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Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies

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Abstract In most maize-growing areas yield reductions due to drought have been observed. Drought at flowering time is, in some cases, the most damaging. In the experiment reported here, trials with F_3 families, derived from a segregating F_2 population, were conducted in the field under well-watered conditions (WW) and two other water-stress regimes affecting flowering (intermediate stress, IS, and severe stress, SS). Several yield components were measured on equal numbers of plants per family: grain yield (GY), ear number (ENO), kernel number (KNO), and 100-kernel weight (HKWT). Correlation analysis of these traits showed that they were not independent of each other. Drought resulted in a 60% decrease of GY under SS conditions. By comparing yield under WW and SS conditions, the families that performed best under WW conditions were found to be proportionately more affected by stress, and the yield reductions due to SS conditions were inversely proportional to the performance under drought. Moreover, no positive correlation was observed between a drought-tolerance index (DTI) and yield under WW conditions. The correlation between GY under WW and SS conditions was 0.31. Therefore, in this experiment, selection for yield improvement under WW conditions only, would not be very effective for yield improvement under drought. Quantitative trait loci (QTLs) were identified for GY, ENO and KNO using composite interval mapping (CIM). No major QTLs, expressing more then 13% of the phenotypic variance, were detected for any of these traits, and there were inconsistencies in their genomic positions across water regimes. The use of CIM allowed the evaluation of QTL-by-environment interactions (Q × E) and could thus identify "stable" QTLs across drought environments. Two such QTLs for GY, on chromosomes 1 and 10, coincided with two stable QTLs for KNO. Moreover, four genomic regions were identified for the expression of both GY and the anthesis-silking interval (ASI). In three of these, the allelic contributions were for short ASI and GY increase, while for that on chromosome 10 the allelic contribution for short ASI corresponded to a yield reduction. From these results, we hypothesize that to improve yield under drought, marker-assisted selection (MAS) using only the QTLs involved in the expression of yield components appears not to be the best strategy, and neither does MAS using only QTLs involved in the expression of ASI. We would therefore favour a MAS strategy that takes into account a combination of the "best QTLs" for different traits. These QTLs should be stable across target environments, represent the largest percentage possible of the phenotypic variance, and, though not involved directly in the expression of yield, should be involved in the expression of traits significantly correlated with yield, such as ASI.

Key words Anthesis-silking interval · Drought · Marker-assisted selection · Quantitative trait loci · Tropical maize

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Introduction

Environmental factors strongly influence the yields of cultivated crops. In maize, drought is one of the major factors limiting biomass and seed production. During the last 50 years considerable effort has been devoted to improving yield performance through breeding, and to understand the mechanisms involved in drought tolerance (e.g., Jensen 1971; Edmeades et al. 1992). Drought is particularly acute in developing countries, where irrigation facilities are often lacking and where rainfall represents the main source of crop-available water

(Edmeades et al. 1997). The risk of drought is highest at both the start and the end of the growing season. However, because maize is a monoecious plant in which male and female flowers are physically separated by up to 1 m, it is particularly susceptible to drought and high-temperature conditions during flowering (Johnson and Herrero 1981; Westgate and Bassetti 1990). Losses in grain yield are particularly severe when drought stress occurs at this stage (Claassen and Shaw 1970; Grant et al. 1989). A delay in silk emergence relative to anthesis – a long anthesis-silking interval (ASI) – often results from drought stress at flowering, and this silk delay is correlated with lowered grain yield (Westgate and Boyer 1986; Bolaños and Edmeades 1993). This is not surprising since the establishment of final kernel number occurs in a 2-week period following flowering (Claassen and Shaw 1970).

Conventional breeding for yield improvement under drought conditions is time consuming and laborious, because carefully managed field conditions are required. In addition, there is a decrease in the genetic variance and heritability of yield components that parallels an increase in environmental stress (Blum 1988). Moreover, when drought induces a reduction in yield, this decrease depends on two factors having combined effects, making selection more complex (Edmeades et al. 1989). The first of these is the drought susceptibility of the plant, and the second the spillover effects of yield potential, that is the higher probability that a plant performing very well under well-watered conditions will also perform well under drought, even if the relative yield reduction for this plant is large. Considering these limitations to efficient selection, and the fact that only one relatively rainfree crop season per year is available for selection in most tropical countries, the use of molecular markers could provide a useful tool to complement phenotypic selection. Several quantitative trait loci (QTLs) involved in the expression of different yield components have already been detected in maize (Stuber et al. 1987; Stuber et al. 1992; Zehr et al 1992; Beavis et al. 1994; Schön et al. 1994; Stromberg et al. 1994; Veldboom and Lee 1994; Ajmone-Marsan et al. 1995; Berke and Rocheford 1995; Ragot et al. 1995; Ajmone-Marsan et al. 1996), but none of these studies were conducted under conditions of controlled water stress in the field. In the first part of the present study (Ribaut et al. 1996), QTL analyses of flowering parameters, male sterility and ASI are reported, and the use of ASI QTLs in a marker-assisted selection (MAS) scheme to improve drought tolerance is discussed.

The objectives of the second part of the study, reported herein, were: (1) to identify the QTLs involved in the expression of yield and two yield components, namely ear and kernel number; (2) to determine phenotypic correlations between these traits, the 100-kernel weight and ASI; and (3) to discuss the efficiency of using yield-component QTLs in a MAS scheme to improve drought tolerance in maize.

Materials and methods

Details of plant material, experimental design, and trial descriptions were presented previously (Ribaut et al. 1996).

Field measurements

Field data were obtained from trials of F_3 families, derived from a segregating F_2 population derived from two tropical maize inbred lines, $Ac7643S_5$ and $Ac7729/TZSRWS_5$, respectively named P_1 and P_2. With a short ASI, P_1 is drought tolerant, while P_2 with a long ASI, is drought susceptible. Trials were conducted using an alpha (0,1) lattice design (Patterson and Williams 1976) under well-watered conditions (WW) in the winter plant cycle of 1992 (92A) and 1993 (93A), under intermediate stress (IS) in the winter plant cycle of 1992 (92A) and 1994 (94A), and under severe stress (SS) in the winter plant cycle of 1994 (94A). The planting density in each environment was 60 000 plants ha^{-1}. The number of ears (ENO) was recorded per plot (ten plants) and included all ears having one grain or more. Harvested ears were air-dried for 3 days in a commercial airflow drier (40°C), where the moisture for all the samples reached 11%. GY was expressed in this study at 11% of moisture. The ears were then shelled, and the grain weighed to obtain the grain yield per plot (GY per ten plants). A random sample of 100 kernels from each plot was dried to constant weight at 80°C and weighed (100-kernel weight, HKWT).

Data analysis

The analysis of the alpha (0,1) lattice design for the recovery of interblock information, considering the blocks within replicates as random effects, was done using the PROC MIXED procedure of SAS (SAS 1988). With this analysis, the adjusted means of the F_3 families were obtained in each environment and for the different field traits. The total parental value for the different traits was obtained from the mean of the nine adjusted means of each parental line (Ribaut et al. 1996). The kernel number per plot (KNO) was calculated by dividing the GY by the average weight per kernel for each replication. Simple Pearson correlations among the traits, within or across the different water regimes, were calculated on the adjusted means of the families. The heritability of each trait across IS and SS conditions (cycle 94A) was calculated as described previously (Ribaut et al. 1996), and the corresponding confidence intervals were calculated as described in Knapp et al. (1985). Two indices of drought tolerance (DTI and DTI′) were calculated, based on the index proposed by Fischer and Maurer (1978), in an attempt to estimate the drought tolerance of the families for GY, without spillover effects of yield potential:

$$\mathrm{DTI} = \frac{\mathrm{GY(SS)}}{\mathrm{GY(WW)}} \quad \text{and} \quad \mathrm{DTI}' = \frac{\mathrm{GY}'\mathrm{(SS)}}{\mathrm{GY(WW)}},$$

where GY(SS) and GY(WW) represent the grain yield of the F_3 families under severe stress and well-watered conditions, respectively, and GY′(SS) is the grain yield under severe stress conditions corrected for differences due to variation in male-flowering date (MFLW) of each family. The GY′(SS) per family was calculated as:

$$\mathrm{GY}'\mathrm{(SS)} = \mathrm{GY(SS)} + (90.0 - \mathrm{MFLW})(-24.0),$$

where 90.0 represents the MFLW mean of all the F_3 families (Ribaut et al. 1996), and -24.0 (g) the mean decrease of GY per additional day to MFLW under SS.

RFLP mapping and QTL detection

The linkage map was obtained as previously described (Ribaut et al. 1996). The identification of QTLs involved in the expression of the different traits was performed using composite interval mapping (CIM, Zeng 1994). This method is based on mixture models and maximum-likelihood techniques, using markers as co-factors under certain conditions. The approach can be divided into two steps. The first one is simple interval mapping (SIM) over the whole genome. Based on the results of the SIM analysis, markers closely linked to the QTLs, one marker per QTL position, were identified as co-factors. Using these co-factors to reduce the residual variation throughout the genome and to increase the power of QTL identification, new QTLs may be identified or previous QTL peaks may shift in position. In these cases a new set of co-factors was defined in the model and the step was repeated until no new QTLs could be detected. In the second step, additional markers flanking the tested interval were used as co-factors to block the effects of possible QTLs linked to the interval of interest and to potentially identify "ghost" QTLs. This was done at all marker intervals throughout the genome; the chosen distance between the tested interval and a co-factor defined a "window" for testing the presence of a QTL in that interval. Two different window sizes, 30 cM and 20 cM, were successively used. All QTLs presented in this paper are considered putative, but will be referred to simply as QTLs in the text that follows. The presence of a QTL was declared significant if the LOD threshold value was > 2.5 in at least one test environment (Ribaut et al. 1996). Allelic effects, additivity and dominance, at each significant QTL were obtained directly from the output of the CIM program. A joint analysis of phenotypic data obtained from the two levels of water stress was conducted using CIM to evaluate the effects of environmental interactions on QTL identification ($Q \times E$) (Jiang and Zeng 1995). Finally, multiple regression was performed for the phenotypic values at all markers closely linked to a QTL position (one per QTL), including both additivity and dominance effects, to evaluate the total percentage of phenotypic variation accounted for by all the identified QTLs.

Results

Field-data analyses

From WW to drought conditions, marked reductions in the F_3 family mean and in the spread of the distribution were observed for GY, ENO, KNO, and to a lesser extent for HKWT (Table 1). GY fell by about 60% under both IS and SS conditions, and the three yield components contributed to this decrease. The comparable reduction in ENO was around 36%, 50% for KNO, and 20% for HKWT. The parental values for GY under IS and SS conditions were significantly lower than the overall mean of the F_3 families. This result, which is most likely due to the different level of inbreeding between the parental lines and the F_3 families, suggested dominance or overdominance effects for several genes involved in the expression of GY.

Except for HKWT, drought stress reduced the genetic variance of the yield components and therefore the power of QTL detection under drought. This is in sharp contrast with the increased genetic variance observed for female flowering and ASI under drought (Ribaut et al. 1996). The heritability calculated across the two stress levels, however, remained high for these yield components (Table 1). This might reflect the efficiency of the alpha (0,1) lattice design in reducing the variance of the error terms of each experiment and the $G \times E$ interaction (Patterson and Williams 1976). By their nature, GY, ENO and KNO are not independent, as confirmed by the high level of correlations (Table 2).

Table 1 Means ($\pm$ SE) of parental lines (P_1 and P_2) and F_3 families, genetic variance (Gen var.) and broad-sense heritability (h^2) with 90% confidence intervals for grain yield (GY), ear number (ENO), kernel number (KNO) and 100-kernel weight (HKWT) evaluated under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Trait	Level	Mean			Range F_3	Gen. var. F_3	h^2 IS/SS
		P_1	P_2	F_3			
GY (g/10 plants)	WW			1052 ± 13	533/1770	42 089**	
GY (kg ha^{-1})	WW			6996 ± 86			
GY (g/10 plants)	IS	179 ± 16	145 ± 15	419 ± 9	42/849	20 956**	0.66
							0.61–0.74
GY (kg ha^{-1})	IS			2786 ± 60			
GY (g/10 plants)	SS	221 ± 14	89 ± 16	411 ± 10	68/887	22 323**	
GY (kg ha^{-1})	SS			2733 ± 67			
ENO (10 plants)	WW			13.0 ± 0.2	6.2/19.8	6.5**	
ENO (10 plants)	IS	8.0 ± 0.3	6.1 ± 0.3	8.3 ± 0.1	3.2/12.7	2.1**	0.58
							0.49–0.67
ENO (10 plants)	SS	8.0 ± 0.2	4.4 ± 0.4	8.3 ± 0.1	3.2/14.1	2.6**	
KNO (10 plants)	WW			3475 ± 44	1858/5693	467 969**	
KNO (10 plants)	IS	801 ± 60	618 ± 61	1703 ± 34	344/3536	277 798**	0.66
							0.58–0.73
KNO (10 plants)	SS	931 ± 47	358 ± 77	1638 ± 34	321/3215	276 572**	
HKWT (g)	WW			30.5 ± 0.2	20.0/41.0	10.9**	
HKWT (g)	IS	22.0 ± 1.5	23.5 ± 1.3	24.1 ± 0.2	14.7/31.8	9.2**	0.73
							0.69–0.80
HKWT (g)	SS	22.2 ± 1.8	22.5 ± 1.9	24.4 ± 0.2	12.3/35.3	14.0**	

** Significant at the 0.01 probability level

Table 2 Linear correlations (Pearson) between anthesis-silking interval (ASI), grain yield (GY), ear number (ENO), kernel number (KNO) and 100-kernel weight (HKWT) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Item	WW				IS				SS			
	ASI	GY	ENO	KNO	ASI	GY	ENO	KNO	ASI	GY	ENO	KNO
GY	− 0.07				− 0.40**				− 0.39**			
ENO	− 0.15*	0.50**			− 0.40**	0.69**			− 0.55**	0.64**		
KNO	− 0.17**	0.88**	0.56**		− 0.43**	0.95**	0.68**		− 0.43**	0.94**	0.70**	
HKWT	− 0.17*	0.22**	− 0.17**	− 0.26**	− 0.10*	0.53**	0.33**	0.27**	− 0.06	0.50**	0.09	0.20**

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level

Under WW conditions, the magnitude of linear correlations between the traits was always lower than under drought. Across the three water regimes, GY was highly correlated with KNO and ENO, and KNO with ENO. Under WW conditions HKWT showed low correlation with the other traits, but the level of these correlations increased under IS conditions. Thus, HKWT is not strongly dependent on changes in ear or grain number per family. Results from Tables 1 and 2 confirm that water stress before and during flowering affected mainly the kernel number and to a lesser extent the size of the kernels (Hall et al. 1981). Under WW conditions, ASI was not correlated with GY and only weakly correlated with other yield components. Under stress conditions, the correlations of ASI with each yield component became highly significant, with the exception of HKWT.

Drought tolerance

To better represent the complex phenomenon of yield reduction under drought, different linear regressions are represented graphically in Fig. 1. A "direct" measure of yield reduction induced by water stress was calculated as the difference between the GYs under WW and SS conditions, and the drought-tolerance index, DTI, was calculated as the ratio between the GYs under WW and SS conditions. By definition, yield reduction and DTI are not independent of GY under both WW and SS conditions, and thus, some correlation among them was expected.

The yield reduction was highly positively correlated with GY under WW conditions (Fig. 1 A) and, to a lesser extent, negatively under drought (Fig. 1 C). Thus,

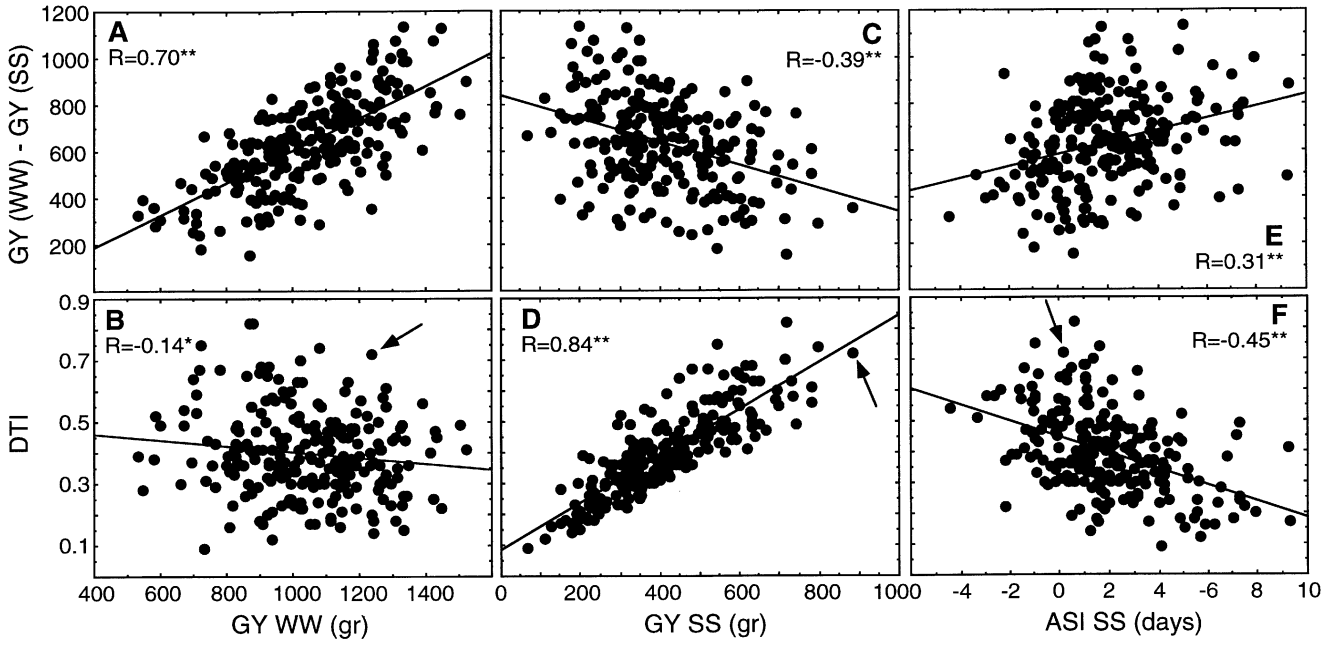

Fig. 1 Linear regression of yield reduction induced by drought [GY(WW)-GY(SS)] and drought-tolerance index (DTI) on grain yield (GY) under well-watered conditions (**A** and **B**), on grain yield under severe stress conditions (**C** and **D**), and on the anthesis-silking interval (ASI, **E** and **F**). The *arrow* in **B**, **D** and **F** indicates the performance of one family (35)

the families performing best under WW conditions had the most marked yield reduction under drought. These families had comparatively low yields under stress conditions, since the yield reduction due to SS conditions was inversely proportional to the performance under drought (Fig. 1 C). The correlation of DTI with GY under WW conditions was slightly negative (Fig. 1 B); however, DTI was very positively correlated with GY under drought (Fig. 1 D). This demonstrates that, in this experiment, there was no positive correlation between drought tolerance and GY under WW conditions. The fact that the DTI never exceeded 1.0 demonstrates also that none of the families performed better under drought than under WW conditions. The ASI was significantly correlated with yield reduction (Fig. 1 E) and DTI (Fig. 1 F).

The drought-tolerance index used here does not consider effects of time to flowering, itself associated with drought escape, which is usually negatively correlated with a yield decrease. In this experiment, GY and male-flowering data (MFLW) under drought were significantly correlated ($r = -0.31$). For this reason, a second index (DTI′) was calculated using GY under drought adjusted for MFLW. This modification of the index produced some changes in the ranking of various families, but the magnitudes of the correlations of DTI′ with GY or ASI were not significantly altered. It was decided, therefore to present graphically the results for DTI, because the differences in time to flower between the different families is one of the important criteria taken into account during selection (Bolaños and Edmeades 1993).

The F_3 families of interest to breeders are those that performed well under both well-watered and stress conditions. As an example, family 35 was highlighted (arrows) in Fig. 1 (B, D and F). This F_3 family was one of the most drought tolerant, with a DTI of 0.72, it performed well under well-watered conditions (1240 g/10 plants, or 8246 kg/ha), and was the best under drought (886 g/10 plants, or 5890 kg/ha). As expected, based on the correlation between ASI and GY, this family had a short ASI under drought (0.16 days). We will refer later to the allelic composition of this family at different QTLs for ASI and GY.

QTLs detected

The same genetic map presented in detail by Ribaut et al. (1996) was used to identify the QTLs involved in the expression of the traits reported here. The location and characteristics of the QTLs identified using CIM (composite interval mapping) for the three major yield components are presented in Tables 3, 4 and 5. The number of QTLs identified per trait was between one and seven under the three water regimes, with less QTLs identified under SS conditions. The reproducibility of different QTLs identified across the three water regimes was low: one QTL for GY on chromosome 10, perhaps one for ENO on chromosome 1, and none for KNO. Taking into account only the two stress regimes, the consistency of the QTLs remains very low: with the same QTLs for GY and ENO on chromosomes 10 and 1, and one for KNO on chromosome 9. When a QTL was identified across several water regimes, the direction of the allelic contribution was consistent.

As a result of this inconsistency, both in the number and location of detected QTLs across the three water regimes, there were important discrepancies in the percent phenotypic variation explained by these QTLs across water regimes. The total of the phenotypic variance expressed under drought for the three yield components was never higher than 27% under IS and 17% under SS conditions, with especially low values for the expression of ENO (17% and 6% under IS and SS, respectively).

By comparing QTLs per water regime, several genomic regions involved in the expression of more than one trait were identified. This was observed under IS conditions for GY and KNO, with two QTLs in common (Tables 3 and 5), and for ENO and KNO with one QTL on chromosome 9 (Tables 4 and 5). Under SS conditions, one genomic region on chromosome 10 was involved in the expression of both GY and KNO, and another on chromosome 1 was involved in the expression of both GY and ENO. Under a specific environment, the identification of several common genomic regions involved in the expression of GY, ENO and KNO reflect well the highly significant level of linear correlation observed between these three traits under the different water regimes (Table 2).

As expected from the frequency distributions of the traits and the observed mean values of the two parental lines, dominant and overdominant effects were observed at several QTL positions.

QTL-by-environment interaction

By combining data sets from the two target stress environments (IS versus SS) it was possible with CIM to identify a subset of significant QTLs. Under a chosen threshold of QTL-by-environment interaction ($Q \times E$), the QTLs can be considered as "stable" across the two stress levels; above that value, the QTLs are considered to be too dependent on a particular stress environment and thus of little use for MAS to improve drought tolerance.

Stable QTLs detected during the combined analysis of IS and SS environments fall into two groups. On the one hand, there are those that had already been detected at the same position in the analysis of the separate data sets. These usually have the highest LOD scores (e.g., the QTL detected on chromosome 10 for GY). On the other hand, there are those for which the two LOD scores in the separate analyses are such that one is not

Table 3 Genetic characteristics of QTLs involved in the expression of grain yield (GY) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions. The composite interval mapping (CIM) method was used for QTL identification

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b] (gr)	Dominance (gr)	Direction[c]	Total additivity	Phenotypic variance[a] (%)
WW 92A	1	168	*umc33a*	4.18	86	54	P2	172	9.0
	2	86	*csu133*	3.15	− 71	− 22	P1	142	5.9
	8	134	*umc30a*	3.04	17	216	P2	34	6.9
	10	48	*npi223b*	2.50	49	118	P2	98	5.5
				10.88					20.0
IS 94A	1	154	*umc119*	5.25	70	31	P2	140	11.8
	1	229	*bnl6.29b*	3.04	− 35	103	P1	70	6.1
	4	14	*umc123*	2.71	49	14	P2	98	5.7
	7	74	*bnl15.07b*	2.66	− 6	124	P1	12	4.6
	10	59	*umc64*	2.79	45	40	P2	90	6.8
				14.91					26.3
SS 94A	1	82	*umc53b*	2.51	− 39	78	P1	78	5.0
	4	114	*umc104a*	2.69	− 37	84	P1	74	5.1
	6	57	*csu111b*	2.88	− 56	− 82	P1	112	8.9
	10	60	*umc64*	4.64	67	− 12	P2	134	10.1
				8.70					16.3
IS + SS	1	82	*umc53b*	2.73	− 38	88	P1	76	5.6
	1	156	*umc119*	4.01	54	58	P2	108	7.7
	6	57	*csu111b*	2.80	− 46	− 39	P1	92	5.5
	8	73	*umc120a*	2.60	− 38	− 24	P1	76	4.5
	10	61	*csu86*	4.10	65	8	P2	130	9.8
				14.56					25.8

[a] Totals of the LOD score and the percentage of phenotypic variance accounted for were determined in a multiple-QTL model
[b] Additive effects are associated with the allele from the susceptible line (P_2). A positive value means that the P_2 allele increases the numeric value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numeric value of the trait

Table 4 Genetic characteristics of QTLs involved in the expression of ear number (ENO) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions. The composite interval mapping (CIM) method was used for QTL identification

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b]	Dominance	Direction[c]	Total additivity	Phenotypic variance[a] (%)
WW 92A	1	27	*csu111*	3.31	− 0.89	0.94	P1	1.78	7.1
	1	241	*umc161a*	2.99	− 0.81	0.26	P1	1.62	5.2
	2	93	*umc8b*	4.61	− 0.98	− 0.72	P1	1.96	8.0
	4	82	*umc156a*	4.31	− 0.96	0.82	P1	1.92	7.9
	7	75	*bnl15.07b*	2.70	0.66	1.18	P2	1.32	5.3
	8	130	*umc30a*	3.56	− 0.73	1.36	P1	1.46	6.0
	9	54	*umc105a*	6.43	1.04	0.24	P2	2.08	8.6
				24.76					39.8
IS 94A	1	65	*umc11*	2.54	− 0.23	1.34	P1	0.46	6.2
	3	44	*umc50*	3.10	0.55	0.36	P2	1.10	7.3
	6	90	*csu60*	3.12	0.15	1.64	P2	0.30	8.2
	9	69	*umc114*	2.76	0.50	0.14	P2	1.00	5.8
				9.22					17.2
SS 94A	1	77	*umc11*	2.66	− 0.22	1.40	P1	0.44	5.8
				2.66					5.8
IS + SS	1	73	*umc11*	2.51	− 0.29	1.00	P1	0.58	4.5
	7	11	*csu34a*	2.67	− 0.41	0.30	P1	0.82	4.0
	9	69	*umc114*	2.71	0.58	0.14	P2	1.16	7.2
				7.46					14.2

[a] Totals of the LOD score and the percentage of phenotypic variance accounted for were determined in a multiple-QTL model
[b] Additive effects are associated with the allele from the susceptible line (P_2). A positive value means that the P_2 allele increases the numeric value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numeric value of the trait

Table 5 Genetic characteristics of QTLs involved in the expression of kernel number (KNO) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions. The composite interval mapping (CIM) method was used for QTL identification

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b]	Dominance (gr)	Direction[c]	Total additivity	Phenotypic variance[a] (%)
WW 92A	1	169	*umc33a*	4.40	310	− 52	P2	620	9.9
	1	245	*umc161a*	2.50	− 207	− 172	P1	414	4.8
	7	75	*bnl15.07b*	2.61	184	272	P2	368	4.6
				9.38					17.5
IS 94A	1	158	*umc119*	4.30	239	194	P2	478	11.1
	3	87	*bnl8.01*	3.65	79	726	P2	158	12.9
	4	10	*umc123*	3.12	206	86	P2	412	7.7
	9	62	*bnl3.06*	3.01	133	431	P2	266	7.3
				11.35					20.7
SS 94A	9	75	*umc114*	2.68	131	435	P2	262	7.3
	10	54	*umc64*	2.65	161	200	P2	322	5.6
				4.55					8.9
IS + SS	1	158	*umc119*	3.44	210	110	P2	420	7.6
	4	18	*umc123*	2.80	190	76	P2	380	6.1
	9	72	*umc114*	2.81	140	397	P2	280	6.6
	10	56	*umc64*	3.05	165	212	P2	330	5.5
				10.48					19.3

[a] Totals of the LOD score and the percentage of phenotypic variance accounted for were determined in a multiple-QTL model
[b] Additive effects are associated with the allele from the susceptible line (P_2). A positive value means that the P_2 allele increases the numeric value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numeric value of the trait

significant but the other is, or else both are just below significance. Examples include a QTL on chromosome 6 for GY (LOD = 2.88 under SS vs 1.45 under IS) and one on chromosome 7 for ENO (LOD = 1.78 under SS vs 2.01 under IS). The LOD of these "new" QTLs was in general just above the threshold value, and the percentage of phenotypic variance that they accounted for was generally low (around 6%).

In the first part of this study (Ribaut et al. 1996), the identification of QTLs for ASI under different water regimes was presented. ASI was calculated per plant as the difference in days between silk emergence and pollen shedding. Five out of the six QTLs detected for ASI were consistent across both IS and SS conditions, using SIM (Mapmaker/QTL). We have re-run the analysis of ASI using CIM in order to be able to compare the results with those reported here for GY. Using CIM, one new ASI QTL was detected on the short arm of chromosome 1. The comparison between ASI and GY QTL locations is shown in Fig. 2. Four genomic regions were involved in the expression of both traits: on chromosomes 1, 6, 8 and 10. At a given position, the distance between QTL peaks for the two traits never exceeded 20 cM. The second QTL detected for each trait on chromosome 1 had peaks 57 cM apart, a distance we consider to be too large to consider these as a common QTL. For three out of the four common genomic regions, the parental line which contributed to

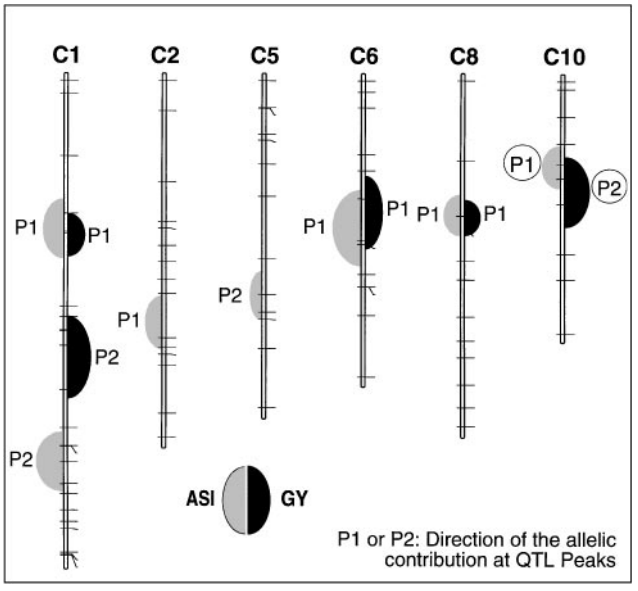

Fig. 2 Location on maize chromosomes of anthesis-silking interval (ASI) and grain-yield (GY) QTLs detected using CIM based on the combined data sets from both intermediate and severe-stress field conditions. Genomic regions responsible for the expression of ASI (left) and GY (right) are represented by *ellipses* for LOD scores higher than 2.0. The width of the ellipse is proportional to the percentage of phenotypic variance explained by that QTL. The parental line contributing the allele for a short ASI or a better yield is indicated for each QTL

reduced ASI (P_1) contributed also to increased GY. However, on chromosome 10, at one of the most important QTLs for GY and KNO, the allele from P_1 contributed to a reduction of ASI but also of yield. This result can be used to explain partially why the linear correlation between ASI and GY failed to exceed -0.39 under SS conditions. In fact, by removing the allelic effect at the marker closest to the peaks of ASI and GY QTLs (*umc64*), the linear correlation between ASI and GY increased to -0.47, which represents an increase of 20%. This kind of result underlines the great importance of checking the "direction" of the allelic contributions at common or linked QTLs.

Discussion

QTLs for yield and yield components under WW conditions

The identification of QTLs involved in the expression of several yield components in maize have already been the subject of a number of studies. A summary of different studies conducted before 1994 was presented by Beavis (1994). Under normal growing conditions several authors mentioned a certain stability of some yield QTLs across locations and/or years (e.g., Stuber et al. 1992; Ajmone-Marsan et al. 1995, 1996). However, comparing studies, Beavis (1994) underlines and analyses the lack of congruency of yield-QTLs among three different studies conducted with independent progeny from the same cross (B73 × Mo17). In our study, two QTLs for grain yield were identified at the same position on chromosomes 2 and 10 across the 92A and 93A WW trials. Based on results from the literature, and our own results, QTLs for GY have now been identified on all ten maize chromosomes. In a number of studies, some individual QTLs accounted for a high percentage of the phenotypic variance: 27% on chromosome 3L (Ragot et al. 1995), 35% on chromosome 5S (Stuber et al. 1992), 24% on chromosome 6S (Ajmone-Marsan et al. 1995), 35% on chromosome 6L (Veldboom and Lee 1994) and 23% on chromosome 9S (Beavis et al. 1994). The fact that these major yield-QTLs mapped at different genomic positions underlines the inconsistency of yield-QTLs across different temperate materials. In our study, no QTL with such major effects was identified under WW or drought conditions.

QTLs for yield components across different water regimes

Taking advantage of some unusually hot seasons in the U.S. (e.g., 1988), some authors have already presented results obtained under water stress conditions, although the timing and intensity of stress were relatively uncontrolled. Stromberg et al. (1994) found eight loci significantly associated with yield under stress using F_2 testcrosses. From the F_2 plants they developed $F_2:S_4$ families, and ten loci were significantly associated with yield in $F_2:S_4$ testcrosses. Only one of these ten loci was also significant in the F_2 testcrosses. They hypothesize that the lack of consistency across years may be due to differences in water deficits: a dry year without irrigation in 1988 (F_2) versus a very wet year in 1990.

In a study dealing with data from 1987 and 1988, Beavis and Keim (1996) augmented results previously published (Beavis et al. 1994). The authors reported that despite the variable growing conditions between 1987 and 1988, most QTLs identified were consistent across stressful and non-stressful environments, although there was a significant Q × E interaction at one of the yield-QTLs. Working under controlled stress conditions, Stuber (1996) presented results from an experiment using a $2 \times 2 \times 2$ factorial field design of soil moisture levels, soil nitrogen levels and planting densities across 3 years in two locations. He observed that QTLs for yield were similar under stress and normal conditions, and concluded that one can use QTLs identified in non-stress environments to breed for stress resistance. It is difficult for us to comment on such results, since no complete paper describing them has been published at this time.

In the present study, QTLs detected for yield components were clearly not stable, in terms of their genomic location, across different water regimes, and the percent of phenotypic variance explained by different sets of QTLs under different field conditions also varied widely. Several arguments can be put forward to explain these results. The most important consequence of water stress is certainly a decrease in yield, coupled with an increase in the G × E interactions (e.g., Blum 1988). This is accompanied by changes in the relative ranks of the families under well-watered and drought conditions. From different experiments conducted in 1987 and 1988 in Mexico on 200 S_1 families, selection of the ten best families under WW and drought conditions demonstrated that only 10–20% of the selected families were common between the two water regimes (Edmeades, unpublished data 1988). In the present study, no positive correlation between drought tolerance (DTI) and the yield performance of F_3 families under WW conditions was observed, and the linear correlation between yield under WW and SS conditions was 0.31. These results demonstrate that selection conducted solely under WW conditions would not provide the most efficient means to improve yield under drought.

Considering inconsistencies in yield performance across WW and drought environments, the identification of different QTLs under different water regimes should be expected. From a genetic point of view, it is logical to imagine that a large number of genes are

involved in the determination of yield. Grain yield is really the final product of plant development, and each physiological mechanism involved in plant development should affect yield to varying degrees. The large number of major phenotypic and physiological changes (such as female flowering, ASI, plant height, water content, cell growth or hormone content), demonstrates the complexity of the plants' response to water stress, and it is expected that the number of QTLs involved in the expression of yield will be large. Of course the stress level is very important, and the role of facultative traits (ASI, osmotic adjustment, hormone content, etc.) depends on the stress intensity, affecting the identification of new sets of QTLs. This in turn might reduce the chance of identifying major QTLs across water regimes, and make yield-QTL detection above a given threshold of probability dependent on specific environmental conditions. In this respect, Beavis (1994) explained the inconsistency of yield-QTLs across experiments using progenies derived from the same cross, by the fact that yield should be under the control of a large number of small-effect QTLs segregating in the genome. In this case, the expectation will be to identify independently a few of these QTLs affecting yield in every experiment, an hypothesis supported by our results. We therefore conclude that the improvement of drought tolerance in maize using molecular markers should be efficient when the identification of QTLs of interest was achieved under drought conditions. Moreover, the QTL inconsistencies, especially across water-stress levels (our target environments), demonstrate that only a few yield-QTLs will be helpful in a MAS strategy and that the use of QTLs involved only in the expression of yield components in a MAS scheme will not result in rapid gains in yield under drought.

MAS strategies: a complementary breeding tool

Drought is an unpredictable climatic phenomenon, varying in timing and intensity. Selected plants have therefore to be able to perform well under both WW and drought conditions, thus complicating the breeding strategy. For this reason, breeders generally evaluate under drought only material identified as performing well under WW conditions. Selection under drought, however, has its drawbacks. First, the efficiency of selection generally decreases due to a decrease in the heritability of grain yield (e.g., Blum 1988), this decrease being related mainly to a decrease in genetic variance (Table 1) which may be accompanied by an increase in error variance (Bolaños and Edmeades 1996). Secondly, selection under drought is costly and time consuming since irrigation facilities are needed and only one dry crop cycle per year is generally available in the tropics. When taking into account undesirable rainfall, 10 years are probably needed to successfully conduct seven cycles of selection under drought in Mexico for example. Marker-assisted selection could thus play a complementary role to conventional breeding.

Based on the results from this study, the use of only QTLs involved in the expression of yield components in a MAS scheme has already been questioned, due to their inconsistency across two stress levels and the small proportion of phenotypic variance that they explain under drought. In general, breeders do not base their selection under stress on GY only, but include secondary traits of interest to construct a selection index to help improve the efficiency of selection (e.g., Bänziger and Lafitte 1997). For example, in a number of experiments carried out at CIMMYT with maize under drought, the heritability of ASI was similar to or higher than that of GY, while the genetic correlation between ASI and GY ranged from -0.4 to -1.0 (Bolaños and Edmeades 1996). These results demonstrated the potential usefulness of ASI as a secondary selection trait for yield improvement under water stress at flowering.

In the design of the best-possible breeding strategy using MAS, additional traits and criteria have to be considered. For each trait of interest, some of the criteria are the number of QTLs detected, the percentage of phenotypic variation that they explain, the total percentage of the genome that they represent, their stability across different environments, and the QTLs each trait has in common with those of yield components. Based on these criteria, ASI appears to be the most useful secondary trait among those examined so far. By comparing the common QTLs for ASI and GY, it is apparent that a MAS scheme based only on ASI-QTLs is not the most efficient way to improve yield under drought. Indeed, on chromosome 10, selection for short ASI will be accompanied by a selection for yield reduction (Fig. 2). Thus, just as breeders use an index of selection combining different traits (e.g., Bolaños and Edmeades 1993), the best strategy for using molecular markers should combine selection for QTLs involved in the expression of key traits, for example ASI and GY. For GY, only two QTLs on chromosomes 1 (second one) and 10 appear useful. This choice is reinforced by the fact that these two QTLs were linked to the two QTLs for KNO under drought, and would thus also indirectly include this trait in selection. To illustrate these points, consider the case of family 35 which had the highest yield under drought. At different ASI QTLs, this family had alleles contributing to the reduction of ASI, though on chromosome 10 it was homozygous for the alleles contributed by P_2 long ASI and higher yield.

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