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M. Sari-Gorla · P. Krajewski · N. Di Fonzo
M. Villa · C. Frova

Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering

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Abstract Drought is a serious agronomic problem, and one of the most important factors contributing to crop yield loss. In maize grown in temperate areas, drought stress occurs just before and during the flowering period; consequently, tolerance to water stress in this species is largely determined by events that occur at or shortly after flowering. The purposes of our investigation were: (1) to identify the chromosomal regions where factors conferring drought tolerance for traits related to plant development and flowering are located and (2) to compare these regions with those carrying QTLs controlling these traits, in order to get indirect information on the genetic and physiological basis of maize response to water stress. To this aim, we performed a linkage analysis between the expression of male and female flowering time, anthesis-silking interval (ASI), plant height and molecular markers. The experiment was carried out under two environmental conditions, well-watered and water-stressed, on a maize population of 142 recombinant inbred lines obtained by selfing the F_1 between lines B73 and H99 and genotyped by RFLP, microsatellites (SSR) and AFLP markers, for a total of 153 loci. Linkage analysis revealed that, for male flowering time and plant height, most of the QTLs detected were the same under control and stress conditions. In contrast, with respect to female flowering time and ASI diverse QTLs appeared to be expressed either under control conditions or under stress. All of the QTLs conferring tolerance to drought were located in a different chromosome position as compared to the map position of the factors controlling the trait per se. This suggests that plant tolerance, in its different components, is not attributable to the presence of favourable allelic combinations controlling the trait but is based on physiological characteristics not directly associated with the control of the character.

Key words *Zea mays* L · Maize · Drought
Molecular markers · Flowering · Linkage analysis

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M. Sari-Gorla (✉) · M. Villa · C. Frova
Department of Genetics and Microbiology, University of Milan, Via Celoria 26, I-20133 Milan, Italy
Fax: +39 02 2664551
E-mail: mirella.sarigorla@unimi.it

P. Krajewski
Institute of Plant Genetics, Polish Academy of Science, Strzeszynska 34, PL-60-479 Poznan, Poland

N. Di Fonzo
Institute for Cereal Crops, Section of Foggia, Strada Statale 16, km 675, I-71100 Foggia, Italy

Introduction

Drought is a serious agronomic problem, being one of the most important factors contributing to crop yield loss in marginal lands and affecting yield stability in temperate areas. It is a multidimensional stress, affecting plants at various levels of their organization over space and time, so that the physiological responses to it are complex and often unpredictable. However, in most species water stress has been observed to have some specific effect. In maize, a major effect of water stress is a delay in silking, resulting in an increase in the ASI (anthesis-silking interval), which is an important cause of yield failures (Bolanos and Edmeades 1993; Byrne et al. 1995). This trait was shown to be highly correlated with grain yield, in particular kernel number (Gutierrez-Rodriguez et al. 1998) and ear number per plant, while anthesis date is virtually uncorrelated with other traits (Chapman et al. 1997).

In maize grown in temperate areas, drought stress occurs just before and during the flowering period; therefore, tolerance to water stress is largely determined by events that occur at or shortly after flowering (Lafitte and Edmeades 1995). In a maize population selected for traits involved in drought tolerance,

flowering was substantially modified (Bolanos et al. 1993): ASI was reduced, and this accounted for most of the variation in grain yield. In particular, selection increased kernel number, while kernel weight remained unchanged. Also, plant water status was unchanged, suggesting that the increase in yield was due solely to an increased partitioning of material assimilated by the ear. This was confirmed by the genetic correlation between grain yield and ASI: it was weak under well-watered conditions, but approached -0.6 under severe stress. This may indicate that variation in grain yield under stress is due to variation in ear-setting processes related to biomass partitioning at flowering, and much less by factors putatively linked to crop water status (Bolanos and Edmeades 1996). There are indications that the stop in embryo growth under limited water conditions is due to a decrease in sucrose flux and an altered carbohydrate metabolism in the ovaries (Zinsellmeyer et al. 1995). Coupled with a low level of reserves, the failure to utilize available sugars at a low ovary water potential would severely inhibit assimilate flux to the ear and render kernel set highly vulnerable to a water deficit during pollination (Schussler and Westgate 1995).

A limited water supply affects plant development: drought often delays developmental events so that plant height is reduced. Consequently, yields are also affected since the plant needs to reach a sufficient stature to have adequate photosynthate.

Some of the devastating effects of drought on crop species could be overcome by exploiting existing genetic variation in drought tolerance in order to develop genotypes better adapted to cope with water stress. However, this goal cannot be achieved without first taking some major hindrances into account. Since there are no traits conferring global drought tolerance, it is necessary to choose the traits to select, but this choice is made difficult by the complexity of designing a drought-resistant ideotype. In fact, it requires a knowledge of the potential capacity of the plant, its constitutive traits and its adaptive response to the level and timing of the stress, all in the context of a final productivity (Blum 1996). Improvement of the target traits has to be reached without breaking the genetic architecture of other adaptive traits. Various studies suggest that different sets of alleles and possibly different loci are being expressed under different environmental conditions (Veldboom and Lee 1996) and, in particular, it is unknown if plant tolerance is due to the presence of specific favourable alleles controlling the trait of interest, or if different genes, not directly involved in the control of that particular trait, are responsible for tolerance.

The purposes of the investigation presented here were: (1) to identify the chromosomal regions where factors conferring drought tolerance are located and (2) to compare these regions with those carrying quantitative trait loci (QTLs) controlling flowering components and plant height in order to get indirect information on the genetic and physiological basis of the response of maize to water stress. To this aim, we used a linkage analysis between the expression of the characters and molecular markers. The strategy is based on linkage disequilibrium, due to physical linkage, between alleles at a marker locus and alleles of a linked genetic factor controlling the trait. Linkage is revealed by a significant association between the marker allelic composition and the expression of the trait.

Materials and methods

Most aspects of the materials and methods used in this study have been previously described (Frova et al. 1999). These include plant material, field design, data analysis and QTL mapping. Thus, this information is only briefly reported here.

Plant material

A population of 142 recombinant inbred lines (RIL), obtained by 11 generations of selfing of the F_1 between parental lines B73 and H99, was used (Sari-Gorla et al. 1997b; Frova et al. 1999). The population was genotyped by restriction fragment length polymorphism (RFLP), microsatellites (SSR, simple-sequence repeats), and amplified fragment length polymorphism (AFLP) markers, for a total of 153 loci that were arranged in a genetic linkage map using the MAPMAKER programme (Lander et al. 1987).

Field measurements

The experiment was carried out in 1997 in Foggia (Southern Italy), which is characterized by a hot, dry climate. Two replications of 15 plants for each genotype were made for each treatment: well irrigated and stressed. Water stress was applied starting from flowering time onwards.

Male flowering time (MFT), female flowering time (FFT), anthesis-silking interval (ASI) and plant height (PH) were evaluated under control conditions (well irrigated) and under water stress. MFT and FFT were recorded as the number of days from sowing to anther extrusion from the tassel glumes (MFT) or to visible silks (FFT) of 50% of the plants per plot. ASI was computed as the difference between MFT and FFT for each plot. Plant height was measured (cm) after flowering was completed, from the soil surface to the tip of the central spike of the tassel.

A tolerance index (TI) for drought was calculated as T/C, where T is the value of the trait expressed under stress, and C represents the value evaluated under control conditions.

Data analysis

The data were submitted to ANOVA using the PROC GLM in SAS (SAS Institute 1998). Simple Pearson correlation among the traits was calculated on the adjusted means of the lines (PROC CORR, SAS). Broad-sense heritability (h^2_B) of the traits was estimated.

Association between the trait expression and the allelic composition at marker loci was evaluated by simple regression analysis using the SAS GLM procedure, and by a least square interval mapping

(IM) method programmed in Genstat (Sari-Gorla et al. 1997a). In brief, the first step is the selection of significant markers by forward selection; in this way the most important markers are selected first and serve as co-variates in the model. At the second step, mapping, correlation between markers is taken into account; a very strict testing criterion is used in order to minimize the probability of detecting a false QTL. The QTLs detected have to show significant improvement of the fit of the model in the presence of all other selected markers (except for the flanking ones). The result of the mapping can be viewed as a confirmation of the selected markers using additional, more precise information about the position of the QTL relative to the marker.

Results

Trait analysis

ANOVA, applied to the four traits (not reported), revealed a highly significant effect of the water stress, as expected, and a differential response of the lines to drought that was highly significant for all traits but MFT.

Characteristics of the parental lines and of the RI population are reported in Table 1. The B73 and H99 lines differed considerably in MFT, with H99 flowering much earlier than B73. H99 also had earlier FFT than B73; under stress, a slight delay in silking was observed.

When compared with H99, B73 had a smaller ASI, which increased under drought; on the other hand, the mean of the trait in H99 did not change. Linear regression of the population drought-tolerance index (TI) on ASI under well-watered conditions gave a highly significant negative coefficient of regression (data not shown). Because of the way in which the tolerance index was computed (trait value under stress, divided by trait value under control condition), a high value of the index for PH indicates a greater tolerance (small reduction in plant height under stress), whereas for MFT, FFT and ASI the meaning of TI is the opposite: high values indicate susceptibility to drought (delay in flowering time and increase in ASI under stress). Thus, these results surprisingly indicate that lines characterized by low ASI values are less tolerant to drought than genotypes with a higher value of the trait. Regression of TI for ASI on MFT under the control environment revealed that early flowering lines are more tolerant to drought, in that they have a low increase of the ASI under stress (highly significant, positive regression coefficient). The same relationship was observed between TI for ASI and PH under WW conditions. However, no relationship between ASI tolerance and FFT was detected.

The B73 inbred was much taller than H99. Water stress reduced height considerably more in B73 than in H99 and the RIL population. Regression of TI for this trait on PH in the well-watered treatment indicated that shorter RILs are more tolerant, i.e. are less reduced in height under stress, than tall lines (negative regression coefficient).

Correlation between the traits under well-watered or stress conditions, and between TIs, is reported in Table 2. Male and female flowering time were highly correlated in both treatments and tolerance index. Under well-watered conditions, lines flowering later had shorter ASI. The strength of this relationship decreased under stress; lines having small variations in ASI under stress had a short delay in MFT. ASI was positively associated with FFT: as expected, lines flowering late have larger ASI values. PH was positively correlated with MFT only under well-watered conditions; there is no relationship between PH and FFT when evaluated in both water regimes, but their TIs are negatively correlated, indicating that lines more

Table 2 Correlation between the traits under well-watered (WW) or stress (WS) conditions and between drought tolerance indices (TI) (MFT male flowering time · FFT female flowering time · ASI anthesis-silking interval · PH plant height). $n = 142$

Trait	Stress condition	FFT	ASI	PH
MFT	WW	0.69*	− 0.28*	0.29*
	WS	0.78*	− 0.09	0.13
	TI	0.41*	− 0.23*	− 0.16
FFT	WW		0.45*	0.06
	WS		0.47*	− 0.08
	TI		0.36*	− 0.43*
ASI	WW			− 0.26*
	WS			− 0.22*
	TI			− 0.26*

* $P < 0.01$

Table 1 Characteristics of parental lines and RIL population evaluated under well-watered (WW) or stress (WS) conditions (MFT male flowering time · FFT female flowering time · ASI anthesis-silking interval · PH plant height)

	MFT (days)		FFT (days)		ASI (days)		PH (cm)	
	WW	WS	WW	WS	WW	WS	WW	WS
Mean B73	92.5 ± 3.0	91.0 ± 2.0	96.0 ± 1.0	98.0 ± 2.0	3.5 ± 0.5	5.0 ± 0.0	215 ± 5.0	175 ± 5.5
Mean H99	80.5 ± 0.5	82.5 ± 2.5	92.5 ± 0.5	93.5 ± 0.5	12.0 ± 0.0	11.0 ± 2.0	115 ± 5.0	110 ± 5.0
Mean RILs	89.1 ± 1.8	88.0 ± 1.9	97.1 ± 1.7	97.6 ± 2.0	8.2 ± 1.5	9.7 ± 1.5	152 ± 3.9	44 ± 3.9
Range RILs	79–101	80–99	87–118	88–111	3.5–23	4.5–21	110–187	100–175
h^2_B	0.68		0.58		0.40		0.69	

tolerant for PH are more tolerant for FFT as well. PH is negatively correlated with ASI: tall lines have short ASI, and lines revealing small variation in stature under stress have a small increase in ASI as well.

QTL mapping

In Table 3 the results concerning the detection of QTLs for MFT, FFT, ASI and PH, respectively, are reported. For each trait the table provides information on the chromosome carrying QTLs, the nearest significant marker, the sign of the QTL effect and its coefficient of determination (R^2). R^2 represents the proportion of the phenotypic variation of the trait explained by allelic substitution at the given molecular marker locus. Total R^2 for each trait measures the proportion of phenotypic variation accounted for by the markers found to be significant in the forward selection step of the analysis. This explains the case (Table 3) in which no QTLs were found at the second step of the analysis. Markers are indicated as m1, m2, …, according to their position on chromosomes, from chromosome 1 to chromosome 10; correspondence with the true marker names is given in the accompanying paper (Frova et al. 1999).

The data were submitted to two different methods of analysis: interval mapping (IM) and simple linear regression. IM is much more restrictive than regression and, in fact, a higher number of significant loci were detected by the latter procedure; in particular, QTLs that were identified only in one treatment with the first approach, were also deemed to be expressed in the other treatment by simple regression. The choice to use relatively rigid criterion for testing was indicated by the fact that, in addition to considering many positions on each chromosome, we also considered many traits, which increases the number of hypotheses tested simultaneously.

Five markers were judged to be significantly associated with MFT under well-watered conditions, including 1 QTL on chromosome 1, 2 on chromosome

Table 3 QTLs involved in the expression of male flowering time (MFT), female flowering time (FFT), anthesis-silking interval (ASI) and plant height (PH) under well-watered (WW), stressed conditions (WS) and drought tolerance (TI)

Trait	Chromosome	WW			WS			TI		
		Marker (shift in cM)[a]	Sign[b]	R^2	Marker (shift in cM)	Sign	R^2	Marker (shift in cM)	Sign	R^2
MFT	1	m11	+	5.1	m11 (+1.4)	+	4.7			
	2	m28 (+4.7)	−	5.9						
		m37	+	4.6	m37	+	9.1			
	4							m66 (+2.8)	+	9.5
	7	m108	+	11.0	m108	+	13.0			
	8	m125 (−4.0)	−	12.5	m125 (−2.0)	−	5.4			
Total				51.4			58.2			25.9
FFT	7				m108	+	7.5			
	9				m135 (−2.6)	−	6.2			
Total				28.3			36.8			23.2
ASI	1	m7	+	5.1						
	2	m28	+	6.1				m28 (+3.7)	−	12.1
	5				m85 (+6.2)	+	9.4			
	7				m107	−	7.6			
		m115	−	5.8						
		m119	+	7.8						
	8	m125 (+5.0)	+	4.4						
		m129 (−2.5)	+	5.9						
	9	m134	−	4.9						
					m135 (−5.6)	−	6.4			
Total				49.5			32.3			40.3
PH	1	m5 (−2.2)	−	13.3	m5 (−9.2)	−	7.4			
		m11 (+2.4)	+	7.3	m11 (−5.6)	+	5.6			
	2	m27 (+4.9)	−	11.7	m27 (−1.1)	−	7.1			
	7							m106	−	13.4
	8	m127 (−5.2)	−	14.4	m127 (−3.2)	−	8.2			
Total				54.7			42.4			18.5

[a] (Shift in cM) gives the position of the QTL relative to the markers: −, to the left; +, to the right (no shift means a shift < 1.0)
[b] Sign of the effect indicates which parental allele increases the trait value: +, allele contributed by parental line H99; −, contributed by B73
[c] Coefficient of determination (see Results)

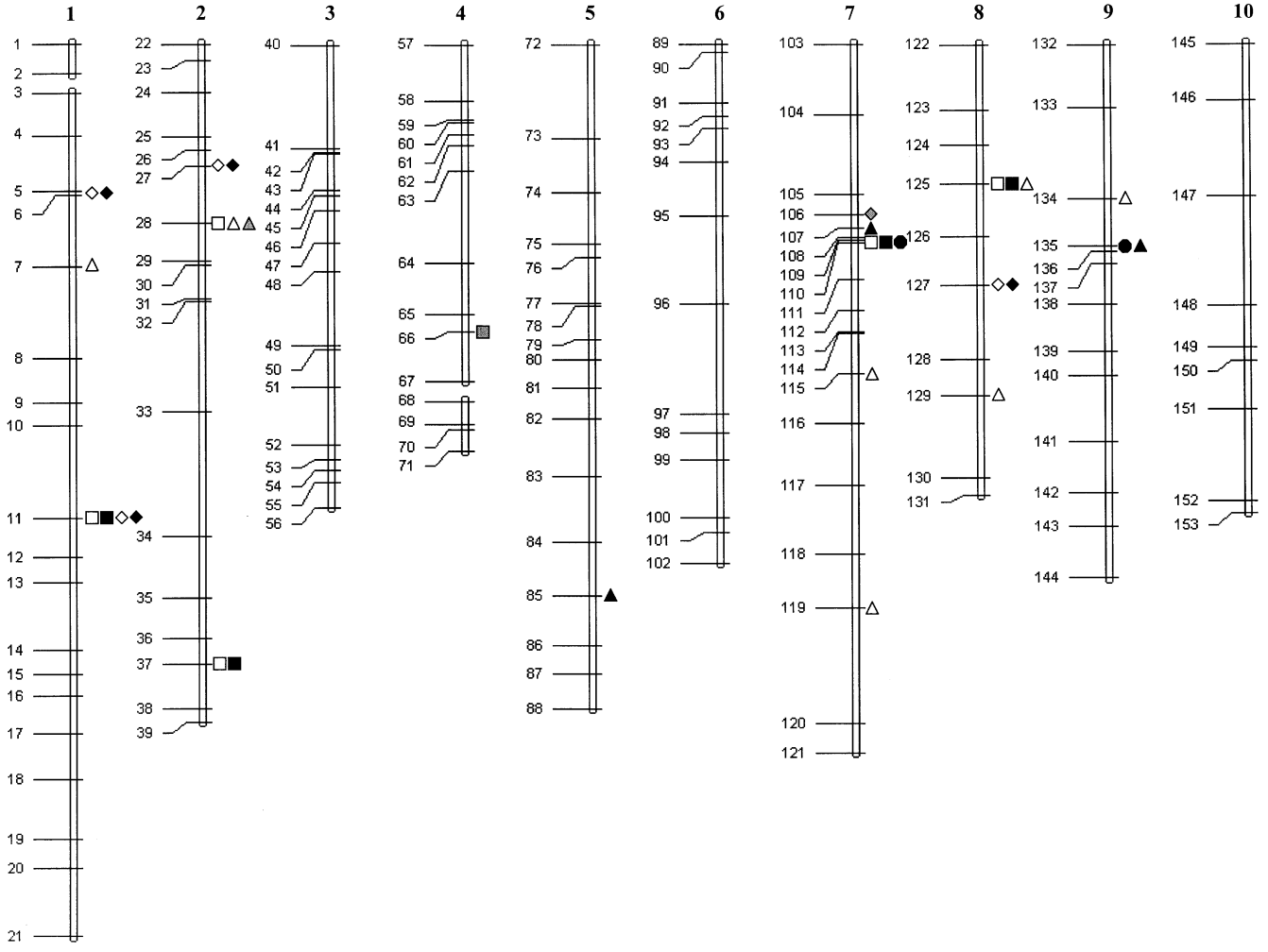

Fig. 1 Linkage map of molecular markers. The *numbers* refer to the marker loci. Those detected as significantly associated with factors controlling each character in well-watered or stressed environment and with drought tolerance index are identified by *empty*, *black* or *grey* symbols, respectively. □ MFT, ○ FFT, △ ASI, ◇ PH

2, 1 on chromosome 7 and 1 on chromosome 8. All were also expressed under stress, except for the QTL linked to m28, which was detected only in the well-watered treatment. R^2 for some markers was quite high; for instance, marker 108 explained 11% of the trait variation under control conditions and 13% under stress. Both parental lines contributed alleles increasing the trait. One QTL for drought tolerance was detected on chromosome 4, in a different region than the previous ones. The proportion of phenotypic variation accounted for by the markers included in the model was 51.4% under control conditions, 58.2% under stress and 25.9% for drought tolerance.

Two QTLs involved in the expression of FFT were detected; these were expressed only under stress conditions.

Seven QTLs involved in ASI determination were expressed under control conditions and 3 under stress; the two groups were located in different map positions. Only one chromosomal region was revealed to carry QTLs for drought tolerance; the favourable allele was contributed by H99. The amount of ASI variation explained by the model was 49.5% under WW conditions, 32.3% under stress and 10.4% for drought tolerance index.

Four QTLs for PH were expressed under both well-watered conditions and under stress; 1 QTL for tolerance was localized on chromosome 7, in a different position than the other ones. The B73 parent contributed most of the alleles associated with increased plant height.

In Fig. 1, the chromosome location of QTLs detected for all the traits is reported. The different symbols indicate the position of QTLs detected for the different traits, and the number refers to the marker map position. One region on chromosome 7 carries factors involved in the control of both male and female flowering time expressed under stress; the alleles increasing the character were contributed by H99. One region on chromosome 8 was revealed to carry QTLs for MFT and ASI (in the well-watered treatment); the allele provided by B73 seemed to increase MFT and decrease ASI. A similar situation was observed on chromosome

2 (m28). A region on chromosome 9 carries QTL for FFT and ASI: the H99 alleles increased FFT, but decreased ASI. On chromosome 1, a QTL involved in MFT and PH was detected; the B73 allele increased the traits.

One QTL for tolerance in terms of MFT was detected on chromosome 4; the allele producing flowering delay under stress was carried by the H99 line. On the contrary, the QTL involved in tolerance for ASI on chromosome 2 revealed that the B73 allele increased the trait under stress. One QTL involved in PH tolerance was identified; the allele conferring tolerance (low reduction in plant height under stress) was associated with the B73 parental line.

In some cases, the QTLs' most probable positions with respect to the nearest marker are not exactly coincident for the same trait and the same marker under WW and WS conditions. Taking into account that the QTL position estimated by mapping has sampling error, the results could be interpreted as the presence of a single QTL expressed across both treatments (more probable) or two different QTLs, one of which is expressed in WW, the other in WS treatment. On the other hand, for practical purposes, such as MAS (marker-assisted selection), it is important that both such loci (if they are two) are closely linked to a given marker that has to be considered important for selection.

Discussion

In this study, linkage analysis between traits related to plant development and flowering and molecular markers was performed. The population of RILs tested was obtained by crossing lines B73 and H99: B73 has late male and female flowering time, short ASI and tall plant height, whereas H99 has early flowering time, longer ASI and short height; thus, it could be expected that B73 would be more drought tolerant than H99. However, in H99, unlike in B73, ASI did not increase and PH was barely reduced under stress. Furthermore, the regression of the tolerance index for ASI and for PH on the respective values of the character evaluated under the well-watered condition revealed that, in the RIL population, lines having a long ASI and low stature were more tolerant under drought stress; regression of TI for ASI on MFT indicated that early flowering lines have a small increase in ASI under stress. These characteristics resembled those of the H99 parent. These results can be interpreted within the framework that the treatment was applied at the beginning of anthesis; under these conditions, early flowering is an important characteristic, since it allows the avoidance of water stress. On the other hand, the treatment applied reflected the environmental conditions of temperate areas, where drought stress does not occur during all of the plant life cycle but, usually, from male flowering time onwards.

If we also take into account the results concerning drought effects on yield components (Frova et al. 1999), the findings by Bolanos and Edmeades (1996) are confirmed: the correlation between ASI and grain yield is much higher under stress conditions than in well-watered environment. In particular, the coefficient of correlation between ASI and ear weight was -0.30 under well-watered conditions but was -0.59 under stress; between ASI and kernel weight it increased from -0.27 to -0.59, respectively, and between ASI and kernel number, from -0.25 to -0.56, respectively.

Linkage analysis revealed that, for MFT and PH, most of the QTLs detected were the same under both control and under stress conditions. The alleles increasing MFT value and its TI were contributed by both the parents; thus, even though H99 is an early flowering genotype, it also carries alleles with opposite effects. For PH, H99 contributed only some alleles increasing the trait, under both water regimes, as expected. A different picture characterizes FFT and ASI: QTLs appeared to be specific for the two water regimes. This could be explained in terms of a genotype $\times$ environment interaction; thus, these characters appeared to be less stable than MFT and PH. For ASI, most of the alleles increasing the trait under well-watered conditions were from H99, but under stress, alleles from B73 also were expressed; moreover, the allele conferring tolerance for this trait was that of line H99.

When we consider, the QTLs detected for all the traits, blocks of common loci for different traits are revealed. When the alleles are contributed by the same parent, two possibilities can be inferred: pleiotropy or closely linked QTLs. Due to the QTLs wide confidence interval, it is very difficult to distinguish linkage from pleiotropy (Kearsey and Farquhar 1998). However, with regard to QTLs putatively involved in male and female flowering time, or in MFT and PH, due to the physiological meaning of these traits, which is dependent on plant maturity and development, it is reasonable to postulate the presence of pleiotropic alleles. In any case, these data can explain the observed correlation between traits.

All the QTLs detected on the basis of tolerance indices were located in different chromosome positions than the map position of the factors controlling the trait. The first QTLs are putative factors conferring tolerance to drought; the latter QTLs are putative genes involved in the determination of the characters. The fact that the two categories of QTLs are not coincident suggests that plant tolerance, in its different components, is not attributable only to the presence of favourable allelic combinations at loci controlling the trait itself but is also based on physiological characteristics not directly associated with the control of the character. Thus, the chromosomal regions to be monitored in a MAS experiment should be those where

QTLs for tolerance are located. This choice could allow selection for drought tolerance without any modification of the architecture of the genetic system controlling the trait.

Flowering and ASI QTLs have been identified in maize in a study by Veldboom and Lee (1996), carried out on a population of F_{2-3} lines derived from the cross of inbreds Mo17 and H99. The latter was one parent of the RI populations used in the present study. Thus, even though the stress investigated in the Veldboom experiment was not drought, but simply a non-optimal climatic condition occurring in 1990, and that the molecular markers used for mapping were not the same in the two cases, it is worth comparing the two series of results. Both analyses detected chromosomal regions where some QTLs were positioned: for MFT and PH on chromosome 1; 1 QTL for PH and 1 for MFT on chromosome 2; 1 locus associated to MFT and FFT and 1 to ASI on chromosome 7; 1 QTL for ASI on chromosome 8. In this position, Ribaut et al. (1996) also identified a QTL for ASI. A QTL for the same trait was also detected by these authors on chromosome 5, linked to the same marker (UMC68) we found to be associated to ASI. Also, the putative QTL for MFT identified in the present work and by Veldboom and Lee (1996) on chromosome 1 could correspond to the one detected by Ribaut et al. (1996) in the same region. The two inbreds used as parental lines of the F_2 population analyzed in this study were maize tropical lines, thus quite different in terms of genetic background from the genotypes we used in the present study. The stress studied was drought, but the climatic conditions in Mexico are considerably different from those of Southern Italy, where drought is normally associated with higher temperatures. The identification of some QTLs important for stress tolerance in the present study and in those made by Veldboom and Lee (1996) and by Ribaut et al. (1996), in spite of the differences between experiments, could indicate that the factors identified represent important genetic components of the trait.

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