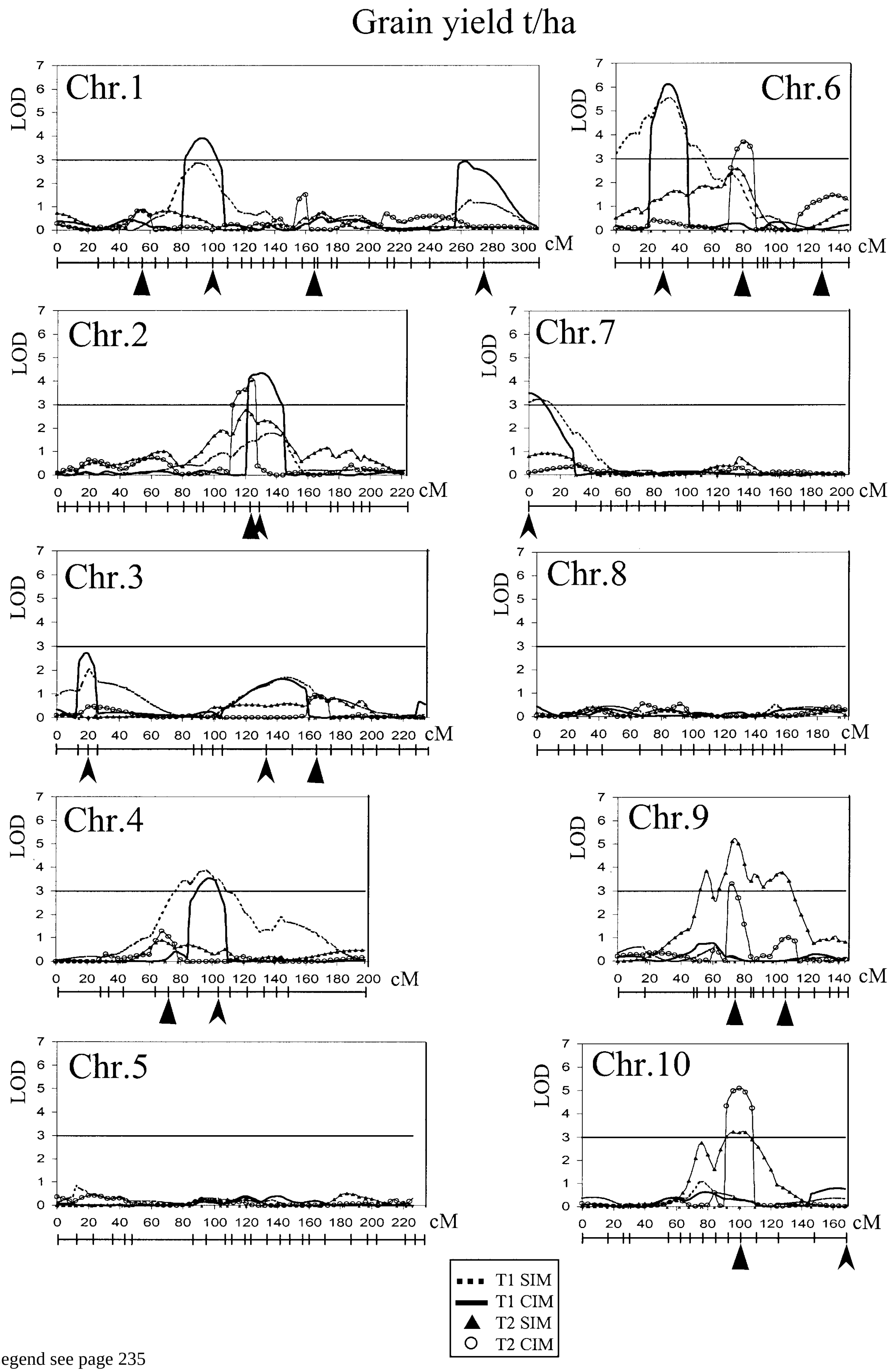

Fig. 2 Legend see page 235

In the Mo17 TC, the CIM algorithm confirmed effects and locations of the three QTLs identified by SIM on Chromosomes 4, 6, and 7 (Table 2). In addition two other putative QTLs were identified by CIM on Chromosome 1S and 2L, where SIM detected peaks below the significance threshold (Fig. 2). They had LOD scores of 3.9 and 4.3 and the respective QTLs accounted for 7.8% and 8.6% of σ^2_{p}. A model fitting all five QTLs simultaneously explained 30.1% of σ^2_p. The sum of absolute α-effects amounted approximately to 3 t/ha. In the A1 TC, the effects and location of the QTL on Chromosome 10 were confirmed (Table 3). Conversely, a single peak was revealed by CIM on Chromosome 9 (LOD 3.3; σ^2_p 6.5%); its location corresponds to that of the more-centromeric peak identified by SIM. The CIM analysis revealed two additional QTLs on Chromosomes 2L and 6L (LODs 4.0 and 3.7, and 7.9% and 7.2% of σ^2_{p}, respectively). In the same regions peaks that did not reach the significance threshold were identified by SIM (Fig. 2). The simultaneous fitting of all four QTLs explained 27.2% of σ^2_p. In agreement with data observed in the Mo17 TC, the total effect of allele substitution in the A1 testcross was higher with CIM than with SIM (2.60 vs 1.50 t/ha).

Although for grain yield CIM analysis revealed nine putative QTLs in the two series of TCs, only one of them, on Chromosome 2L, was in common between the two testers. The QTLs found in the two series of TCs on Chromosome 6, clearly have different locations by the criterion of non-overlapping support intervals. Alleles conferring superior grain yield alleles were contributed by P1 at only three QTLs, detected on Chromosomes 4 (centromeric region) and 7 (distal region) in the Mo17 TC, and on Chromosome 2 in the A1 TC, while for the other six QTLs the superior alleles were contributed by P2.

Dry matter concentration

For this trait SIM analysis identified five putative QTLs in the TC with Mo17 (Table 2). These were positioned on Chromosomes 1 (two QTLs in the centromeric region), 2 (two QTLs: on the short arm and in the centromeric region), and on 3L. For the A1 TC, two putative QTLs located on Chromosome 2 (centromeric region) and 8L were detected (Table 3). A single QTL shared between TCs was found on Chromosome 2 (centromeric region); for this QTL, LOD peaks were located in flanking marker brackets and showed an overlapping confidence interval (Fig. 3). For this trait, σ^2_p accounted for by individual loci ranged from 7.7% to 9.0% and from 7.0% and 14.1% in Mo17 and A1 TC, respectively. Despite the different number of significant QTLs identified in the two series of TC progenies, a similar percentage of the total σ^2_p was attributable to the QTL detected in the two TCs (26.1% in Mo17 and 21.8% in A1). Allele substitution effects ranged between 0.26% (QTL on Chromosome 1S in Mo17 TC) and 1.31% of dry matter concentration (QTL on Chromosome 2 in A1 TC) and summed up to 2.29% and 2.25% in the Mo17 and A1 TCs respectively.

The CIM analysis of data from the Mo17 TC confirmed the effects and locations of three out of the five QTLs identified by SIM (QTL on Chromosomes 1, 2, and 3), while the other two QTLs, detected on Chromosomes 1S and 2S by the SIM algorithm, decreased below the threshold level, and were not revealed by CIM (Table 2; Fig. 3). Moreover, four additional QTLs were detected by CIM, on Chromosome 1 (centromeric region), 5 (one QTL in the centromeric region and the other on 5L), and 6L. A simultaneous fit of all seven QTLs accounted for 40.1% of σ^2_p. The sum of absolute α-effects was 4.19%. In the A1 TC, effects and locations of the two QTLs identified by SIM were confirmed. In addition, CIM revealed four additional QTLs in regions where no significant QTLs were identified by SIM analysis. These QTLs were positioned on Chromosomes 3L, 5L and 10L, respectively, and in the centromeric region of Chromosome 7, individually conferring between 6.1% and 7.5% of $\sigma^2_{p,}$. The simultaneous fit of all six QTLs accounted for 36.7% of σ^2_p. The total effect of allele substitution in A1 testcrosses was higher with CIM than with SIM (4.89 vs. 2.25%).

For dry matter concentration, CIM analysis identified altogether 13 putative QTLs in the two TCs; only one of them was in common between the two testers (Chromosome 2 centromeric region). Some QTLs for dry matter concentration found on the same chromosome (i.e. on Chromosomes 3 and 5) in the two TC experiments, were mapped definitively to different locations. Alleles conferring superior dry matter concentration were contributed equally by P1 (6 loci) and P2 (7 loci).

Test weight

Six QTLs for grain test weight were detected in the Mo17 TC by means of SIM analysis (Table 2). LOD scores ranged from 3.1 to 5.2. The respective putative QTLs accounted for between 6.2% (Chromosome 1) and 10.1% (Chromosome 9) of σ^2_p . In the TC with A1, four QTLs for grain test weight were identified by means of SIM analysis (Table 3). Two QTLs were located on the centromeric region of Chromosome 2S and the long arm of Chromosome 9. The QTL peak in the centromeric region of Chromosome 9 found in the Mo17 TC is located in marker brackets flanking the QTL identified in the A1 TC (Fig. 4). The overlapping support intervals and the signs of allele substitution effects suggested the possibility of having identified the same QTL. σ^2_p contributed

Fig. 3 Results of the analysis performed for dry matter concentration by simple interval mapping (*SIM*) and composite interval mapping (*CIM*) with tester T1 (Mo17) and tester T2 (A1). Markers are indicated by their progressive numbers on each chromosome. The *solid triangles*, ▲ and ⮝, denote the markers used as cofactors by CIM with testers T1 and T2, respectively ▶

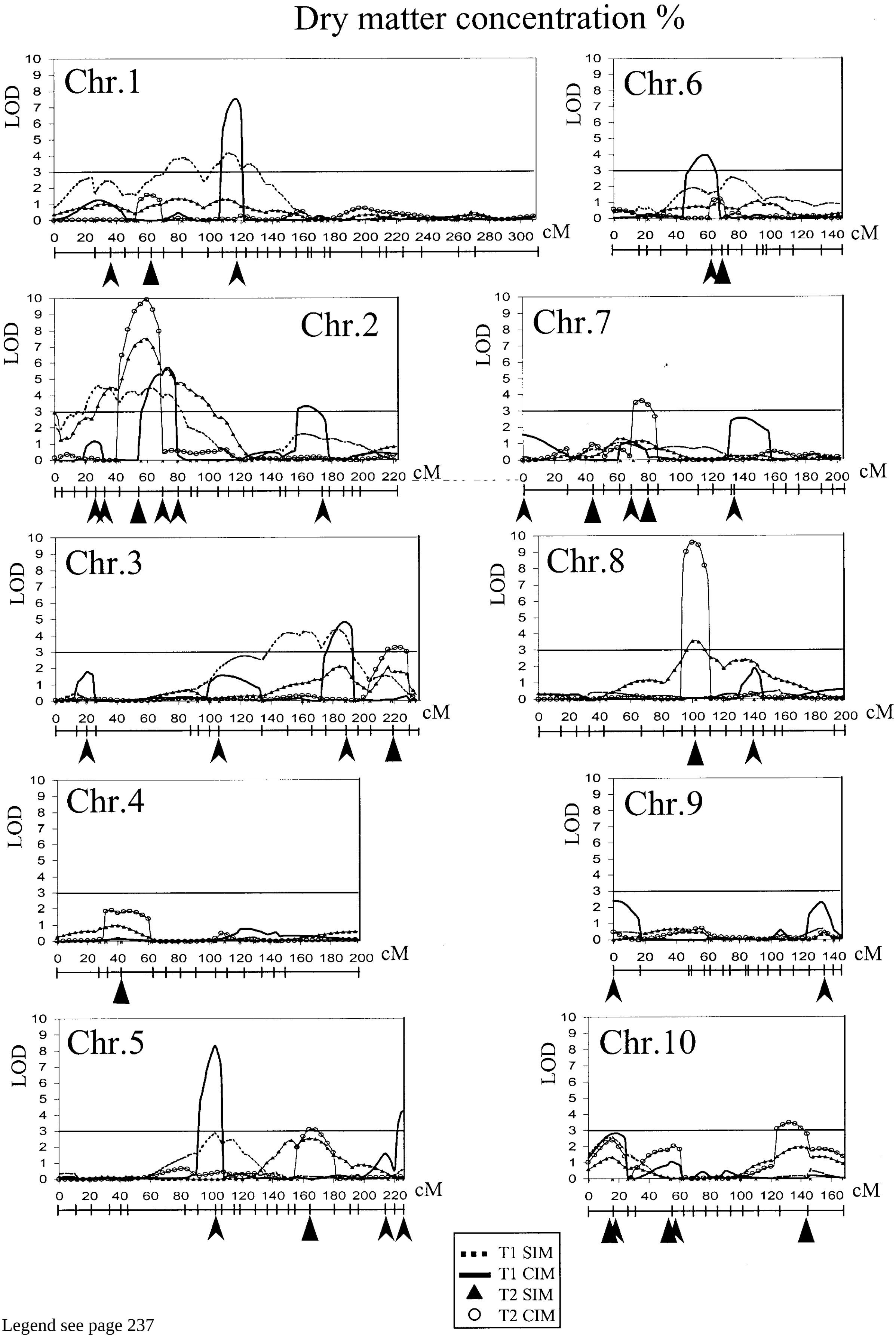

Fig. 3 Legend see page 237

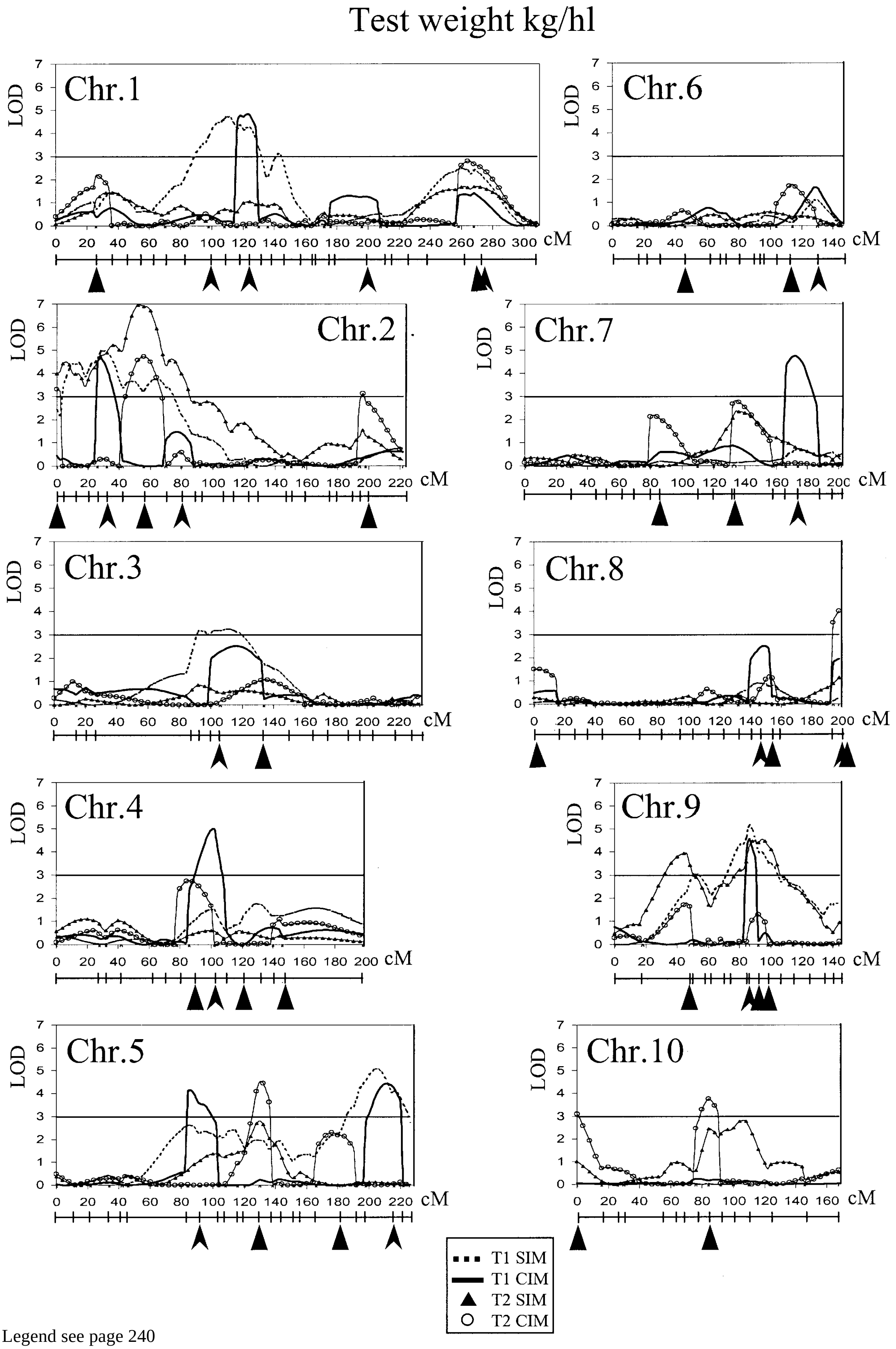

Fig. 4 Legend see page 240

Table 4 Number of putative QTLs detected and in common (∩) for maize TC progenies of F_3 lines with tester T1 and T2 by using an AFLP-integrated (this study) and RLFP (Ajmone Marsan et al. 1995) maps

Tester/marker	Grain yield		Dry matter concentration		Test weight		Sum	
	AFLP[a]	RFLP	AFLP	RFLP	AFLP	RFLP	AFLP	RFLP
T1	5	2	7	2	7	6	19	10
T2	4	4	6	2	7	2	17	8
T1 ∩ T2	1	1	1	1	0	2	2	4
T1 AFLP ∩ T1 RLFP	2		2		5		9	
T2 AFLP ∩ T2 RFLP	2		2		2		6	

[a] QTLs identified with CIM analysis

by a single QTL ranged between 7.8% and 13.1% in the A1 TC (Table 3). A higher percentage of the total σ^2_p (35.1%) was accounted for by QTLs in the Mo17 TC compared with the QTLs identified in the A1 TC (22.8%). Allele substitution effects ranged from –1.15 kg/hl (the QTL on Chromosome 9 in the Mo17 TC) to 1.18 kg/hl (the near-centromeric QTL on Chromosome 5 in the Mo17 TC) and summed up to 5.58 kg/hl and 3.01 kg/hl in the Mo17 and A1 TCs, respectively.

The CIM analysis confirmed the effects and locations of three of the six putative QTLs identified by SIM in Mo17 (Chromosomes 2, 5, and 9). In addition, CIM revealed the presence of a significant QTL on Chromosome 1 (9.4% of σ^2_p explained), located midway between two significant QTLs found by SIM analysis, while for Chromosome 3 (centromeric region) cofactor analysis decreased the QTL peak to below the significance threshold. Moreover, CIM identified three QTLs that were not detected by SIM on the centromeric regions of Chromosomes 4, 5 and 7L. These QTLs individually explained more than 8% of σ^2_p. Interestingly, in two of these regions (Chromosomes 4 and 7), CIM analysis detected putative QTLs, while SIM showed an almost flat LOD profile. The simultaneous fit of the seven QTLs accounted for 41.4% of σ^2_p. The sum of the absolute α-effect amounted to 7.51 kg/hl.

In the A1 TC, the effects and locations of the QTLs on Chromosome 2 were confirmed, although the LOD peak moved to flanking marker intervals compared to SIM (Table 3). Conversely, the peak profile revealed by CIM on Chromosome 9 was low; the two QTLs found on this chromosome by the SIM algorithm were not significant (Fig. 4). In addition, CIM analysis detected five additional putative QTLs, located on Chromosomes 2L, 5 (centromeric region), 8L and 10 (two QTLs: short arm and centromeric region); they explained individually between 6.1% and 9.1% of σ^2_p. The total effect of allele substitution in the A1 TC was 5.64 kg/hl. A model fitting simultaneously all six QTLs explained approximately 34% of σ^2_p. For test weight no QTL was consistently identified across testers by the mean of the CIM analysis. A possible common QTL could be tentatively identified on Chromosome 4; in fact, in the A1 TC a peak almost reaching the LOD threshold was found corresponding to a significant QTL identified in Mo17. Superior test-weight alleles were contributed almost equally by the P1 (eight loci) and P2 (six loci) parents.

◀ **Fig. 4** Results of the analysis performed for test weight by simple interval mapping (*SIM*) and composite interval mapping (*CIM*) with tester T1 (Mol17) and tester T2 (A1). Markers are indicated by their progressive numbers on each chromosome. The *solid triangles,* ▲ and ⮝, denote the markers used as cofactors by CIM with testers T1 and T2, respectively

Number of QTLs in common among the TCs

Table 4 summarises the number of putative QTLs detected, and in common, for maize TC progenies of the same set of F_3 lines with testers T1 and T2 by using AFLP maps and the previous RFLP maps (Ajmone-Marsan et al. 1995). It is evident that for all traits and both testers AFLP markers detect the majority of the QTLs in comparison to RFLP markers, and only a fraction of them were detected by both marker systems.

Discussion

Successful application of genetic markers in crop improvement depends on the availability of saturated linkage maps and the ability to analyse large populations efficiently and inexpensively (Dudley 1993; Tanksley 1993; Stuber 1994). The AFLP technique has been demonstrated to be a highly reliable and reproducible marker system because of the ability to screen large numbers of DNA samples, and to simultaneously detect a large number of marker loci in a single assay within a short period of time (Vos et al. 1995). In addition to the type of molecular marker used, the efficiency of QTL detection depends on several factors such as the biometrical approach employed for QTL mapping, the type of mapping population, the marker density, the sample size of the population, and the heritability of the traits under investigation (Melchinger 1998). Accordingly, as a first step in exploiting AFLPs in a maize genome mapping program, and in the long-term for MAS and positional cloning, we have used AFLPs to identify QTLs for grain yield and grain-related traits in maize F_3 lines using different testers and biometrical procedures.

The efficiency of generating AFLP markers appears to be substantially higher than that of RFLP mapping in the same population (Ajmone-Marsan et al. 1995), and the speed at which they can be generated shows a great potential for application in MAS. The appropriate selection of primer combinations that generates a high level of polymorphism with markers well-distributed over the genome plays a crucial role. Furthermore, AFLP markers cover linkage group regions left uncovered by RFLPs, in particular telomeric regions previously almost depleted of markers. The addition of telomeric markers to our map has permitted new QTLs to be identified, e.g. the QTL for grain yield on Chromosome 7, and allowed us to map telomeric QTLs more accurately. Without telomeric markers, the QTLs affecting grain yield on Chromosome 6 were assumed to be the same in the two TCs whereas, by adding telomeric markers, the QTL moves clearly in a different position in the A1 TC, suggesting that this QTL does not correspond to the one detected in the Mo17 TC. By increasing the marker density to below 20 cM the power of QTL detection in early generations is only marginally improved (Darvasi et al. 1993). Furthermore, although simulation studies have indicated that population size and heritability appeared to be major determinants of the power of QTL detection (Beavis 1994; Utz and Melchinger 1994), a higher marker density filling gaps in the RFLP map improves the accuracy of QTL mapping, as reported in the current study. This can be useful for the resolution of linked QTLs, especially in advanced generations, because of increased opportunities for recombination to be detected.

The present study provided an opportunity to compare different statistical methodologies for QTL mapping, i.e. simple interval mapping (SIM; Lander and Botstein 1989) and the more recently described composite interval mapping (CIM; Jansen and Stam 1994; Zeng 1994). The advantage of CIM is that, when testing for the putative QTL in an interval, one uses other markers as covariates to control for other QTLs to reduce the residual variance, thus improving the detection power. Both methods rely on the same data set and employ the same LOD threshold for QTL detection, providing a suitable comparison. According to theoretical considerations and simulation results (Jansen and Stam 1994; Utz and Melchinger 1994; Zeng 1994) the CIM approach as employed in this study should have greater power for the detection of QTLs, and provides more precise and accurate estimates of QTL positions and effects, than SIM. Our results are in good agreement with this expectation, although further research is needed to verify these observations. By the use of selected cofactors, which absorb a major part of the background noise due to other putative QTLs, CIM has allowed the detection of a larger number of QTLs. In some cases CIM also simply increased existing LOD peaks beyond the threshold values (i.e. peaks on Chromosome 5 for dry matter concentraton). In other situations CIM detected significant QTLs where SIM LOD profiles were almost flat (e.g. the test-weight QTL on Chromosome 4 in Mo17 TC). Furthermore, CIM reduces the significance of QTLs overestimated by SIM (e.g. the test-weight QTL Chromosome 3, Mo17 TC), In addition, the R^2 values for the simultaneous fit were always higher with CIM and showed a higher estimated value of substitution effects. Hence, advanced statistical methods promise to make an important contribution for improving the prospects of MAS without any additional cost.

Zehr et al. (1992) recognized that for marker-QTL associations to be most useful in plant breeding, they should be consistent across populations within a heterotic group, and across different genetic backgrounds of inbred line testers. The experimental mating design adopted in our experiment was based on two different tester lines. In this study we found that QTLs revealed by one tester may not be detected with the second one. These findings indicate that the allelic compositions of a tester line determine whether a QTL segregating in a population will be detected. In fact, the number of QTLs associated with grain yield and yield-related traits detected in this study largely depends on the tester under study. The differential effect of the two testers on TC performance is confirmed by the intermediate genotypic and phenotypic correlation coefficients for all traits of the TC progenies. These findings are in good agreement with previous studies reporting that the identification of QTLs in testcrosses is determined by the variance created by the cross and the line by tester interaction of alleles (Schön et al. 1994; Ajmone Marsan et al. 1995; Melchinger et al. 1998).

The identification of only a few common QTLs between both testers observed for grain yield and grain-related traits can be explained by several causes, the most important being related to gene action. Studies on maize grain yield exhibited a high degree of dominance (Hallauer and Miranda 1988); in addition, further studies have indicated a large proportion of QTLs with dominance and overdominance or pseudo-overdominance effects (Stuber et al. 1992; Beavis et al. 1994; Bohn et al. 1996; Cockerham and Zeng 1996). Therefore, inconsistent QTL results among testers can be explained by a masking effect of the tester allele. If a QTL is detected for tester T1, but tester T2 carries an allele fully dominant over the alleles carried by P1 and P2, no QTL will be detected in the TC progenies. Overdominance of the parental line over tester alleles can also lead to divergent results between two TC experiments unless both testers carry the same allele. For grain yield, dominance or overdominance was found in maize to be the most usual mode of gene action (Abler et al. 1991; Stuber et al. 1992; Veldboom et al. 1994; Bohn et al. 1996), while no or only a few significant epistatic interactions were detected (Stuber et al. 1992; Schön et al. 1994; Melchinger et al. 1998). From this point of view, it can be stressed in agreement with the theoretical indications proposed by Hull (1947) that the most efficient tester for evaluating maize lines would be the one that is homozygous recessive at all loci. However, experimental evidence has indicated that an unrelated elite-line tester was as effective as the related low-performance testers (Hallauer 1990). Another reason for inconsistency across TCs is the low power of QTL detection.

In conclusion, our results and those reported by other authors (Melchinger et al. 1998) suggest that the choice of tester for identifying QTL alleles for use in improving an inbred is critical and that the expression of QTL alleles identified may be tester-specific. This would require an evaluation of TC progenies with several testers, unless the tester is definitively a fixed parent of the hybrid, as is often required for applied breeding programs. Although the use of MAS seems a promising approach, its application may become quite expensive if QTL expression is tester-specific; this suggests that the use of MAS should be evaluated before starting an applied breeding program. The successful transfer of QTLs for grain yield by MAS, resulting in "enhanced" elite lines of maize with improved testcross performance, was shown by Stuber (1994).

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