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Mapping quantitative trait loci controlling silking date in a diallel cross among four lines of maize

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Abstract We describe and apply an interval mapping method for quantitative trait locus (QTL) detection using F_3 and testcross progenies derived from F_2 populations obtained from a diallel cross among four elite lines of maize. Linear model-based procedures were used for the test and estimation of putative QTL effects together with genetic interactions including epistasis. We mapped QTL associated with silking date and explored their genetic effects. Ten QTL were detected, and these explained more than 40% of the phenotypic variance. Most of these QTL had consistent and stable effects among genetic backgrounds and did not show significant epistasis. QTL-by-environment interaction was important for four QTL and was essentially due to changes in magnitude of allelic effects. These results show the efficiency of our method in several genetic situations as well as the power of the diallel design in detecting QTL simultaneously over several populations.

Keys words QTL · RFLP markers · Interval mapping · Diallel · Maize

Introduction

Many powerful methods using information from pairs of neighbouring markers have been proposed for the mapping of quantitative trait loci (QTL) in classical designs. These designs usually involve segregating populations derived from an initial cross between two inbred lines. We have shown (Rebaï et al. 1995) that the interval mapping maximum likelihood method of Lander and Botstein (1989) and the linear approach (Knapp et al. 1990; Haley and Knott 1992) have similar powers for large population sizes. However, the linear approach provides models which are easier to generalize to complex experimental designs.

The detection and mapping of QTL have been reported in maize for several agronomic traits (Stuber et al. 1992; Koester et al. 1993; Schön et al. 1994 and many others). These estimates of QTL effects are based on a range of reference populations derived from biparental crosses between elite lines. As one might expect, the use of several connected populations deriving from crosses between more than two lines provides an interesting and a powerful approach by which to study the stability of QTL in different genetic backgrounds (Rebaï et al. 1994a). For instance, one can use populations derived from diallel or factorial mating designs to characterize QTL effects over a large set of related populations and study their consistency and their stability among these populations.

In this paper we apply the interval mapping method developed by Rebaï et al. (1994a) to detect QTL for silking date in maize using populations derived from crosses between four inbreds lines. Results are discussed, and some refinements are proposed for the resolution of QTL and the dissection of their effects.

Materials and methods

Experimental procedure

The experimental materials were developed by first intercrossing four elite maize inbred lines (denoted L_1 to L_4) in a diallel scheme with no selfings or reciprocals. Lines were chosen from different combining ability groups: "flint European" (L_1, L_3) and "American dent" (L_2, L_4). Six F_2 populations were obtained with 100 individuals in each, except for the crosses $L_1 \times L_2$ and $L_3 \times L_4$ where 200

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individuals were derived. The 800 F_3 families obtained by selfing each F_2 individual were scored for several characters and crossed to the two non-parental lines as testers (e.g. F_3 from $L_1 \times L_2$ are crossed with L_3 and L_4). Testcross progenies (denoted TC), so obtained, were phenotyped in field evaluations in four environments in 1993. A total of 1,600 progenies for the 12 TC hybrid types were grown in each of the locations in a randomized complete block design. Means adjusted for block effects were then calculated using an analysis of variance model. Locations (denoted E1 to E4) were all in northern France. Plants in E1 were not irrigated and suffered from drought. Earliness was evaluated by the silking date in days from January 1 and measured on both F_3 and TC progenies. F_3 populations will be denoted as P_{ij}, the indices being relative to the parents, and TC as P_{ijt}, with index t being relative to the tester.

Genotypes of F_2 individuals were scored for 101 restriction fragment length polymorphism (RFLP) and eight isozyme loci at Rustica Prograin Génétique, Toulouse laboratory using techniques reported by Helentjaris et al. (1985) and Stuber et al. (1988). The RFLP markers used are public probes from UMC and BNL libraries (see e.g. Gardiner et al. 1993).

Linkage analysis of genetic markers

There are basically two alternatives for the building of a marker linkage map using simultaneous information from the six F_2 populations. The first is an interactive approach using MAPMAKER (Lander et al. 1987) as described by Beavis and Grant (1991). The second consists of employing the JOINMAP software (Stam 1993), which is a satisfactory tool for the construction of integrated genetic maps using data from several populations. This latter procedure is the more convenient and is a good compromise between statistical rigour and computational speed and was used in our study. However, the ordering of markers in linkage groups was controlled by likelihood computations in MAPMAKER to guarantee their optimality. Linkage groups were determined using pairwise analyses of JOINMAP with a threshold of 6.0 for the test of the independence of pairs of markers. Ten linkage groups were finally obtained, and 2 markers remained unmapped.

QTL analysis using interval mapping

The approach is described in Rebaï et al. (1994a), but the main ideas are summarized here. Consider an F_2 population derived from the cross $L_i \times L_j$ in which marker alleles are indexed in i and j, respectively. Consider 2 linked markers A and B and a putative QTL Q between them, then we have nine marker classes with expectations (θ_l, $l = 1..9$) that can be expressed as linear functions of QTL parameters. The QTL is assumed to have four alleles, one for each of the four parental lines, so that in the six F_2 populations we have four homozygous and six heterozygous QTL genotypes. The parameters involved in the model are: $\mu_{ij(t)}$ (6 and 12 parameters in the F_3 and TC, respectively), the genetic background-dependent mean of the cross $ij(t)$ (index t is for the tester); a_i, the additive effect of the allele Q_i of the QTL (4 parameters); and d_{ij} is the dominance effect between Q_i and Q_j (6 parameters). We have:

$$\theta_l = \mu_{ij} + \alpha_1 a_i + (2 - \alpha_1) a_j + \alpha_2 d_{ij} \quad \text{for } F_3$$

$$\theta_l = \mu_{ijt} + \alpha_3 (a_i + d_{it}) + (1 - \alpha_3)(a_j + d_{jt}) + a_t \quad \text{for TC}$$

where the coefficients α are nonlinear functions of the recombination rates between the markers and the QTL and can be easily expressed in terms of 2 parameters, s and t, defined by: $s = r_1 r_2/(1 - p)$, $t = r_1(1 - r_2)/p$ with $p = r_1 + r_2 - 2r_1 r_2$. r_1, r_2 and p are recombination rates between loci $A - Q$, $Q - B$ and $A - B$, respectively. If we suppose that p is known from the linkage map we have only 1 parameter of position (e.g. r_1), which we denote by x. Then, we get: $s = x(p - x)/(1 - p)(1 - 2x)$ and $t = x(1 - p - x)/p(1 - 2x)$ where x characterizes the test position within the interval considered relative to the left marker. The general model, for F_3 progenies, is then:

$$Y_{ijk} = \sum_{l=1}^{9} \theta_l g_l + e_{ijk}. \tag{1}$$

where $Y_{ijk.}$ is the phenotypic mean of F_3 individuals coming from the k^{th} F_2 individual derived form the cross $L_i \times L_j$; $e_{ijk.}$ is the error term of expectation 0 and variance σ^2, including environmental and other QTL effects; g_l are variables indexing the marker classes ($g_l = 1$ if the individual belongs to the class l, and 0 otherwise). The model for TC progenies is equivalent but with different expressions of θ_l.

At every position, model 1 is linear and ordinary least squares could be used to estimate the parameters. We have a total of 16 and 22 parameters where only 15 and 17 are estimable in F_3 and TC, respectively. All $\mu_{ij(t)}$ and three a_i (e.g. a_1, a_2 and a_3) are estimable for both progenies, and we use the constraint $\sum_{i=1}^{4} a_i = 0$. For dominance, there are six estimable d_{ij} in F_3 but only two d_{it} in TC because only the six heterozygous QTL genotypes are observed in TC progenies. For these progenies the following constraints were used:

$$\sum_{j=1 \neq i}^{4} d_{ij} = 0 \quad \text{for each } i = 1.4$$

Note that d_{ij} and d_{it} do not have the same meaning. d_{it} represents an interaction between the effects of F_3 alleles and the tester alleles. It is estimable only because the testers are also involved as parents in the initial diallel cross.

In Rebaï and Goffinet (1993, 1996) we proposed to use two tests for the presence of QTL. The first (noted T_1) is a global effects test (additivity and dominance), and the second (noted T_2) is a restricted test which tests only additive effects assuming the absence of dominance. We have then shown that T_2 could be more powerful than T_1 if dominance effects are small relative to additive ones. Both tests will be used in the analyses in this paper.

Algorithm and programming

At each testing position the models described could be written as: $Y = X\beta + e$, where Y is the $(n,1)$ vector of observations, X is the (n, r) incidence matrix of the model, β is the $(r, 1)$ vector of parameters and e is the $(n, 1)$ vector of residuals supposed to have a normal distribution with mean $\mathbf{0}$ and covariance matrix $\sigma^2 \mathbf{I}$, X and β can be decomposed as: $X = [X_0 \,|\, X_1 \,|\, X_2]$ and $\beta' = [\beta'_0 \,|\, \beta'_1, |\, \beta'_2]$ where β_0, β_1 and β_2 are vectors of $\mu_{ij(t)}$, a_i and d_{ij}, respectively, and X_0, X_1 and X_2 the corresponding submatrices. Elements of X_0 are 0 or 1 according to the cross to which the individual belongs, and those of X_1 and X_2 are coefficients of the a_i and d_{ij} which are calculated at each position x according to the marker interval considered. Tests and estimations are then computed as described in Rebaï et al. (1994a). As four parent lines are involved, two or three of these could share the same allele at any given locus. Thus, a significant number of markers would not be polymorphic in some crosses. When this is the case, i.e. when 1 (or both) of the markers flanking a given position is not informative for some individual (genotype unknown or missing), the elements of X will be calculated using the genotype(s) of the closest informative marker(s) to the position under study. If the non-informative marker is the first or the last on the chromosome, expectations of individuals concerned are written only for the three classes of the closest informative marker. It follows that, at every position in the genome, the full model could be applied and all parameters remain estimable but with a variable quality of estimation (sampling variance) depending on the density of markers and their polymorphism. A more precise description of this algorithm, including the possible use of dominant markers, is given in Rebaï (1995).

The linear-based interval mapping method already described was programmed using the Interactive Matrix Language (IML) of the SAS institute (1985) which permits easy calculations of test statistics and parameter estimates using standard matrix algebra. Test profiles are then drawn according to the testing position on the chromosome, and a QTL is declared when the test statistic (T_1 or T_2) exceeds a predetermined threshold. Thresholds for the tests used (at the 1% significance level) were calculated for each chromosome using the marker map and the approximations described in Rebaï et al. (1994b). The likely QTL location is defined by the position corresponding to the maximum value of the test statistic, and QTL effects are given for that position. Support intervals were obtained as proposed by Lander and Botstein (1989), by taking, as bounds, the points which correspond to the equivalent of one LOD test unit under the maximum. This procedure gives quite good confidence intervals (near 95%) for QTL with large effects (Mangin et al. 1994). The global effect of the QTL could be expressed using the partial coefficient of determination r^2 of the QTL parameters in the model. The r^2 could be expressed as the ratio between sum of squares due to the QTL and the total sum of squares (adjusted to the means $\mu_{ij(t)}$) or equivalently as:

$$r_2 = \frac{\sigma_a^2 + \sigma_d^2}{\sigma^2 + \sigma_a^2 + \sigma_d^2} = \frac{\sigma_q^2}{\sigma^2 + \sigma_q^2}$$

where σ_a^2, σ_d^2 and $\sigma_q^2 = \sigma_a^2 + \sigma_d^2$ are the additive, dominance and total variance due to the QTL respectively. We have shown that σ_q^2 could be expressed as a linear function of the square of the allelic effects of the QTL:

$$\sigma_q^2 = c_a \sum_{i=1}^{4} a_i^2 + c_d \sum_{i,j=1>i}^{4} d_{ij}^2$$

where $c_a = 1/3$ and $1/6$ and $c_d = 1/24$ and $1/8$ for F_3 and TC, respectively (Rebaï 1995). $\hat{r}^2$ is then calculated using the estimates of the parameters at the likely position of the QTL. Simulation results showed the good precision and power of our method for a large variety of marker informativeness and QTL effects (Rebaï 1995). The global variance explained by all the QTL was calculated as the sum of r^2 of individuals QTL.

Approximate mapping of multiple QTL

In interval mapping studies, the test profile can present two or more distinct peaks, suggesting the presence of multiple QTL. If the peaks are far apart, in a way such that their support intervals do not overlap, we assume the presence of two QTL. In this case, the positions and effects of these QTL are likely to be biased. For chromosomes showing this phenomenon we applied the procedure proposed by Lincoln and Lander (1990). It consists in fixing the most important QTL (by correcting the trait with its effects) and making a second scan of the chromosome. This may be a good approximation when QTL are well-separated (say more than 50 cM from each other). For chromosomes showing close significant peaks a multiple QTL search with markers as cofactors (Jansen and Stam 1994) can be used. The application of this approach to multiple populations is under study.

Analyses of genetic interactions

The test of the interaction between the cross mean values ($\mu_{ij(t)}$) and the QTL effects a_i (possibly d_{ij}) is a very important way to study the stability of the expression of QTL in different genetic backgrounds. The additive-by-additive epistasis (denoted EAA), defined as the interaction effect between the a_i parameters of two independent QTL, could also be investigated but would require the estimation of a large number of parameters.

To simplify the task and to avoid working with cumbersome models we chose to test these interaction effects with individual marker models. Instead of considering the QTL itself, we consider the most closely linked marker(s) to it. Inferences about the interactions between pairs of independent markers or between a marker and the genetic background could then be easily done. For instance, to test QTL by genetic background interaction (denoted GBI) in F_3 (for any given marker M), we use the following model:

$$Y_{ijk.} = \mu_{ij} + 2a_i + 2\gamma_{iji} + e_{ijk.} \quad \text{if } G(k) = M_i M_i$$

$$Y_{ijk.} = \mu_{ij} + a_i + a_j + d_{ij} + \gamma_{iji} + \gamma_{iji} + e_{ijk.} \quad \text{if } G(k) = M_i M_j$$

$$Y_{ijk.} = \mu_{ij} + 2a_j + 2\gamma_{iji} + e_{ijk.} \quad \text{if } G(k) = M_j M_j$$

where $G(k)$ is the genotype of the kth F_2 individual for marker M, and γ_{iji} is the interaction effect $\mu_{ij} * a_i$. There are 12 parameters γ but only 3 are estimable for a marker having four alleles. When the marker is not informative in all the populations flanking markers are used. The F test statistic for significance of parameters γ is calculated for all the m markers of the chromosome considered. If the P value of the test for at least 1 of the markers is less than α/m, where α is the level desired (we took $\alpha = 10\%$) then GBI is declared significant for the QTL. The test for TC progenies is based on a similar model but involves more parameters.

The model for A × A epistasis implies 2 markers and is more complicated (Rebaï 1995). For markers having all four alleles, the test of EAA has 20 and 36 *df* in F_3 and TC, respectively. In practice, we chose at least 1 marker per chromosome (the nearest to the QTL or the one with the highest test value if no QTL is declared present) and perform $n_t = c(c-1)/2$ tests (supposed independent), where c is the number of markers considered. We therefore used a per test level of α/n_t.

For these interaction effects one could use a standard analysis of variance with population type and marker genotypes as factors. But, as the frequency of missing data could be important for a large number of markers, the results would be less precise than the approach described above.

Analyses of QTL by environmental interactions

Individual marker analysis was used to assess the interaction between QTL and locations in the TC. An analysis of variance was carried out with a model including three factors: population type, marker genotype and locations (4 levels) and their pairwise interactions. However, QTL which are not detected in all locations express necessarily QTL-by-environment interaction (noted QEI). QEI can also occur due to changes in magnitude or sign of QTL effects across environments.

In all the interaction studies one needs to know which QTL could be considered as the expression of the same gene or group of linked genes. The decision rule was the overlapping of support interval, that is to say: if the support intervals (SI) of two QTL overlap, then we consider that it is the same QTL. This is a rough criterion to be used with caution.

Results

Genetic map

Over the 107 mapped markers 30% had four alleles, 35% had three alleles and 31% two alleles. Four markers were genotyped in only one population. Populations P_{12} and P_{13} had respectively the highest and the smallest number of polymorphic markers, which is in good agreement with the origin of and the genetic

distances between the parental lines. Ten linkage groups were obtained and the total length of the map was near 1,730 cM (using Haldane's mapping function) with a marker each 16 cM on average, ensuring an 80% coverage of the maize genome (Fig. 1). Some genomic regions, like those on chromosome 10 (where only 3 markers were mapped), were not well marked. For markers mapped in all the six populations, heterogeneity of recombination was tested as proposed by Beavis and Grant (1991). Only few markers showed strong evidence for heterogeneity. This was the case of the terminal markers of chromosome 1, bnl8.29a and bnl6.32, where the test was significant at the 0.1% level.

QTL for silking date in F_3 populations

Six QTL explaining 38.5% of the phenotypic variance were detected with both T_1 and T_2 tests (except the QTL of chromosome 3 detected by T_2 alone). Allelic effects of these QTL were globally in agreement with the expected earliness of the parent lines (Table 1). In particular, lines L_1 and L_2 carry respectively, negative (earliness) and positive alleles for four QTL out of six. The alleles had global effects of -2.66 and $+5.92$ days on the mean for lines L_1 and L_2, respectively. Dominance was small for most QTL except for those of chromosomes 1 and 7 where overdominance was found between alleles of $L_1 \times L_2$ and $L_1 \times L_4$, respectively, in the direction of the positive allele.

QTL mapping was also achieved in each single F_3 population. Results for chromosomes 1, 7 and 10, given in Table 2, show that the QTL which are globally detected in the diallel are also detected in at least one of the F_3 populations. Their effects in these populations are in good agreement with the allelic effects globally estimated; i.e. if $\hat{a}_i - \hat{a}_j$ is quite large, the QTL is detected in population P_{ij} with additive effect a of line L_i

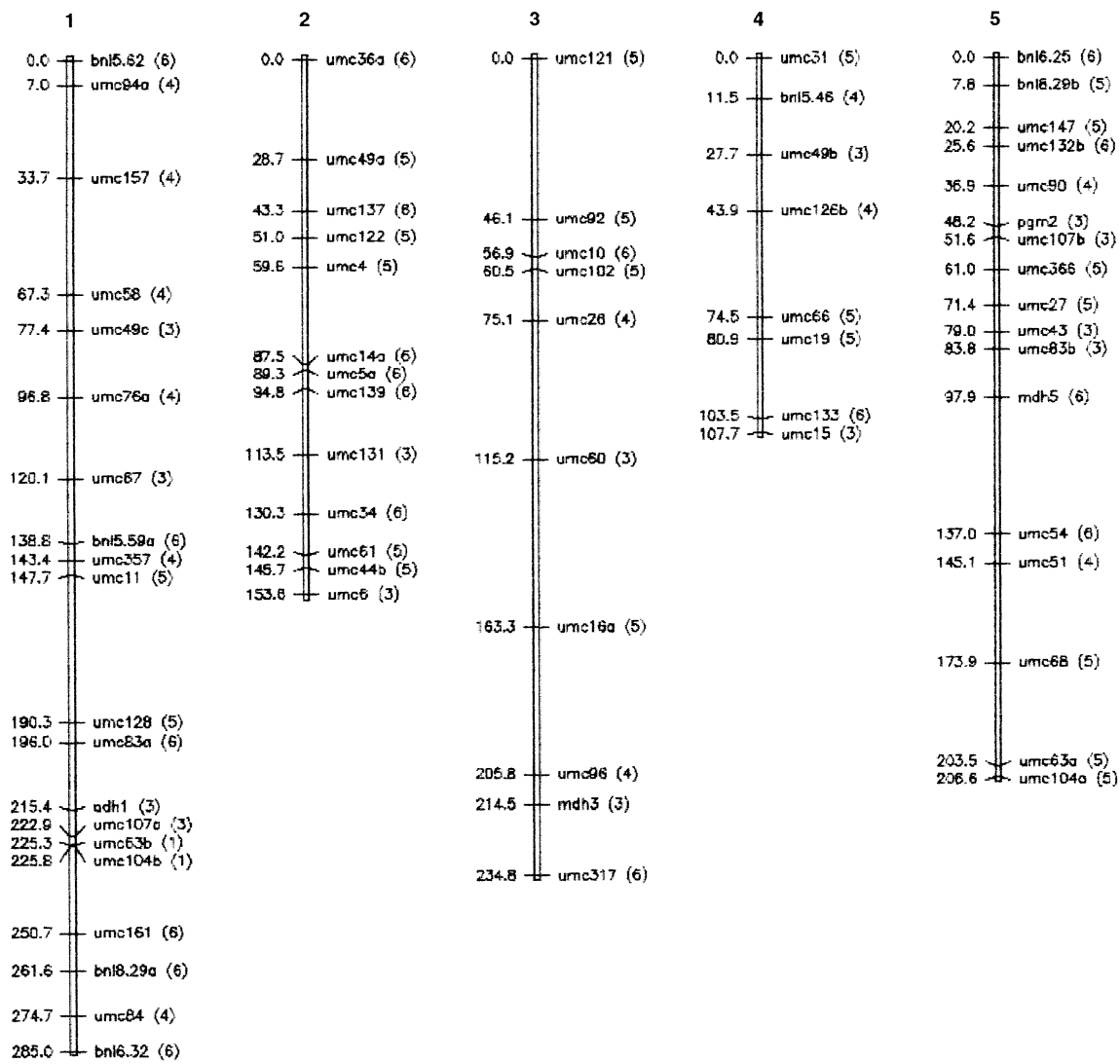

Fig. 1 For legend see page 455

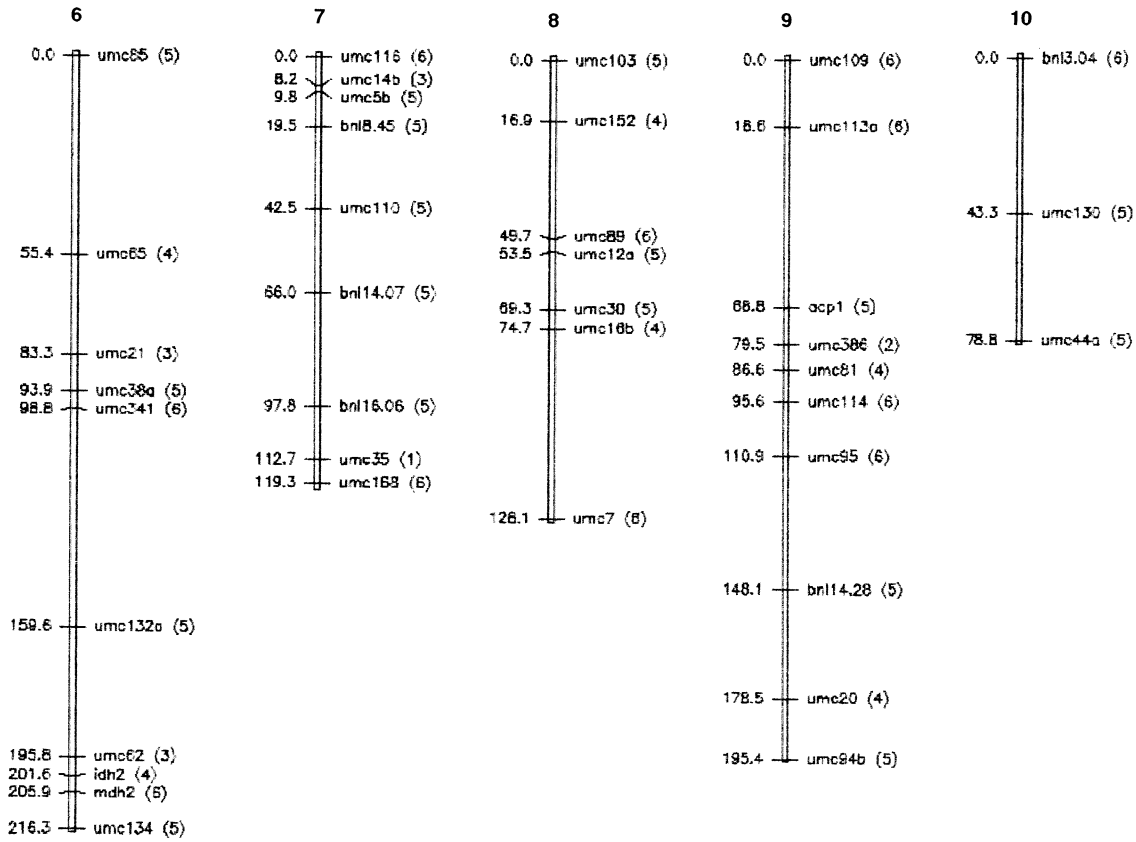

Fig. 1 Maize chromosome map showing the locations of the seven isozyme and 100 RFLP markers calculated using JOINMAP from F_2 genetic data in this study (Stam 1993). Cumulative distances in centiMorgans (cM) are given at the *left side* of the chromosome bar. The number between *parentheses* indicates the number of crosses where the marker is polymorphic

having the same sign as a_i. For chromosome 1 the QTL were detected in different positions in four populations. This can be due to the presence of two QTL or to a significant heterogeneity of recombination among populations. In fact, the test of heterogeneity was found to be significant for the markers surrounding the QTL, whereas the approximate multiple QTL search failed to detect a second QTL (results not shown). Although $\hat{a}_1 - \hat{a}_3 = 1.88$, the QTL on chromosome 10 was not detected in population P_{13}. This is probably due to the small population size (70 individuals) and to the fact that the terminal marker, umc44a, is not polymorphic in P_{13}. These two factors reduce the power of QTL detection tests.

No convincing evidence for genetic interactions was found: only chromosome 1 showed a significant GBI (genetic background interaction) effect on umc76a, and a highly significant A × A epistasis was observed between chromosomes 1 and 10 (markers umc76a-umc44a, $P = 0.0005$). Another significant epistasis ($P = 0.0003$) was found between chromosomes 2 (marker umc5a, where no QTL was found) and 3 (marker umc10) probably indicating the presence on

Table 1 QTL detected for silking date in F_3

Chromosome	Position[a] (SI)	r^2 (%)	a_1^b	a_2	a_3	a_4	σ_a^2/σ_d^2
1	127 (117–135)	9.5	− 0.43	1.22	− 1.14	0.35	0.21
3	46 (33–60)	3.2	0.19	− 0.17	− 0.76	0.74	0.12
4	65 (58–77)	7.2	1.03	− 0.12	0.26	− 1.16	0.13
7	113 (96–119)	7.1	− 0.57	0.30	− 0.78	1.05	0.28
8	39 (30–53)	4.8	− 0.63	1.09	ns	− 0.45	0.09
10	64 (58–75)	6.7	− 0.92	0.64	0.96	− 0.68	0.03

[a] Position in centiMorgans from the first marker on chromosome with support interval
[b] a_i is the additive effect of the QTL allele of line L_i, dominance effects d_{ij} are not shown
[c] Not significantly different from 0

Table 2 QTL detected in each F_3 population for silking date

Chromosome	Population	Position (SI)	r^2 (%)	a[a]	d[b]
1	P_{12}	111 (104–118)	9.0	− 0.97	0.85
1	P_{23}	145 (135–155)	5.0	0.77	− 0.68
1	P_{24}	223 (213–230)	4.0	− 0.27	− 3.04
1	P_{13}	200 (193–205)	6.3	0.30	− 3.33
7	P_{12}	113 (95–125)	3.0	− 0.59	− 0.19
7	P_{14}	112 (100–119)	5.0	− 0.60	2.2
7	P_{34}	111 (99–121)	6.7	− 0.92	0.57
10	P_{12}	68 (53–73)	5.1	− 0.84	− 1.2
10	P_{34}	71 (61–79)	4.0	0.79	− 1.2

[a, b] Additive and dominance effects of QTL, respectively

Table 3 QTL detected for silking date in TC[a]

Location	Position (SI)	r^2 (%)	a_1	a_2	a_3	a_4	σ_a^2/σ_d^2
Chromosome 1							
E1	126 (110–136)	10.4	− 0.29	1.14	− 0.94	ns	0.27
E2	133 (127–163)	10.9	− 0.66	1.47	− 0.67	ns	0.13
E3	132 (126–142)	8.1	− 0.35	1.08	− 0.63	ns	0.06
E4	159 (141–174)	6.0	− 0.52	0.30	− 0.39	0.61	0.05
Chromosome 2							
E1	133 (102–153)	3.0	− 0.65	0.30	0.17	0.18	0.60
E2	148 (140–153)	3.2	− 0.67	0.42	ns	0.24	0.33
E3	150 (145–153)	3.2	− 0.57	0.45	ns	0.17	0.25
E4	151 (147–153)	3.9	− 0.12	0.50	ns	− 0.34	0.60
Chromosome 3							
E1	94 (56–113)	12.3	ns	− 0.24	− 0.91	1.22	0.66
E2	51 (27–67)	4.9	0.24	− 0.10	− 0.91	0.77	0.0
E3	31 (18–45)	8.7	ns	− 0.27	− 0.77	1.13	0.0
E4	36 (23–51)	4.9	ns	ns	− 0.64	0.51	0.0
E1	211 (200–220)	3.8	− 0.24	ns	− 0.55	0.79	0.01
E2	190 (171–220)	5.7	0.24	0.23	− 1.12	0.65	0.0
E3	217 (208–226)	4.2	− 0.14	0.17	− 0.68	0.65	0.0
E4	214 (206–224)	2.6	ns	0.16	− 0.47	0.39	0.0
Chromosome 4							
E1	89 (48–104)	5.0	0.78	− 0.38	0.20	− 0.60	0.13
E3	90 (63–104)	3.3	0.55	0.18	− 0.10	− 0.63	0.01
E4	86 (44–96)	5.1	0.38	0.23	0.11	− 0.72	0.01
Chromosome 5							
E1	63 (38–118)	3.4	ns	− 0.76	0.39	0.30	0.02
E2	65 (40–93)	4.9	− 0.44	− 0.74	0.46	0.71	0.03
Chromosome 7							
E2	13 (0–35)	5.3	− 0.29	− 0.66	ns	0.98	0.03
Chromosome 8							
E2	10 (0–53)	4.0	− 0.42	0.84	ns	− 0.40	0.15
E4	40 (26–52)	4.9	− 0.52	0.59	0.11	− 0.19	0.13
Chromosome 9							
E4	47 (31–61)	8.1	ns	0.56	0.31	− 0.86	0.08
Chromosome 10							
E1	48 (30–78)	3.6	− 0.80	0.24	0.45	0.11	0.04
E2	60 (43–78)	7.1	− 1.03	0.15	1.12	ns	0
E3	53 (20–61)	4.8	− 0.69	0.25	0.56	− 0.12	0.21
E4	58 (46–78)	5.3	− 0.56	0.25	0.58	− 0.26	0.03

[a] Same notations as in Table 1

chromosome 2 of a QTL having a small effect not detectable by the experiment.

QTL for silking date in TC populations

We detected globally ten QTL among which five were found in all locations, one in three locations, two in two locations and two in one location (Table 3). The number and effects of QTL in the locations are shown below:

Locations	E1	E2	E3	E4
Number of QTL	7	8	6	8
Variance explained R^2 (%)	41.5	46.0	32.3	45.7

Most QTL showed an additive action with an exception for chromosome 2, having major dominance effects over the four locations between alleles 1 and 3 ($d_{13} \simeq 0.25$) and 1 and 4 ($d_{14} \simeq -0.32$). QTL on chromosomes 1, 2 and 10 were quite stable over environments with respect to the positions and effects. The other QTL (except that of chromosome 4) had allelic effects which differed in magnitude but not in sign indicating that the QTL-by-environment interaction was due essentially to a reduction in the QTL effect (which sometimes vanished) in some environments. The QTL on chromosome 9, having a large additive effect, seemed to be environment-specific. Two QTL were found on chromosome 3 about 120 cM apart and linked in coupling phase.

The application of the approximate multiple QTL procedure is shown as an example for environment E2 (Fig. 2). After fixing the first QTL at position 52 cM, the second was found at 194 cM with smaller effect. The allelic effects of the second QTL, the first being fixed, were not significantly different from those of Table 3. Its global effect was reduced by about 1%. This is probably due to the large distance and the presence of good informative markers between the two QTL, so that they only slightly affect their individual estimation.

GBI was significant for chromosomes 1 (umc49c) and 5 (pgm2), and epistasis between chromosomes 1 (bnl5.59a) and 3 (umc60) was the only significant pairwise interaction ($P = 0.0032$). Five of the ten QTL detected, including those detected in three locations or less, showed a significant QE interaction. The study of the test profiles in the environments where no QTL was detected showed that they had the same shapes with peaks in the same regions as those identified for the detected QTL but which remained below the threshold value. This indicates that most of the interaction is due to the disappearance of the allelic effects. The effect of allele L_4 for QTL of chromosomes 2 and 10 showed changes in sign over locations with a strong negative action in location E4.

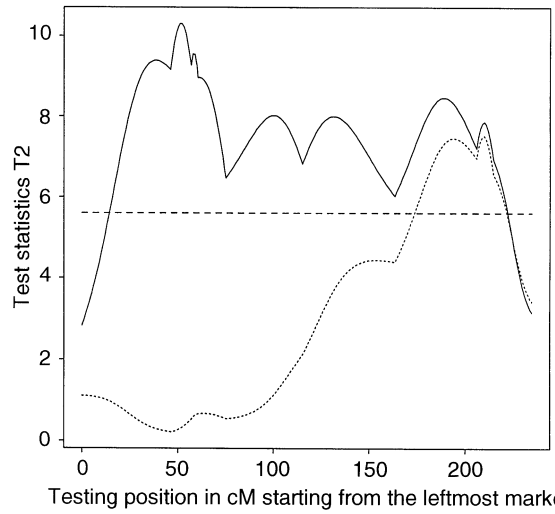

Fig. 2 Test statistics T_2 in TC progenies and environment E2 for chromosome 3. The *solid-line curve* is the global scan showing two major peaks. The *dashed curve* is the result of a second scan after fixing the first QTL (*peak*) from the left at position 52 cM. The *straight line* is the threshold value of the test

Discussion

Comparison of F_3 and TC progenies

By examining Tables 1 and 3, we note that nearly all the QTL detected in F_3 for silking date were also detected in TC at similar positions (SI do overlap) and with allelic effects having the same signs. The only exception is for chromosome 7, where it is likely that two different QTL were identified in F_3 and TC (Fig. 3). Allelic effects of line L_2 for chromosome 7 had opposite signs in the two progenies with a large dominance between $L_2 \times L_3$ observed in F_3 that was not significant in TC.

Three other QTL were found only in TC on chromosomes 2, 3 and 5, and these had small effects (nearly 4%). This indicates the greater power of TC progenies for QTL detection. This result is in disagreement with the proposition that the QTL detected in a testcross are a subset of those found in F_3 because of the masking effect of the tester's dominant alleles which tend to reduce the genetic variability (only six genotypes are observed here in TC vs. 10 in F_3). However, one should keep in mind that F_3 individuals of allogamous species (because of inbreeding depression) are more sensitive to environmental stresses and could be seriously affected by external limiting factors.

Dominance

Additive gene action predominated at most QTL. Dominance effects were relatively small in both F_3 and

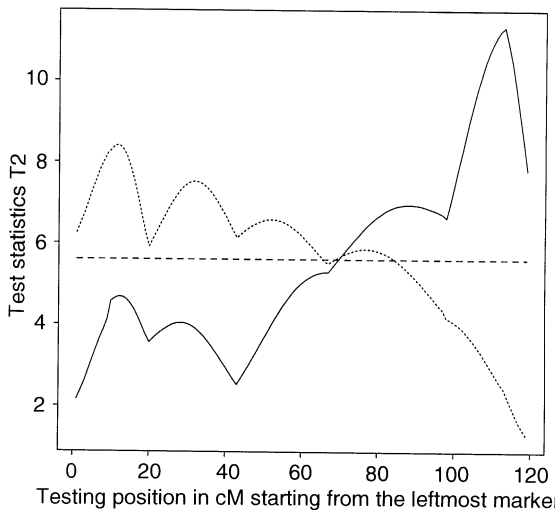

Fig. 3 Test statistics T_2 in F_3 progenies (*solid line*) and TC in environment E2 (*dashed line*) for chromosome 7. The *straight line* is the threshold value of the test

TC progenies with the dominance ratio (σ_d^2/σ_a^2) varying from 0 to 0.6 with a large majority below 0.2. Globally, over all detected QTL the dominance ratio was about 0.15 in F_3 and from 0.10 in E4 to 0.28 in E1 (significantly larger than at the other three locations) in TC. Although this ratio is small, dominance and overdominance were observed between specific combinations of alleles for some QTL. However, as indicated in the Material and methods section, F_3 progenies (but not TC) allow the estimation of all the six dominance parameters whereas with TC we can only estimate two parameters.

Dominance has a direct effect on QTL detection in the diallel. In this study, more than 20% of the detected QTL were found by the additive test T_2 alone and only 8% by T_1 (global test) alone. Small additive QTL are not detected by T_1 and those expressing a large dominance not by T_2. QTL with moderate or major effects (5% or more) are, in general, detected by both tests. It seems that for most of the experimental situations we encountered here (lack of dominance), T_2 is more powerful than T_1, especially in F_3. In practice, one should use both tests to ensure a good power in detecting QTL.

Genetic interaction

There is a lack of evidence for QTL-by-genetic background interaction for earliness. Charcosset et al. (1994), using recombinant inbreds derived from a diallel cross between three lines, found major GBI effects for earliness, especially on chromosomes 1 and 8. Dudley (1993) cited some results which tend to show that the importance of GBI effects is related to the complexity of the trait. QTL influencing highly polygenic traits (such as yield) are more likely to be involved in interaction networks.

Digenic additive epistasis was rarely found. This illustrates the small power of the tests for detecting such interactions, which involve a large number of parameters (especially in complex designs). Only strong epistasis effects are thus detected.

QTL-by-environment interaction

More than 50% of the QTL detected on testcross progenies showed significant QTL-by-environment interaction. However, this interaction was essentially due to a change in the magnitude of the allelic effects rather than an inversion of their signs. Previous studies (Koester et al. 1993; Schön et al. 1994, Stuber et al. 1992) have shown that maize QTL can be consistent across environments and suggested that major QTL could be reliably detected in few environments. We report here results from four locations with the understanding that earliness is influenced by environment. Nevertheless, "major" QTL of plant maturity were consistently mapped in the same regions (chromosomes 1, 8 and 10) as those reported in the literature (Koester et al. 1993, Veldboom et al. 1994).

Conclusion

Identification of QTL affecting agronomically important traits is a key step in the understanding of genetic phenomena and the efficient use of markers in plant improvement. The simultaneous study of several crosses for QTL mapping is a new approach which sheds light on the genetic mode of action and the sensitivity of QTL to genetic background and environment. In this paper, we have mapped QTL for silking date (earliness) and evaluated their phenotypic effects and their stability. The diallel scheme we used has suggested some new conslusions about the genetics of QTL. It allowed the consistent characterization of QTL over several populations and the estimation of allelic effects for more than two lines as well as the interaction relations between these alleles. Detailed understanding of QTL effects will now require a fine mapping via the use of more precise procedures, like multiple QTL methods.

We are presently supporting QTL validation efforts for traits associated with yield, based on marker-assisted breeding by complementary matings and the selection of genotyped individuals carrying favourable alleles of QTL.

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