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ORIGINAL PAPER

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# Quantitative trait loci for grain moisture at harvest and field grain drying rate in maize (*Zea mays*, L.)

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**Abstract** Hybrids with low grain moisture (GM) at harvest are specially required in mid- to short-season environments. One of the most important factors determining this trait is field grain drying rate (FDR). To produce hybrids with low GM at harvest, inbred lines can be obtained through selection for either GM or FDR. Thus, a single-cross population (181 $F_{2:3}$-generation plants) of two divergent inbred lines was evaluated to locate QTL affecting GM at harvest and FDR as a starting point for marker assisted selection (MAS). Moisture measurements were made with a hand-held moisture meter. Detection of QTL was facilitated with interval mapping in one and two dimensions including an interaction term, and a genetic linkage map of 122 SSR loci covering 1,557.8 cM. The markers were arranged in ten linkage groups. QTL mapping was made for the mean trait performance of the $F_{2:3}$ population across years. Ten QTL and an interaction were associated with GM. These QTL accounted for 54.8 and 65.2% of the phenotypic and genotypic variation, respectively. Eight QTL and two interactions were associated with FDR accounting for 35.7 and 45.2% of the phenotypic and genotypic variation, respectively. Two regions were in common between traits. The interaction between QTL for GM at harvest had practical implications for MAS. We conclude that MAS per se will not be an efficient method for reducing GM at harvest and/or increasing FDR. A selection index including both molecular marker information and phenotypic values, each appropriately weighted, would be the best selection strategy.

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## Introduction

Grain moisture (GM) at harvest is of primary importance for maize (*Zea mays* L.) production and breeding, since crops with low GM at harvest reduce the economic impact of artificial drying (Dijak et al. 1999; Sweeney et al. 1994). In mid- to short-season environments, the rapid decrease in temperature and increase in relative humidity toward the end of the growing season establish a compromise between full utilization of the heat units available for maize growth and GM loss during the drying period.

Field grain drying rate (FDR) and maturity group are critical for determining final GM at harvest. FDR is defined as the rate of GM loss during the drying period, that is, from physiological maturity to grain harvest. After physiological maturity, GM loss is associated only with drying, since no further changes in kernel dry weight occur. During this period, weather variables related to air humidity or atmospheric demand (Schmidt and Hallauer 1966) and several plant morpho-physiological traits (Crane et al. 1959; Troyer and Ambrose 1971) would be expected to exert control over the FDR (Brooking 1990).

In previous reports the genetic basis for GM at harvest and FDR was established (Purdy and Crane 1967; Sentz 1971; Zhang et al. 1996). Thus, selection of inbred lines to produce hybrids with low GM at harvest is possible through either selection for GM at harvest or FDR (Sweeney et al. 1994). Selection for GM would be easier to implement in breeding programs. In fact, selection of inbred lines based on low GM at 45 days after pollination was effective in reducing GM at harvest (Cross 1985; Freppon et al. 1992). Nevertheless, yield and GM at harvest are positively correlated (Hallauer and Miranda 1988; Mather and Kannenberg 1989) because both variables are associated with the maturity group (Lauer et al. 1999). Selection of genetic materials with high FDR would allow breeders to obtain high-yielding hybrids diminishing the negative effects of increased maturity on GM at harvest. However, evaluating FDR is a labor-intensive process and, therefore, not practical in large segregating populations (Sweeney et al. 1994).

Most genetic investigations on GM at harvest and FDR thus far, have made use of conventional breeding and biometric procedures to dissect the variation observed in these traits, allowing researchers to obtain only general information about their inheritance (Simko et al. 1997). The availability of DNA-based marker technologies, on the other hand, has provided new tools for analyzing and dissecting complex inherited traits. With the aid of molecular markers, it is possible to identify the chromosomal regions that are associated with the inheritance of a trait of interest (QTL) as a starting point for marker assisted selection (MAS; Papst et al. 2004).

The correspondence between QTL for GM at harvest in $F_{2:3}$ populations or later generations and their respective testcross has been previously reported (Austin et al. 2000; Beavis et al. 1994; Melchinger et al. 1998; Mihaljevic et al. 2005). However, there are no records in the literature regarding QTL for FDR.

The objective of this study is to locate QTL affecting GM at harvest and FDR in a segregating population.

## Materials and methods

### Plant materials

Two near-homozygous inbred lines, property of Monsanto, were selected as parents based on their similitude in time to flowering and their difference in GM at harvest. The maternal (A) and paternal (B) inbred lines are considered to be part of the *Iodent* and *Argentine Flint* heterotic groups, respectively (Troyer 1999; J.C. Cerono, personal communication). Lines A and B were crossed in summer 2000 in Monsanto Research Station at Camet, Buenos Aires, Argentina (37°52′S, 57°21′W). Three $F_1$ plants were self-pollinated in 2001 in Monsanto's winter nursery at Hawaii. The seed of the $F_2$ population was sown at Camet in summer 2001 and 181 unselected individual $F_2$ plants were self-pollinated to create their respective $F_{2:3}$ progenies.

### Field design and phenotypic measurements

Parental lines were sown in November 2, 2003 and November 5, 2004 at Camet, using a randomized complete block design with four blocks. From 20 days after silking (50% of plants in the row having extruded silks), five ears were collected at weekly intervals until harvest. Ten kernels from the lower third of each ear were weighted and dried to constant weight in a force-air oven at 60°C to obtain kernel dry weight. GM was calculated as (1-dry weight/fresh weight)×100. GM at physiological maturity was estimated according to Brooking (1990).

The 181 $F_{2:3}$ progenies were sown on October 25, 2002 and on November 2, 2003 at Camet, with a final plant density of 6 plants $m^{-2}$. In 2002, the $F_{2:3}$ population was sown using an augmented complete block design (Federer 1998) with four blocks and two checks (a commercial hybrid and an inbred line). The experimental design in the 2003 trial was an incomplete block with two replications and four blocks per replication. Each replication was planted as an augmented complete block design with six checks (three commercial hybrids and three inbred lines). In both years, the single-row plots were 6 m long with 0.70 m spacing between rows.

We considered that an $F_{2:3}$ progeny reached physiological maturity when GM was 350 g $kg^{-1}$ (Brooking 1990; Dijak et al. 1999) and that harvest date was the moment at which the check hybrids reached a GM below 200 g $kg^{-1}$. FDR for each $F_{2:3}$ progeny was calculated as a linear regression of GM across days from physiological maturity to harvest (approximately from March 10 to April 20).

All moisture measurements were recorded using a hand-held moisture meter as the one used by Freppon et al. (1992). For each $F_{2:3}$-progeny row, four randomly taken plants were tagged at silking as in Magari et al. (1996) and Zhang et al. (1996). Two GM measurements were recorded and averaged in each of these four plants at weekly intervals until harvest. The hand-held moisture meter was calibrated with different plant materials, including parental lines, using the oven method as a reference (Kang et al. 1978). Ears with different GM were measured with the hand-held moisture meter, labeled and harvested. Ten to 20 kernels from the lower third of each ear were weighted and dried to constant weight in a force-draught oven at 60°C to obtain kernel dry weight. GM was calculated as previously indicated.

### Statistical analysis

Adjusted entry means for each trait were corrected for block effects according to Federer (1998) and Scott

and Milliken (1993) in 2002 and for incomplete block effects in 2003 (Cochram and Cox 1957). Analysis of variance was conducted within and across years for each trait separately, and estimates of genotypic ($\sigma^2_g$), genotypic × environment ($\sigma^2_{gxe}$) and error ($\sigma^2$) variance components were obtained, considering all effects in the statistical model as random. To perform the combined analysis, each replication in 2003 trial was considered as an individual augmented complete block design. Heritability values ($h^2$) for each trait were calculated on an entry mean basis (across years, Hallauer and Miranda 1988) and on a plot basis (for each year). Exact 90% confidence intervals for heritability values on an entry mean basis were calculated (Knapp et al. 1985). Simple phenotypic correlations were obtained using the adjusted means. Distribution of phenotypic means for both traits was evaluated on the basis for the $W$ statistic (Shapiro and Wilk 1965).

### Genetic mapping

The genotypic data used to construct the genetic linkage map and to carry out the QTL analysis were provided by the Molecular Biology Laboratory of Monsanto at Camet Research Station. The SSR data was obtained with DNA samples isolated from a bulk of leaf tissue from the $F_{2:3}$-generation plants using a modified version of Dellaporta et al. (1983) protocol (D. Bernacchi, personal communication). The genetic linkage map was constructed using MAPMAKER v3.0 (Lander et al. 1987) as described in detail by Lincoln et al. (1992). Marker segregation was evaluated with the Chi-square test.

### QTL analyses

QTL mapping was made separately for each trait using the mean performance of the $F_{2:3}$ progenies across environments (hereafter "the mean environment") as a measure of the phenotype of a progeny.

To identify single and interacting QTL associated with GM at harvest and FDR, the multi-stage analysis of Sen and Churchill (2001) was employed. All computation for this method were performed using the add-on software package R/qtl v0.98 (Broman et al. 2003), under the statistical language/environment R v1.9 (R Development Core Team 2004). In the first stage, a genome scan was performed employing interval mapping (IM) every 2 cM with the pseudo-marker algorithm (64 imputations, Lyons et al. 2003). The results of the previous analysis were compared to the output of PLABQTL software (Utz and Melchinger 1996), which employs composite IM (CIM; Zeng 1994). Significance thresholds were determined by permutation tests ($n = 1{,}000$ permutations; Churchill and Doerge 1994); considering a significant and a suggestive locus when the LOD statistic exceeded the 95th ($P < 0.05$) and the 63rd ($P < 0.37$) percentile of the permutation distribution, respectively (Wittenburg et al. 2002). For each detected QTL, one- and two-LOD support intervals were calculated (Lander and Botstein 1989; van Ooijen 1992). Since in this study we used a $F_{2:3}$ population for trait evaluation, the estimates of dominance effects were doubled. The scale based on the dominant/additive ratio proposed by Edwards et al. (1987) was used to classify gene action. Detected QTL were identified with a code consisting of the linkage group number and the absolute position of the QTL (cM) in the linkage group separated by a dash.

In the second stage, we performed a genome scan assuming a two-QTL model and an interaction term. All marker pairs were tested by scanning at 5-cM intervals (16 imputations, Lyons et al. 2003). From this analysis three statistics were calculated: (a) a joint LOD, (b) a LOD score for interaction ($LOD_{int}$) and (c) a partial LOD score for each single QTL in the pair (LODp). If a QTL pair was significant, subsequent tests were performed to distinguish between QTL interactions, two strong loci acting additively or simply from one locus with strong effects carrying along a locus of weak effects (Sugiyama et al. 2001). Empirical thresholds for the joint and the interaction statistics were determined by permutation tests (Churchill and Doerge 1994). For LODp we used a threshold of 2.4. Although this threshold is arbitrary, according to Lander and Botstein (1989) it is equivalent to a 0.05 level of significance. Complementarily, epistatic effects among pairs of marker loci were assessed with two-way analyses of variance using the SAS program EPISTACY (Holland 1998). Marker additive or dominance effects were calculated as in Edwards et al. (1987).

The third stage integrated into a multiple regression model all the QTL detected in the first two stages. A backward analysis was performed to discard those QTL that were not significant in the model ($P < 0.1$). The percentage of the phenotypic variation explained by each single QTL or interaction and all QTL considered together were estimated within the multiple-QTL model. The proportion of genotypic variation explained by the model was estimated as the ratio between the percentage of the phenotypic variation explained by all QTL and the $h^2$ of the trait.

## Results

### Climatic data

For the whole drying period the reference evapotranspiration ($ET_0$), which is a good estimator of the atmospheric demand (Allen et al. 1998), was 1.98 mm day$^{-1}$ in 2002 and 2.58 mm day$^{-1}$ in 2003. The long-term average over 32 years was 2.47 mm day$^{-1}$.

## Hand-held moisture meter calibration

The linear regression between GM determined by the oven method and that measured with the hand-held moisture meter accounted for 89% of the variation in GM in the range between 125 and 406 g $kg^{-1}$ ($n = 65$, $P < 0.001$). The slope of the linear regression was close to one and the intercept was 14.3 g $kg^{-1}$. There were no differences in the relationship between the hand-held moisture meter and the oven method readings when tested separately for each plant material. Given that the variance of the error term was fairly constant in the range of measurements and the slope of the calibration curve was close to one, no corrections were made to the hand-held moisture meter data.

## Trait characterization and variance components

The maternal line (A) presented lower GM at harvest and higher FDR than the paternal line (B, Table 1). Thermal times to flowering and to physiological maturity were similar for the two lines. These results were the same when the 2 years were analyzed separately (data not shown). GM at physiological maturity was $343 \pm 21$ g $kg^{-1}$ (90% confidence interval) for line A, and 360 g $kg^{-1}$ with a 90%-confidence interval $\pm$ 45 g $kg^{-1}$ for line B.

Means for FDR of the $F_{2:3}$ progeny fitted a normal distribution according to $W$-test. Deviation from normality was observed for GM at harvest toward lower values. A $\log_{10}$ transformation was applied to GM at harvest data to attain a normally distributed trait prior to QTL analysis. Such transformation did not alter QTL mapping results as was also observed by Beavis et al. (1994) and Simko et al. (1997). Therefore, the original non-transformed data are presented.

Heritability values on a plot-mean basis for FDR were 0.88 and 0.49 in 2002 and 2003, respectively. For GM at harvest, $h^2$ on a plot basis were 0.98 in 2002 and 0.74 in 2003. Heritability values on an entry basis were 0.79 and 0.84 for FDR and GM at harvest, respectively (Table 1). The genotype × environment interaction term was significant for GM at harvest ($P = 0.0142$) but not for FDR ($P = 0.88$).

Phenotypic correlations between years were 0.49 ($n = 174$, $P < 0.01$) for GM at harvest and 0.36 ($n = 151$, $P < 0.01$) for FDR. The correlation between GM at harvest and FDR in 2003 was −0.51 ($n = 163$, $P < 0.01$) and close to zero in 2002 ($n = 164$, $P = 0.872$). The correlation between both traits in the mean environment was rather low (−0.19) but highly significant ($n = 175$, $P = 0.012$).

## Genetic linkage map and marker segregation

A total of 122 SSR loci covered 1,557.8 cM with an average interval of 13.9 cM. Ninety-five percent of the intervals between markers were below 20 cM. The markers were arranged in ten linkage groups, which is the haploid number of chromosomes in maize (Fig. 1). These data are in good agreement with other linkage maps for maize (Austin et al. 2000; Bohn et al. 1996).

Genotypic classes at 15 loci (12.3%) deviated significantly from the expected Mendelian ratios (1:2:1) according to Chi-square analyses. These loci were scattered along the genome, suggesting some errors in marker genotyping as proposed by Vogl and Xu (2000). A rough estimation of the error rate rendered a value of 1.6%. As suggested by Lincoln and Lander (1992), such an error rate may have only slight effects on a sparse genetic map as the one used in this study.

## Identification of single QTL

The empirical LOD thresholds for GM were 3.3 (significant locus) and 2.4 (suggestive locus). Six putative QTL were associated with GM at harvest, accounting for 37.8% of the phenotypic variation and 45% of the genotypic variation (Table 2). Each QTL explained between 10.4 and 19.7% of the phenotypic variation. Except for QTL 8/64, the maternal line contributed the favorable alleles to reduce GM at harvest. Of the six

**Table 1** Grain moisture (GM) at harvest and field grain drying rate (FDR) means, variance components and broad-sense heritabilities across years

| Trait | Means[a] | | | Range | Variance components[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Line A | Line B | $F_{2:3}$ progeny | $F_{2:3}$ progeny | $\sigma^2_g$ | $\sigma^2_{gxe}$ | $\sigma^2$ | $h^{2c}$ | 90% CI on $h^{2d}$ |
| GM at harvest (g $kg^{-1}$) | 143.7 (6.31) **a** | 258.2 (6.31) **b** | 205.6 (0.65) | 146.0–300.0 | 530.08 | 215.08 | 248.99 | 0.84 | 0.81–0.88 |
| FDR (g $kg^{-1}$ $day^{-1}$) | 7.97 (0.32) **a** | 4.67 (0.32) **b** | 5.74 (0.04) | 3.87–7.90 | 0.308 | 0.008 | 0.969 | 0.79 | 0.74–0.83 |

[a]Mean values for maternal (Line A), paternal (Line B) and $F_{2:3}$ progenies. For parental lines, mean values are the average of 2003 and 2004 years. For $F_{2:3}$ progenies, mean values are the average of 2002 and 2003 years. Values between brackets are standard errors. Means followed by different letters within rows are different ($P < 0.01$) according to Tukey test

[b]$\sigma^2_g$ = genotypic variance, $\sigma^2_{gxe}$ = genotype × environment interaction variance, $\sigma^2$ = error variance

[c]Broad-sense heritability

[d]90% CI on $h^2$ = 90% confidence interval on heritability calculated according to Knapp et al. (1985)

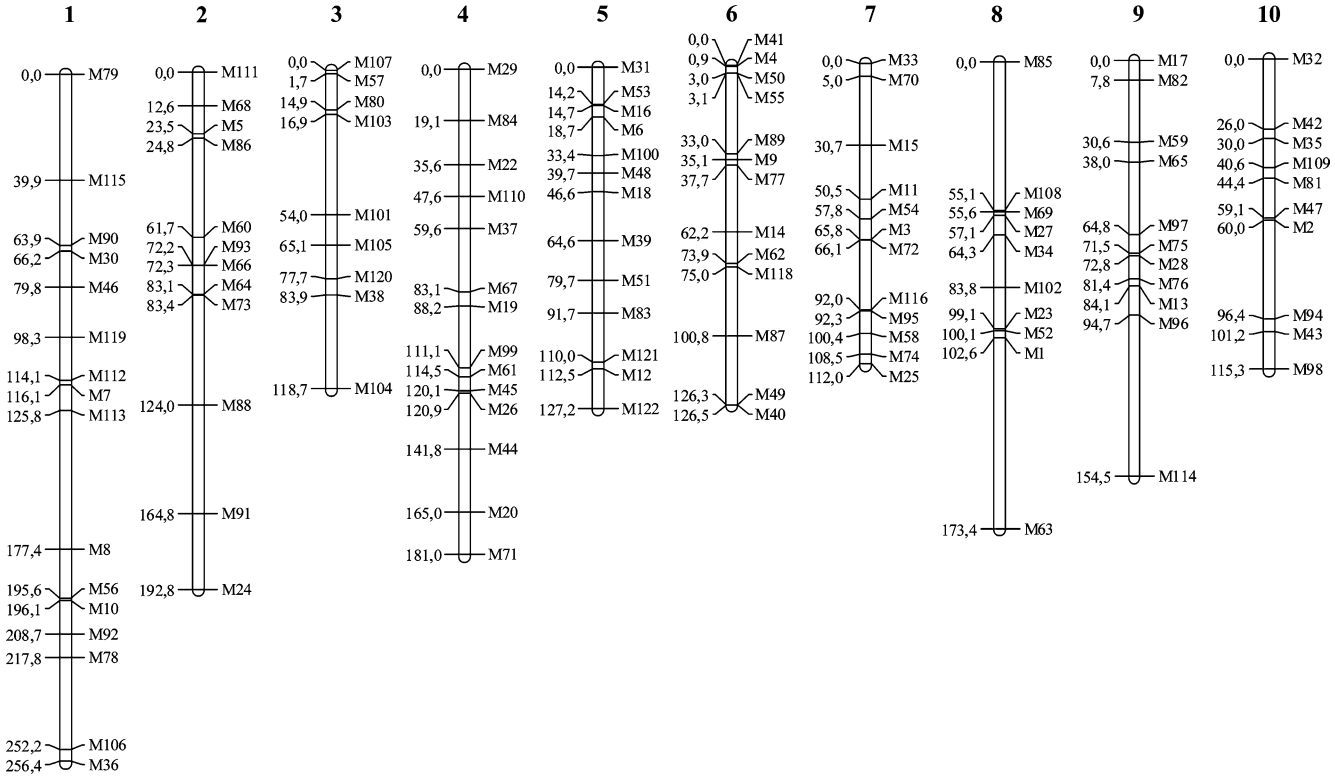


**Fig. 1** Linkage map based on 122 SSR marker loci. *Right of bars* marker loci coded with the prefix M and an arbitrary number. *Left of bars* absolute positions (cM). Graphs made with Map Chart (Voorrips 2002)

QTL, three of them presented a dominant type of gene action. In the remaining QTL, gene action was additive or partially dominant and overdominant.

For FDR the significant and suggestive LOD empirical thresholds were 3.2 and 2.4, respectively. A significant QTL in linkage group 5 and two suggestive

**Table 2** Parameters of QTL for grain moisture at harvest and field grain drying rate for the $F_{2:3}$ population for the mean performance of each trait across years 2002 and 2003

| Trait | QTL[a] | Linkage group | Position (cM)[b] | Support interval (cM)[c] | LOD | $R^{2d}$ | $a^e$ | $d^f$ | Gene action[g] | Direction of response[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| Grain moisture at harvest (g kg$^{-1}$) | 1/194 | 1 | 194 | (180–216) (160–236) | 3.65 | 14.1 | 7.79 | −15.5 | OD | A |
| | 2/132 | 2 | 132 | (108–162) (86–174) | 5.69 | 19.2 | 12.9 | −1.04 | A | A |
| | 5/0 | 5 | 0 | (0–12) (0–18) | 2.95 | 10.8 | 8.73 | −2.6 | A | A |
| | 8/64 | 8 | 64 | (60–72) (55–78) | 4.74 | 19.7 | −5.94 | 5.2 | D | B |
| | 9/34 | 9 | 34 | (24–46) (18–60) | 4.33 | 16.7 | 9.7 | 8.7 | D | A |
| | 10/26 | 10 | 26 | (20–58) (12–80) | 3.72 | 10.4<br>37.8[i] | 6.79 | −5.7 | D | A |
| Field drying rate (g kg$^{-1}$ day$^{-1}$) | 1/108 | 1 | 108 | (98–184) (74–230) | 3.18 | 8.0 | −0.30 | −0.40 | OD | A |
| | 5/0 | 5 | 0 | (0–44) (0–58) | 4.61 | 14.2 | −0.40 | 0.10 | A | A |
| | 8/64 | 8 | 64 | (56–104) (0–173) | 2.54 | 7.0<br>16.4[i] | −0.10 | 0.40 | OD | A |

[a]Linkage group/absolute position in centi Morgan
[b]Position of highest LOD score
[c]One- and two-LOD, respectively
[d]Coefficient of determination: percentage of phenotypic variance explained by the QTL
[e]Additive value. Negative sign = increase of the mean value of the trait due to the female parent alleles. Positive sign = increase of the mean value of the trait due to the male parent
[f]Dominant value. A positive sign means dominance for higher value of the trait. A negative value means dominance for lower value of the trait
[g]*A* Additive or partial dominance ($0 < |d/a| < 0.55$); *D* partial dominance or dominance ($0.55 < |d/a| < 1.20$); *OD* overdominance ($|d/a| > 1.20$). Based on the scale of the $|d/a|$ ratio (Edwards et al. 1987)
[h]Parent whose additive value of a QTL allele provided the favorable allele for a given trait
[i]Total coefficient of determination: estimate of total phenotypic variance obtained from the simultaneous fit of all putative QTL for a given trait

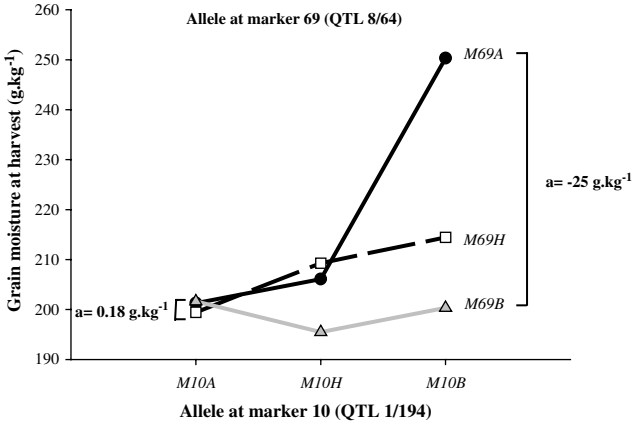


**Fig. 2** Interaction plot between marker 10 (M10) and marker 69 (M69) alleles for grain moisture at harvest. Both markers are representative of QTL 1/194 and 8/64, respectively. Letters *A* (maternal), *H* (heterozygote) and *B* (paternal) represent the alleles of each marker. *a* Additive effect of M69 for each allele of M10

QTL in linkage groups 1 and 8 were found. Each QTL accounted from 7 to 14.2% of the phenotypic variation. Together, the three QTL accounted for 16.4 and 20.7% of the phenotypic and genotypic variation, respectively. For QTL 1/108 and 8/64, gene action was overdominant, while for QTL 5/0 it was additive. The maternal line contributed all the favorable alleles to increase FDR (Table2).

The results of this analysis were similar whether we used IM or CIM. The only differences were in the positions of the QTL detected in linkage groups 2 and 8 for GM at harvest and in linkage group 1 for FDR.

### Identification of QTL pairs and interacting QTL

The simultaneous search for QTL pairs confirmed the results of the first scan in both traits but it also detected other regions potentially associated with the traits.

For GM at harvest, seven additional QTL were detected. According to LODp values, regions 3/80, 4/0 and 6/25 were included in the final model for further testing. QTL 2/132 would actually be two linked QTL in positions 100 and 145. None of the interactions tested were above the suggestive $LOD_{int}$ threshold. The highest $LOD_{int}$ was between regions 1/194 and 8/64. With EPISTACY, a significant interaction was detected between marker (M) 10, located at position 196.1 in linkage group 1, and M69, located at position 55.6 in linkage group 8 (Fig. 1), that may be representative of this interaction, according to the two-LOD support interval (Table 2). The partition of the interaction variance revealed that it was additive by additive (Fig. 2). Even though the interaction was not significant in the joint analysis, since it involved two significant loci and was detected with EPISTACY, we decided to test its contribution in the multiple QTL model.

For FDR, six new regions were detected. The LODp values for these regions were not significant. However, regions 2/190 and 3/115 had a LODp value close to 2.4; therefore, they were considered for testing in the final model. QTL 5/0 would be actually two linked QTL in positions 0 and 40. A significant interaction was detected between regions 4/115 and 6/75 and a nearly suggestive interaction was found between QTL 1/108 and region 8/110. Using EPISTACY we detected an interaction between M119 in position 98.3 (linkage group 1) and M1 in position 102.6 (linkage group 8) that may be representing interaction between QTL 1/108 and 8/110. For QTL 4/115 and 6/75, an interaction was detected between M61 and M118 located at the exact positions of the QTL (Fig. 1). The partition of the interaction variance suggested that both interactions were additive by dominant. These two interactions were included in the final model for further testing.

### Multiple QTL model to detect main and interacting QTL

The multiple regression analysis for GM at harvest indicated that ten QTL and an interaction were significantly associated with the trait (Table 3). Each QTL or interaction accounted from 1.35 to 11.65% of the phenotypic variation. Both loci in linkage group 2 were individually associated with GM at harvest. The final model for GM at harvest accounted for 54.8% of the phenotypic variation and 65.2% of the genotypic variation.

For FDR, the multiple-QTL model accounted for 35.7 and 45.2% of the phenotypic and genotypic variation, respectively. The analysis suggested that QTL 5/0 and 5/40 were individually associated with the trait and that QTL 3/115 may not be considered in the model. Therefore, eight QTL and two interactions were included in the final model. Each individual QTL or interaction explained between 2.73 and 7.04% of the phenotypic variance (Table 3).

## Discussion

### Methodology for measuring FDR

The availability of a reliable methodology to measure GM across the drying period under field conditions is a bottleneck in selection for FDR (Kang et al. 1978). The hand-held moisture meter has been reported to be useful for selecting and evaluating genetic materials (Freppon et al. 1992; Kang et al. 1978; Zhang et al. 1996). Our calibration results supported the use of the hand-held moisture meter as an estimator of the actual GM and FDR. However, the estimation of FDR has an additional error related to the calculation of the linear regression. This could partially explain the low per-

centage of the phenotypic variation accounted for by the final model and each QTL individually (Table 3).

## Parental lines and heritabilities

The parental lines had highly significant differences in GM at harvest and FDR (Table 1) but not in thermal time to physiological maturity. As stated by Lander and Botstein (1989), the high contrast between parental lines performance for GM at harvest and FDR would theoretically increase the genetic segregation of loci influencing these traits in the $F_{2:3}$ population. Conversely, segregation for physiological maturity date would not be expected in this population. However, we observed some variation in the $F_{2:3}$ population for the latter trait (data not shown) that could have influenced GM at harvest. We are currently conducting more research in this regard.

Based on the classification of Hallauer and Miranda (1988), $h^2$ values on an entry basis could be considered high for both traits. The values obtained in this study were within the range reported in the literature, either for GM at harvest (Austin et al. 2000; Beavis et al. 1994; Mihaljevic et al. 2005; Hallauer and Miranda 1988) or FDR (Kondapi et al. 1995; Purdy and Crane 1967).

## QTL for GM at harvest and FDR

The difference in $ET_0$ between the 2 years represented two contrasting environments for evaluating GM at harvest and FDR. As suggested by previous studies (Austin et al. 2000; Leon et al. 2001; Veldboom and Lee 1996) the mean environment is adequate for detection of QTL with consistent effects across environments. In the $F_{2:3}$ population under study, we detected ten QTL and an interaction for GM at harvest (Table 3). Previous studies have reported QTL for GM at harvest (Austin et al. 2000; Beavis et al. 1994; Melchinger et al. 1998; Mihaljevic et al. 2005; Ragot et al. 1995). The number of QTL found in these studies ranged from 1 to 17 and the variation accounted for by individual QTL varied from 0.8 to 28.5%. In four of these studies, QTL mapping was performed in testcross populations. However, as shown by Beavis et al. (1994) and Mihaljevic et al. (2005), there is some congruency between the QTL detected in the line populations and their respective testcross populations. Hence, to some extent, the results of the different studies are comparable with ours. In all these studies but one (Ragot et al. 1995), at least one QTL was found in linkage groups 1, 2, 7 and 8. As shown in Table 3, we also detected QTL for GM at harvest in linkage groups 1, 2 and 8. The consistency of QTL detected in the same linkage group from such different studies and environmental conditions suggests that one or more genes of importance for the trait may be located in these linkage groups. Although the LOD score for the interaction between QTL 1/194 and 8/64 was below the threshold, the inclusion of the term in the multiple regression model significantly contributed to explain the observed variation (Table 3). No interacting QTL have been reported in the literature for GM at harvest. Traditionally, this was attributed to the lack of statistical resolution necessary for detecting interactions, because of the study of rather small populations or the use of sparsely distributed genetic markers (Paterson et al. 1991). The fact that we have detected interaction in this population may remark the importance of model selection in QTL mapping (Broman and Speed 2002; Zeng et al. 1999).

We detected eight QTL and two interactions for FDR. No previous reports were found in the literature

**Table 3** Multiple regression models for grain moisture at harvest and field grain drying rate

| Grain moisture at harvest (g kg$^{-1}$) | | | | | Field drying rate (g kg$^{-1}$ day$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| QTL[a] | *df*[b] | Adj SS[c] | LOD | $R^{2d}$ | QTL | *df* | Adj SS | LOD | $R^2$ |
| 1/194 | 6 | 8069.5 | 6.51 | 7.06*** | 1/108 | 6 | 6.90 | 4.54 | 7.04** |
| 2/100 | 2 | 2821.1 | 2.40 | 2.47** | 5/0 | 2 | 4.92 | 3.30 | 5.02** |
| 2/145 | 2 | 2585.3 | 2.21 | 2.26* | 5/40 | 2 | 2.67 | 1.83 | 2.73* |
| 5/0 | 2 | 4945.3 | 4.12 | 4.33*** | 8/64 | 2 | 2.89 | 1.97 | 2.95* |
| 8/64 | 6 | 13317.5 | 10.23 | 11.65*** | 2/190 | 2 | 3.47 | 2.35 | 3.54** |
| 9/34 | 2 | 8305.2 | 6.69 | 7.27*** | 4/115 | 6 | 6.56 | 4.34 | 6.70** |
| 10/26 | 2 | 4364.0 | 3.66 | 3.82*** | 6/75 | 6 | 5.97 | 3.97 | 6.10* |
| 3/80 | 2 | 1981.0 | 1.70 | 1.73* | 8/110 | 6 | 4.23 | 2.85 | 4.32$^{\dagger}$ |
| 4/0 | 2 | 3618.3 | 3.05 | 3.17** | 4/115*6/75 | 4 | 5.52 | 3.68 | 5.64** |
| 6/25 | 2 | 1539.9 | 1.33 | 1.35$^{\dagger}$ | 1/108*8/110 | 4 | 3.07 | 2.09 | 3.14$^{\dagger}$ |
| 1/194*8/64 | 4 | 4622.5 | 3.86 | 4.05 ** | | | | | |
| | | | | 54.8[e] | | | | | 35.7[e] |

[a]Linkage group/absolute position in centi Morgan
[b]Degrees of freedom
[c]Adjusted (type III) sum of squares
[d]Coefficient of determination: percentage of phenotypic variance explained by each QTL individually in the model
[e]Adjusted total: estimate of total percentage of phenotypic variance explained by the simultaneous fit of all putative QTL, corrected by the degrees of freedom of the model
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; $^{\dagger}P < 0.1$

regarding QTL for FDR. As previously discussed, a possible explanation for this is the lack of a reliable and non-destructive methodology for measuring GM across the drying period. The analysis performed with EPISTACY revealed a high number of markers interacting for FDR (data not shown). Although many of these marker interactions could be random, they would suggest a high influence of the genetic background on FDR. The lack of genotype × environment interaction for FDR in this study is remarkable because previous reports highlight the strong influence of the environment on the trait (Dwyer et al. 1994; Hallauer and Russell 1961; Magari et al. 1997). In an independent study, using two hybrids sown on five consecutive dates, we found no genotype × environment interaction for FDR (unpublished results).

There was little coincidence in QTL location between GM at harvest and FDR. QTL 5/0 was located at the exact position for both traits. QTL 8/64 was also located at the same position, but with opposite allelic effect between traits. The favorable allele for FDR was derived from the expected parent but this was not the case for GM at harvest. This apparently opposite allelic effect for GM at harvest for the latter QTL has been previously documented in the literature for other QTL and traits (Lee 1995). A third QTL, in linkage group 1 could also be considered as a common QTL, given the superimposition of their one-LOD support interval (Table 2). However, the simultaneous search detected two putative QTL for FDR in linkage group 1 at positions 115 and 160 (data not shown) that could explain the wide support interval for QTL 1/108 (Table 2). Thus, the latter coincidence may be taken cautiously. As mentioned by Lebreton et al. (1995), the identification of common regions may provide a more precise test of whether the traits are causally related or merely vary in association (Simko et al. 1997). The finding of common QTL between traits provides evidence for the former hypothesis. The low coincidence in QTL position between traits, however, suggests that in the $F_{2:3}$ population other factors, such as maturity, may be segregating and influencing both traits separately. Another reason for this low coincidence between traits could be the lack of precision in FDR estimation. This highlights the importance of considering both traits as selection criteria.

## Implications of QTL interaction and prospects for MAS

Quantitative trait loci interaction or epistasis, as defined in quantitative genetics (Mackay 2001), has important practical and theoretical consequences (Mackay 2001; Zhuang et al. 2002). The interaction between QTL 1/194 and 8/64 for GM at harvest involved two major QTL detected in the first scan (Table 2). Since this interaction was additive by additive, we recalculated the additive effects of QTL 8/64 (M69) for each allele of QTL 1/194 (M10; Fig. 2). From these results, it is clear that the estimated additive effects of QTL 8/64 would be highly affected by the interaction. Moreover, based on the results of the first scan (Table 2), in the framework of a MAS program to reduce GM at harvest, we would have selected toward the maternal allele in QTL 1/194 and the paternal allele in QTL 8/64. As shown before, given this combination, the additive effect of QTL 8/64 is practically null and therefore no progress in the trait would be expected with MAS. Considering the high costs and technology input regarding MAS, these epistatic effects should be taken into account. This interaction could also explain the disagreement between IM and CIM in the estimated position of QTL 8/64. The two-dimensional scan allowed us to explain the disagreement in position between mapping methodologies for QTL 2/132 (GM) and 1/108 (FDR) but not for QTL 8/64. One of the limitations of CIM is that it is not directly extendable to analyzing epistasis (Zeng et al. 1999), and according to Zhuang et al. (2002) and Mackay (2001), the estimation of QTL position would be biased if epistasis is not considered. It may be possible then, that the position of the interacting QTL 8/64 was miss-detected with CIM due to the interaction of this QTL with QTL 1/194.

Understanding the complexity of a trait would aid in the selection of an adequate breeding strategy. MAS should be more effective than phenotypic selection when the proportion of the additive variance accounted for by the marker-QTL association is greater than the heritability of the trait (Dudley 1993; Lande and Thompson 1990). Previous reports documented that for GM at harvest (Sentz 1971) and FDR (Kondapi et al. 1995; Purdy and Crane 1967; Zhang et al. 1996) a high proportion of the genetic variance is additive, suggesting that selection through MAS should be effective. However, when a trait is controlled by a relatively large number of genes, then MAS should not be as effective as expected (Dudley 1993; Young 1999). In this study, for both GM at harvest and FDR we found a high number of QTL according to Kearsey and Farquhar (1998). Also, we found interactions between QTL which, as previously discussed, would further complicate MAS. According to these results, the feasibility of MAS for GM at harvest and FDR should be taken cautiously. A selection index including both molecular marker information and phenotypic values, each appropriately weighted (Lande and Thompson 1990), would be the best selection strategy for these traits.

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