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Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval

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Abstract Drought is an important climatic phenomenon which, after soil infertility, ranks as the second most severe limitation to maize production in developing countries. When drought stress occurs just before or during the flowering period, a delay in silking is observed, resulting in an increase in the length of the anthesis-silking interval (ASI) and in a decrease in grain yield. Selection for reduced ASI in tropical open-pollinated varieties has been shown to be correlated with improved yields under drought stress. Since efficient selection for drought tolerance requires carefully managed experimental conditions, molecular markers were used to identify the genomic segments responsible for the expression of ASI, with the final aim of developing marker-assisted selection (MAS) strategies. An F_2 population of 234 individuals was genotyped at 142 loci and F_3 families were evaluated in the field under several water regimes for male flowering (MFLW), male sterility (STER), female flowering (FFLW) and ASI. The genetic variance of ASI increased as a function of the stress intensity, and the broad-sense heritabilites of MFLW, FFLW and ASI were high under stress conditions, being 86%, 82% and 78%, respectively. Putative quantitative trait loci (QTLs) involved in the expression of MFLW and/or FFLW under drought were detected on chromosomes 1, 2, 4, 5, 8, 9 and 10, accounting for around 48% of the phenotypic variance for both traits. For ASI, six putative QTLs were identified under drought on chromosomes 1, 2, 5, 6, 8 and 10, and together accounted for approximately 47% of the phenotypic variance. Under water-stress conditions, four QTLs were common for the expression of MFLW and FFLW, one for the expression of ASI and MFLW, and four for the expression of ASI and FFLW. The number of common QTLs for two traits was related to the level of linear correlation between these two traits. Segregation for ASI was found to be transgressive with the drought-susceptible parent contributing alleles for reduced ASI (4 days) at two QTL positions. Alleles contributed by the resistant line at the other four QTLs were responsible for a 7-day reduction of ASI. These four QTLs represented around 9% of the linkage map, and were stable over years and stress levels. It is argued that MAS based on ASI QTLs should be a powerful tool for improving drought tolerance of tropical maize inbred lines.

Key words Anthesis-silking interval · Drought · Quantitative trait loci · RFLP · Tropical maize

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Introduction

Drought is a common phenomenon in developing countries and, after low soil fertility, represents the second most important cause of yield loss for maize [around 17% annually (Edmeades et al. 1992)]. The plant's response to water stress depends on its metabolic activity, morphology and stage of growth. In maize, when drought stress occurs just before and during the flowering period, a delay in silking is observed resulting in an increase in the length of the anthesis-silking interval (ASI) (Hall et al. 1982; Westgate and Bassetti 1990; Bolaños and Edmeades 1993) This asynchrony between male and female flowering has been associated with a grain-yield decrease under drought (Du Plessis and Dijkhuis 1967; Hall et al. 1981; Westgate and Boyer 1986; Bolaños and Edmeades 1993). Selection for grain yield under drought has often been considered inefficient because of the increase in environmental variance relative to genetic variance, which decreases yield heritability as yield decreases. Under these conditions, selection for secondary traits, such as ASI, which are correlated to grain yield and have relatively high heritability, may increase selection efficiency (Bolaños et al. 1993). The anthesis-silking interval is a relatively simple trait to measure in the field. However, there is only one drought cycle per year in the tropics and conventional selection for ASI

requires carefully managed drought conditions in each cycle, which severely limits its use in many breeding programs. Therefore, identification of genomic segments responsible for the expression of ASI, with the aim towards marker-assisted selection, should be very useful.

Paterson et al. (1988) reported the use of a restriction fragment length polymorphism (RFLP) linkage map for tomato to identify genomic regions responsible for the expression of quantitative trait loci (QTLs). This method allowed the dissection of quantitative traits into their Mendelian components, thus increasing our understanding of their inheritance and gene action. Among plants, maize is particularly well suited for mapping since it has a very high degree of molecular polymorphism (e.g., Burr and Burr 1991). Molecular markers have been extensively mapped in the genome of temperate maize to obtain genetic linkage maps (Helentjaris et al. 1986; Burr et al. 1988; Coe et al. 1988) and to identify QTLs (Edwards et al. 1987, 1992; Stuber 1989; Veldboom et al. 1994). However, few studies on quantitative trait inheritance have been conducted under abiotic stresses. This may be due to various problems encountered under stress conditions, such as the reduced heritability of the trait due to increased environmental effects, or the difficulty in obtaining the correct level of stress to provide adequate expression of the trait of interest. To our knowledge, except for the detection of some markers associated with leaf curling in response to drought (Zehr et al. 1994), this study is the first to report QTLs responsible for the expression of different morphological traits in maize under field water-stress conditions.

The objectives of this study were: (1) to identify the genomic segments responsible for the expression of male flowering (MFLW), male sterility (STER), female flowering (FFLW) and ASI in a segregating population; (2) to estimate the level of phenotypic and genotypic interaction between these different traits; and (3) to determine whether QTLs for ASI could be used in marker-assisted selection (MAS) for the improvement of drought tolerance in maize.

Materials and methods

Plant materials

The two maize inbreds used as parental lines (P_1 and P_2) in this study were tropical S_5 lines derived from Tuxpeño germplasm and supplied by Dr. S.K. Vasal, CIMMYT. P_1, $Ac7643S_5$, was derived from Population 43 (La Posta) and P_2, $Ac7729/TZSRWS_5$, from Population 29 (Tuxpeño Caribe). Compared to P_2, P_1 yields well under drought and has a short ASI, while P_2 is low yielding under drought and has a long ASI. Leaf samples were harvested from 272 F_2 plants (from two selfed F_1 plants), quick frozen in liquid nitrogen, freeze dried, ground and stored at –20°C before DNA extraction. F_3 lines, produced by self-pollinating F_2 plants, were seed-increased by random sib-mating (Gardiner et al. 1993).

Field design

Experiments were conducted under different water regimes over 3 years. Trials were planted in the field on 26th November 1991 (92A), 30th November 1992 (93A) and 16th November 1993 (94A). All experiments were conducted during the dry winter season (November–April) at the CIMMYT Experimental Station in Tlaltizapan, Mexico (18°N, 940 masl). Experiments were evaluated in an alpha (0,1) lattice design with two repetitions for each water regime. In 92A and 93A, 240 F_3 families were planted in 24 blocks with ten plots per block. In 94A, 242 F_3 families and nine entries from each parental line were planted in 26 blocks with ten plots per block. In all cycles, plots consisted of 2.5-m rows (12 plants), with 20 cm between hills and 0.75 m between rows. Plots were overplanted, with two seeds per hill, then thinned to one plant per hill. The first two plants of each plot were considered as border plants and were not used in measurements. Experiments were conducted under three water regimes: well-watered (WW, 92A and 93A), intermediate stress (IS, 92A and 94A), and severe stress (SS, 92A, 93A and 94A). To avoid water contamination during irrigation, six rows of sweet corn were planted to divide trials in equal parts as a function of the water regime. Water was applied by a sprinkler for germination and thereafter by furrow irrigation. All the plants received the first three irrigations (1, 15 and 24 days after planting). After this period, irrigation was applied every 2 weeks to the well-watered regime. The IS treatment was obtained by applying an additional full irrigation at 43 days, and a half irrigation (every two rows) at 57 days. The SS treatment was obtained by applying only the half irrigation at 57 days. No more water was applied until flowering was completed. Directly after the flowering period (around 105 days after planting), all the trials were well irrigated to encourage adequate development of the kernels that had been set.

During 92A rainfall affected the experiment (90 mm of water from January 26 to February 4), so for this cycle we considered the stress level to be only intermediate. In 93A, the SS level was quite severe, killing about 60% of the plants. For this cycle, only the results obtained under WW have been taken into account. In 94A, the desired stress levels were obtained, although there was no WW trial in that season.

Field measurements

For all the trials, male flowering (MFLW) and female flowering (FFLW) were measured on an individual plant basis. Male flowering was recorded as the number of days from sowing to the first anther extrusion from the tassel glumes. Female flowering was the number of days from sowing to the first visible silk. For cycles 92A and 93A, ASI was calculated as the difference between the FFLW and the MFLW family means. In 94A, ASI was calculated per plant, as the difference between FFLW and MFLW for each plant. Male sterility was also registered during the 94A cycle as the presence of a blasted tassel. Plants without MFLW data because of sterility were not taken into account. In 94A, plants without FFLW data because the silks failed to extrude due to drought were taken into account because they were potentially the most susceptible to water stress. Their ASI was estimated by adding one standard deviation value, calculated from all ASI data observed in that particular family (never less than six plants), to the largest ASI observed in that family.

Data analysis

Adjusted means, as well as the genotypic variance per trial, were calculated per family for each trait using the PROC MIXED procedure in SAS (SAS Institute 1988). For cycle 94A data, the parental mean for each trait was calculated based on the adjusted means of the nine replicated entries of both lines, which were included in each replication, taking care that two parental lines were never included in the same block. Using the adjusted means of the F_3 families, simple Pearson correlation coefficients were calculated between the traits. Broad-sense heritabilities (h^2) of MFLW, FFLW and ASI were estimated over the two levels of stress as follows:

$$h^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{\hat{\sigma}_{ge}^2}{e} + \frac{\hat{\sigma}^2}{re}}$$

Where r=number of repetitions, e=number of environments, $\hat{\sigma}^2$=error variance, $\hat{\sigma}^2_g$=genotypic variance and $\hat{\sigma}^2_{ge}$=genotype-environment interaction variance. The variances were estimated using the PROC MIXED procedure.

RFLP analysis

Maize genomic DNA was extracted from the two parental lines and 234 F_2 plants. DNAs were purified, quantified, digested with one of two restriction enzymes (*Eco*RI or *Hin*dIII), separated in agarose gels (0.7%) and transferred to nylon membranes (MSI Magnagraph, Fisher Scientific) by Southern blotting. Labeled probes (digoxigenin-dUTP) were used to detect polymorphism with the antidigoxigenin-alkaline phosphatase-AMPPD chemiluminescent reaction. Details of these protocols are given in Hoisington et al. (1994). Around 200 probes from the University of Missouri Columbia (UMC), the Brookhaven National Laboratory (BNL), and Native Plants Incorporated (NPI), were used to screen the two parental lines. The best polymorphic probes (113) were chosen to construct the linkage map of the F_2 population. Segregation ratios at each marker locus were tested by a chi-square goodness of fit test for the expected Mendelian segregation ratio, 1:2:1 for co-dominant loci and 3:1 for dominant ones. Hybridization blots were read, and genetic data captured and verified by two different readers, using HyperMapdata, a software program developed at CIMMYT.

Mapping and QTL determination

Map positions of polymorphic loci were established by multipoint analysis using the computer program "MAPMAKER" (version 3.0; LOD threshold=3.0 and Theta threshold=0.40) (Lander et al. 1987). Mapping of QTLs was performed using adjusted F_3 family means for the traits measured in the field. The localization of QTLs for each trait was estimated by using "MAPMAKER/QTL" (version 1.1) software (Lander and Botstein 1989). Based on the density of the map, the number of markers, and suggestions given by Lander and Botstein (1989), the presence of a putative QTL was considered significant in this study when the LOD threshold was larger than 2.5. A putative QTL with a LOD threshold between 2.0 and 2.5 was reported only if a putative QTL with a LOD larger than 2.0 was detected (same locus ± 25 cM) under another water regime. Putative QTLs with a LOD threshold under 2.0 were not considered. Percentages of phenotypic variation accounted for by all significant QTLs for each trait and allelic effects (additivity and dominance) at the significant QTL locations were estimated using "MAPMAKER/QTL" software. The total percentage of phenotypic variation accounted for in each trait was determined in a multiple-QTL model that included all of the significant QTLs (Lander and Botstein 1989). Using F_3 family means as phenotypic measurements, the dominance effect given by "MAPMAKER/QTL" had to be multiplied by two to obtain the correct dominance estimation (Mather and Jinks 1971). To support the "MAPMAKER/QTL" analysis, a single-point analysis using one-way ANOVA from the PROC GLM routine in SAS (SAS Institute 1988) was performed.

Results

RFLP linkage map

Around 200 probes were tested on the two parental lines, and 150 (75%) of them revealed at least one polymorphism with one or two of the restriction enzymes used. Multiple loci were detected with 36 probes (two loci, 30 probes; three loci, four probes; and four loci, two probes). To con-

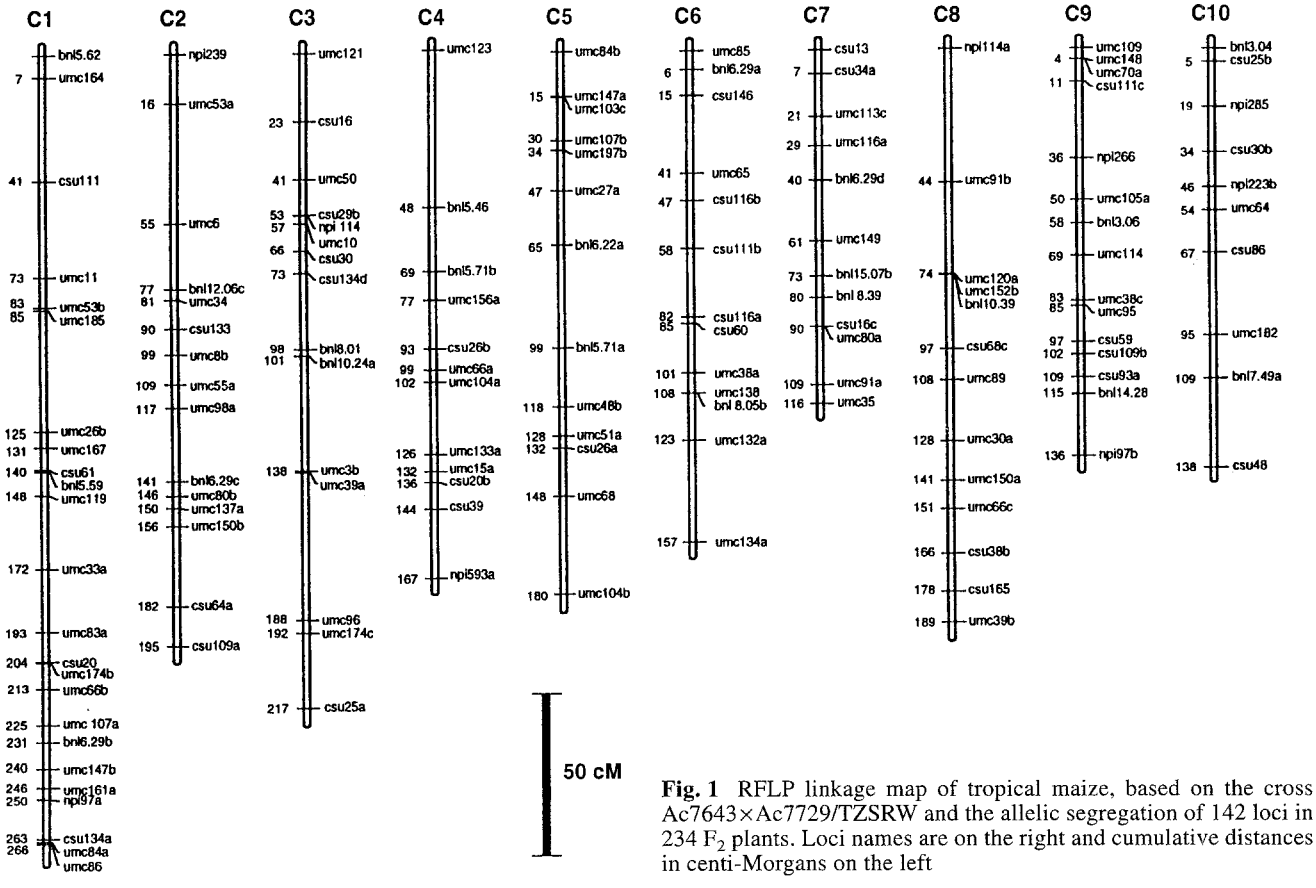

Fig. 1 RFLP linkage map of tropical maize, based on the cross Ac7643×Ac7729/TZSRW and the allelic segregation of 142 loci in 234 F_2 plants. Loci names are on the right and cumulative distances in centi-Morgans on the left

Table 1 Means (±SE) of parental lines and F_3 families, genetic variance (Gen var) and broad-sense heritabilities (h^2) for flowering traits (MFLW and FFLW) and the anthesis-silking interval (ASI) evaluated under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Item	MFLW			FFLW			ASI		
	WW	IS	SS	WW	IS	SS	WW	IS	SS
Mean (P_1)	·	93.0± 0.2	92.8± 0.2	·	92.6± 0.3	92.0± 0.3	·	−0.1±0.3	−0.6±0.2
Mean (P_2)	·	93.1± 0.4	93.4± 0.3	·	98.0± 0.5	100.4± 0.3	·	5.8±0.7	8.2±0.8
Mean (F_3)	93.7± 0.2	90.6± 0.1	90.0± 0.1	92.1± 0.2	92.3± 0.2	91.8± 0.2	−1.5±0.1	1.7±0.1	1.9±0.2
Range (F_3)	83.0/98.1	85.3/95.3	84.6/95.1	83.5/98.1	84.5/101.4	81.6/100.4	−4.8/1.9	−2.5/7.8	−4.4/9.3
Gen var (F_3)	4.2**	2.9**	3.0**	4.2**	6.0**	5.9**	0.9**	2.8**	3.3**
h^2(IS/SS)		0.86			0.82			0.78	

** Significant at the 0.01 probability level

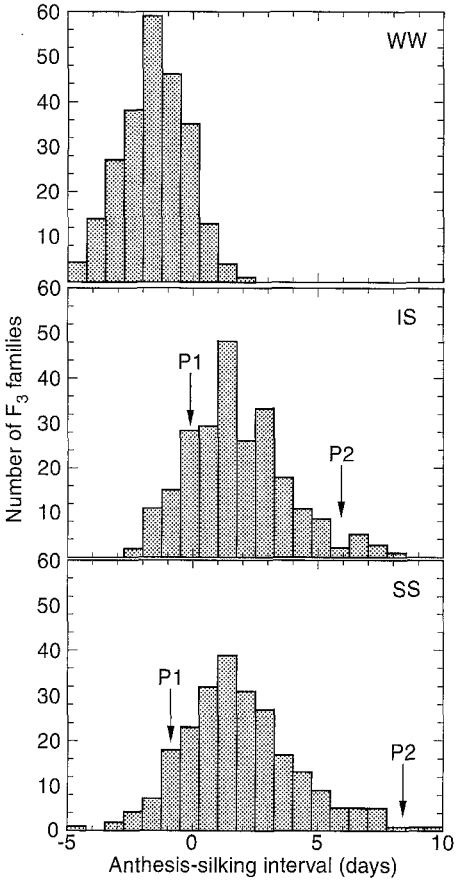

Fig. 2 Distribution of adjusted means of ASI in segregating F_3 families from the cross Ac7643×Ac7729/TZSRW under three water regimes: well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A). Parental mean values (P_1 and P_2), not measured in 92A, are reported under intermediate and severe stress

struct the RFLP linkage map (Fig. 1), 142 loci were included and ten linkage groups were obtained. The total length of the map was 1760 cM with a mean density of 12.5 cM. The length of the longest gap between two loci, located on chromosome 3, was 50 cM. The map is largely in agreement with previously published maps established for temperate maize (e.g., see Maize Genetics Cooperation Newsletter 1994). The order of the markers is consistent with these other maps but, in general, the distances between loci were smaller in this map. Forty-one "new" loci were detected in this cross, mostly as duplications of known ones. Only one highly significant distortion ($P<0.01$) from expected segregation ratios (favoring heterozygotes) was identified at locus *bnl5.46b* on chromosome 4.

Field trait analyses

Owing to the clear success of three of the trials, 92A (WW) and 94A (IS and SS), only the results for these trials are presented in detail in this paper. The rest of the data, for cycles 92A (IS) and 93A (WW), are used as additional evidence supporting our observations.

The alpha lattice design used for all the traits was efficient, since block effects were significant for the three variables measured. Therefore, adjusted means were used both for the estimation of field correlations and for QTL detection. Under drought conditions, the three traits measured showed transgressive segregation (Table 1). For ASI, the range among F_3 families, as well as the mean of these families, increased with stress intensity. For FFLW, the range among F_3 families increased with stress level but the mean of F_3 families remained unchanged, whereas for MFLW a water stress induced a reduction of both the mean and the range among F_3 families. Genotypic variances among F_3 lines were significant ($P<0.01$) for MFLW, FFLW and ASI and, as expected from the range of ASI, the genotypic variance of this trait increased with stress intensity.

The expression of flowering parameters and especially of ASI depended strongly on the water regimes (Table 1, Fig. 1). Since the water-stressed conditions (IS and SS) compared to normal irrigation (WW) represent different field conditions, we decided not to pool the data across the three water regimes, but to combine only the data recorded under stress conditions in order to estimate G×E and heritability. Differences between the F_3 means obtained under IS versus SS for cycle 94A were small, as were the differences between the genotypic variances (Table 1). For MFLW, FFLW and ASI, the estimated G×E components of variance across the two stress levels were not signifi-

Table 2 Linear coorelation (Pearson's) between the anthesis-silking interval (ASI), male flowering (MFLW), female flowering (FFLW) and male sterility (STER, 94A only) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Trait	WW			IS			SS		
	ASI	MFLW	FFLW	ASI	MFLW	FFLW	ASI	MFLW	FFLW
MFLW	−0.29**			0.11			0.07		
FFLW	0.29**	0.83**		0.66**	0.75**		0.63**	0.72**	
STER				−0.01	−0.04	−0.04	−0.03	−0.01	−0.11

** Significant at the 0.01 probability level

cant, and the heritabilities for the three traits were high (Table 1). These high heritability values underlined the stability and the reproducibility of the traits under drought and reflected the high accuracy of the flowering data measured on an individual plant basis. These heritability values are comparable to those observed under WW conditions by Veldboom et al. (1994).

The frequency distributions of ASI in all three trials were relatively normal (Fig. 2). As previously demonstrated (Herrero and Johnson 1981; Westgate and Boyer 1986; Bolaños and Edmeades 1993), drought stress induced an increase of the asynchrony between MFLW and FFLW, and hence increased ASI. Whereas the range of the ASI distribution under WW was about 7 days, it almost doubled under the higher stress level. When the stress level was increased from IS to SS, a reduction in ASI for P_1 and for some resistant families was observed. This increase in the stress level widened the distribution of ASI, by pushing family means toward the extremes of the distribution.

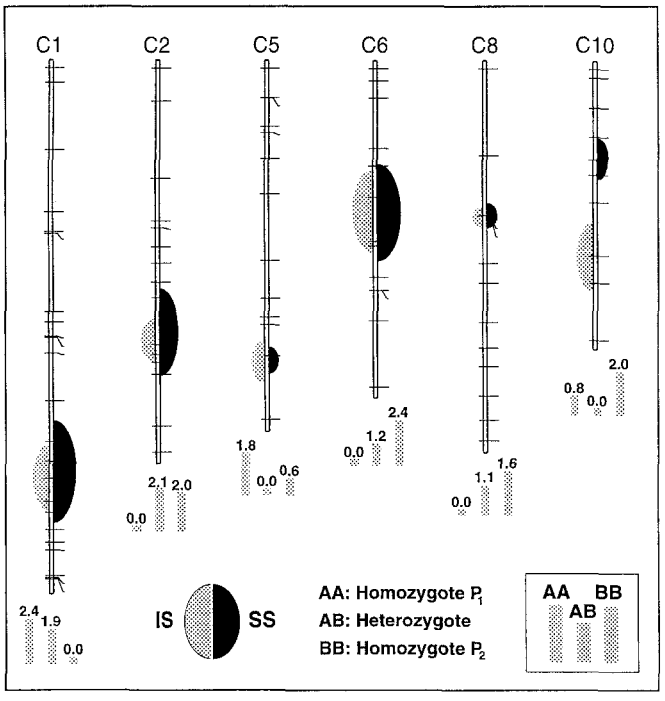

Fig. 3 Location of ASI QTLs detected under intermediate stress (IS) and severe stress (SS) in 94A. Genomic regions responsible for the expression of ASI are represented by ellipses for LOD scores higher than 2.0. The width of the ellipses is proportional to the percentage of phenotypic variance explained by that QTL, and the magnitude of the allelic effects (days) under *SS* conditions is represented in the bar graph for each QTL

Correlations between flowering data and ASI

Linear correlations calculated between the different variables under the three water regimes are presented in Table 2. MFLW and FFLW were highly correlated (>0.70) under all water regimes. In the absence of drought stress, ASI was slightly, but significantly, correlated with MFLW (negatively) and with FFLW (positively). Under stress conditions, ASI was not correlated with MFLW, but was highly correlated positively with FFLW, confirming previous observations that drought causes major delays in silk emergence and has only slight effects on MFLW (Bolaños and Edmeades 1993). To further confirm our result, the MFLW and FFLW means of the 30 families having the shortest and the longest ASI under SS conditions were calculated. No significant difference was observed between the MFLW means of the two groups mentioned above, 89.8 and 90.3 days, respectively, but a marked difference was obtained between the FFLW means of the families presenting the shortest ASI (88.0 days) and the longest ones (93.4 days). These results demonstrated again that ASI is determined largely by variation in FFLW under drought.

A problem in the determination of ASI could be the increase of the stress intensity over time during the flowering period (around 27 days). However, since no correlation was observed between MFLW and ASI under drought, no adjustment of ASI for time of MFLW was considered necessary.

QTL mapping

All the QTLs detected in this study are putative but, to simplify the text that follows, we will use the term "QTL(s)" rather than "putative QTL(s)". Under normal irrigation, the six QTLs detected for MFLW (Table 3) and the six QTLs detected for FFLW (Table 4) accounted for close to 39% of the total phenotypic variance. Under SS, the total percentage of the phenotypic variance explained by the two flowering traits increased up to around 48%, with the identification of new QTLs on chromosomes 1, 2, 5 and 8 for

Table 3 Genetic characteristics of QTLs involved in the expression of male flowering (MFLW) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b] (days)	Dominance (days)	Direction[c]	Total additivity[d] (days)	Phenotypic variance[a] (%)
WW 92A	1	159	*umc119*	3.46	−1.0	0.0	P1	2.0	9.6
	3	46	*umc50*	3.49	−1.0	1.0	P1	2.0	9.2
	4	58	*bnl5.71b*	2.92	−0.9	−0.4	P1	1.8	8.4
	6	86	*csu60*	3.32	−0.8	−0.7	P1	1.6	6.6
	9	125	*bnl14.28*	3.13	0.7	1.8	P2	1.4	9.3
	10	6	*csu25b*	4.58	−0.8	1.3	P1	1.6	9.0
				18.86					40.9
IS 94A	1	83	*umc53b*	3.29	−0.7	−0.8	P1	1.4	6.5
	1	145	*umc119*	3.49	−0.8	0.5	P1	1.6	7.9
	2	131	*bnl6.29c*	3.54	0.2	−3.0	P2	0.4	15.1
	4	59	*bnl5.71b*	2.75	−0.8	0.2	P1	1.6	7.7
	5	7	*umc84b*	2.51	0.5	−1.1	P2	1.0	5.9
	6	86	*csu60*	3.09	−0.7	−0.4	P1	1.4	6.1
	9	100	*csu109b*	5.46	0.8	−1.3	P2	1.6	11.4
				22.64					50.3
SS 94A	1	83	*umc53b*	2.30	−0.5	−1.1	P1	1.0	4.6
	1	145	*umc119*	3.36	−0.6	1.2	P1	1.2	7.6
	2	17	*umc53a*	2.81	0.2	−1.8	P2	0.4	5.7
	2	163	*umc150b*	2.86	0.3	−2.2	P2	0.6	9.0
	4	60	*bnl5.71b*	3.52	−0.9	1.1	P1	1.8	9.1
	5	9	*umc147a*	2.10	0.4	−1.0	P2	0.8	5.1
	8	103	*umc89*	2.50	0.7	0.4	P2	1.4	5.9
	9	103	*csu109b*	4.86	0.7	−1.3	P2	1.4	10.0
				21.64					45.2

[a] Totals of the LOD score and the percentage of phenotypic variance 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 numerical value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numerical value of the trait
[d] Total additivity is equal to twice the absolute value of Additivity [a]

MFLW and on chromosomes 2, 5, 8 and 10 for FFLW. Under no stress, P_1 alleles contributed mainly to a delay in pollen shedding and in silking, but the situation was reversed under drought with a major delaying effect from P_2 alleles. For the expression of ASI (Table 5), four regions were identified under well-watered conditions. These collectively accounted for 33% of the phenotypic variance. Under stress conditions, six QTLs were identified for ASI on chromosomes 1, 2, 5, 6, 8 and 10 (Table 5, Fig. 3), which represented under SS a change of 11 days in ASI. As expected, the susceptible line (P_2) alleles contributed mainly to an increase in the length of ASI over all the trials. When considering the traits over different water regimes, an increase of the stress intensity corresponded generally to an increase in the number of QTLs detected, as well as in the percentage of the variance explained. The large increase in the total percentage of the phenotypic variance (33% to 47%) accounted for by ASI QTLs reflected well the marked increase in the genotypic variance (0.85–3.26, Table 1).

For each trait, the location of some QTLs appeared to be consistent across water regimes. For MFLW, "stable" QTLs were detected on chromosomes 1, 4 and 9; for FFLW they were on chromosomes 1 (two QTLs) and 9; and for ASI on chromosomes 1, 2, and 6. Generally these stable QTLs were identified with the highest LOD score, they accounted for the highest percentage of genetic variance, and the genetic contribution always originated from the same parental line in all the trials.

Analysis of the data from the IS of 92A confirmed the location of QTLs for ASI on chromosomes 1, 2, 5, 6 and 10. The only QTL with a LOD score <2.0 (1.53) was on chromosome 8. The more important QTLs were identified on chromosomes 1 (LOD=3.85), 2 (LOD=3.27) and 6 (LOD=5.95). Thus, over all experimental conditions, the three ASI QTLs on chromosomes 1, 2 and 6, were the most consistent. All the QTLs detected with "MAPMAKER/QTL" under IS and SS, were also detected with a one-way ANOVA ($P<0.01$). In this analysis, the QTLs accounting for the highest percentage of phenotypic variance were on chromosomes 1 (*csu20*), 2 (*bnl6.29c*) and 6 (*csu60*).

Sterility

Male sterility was quantified in the 94A trial only. There was no correlation between sterility and the two flowering traits or ASI under the two stress conditions (Table 2). This

Table 4 Genetic characteristics of QTLs involved in the expression of female flowering (FFLW) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b] (days)	Dominance (days)	Direction[c]	Total additivity[d] (days)	Phenotypic variance[a] (%)
WW 92A	1	99	*umc185*	2.55	–0.9	–0.9	P1	1.8	9.1
	1	163	*umc33a*	6.00	–1.3	–0.3	P1	2.6	15.3
	4	58	*bnl5.71b*	2.41	–0.8	–1.4	P1	1.6	6.9
	8	157	*umc66c*	2.55	0.8	–0.3	P2	1.6	6.3
	9	123	*bnl14.28*	2.20	0.7	1.2	P2	1.4	6.1
	10	5	*csu25b*	2.86	–0.7	1.0	P1	1.4	5.7
				18.13					36.3
IS 94A	1	83	*umc53b*	4.52	–0.9	–1.9	P1	1.8	8.9
	1	162	*umc33a*	5.23	–1.3	0.2	P1	2.6	13.3
	2	172	*umc150b*	2.72	0.6	–2.4	P2	1.2	8.9
	8	77	*bnl10.39*	2.20	0.6	–1.2	P2	1.2	4.4
	9	97	*csu59*	4.36	0.9	–1.4	P2	1.8	9.4
				19.40					38.9
SS 94A	1	75	*umc11*	3.35	0.0	–2.7	P2	0.0	7.4
	1	200	*csu20*	3.08	–1.0	–0.6	P1	2.0	7.1
	2	139	*bnl6.29c*	2.61	1.0	–1.3	P2	2.0	9.1
	2	173	*csu64a*	3.06	0.9	–1.7	P2	1.8	8.8
	4	60	*bnl5.71b*	2.94	–1.0	0.2	P1	2.0	8.0
	5	143	*umc68*	2.52	–0.5	–2.1	P1	1.0	6.0
	8	82	*bnl10.39*	2.26	0.8	–0.0	P2	1.6	5.4
	9	106	*csu93a*	3.09	0.7	–1.7	P2	1.4	6.6
	10	100	*bnl7.49a*	2.53	0.4	–2.4	P2	0.8	6.4
				20.98					48.7

[a] Totals of the LOD score and the percentage of phenotypic variance 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 numerical value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numerical value of the trait
[d] Total additivity is equal to twice the absolute value of Additivity [a]

result showed that tassel blasting was neither correlated with ASI nor with the drought stress tolerance of the F_3 families. In fact, male-sterile plants were found in both parental lines, and climatic conditions such as low air humidity and high temperature most likely influenced the expression of this trait (Bonnett 1960). Both QTL analyses of sterility, using one-way Anova and "MAPMAKER/QTL", yielded a few small QTLs, consistent over stress intensities, located on chromosomes 4 (126 cM), 7 (26 cM) and chromosome 9 (115 cM) (data not shown). Under drought stress conditions, QTLs for ASI expression and male sterility were never linked. However, on chromosome 9, QTLs for male sterility, MFLW and FFLW were detected at the same genetic position. The sterility QTL detected on chromosome 4 is near the location of *Ms41*, a gene for male sterility, while that on the chromosome 7 is near the location of *ms7* (Coe et al. 1988). However, the coincidence between QTL locations and genes for male sterility may not be significant, as there are at least 22 genes for male sterility distributed over the maize genome and described in mutant stocks (Coe et al. 1988). The possibility of a chance coincidence between a gene and a QTL for male sterility cannot be excluded.

Discussion

Correlation and pleiotropism

In this study, linkage between QTLs for different traits was observed and, as already suggested by different authors (Abler et al. 1991; Paterson et al. 1991), the same location of QTLs for different traits should be associated with a correlation of the phenotypic data. Under severe stress conditions, ASI was well correlated with FFLW and these two traits presented four "common" QTLs (chromosomes 1, 2, 5 and 8) (Tables 4 and 5). The anthesis-silking interval was not correlated with MFLW; there was one region in common on chromosome 2 and two linked QTLs at 28 cM on chromosome 8 under SS conditions only (Tables 3 and 5). The highest correlation was observed between FFLW and MFLW data, and four segments involved in the expression of these two traits were identified on chromosomes 1, 2, 4 and 9 (Tables 3 and 4). Except for the QTL on chromosome 2, those QTLs were unlinked with any ASI QTLs. Thus, the expected association between the level of phenotypic correlation and the linkage of QTLs for different traits is verified in this study.

Table 5 Genetic characteristics of QTLs involved in the expression of anthesis-silking interval (ASI) under well-watered (WW, 92A), intermediate stress (IS, 94A) and severe stress (SS, 94A) conditions

Trials	Chromosome	QTL position (cM)	Nearest RFLP locus	LOD score[a]	Additivity[b] (days)	Dominance (days)	Direction[c]	Total additivity[d] (days)	Phenotypic variance[a] (%)
WW 92A	1	201	*csu20*	2.99	−0.5	0.3	P1	1.0	6.7
	2	138	*bnl6.29c*	4.31	0.6	−0.2	P2	1.2	11.4
	6	90	*csu60*	5.00	0.6	−0.3	P2	1.2	11.3
	10	61	*csu86*	2.40	0.4	0.6	P2	0.8	5.8
				15.16					33.0
IS 94A	1	203	*csu20*	3.47	−0.8	0.2	P1	1.6	7.2
	2	134	*bnl6.29c*	2.91	0.8	0.7	P2	1.6	7.2
	5	147	*umc68*	2.20	−0.6	−1.1	P1	1.2	6.9
	6	76	*csu116a*	3.94	0.8	−1.0	P2	1.6	10.8
	8	76	*umc120a*	2.31	0.6	−0.6	P2	1.2	5.0
	10	101	*umc182*	2.96	0.3	−1.9	P2	0.6	7.7
				17.13					37.4
SS 94A	1	206	*umc174b*	5.77	−1.2	0.7	P1	2.4	12.6
	2	130	*bnl6.29c*	4.07	1.0	1.1	P2	2.0	10.1
	5	147	*umc68*	2.32	−0.6	−1.2	P1	1.2	5.0
	6	79	*csu116a*	5.69	1.2	0.0	P2	2.4	13.0
	8	75	*umc120a*	2.59	0.8	0.3	P2	1.6	5.3
	10	43	*npi223b*	2.48	0.6	−1.4	P2	1.2	5.9
				23.98					46.7

[a] Totals of the LOD score and the percentage of phenotypic variance 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 numerical value of the trait
[c] Direction indicates the parental line which contributes to the increase of the numerical value of the trait
[d] Total additivity is equal to twice the absolute value of Additivity [a]

When QTLs for MFLW and FFLW were linked, the sign of the additive component was always the same for the two traits, showing that at these loci, the genetic contribution came from the same parental line for both traits. Therefore, the presence of a QTL for ASI cannot be explained simply by the opposite contribution of a parental line on pollen shedding and silk emergence at one locus.

From this study, it is not possible to establish with assurance the presence of pleiotropic effects affecting several traits. However, considering the related nature of MFLW and FFLW, both depending on plant maturity and development, the presence of pleiotropic genes involved in the expression of both MFLW and FFLW can be postulated. The anthesis-silking interval is calculated as the difference between FFLW and MFLW data. By definition, ASI has to be more or less correlated with FFLW and/or MFLW. Thus, in the case of the four QTLs responsible for the expression of both ASI and FFLW identified in this study, the term "pleiotropic effect" is abusive.

Flowering and ASI QTLs in different studies

Flowering parameters in temperate maize have been studied under well-watered conditions, and QTLs for MFLW and FFLW identified (Phillips et al. 1992; Beavis et al. 1994; Veldboom et al. 1994). Considering the three studies mentioned above and our results under WW conditions, QTLs responsible for the expression of MFLW and FFLW were identified on all ten maize chromosomes, with more consistency on chromosomes 1, 3, 4, 8 and 9 for MFLW, and on 1, 8 and 9 for FFLW. The source of parental inbreds, the size of the progeny, genotype-by-environment interaction effects and the different type of genetic maps used, are most likely responsible for differences in the QTL localization, as suggested by Beavis et al. (1994). Especially in the study of Beavis et al., field tests were grown under a wide range of environmental conditions including natural drought conditions throughout the Corn Belt in 1988, an unusually dry season, making comparisons with other studies even more complex.

The ASI QTLs were also identified by both Beavis et al. (1994) and Veldboom et al. (1994). On chromosome 1, a clear tendency for an ASI QTL was detected by Veldboom et al. (LOD just under 2.0, near *umc37*), corresponding to the genomic location of the ASI QTL detected on the same chromosome in the present study (near *csu20*) over the three water regimes. Beavis et al. also identified a QTL for ASI in the same region (*bnl7.21-umc133*). On chromosome 2, Beavis et al. identified a QTL for ASI at the same position (*bnl6.20*) as in the present study (*bnl6.29c*) under all water regimes.

Over all the trials reported here, the major ASI QTL was identified on chromosome 6 (near *csu116a*). Comparing the peaks of the ASI QTLs detected on chromosome 6 in this study and in the study of Veldboom et al. (between

bnl5.47 and *npi280*), we can conclude that the same genomic region was identified. Zehr et al. (1994) reported the presence of a QTL for ASI under no stress conditions on chromosome 6 (*npi223*), which agrees with the result presented here. Finally, Quarrie (personal communication, 1994), when studying allelic frequency shifts over cycles of selection (C0 to C8) for drought in the Tuxpeño Sequía tropical maize population, detected allelic frequency changes on chromosome 6 in the interval between *csu155* and *umc132*. These results underline the consistent presence of an ASI QTL on chromosome 6 over several studies in which populations segregating for flowering traits were examined. It suggests that this QTL might be "universal" for ASI in maize under both well-watered and stressed conditions. However, Beavis at al. (1994) did not identify a QTL for ASI on chromosome 6 under their experimental conditions. It is possible that in this cross the two parental lines did not segregate for the pertinent genomic region of chromosome 6.

Veldboom et al. (1994) also identified a QTL for ASI on chromosome 8 (between *umc165b* and *umc7*). At the same genomic region (chromosome 8, near *umc66c*) we identified a peak with a LOD score of 2.1, but only under WW conditions so that it is not reported in Table 5, suggesting that there was a strong tendency at this location for an ASI QTL. Under the two different stress intensities, a QTL for ASI was also detected on chromosome 8, but not at the same location (near *umc120*). Beavis et al. (1994) found no QTL on chromosome 8, but did find one ASI QTL on chromosome 9. From these studies, one can conclude that ASI QTLs are relatively consistent in the maize genome.

Marker-assisted selection

In 1980, Stuber et al. observed a significant correlation between changes in isozyme allelic frequencies and changes due to selection for improved grain yield. This result was confirmed later in an open-pollinated maize population, when the same authors reported that selections based solely on manipulations of allelic frequencies at seven enzyme loci significantly increased grain yield and ear number (Stuber et al. 1982). This was effectively the first successful MAS experiment with plants. The potential efficiency and limitations of MAS (e.g., Tanksley and Hewitt 1988; Lande and Thompson 1990) have emphasized the importance of the number of traits involved in the selection, as well as the nature of the genome of the plant and the nature of the trait to be improved. In maize, MFLW and FFLW are easy to measure in the field and ASI can be calculated with precision. Heritability of ASI is high under drought and measurement of ASI is not subjective, as is for example pest resistance scoring. However, the major problems for classical selection under drought are the management of the experimental conditions (e.g., the problems of cycle 92A and 93A in this study) and the fact that only one cycle per year is suitable for selection. Therefore, the use of molecular markers to improve the efficiency of breeding towards better drought tolerance may provide a working alternative

Except for the QTL on chromosome 10, ASI QTLs were consistent over trials under drought. Under SS conditions, the four QTLs with the reduced ASI alleles coming from the tolerant line (P_1) accounted for a reduction in ASI of 7 days. For three out of the four QTLs (chromosomes 2, 6 and 8) with the alleles contributed by P_1, the reduction of ASI was determined to be both additive and dominant. By adding the distance spanning the four QTLs for a LOD2.0 (Fig. 3), the coverage of the genome is 160 cM, which represents around 9% of the total mapped genome. Within these 160 cM, and including the two flanking markers for each QTL, the largest distance between those markers was 30 cM (on chromosome 8). These results indicate that MAS based on ASI QTLs could well succeed in improving drought tolerance in maize lines, without affecting too much the agronomic characters of the target lines. Moreover, since only one common QTL was identified for ASI and MFLW on chromosome 2 across the two stress levels, the transfer of the four QTLs responsible for a short ASI expression should not change significantly the maturity of the recurrent line.

The comparison and correlations between ASI and yield components will be presented and discussed in detail in the second paper of this study (in preparation), in which the efficiency of MAS on yield improvement under drought will also be examined.

To initiate a MAS project for ASI, a backcrossing scheme using Ac7643S_5 (P_1) as the donor line and CML247 as the recurrent line is now underway at CIMMYT. CML247 is an elite tropical line from CIMMYT, with outstanding combining ability and good per se yield potential under well-watered conditions. However, CML247 has a relatively long ASI under water stress, which significantly reduces its yield potential under drought. Identification of QTLs, as described in this study, will be conducted again to confirm the location of P_1 and CML247 alleles contributing for a short ASI in this new cross.

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