Theor Appl Genet (1999) 99:280–288 

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

# Genetic analysis of drought tolerance in maize by molecular markers

## I. Yield components

Received: 19 October 1998 / Accepted: 28 December 1998

**Abstract** Grain yield is a complex trait, strongly influenced by the environment: severe losses can be caused by drought, a stress common in most maize-growing areas, including temperate climatic zones. Accordingly, drought tolerance is one of the main components of yield stability, and its improvement is a major challenge to breeders. The aim of the present work was the identification, in maize genotypes adapted to temperate areas, of genomic segments responsible for the expression of drought tolerance of yield components: ear length, ear weight, kernel weight, kernel number and 50-kernel weight. A linkage analysis between the expression of these traits and molecular markers was performed on a recombinant inbred population of 142 families, obtained by repeated selfing of the $F_1$ between lines B73 and H99. The population, genotyped at 173 loci (RFLPs, microsatellites and AFLPs), was evaluated in well-watered and water-stressed conditions. A drought tolerance index was calculated as the ratio between the mean value of the trait in the two environments. For the traits measured, a highly positive correlation was found over the two water regimes, and more than 50% of the quantitative trait loci (QTLs) detected were the same in both; moreover, the direction of the allelic contribution was always consistent, the allele increasing the trait value being mostly from line B73. Several QTLs were common to two or more traits. For the tolerance index, however, most of the QTLs were specific for a single component and different from those controlling the basic traits; in addition, a large proportion of the alleles increasing tolerance were provided by line H99. The data suggest that drought tolerance for yield components is largely associated with genetic and physiological factors independent from those determining the traits *per se*. The implications of these results for developing an efficient strategy of marker-assisted selection for drought tolerance are discussed.

**Key words** *Zea mays* L · Drought tolerance · Molecular markers · Yield components · Linkage analysis

Communicated by P.L. Pfahler

C. Frova (✉) · M. Villa · M. Sari-Gorla
Department of Genetics and Microbiology, University of Milano, Via Celoria 26, 20133 Milano, Italy
e-mail: Frova@imiucca..csi.unimi.it
Fax: +39 02 2664551

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

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

## Introduction

Maize is the third most important crop worldwide, and is cultivated both in temperate zones, such as the U.S. Corn Belt and the Mediterranean area, and in the tropics, including Mexico, Central and Latin America. In most maize-growing areas, drought or water stress is one of the main environmental factors causing substantial yield reductions, even though temperate and tropical zones differ both in the intensity of the stress (higher in the tropics) and for its variability in different years (larger in temperate zones). For instance, Mediterranean zones are, on average, characterized by dry summers and wet winters and springs, with a rapidly increasing temperature and evaporation during late spring (Passioura 1996). Therefore, during the crucial flowering and seed-set period (July for most corn genotypes cultivated in these areas) the crop should be able to rely on water stored in the soil during spring to provide a good yield. In fact, most of the varieties cultivated in these areas have been selected for high yield under these climatic conditions, i.e. a good water supply during crop maturation. However, substantial periods of drought and high temperatures may occur earlier during the growing season; this causes a delay in the maturation of the crop in an increasingly hot and water limiting environment and, as a consequence, a decrease in yield (Passioura 1996). Thus in

these zones, unless adequate water supply is provided through irrigation, the major problem is poor yield stability and the main goal is not so much to select varieties that perform well under consistent drought, as in the tropics, as it is to breed genotypes able to maintain a good yield over a range of water supply, in other words to be drought-tolerant.

This is not an easy task and for several reasons.

(1) Drought tolerance is a very complex trait, influenced by a broad range of processes spanning both the time scale and the plant organization level. In this regard, crop phenology, that is the timing of its development in relation to temporal changes in water supply, is considered a very important feature of drought tolerance (Passioura 1996). This form of plant adaptation, however, may be ineffective if climate changes are rapid and inconsistent over time.

(2) For many crops, a low genetic correlation is often observed for yield in high- and low-productivity environments, indicating that different sets of genes may be important in conditioning the yield in different environments (Johnson and Geadelmann 1989; Atlin and Frey 1990). This has been confirmed in a recent study aimed at the identification of quantitative trait loci (QTLs) governing yield in tropical maize under drought conditions: consistency both in the number and location of QTLs detected under three water regimes was very low (Ribaut et al. 1997).

The consequence of this information is that selecting for high yield in one particular environment (droughted or non droughted) may not be an effective strategy to obtain yield stability. An alternative would be the identification of genetic factors, not necessarily involved in yield *per se* but specific for drought tolerance, to be introduced into the desired genotypes through a marker-assisted selection program. In this way, an improved performance under drought without affecting productivity in well-watered conditions would be expected. The underlaying assumption is that at least some QTLs for tolerance are different from those for yield *per se* in either environment; this has been shown to be the case, for instance, for maize thermotolerance components (Frova and Sari-Gorla 1994); but, to our knowledge, no such analysis has been performed for drought tolerance.

The aim of the present study was to determine the genetic architecture of drought tolerance in maize, by identifying the number and chromosomal location of QTLs controlling the stability of major yield components. For this purpose, we used linkage analysis between the expression of these traits and molecular markers in two different water regimes.

## Materials and methods

### Plant material

A population of recombinant inbred lines (RILs) consisting of 142 $F_{12}$ families, derived from the cross B73 x H99 was used in this study. The two parental lines and their $F_1$ were also included. The 142 lines were highly homozygous, showing a residual heterozygosity of 0.6% (Sari-Gorla et al. 1997a). The two parental inbreds are elite lines belonging to the Lancaster Sure Crop (H99) and Iowa Stiff Stalk Synthetic (B73) heterotic groups, and are widely utilized in temperate climatic zones.

### Population typing and linkage-map construction

A linkage map based on the same RIL population, that included 100 molecular markers, RFLPs and microsatellites (SSRs), had been previously developed in our laboratory (Sari-Gorla et al. 1997a). This map has now been further saturated with additional SSRs and AFLP markers. DNA extraction from plant material (seedlings) was performed as indicated in Sari-Gorla et al. (1997a). The new SSR all belong to the bngl series, for which primer information and amplification conditions were obtained from B. Burr (Brookhaven National Laboratory). As for AFLP markers, genomic DNA was digested with EcoRI and MseI and ligated to Eco- and Mse-specific adaptors according to Vos et al. (1995). Four primers combinations, E-33/M-51, E-35/M-50, E-38/M-51 and E-40/M-49, were used. The primers sequences, complementary to the Eco- and Mse-adaptors except for the three 3′ nucleotides which differentiate them within each series, were as followings:

E-33 : 5′- GAGTGCGTACCAAATC |AAG - 3′
E-35 : 5′- GAGTGCGTACCAAATC |ACA - 3′
E-38 : 5′- GAGTGCGTACCAAATC |ACT - 3′
E-40 : 5′- GAGTGCGTACCAAATC |AGC - 3′
M-49 : 5′- GTAGAGTCCTGAGTAA |CAG - 3′
M-50 : 5′- GTAGAGTCCTGAGTAA |CAT - 3′
M-51 : 5′- GTAGAGTCCTGAGTAA |CCA - 3′

Pre-amplification, selection and final amplification were performed according to Vos et al. (1995). Primers and adaptors were purchased from Genset SA (Paris, France).

A genetic map including the old and the new markers was constructed by means of the MAPMAKER/EXP program (Lander et al. 1987). One hundred and fifty seven markers fell into a linkage group with at least another marker at LOD 3.0. The order of the markers within each linkage group was established by full multipoint analysis, and tested by the “ripple” command.

### Field design and growth conditions

The trial was carried out in Foggia (Southern Italy) in 1997. This location was chosen because rainfall in late spring and summer is usually very limited or absent, consequently water supply can be tightly controlled by differential irrigation. In particular, in 1997 there was 37.2 mm rainfall in April, 7.6 mm rainfall cumulatively in May, June and July, 35.8 mm in August and 24.4 mm in September.

Two levels of drought treatment were imposed, well-watered and water-stressed, with two blocks for each level. For each block, two rows of 15 plants per genotype were grown at a plant density of four plants/square meter. Within each block, the order of the genotypes was random. All the material was sown on April 18. All blocks were well-irrigated until June 29. After that date, the well-watered trials received five full irrigations, maintaining soil moisture at 30% expressed on a dry weight basis, while stressed blocks received just enough water in order for the plants not to die (soil moisture 16%).

The first antheses and silking were recorded on July 6 and 13 respectively, but the vast majority of the plants flowered between July 13 and 16 (male) and July 22 and 24 (female). Thus water stress was imposed from flowering time on, starting about 2 weeks before anthesis.

### Field-traits evaluations

Ear length (EL), ear weight (EW), kernel weight per ear (KWE), kernel number per ear (KN) and 50-kernel weight (50 KW) were considered as yield-component traits. For each, measurements were taken on mature ears from the ten central plants per plot (five from each row) per genotype. A tolerance index (TI) of each genotype was calculated as a T/C ratio, where T is the mean value of each trait over the two stressed plots and C is the mean value over the two well-watered plots.

### Statistical analysis and QTL identification

All five traits were analysed separately by analysis of variance, using the PROC GLM SAS procedure (SAS Institute 1998). Corrected means for the RILs in well-watered (WW) and water-stressed (WS) conditions were calculated, and the tolerance index was obtained for each line. This procedure gave 15 (5 x 3) new traits which were independently subjected to subsequent analyses. Simple Pearson correlation coefficients among the traits and among the two water regimes were calculated using the corrected means of the RILs (PROC CORR, SAS). Broad sense heritability ($h^2_B$) was estimated over the two levels of water supply, according to Hallauer and Miranda (1981).

QTL number, genomic localization and effects for each trait were estimated by the least square interval mapping procedure programmed in Genstat, as described in a previous paper (Sari-Gorla et al. 1997b). In brief, the method consists of two steps. Firstly, the forward selection algorithm was used to select markers explaining a significant portion of the variation. Then regression interval mapping was done using the model with selected markers serving as co-variates. The significance of the *F*-values for the hypothesis of no QTL was checked by the sequentially rejective Bonferroni procedure, with the linkage-group-wise Type-I error rate equal to 0.2. In this way only the really important markers are confirmed: in order to be retained, these must show a significant improvement for the fit of the model in the presence of all other selected markers. The result of the mapping step can be viewed as a re-checking of the selected markers, and also provides more precise information about the position of the QTLs relative to the nearest marker, here reported as the "shift in cM". The reason for adopting such strict testing criteria relates to the large number of hypotheses tested, due both to the fact that many positions are tested on each chromosome and that many traits are evaluated simultaneously. In this way, only the really important QTLs are detected, thus keeping the risk of false assignements to a minimum. On the other hand, with this procedure, weak QTLs may pass undetected. For this reason, we also analysed the data by one-at-a-time simple regression analysis of the line mean values for each trait on the marker allelic composition; these results are not reported, but some of them are discussed in the text, in particular when they are useful for a better understanding of the general picture. Total $R^2$ were calculated from fitting the model with all markers found significant by the forward selection procedure.

## Results

### Molecular-marker linkage map

Fifteen new microsatellites and four AFLP primer combinations were tested on the two parental lines. Nine SSRs and 65 AFLPs proved to be polymorphic and were used to type the entire population. Although AFLPs are in practice dominant markers, and thus less powerful than the co-dominant RFLPs and SSRs for genotype typing, in the case of RILs this is not very relevant, since the lines are highly homozygous. The 74 new markers were used to saturate our previous map, based on RFLPs and SSRs (Sari-Gorla et al. 1997a), and a new map consisting of 13 linkage groups for a total of 157 molecular markers was obtained (Fig. 1). Despite the addition of the new markers, chromosomes 1 and 4 remained split into two parts. An additional linkage group, named A, consisting of two SSRs and two AFLPs, was obtained. It could not be attributed to any of the ten maize chromosomes because these markers are not mapped in other populations and, in ours, their linkage to markers of known position was not supported by a LOD score higher than 2.0. Sixteen additional markers did not fall into any linkage group. The total length of the map covers about 2260 cM, corresponding to an average distance between markers of 14 cM.

### Field-traits analysis

All the traits revealed a large quantitative variability and a fairly normal frequency distribution in both water regimes (well-watered and stressed) and for tolerance. For example, the frequency distribution of KWE is shown in Fig. 2. Mean values and tolerance for all traits for P1 (B73), P2 (H99) and the RIL population, as well as $h^2_B$ values, are reported in Table 1. The two parental lines were clearly differentiated for most traits and, with the exception of EL, B73 was characterised by higher values than H99, both in well-watered and in stress conditions and also appeared to be more tolerant. Under drought conditions, the RILs population showed a general shift of the distributions towards lower trait values, often with a concomitant shrinking of the range, indicating that water shortage pushed the population towards its negative extreme. The RILs tolerance index, TI, however, consistently showed some values higher than 1 (Table 1), indicating that, for each trait, a certain proportion of the lines performed as well under drought as in well-watered conditions.

The effect of water stress on all traits was highly significant, as were the variances between RI lines and the G×E interaction ($P<0.0001$ for all, ANOVA not reported), indicating a differential response of the genotypes to drought. Broad-sense heritability ($h^2_B$) of the traits, calculated over the two water regimes, was very high, ranging from 0.86 (EL and KWE) to 0.94 (50 KW).

Across the two water regimes, EL, EW, KWE and KN were positively correlated, and the magnitude of the linear correlations increased under water stress (Table 2). These results are not unexpected since these traits are, by nature, not independent. By contrast, 50 KW showed a lower correlation, generally decreasing under water

**Fig. 1** Molecular-markers linkage map representing the ten chromosomes and one additional linkage group (*A*) of 142 maize recombinant inbred lines derived from the cross B73 × H99. Markers are numbered sequentially (right of chromosomes) and their names are reported to the left of chromosomes. RFLP markers belong to the following series: bnl (indicated as *b*), csu, npi (*n*), php (*p*) and umc (*u*). The map length is 2260 cM and the average interval length is 14 cM

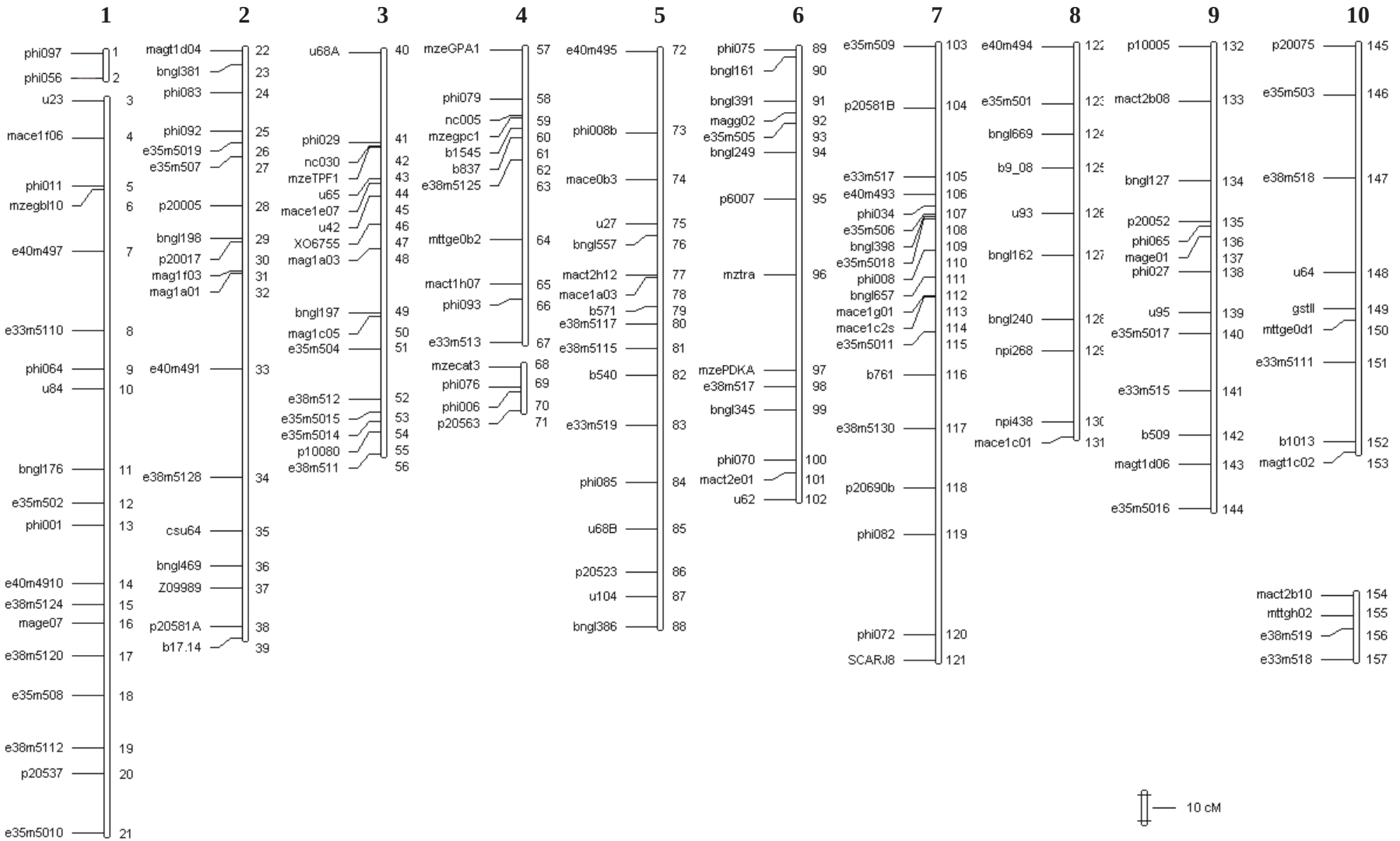
1
2
3
4
5
6
7
8
9
10
phi097
phi056
u23
mace1f06
phi011
mzegbl10
e40m497
e33m5110
phi064
u84
bngl176
e35m502
phi001
e40m4910
e38m5124
mage07
e38m5120
e35m508
e38m5112
p20537
e35m5010
magt1d04
bngl381
phi083
phi092
e35m5019
e35m507
p20005
bngl198
p20017
mag1f03
mag1a01
e40m491
e38m5128
csu64
bngl469
Z09989
p20581A
b17.14
u68A
phi029
nc030
mzeTPF1
u65
mace1e07
u42
XO6755
mag1a03
bngl197
mag1c05
e35m504
e38m512
e35m5015
e35m5014
p10080
e38m511
mzeGPA1
phi079
nc005
mzegpc1
b1545
b837
e38m5125
mttge0b2
mact1h07
phi093
e33m513
mzecat3
phi076
phi006
p20563
e40m495
phi008b
mace0b3
u27
bngl557
mact2h12
mace1a03
b571
e38m5117
e38m5115
b540
e33m519
phi085
u68B
p20523
u104
bngl386
phi075
bngl161
bngl391
magg02
e35m505
bngl249
p6007
mztra
mzePDKA
e38m517
bngl345
phi070
mact2e01
u62
e35m509
p20581B
e33m517
e40m493
phi034
e35m506
bngl398
e35m5018
phi008
bngl657
mace1g01
mace1c2s
e35m5011
b761
e38m5130
p20690b
phi082
phi072
SCARJ8
e40m494
e35m501
bngl669
b9_08
u93
bngl162
bngl240
npi268
npi438
mace1c01
p10005
mact2b08
bngl127
p20052
phi065
mage01
phi027
u95
e35m5017
e33m515
b509
magt1d06
e35m5016
p20075
e35m503
e38m518
u64
gstII
mttge0d1
e33m5111
b1013
magt1c02
mact2b10
mttgh02
e38m519
e33m518
10 cM

**Table 1** Characteristics of parental lines (P1 = B73, P2 = H99) and the RILs population. Ear length (EL) is expressed in cm; ear weight (EW), kernel weight per ear (KWE) and 50-kernel weight (50 KW) are expressed in grams

| Item | EL | | | EW | | | KWE | | |
|---|---|---|---|---|---|---|---|---|---|
| | WW | WS | TI | WW | WS | TI | WW | WS | TI |
| Mean P1 | 14.0 ± 0.2 | 13.9 ± 0.2 | 0.99 | 139.9 ± 5.8 | 126.9 ± 5.2 | 0.91 | 111.0 ± 5.8 | 99.4 ± 5.0 | 0.90 |
| Mean P2 | 16.6 ± 0.3 | 15.2 ± 0.4 | 0.92 | 100.9 ± 3.1 | 78.5 ± 5.1 | 0.78 | 82.9 ± 2.8 | 61.3 ± 4.9 | 0.74 |
| Mean RIL | 15.4 ± 0.4 | 14.3 ± 0.4 | 0.93 | 100.7 ± 6.1 | 79.7 ± 5.9 | 0.79 | 81.2 ± 5.67 | 61.7 ± 5.2 | 0.76 |
| Range RIL | 11.1 – 21.1 | 9.2 – 18.7 | 0.7 – 1.1 | 10.2 – 184.4 | 7.0 – 170.7 | 0.3 – 1.3 | 16.8 – 157.9 | 7.1 – 146.5 | 0.2 – 1.4 |
| $h^2_B$ | 0.86 | | | 0.88 | | | 0.86 | | |
| | KN | | | 50 KW | | | | | |
| | WW | WS | TI | WW | WS | TI | | | |
| Mean P1 | 401.2 ± 23.9 | 386.3 ± 20.7 | 0.96 | 13.9 ± 0.2 | 12.9 ± 0.2 | 0.93 | | | |
| Mean P2 | 331.6 ± 11.8 | 253.8 ± 20.1 | 0.77 | 12.5 ± 0.2 | 12.1 ± 0.1 | 0.96 | | | |
| Mean RIL | 325.6 ± 22.1 | 261.7 ± 21.0 | 0.80 | 12.6 ± 0.3 | 11.9 ± 0.4 | 0.95 | | | |
| Range RIL | 65.7 – 570 | 30.8 – 564 | 0.2–1.2 | 7.7 – 18.9 | 7.6 – 18.3 | 0.7 – 1.4 | | | |
| $h^2_B$ | 0.89 | | | 0.94 | | | | | |

stress, with the other traits and the correlation with kernel number, although weak, was negative. The data presented in Tables 1 and 2 indicated that the traits most affected by water shortage were KN, KWE and EW, while EL and 50 KW were less affected. Thus a selection program should emphasize the first three traits, also considering that KWE is expected to be strongly associated with grain yield. In agreement with what reported for other cereals (Blum 1996), but in contrast to other maize studies (Ribaut et al. 1997), 50 KW is inversely proportional to grain number. Considering drought tolerance, the highest correlations were observed between KWE, KN and EW.

In addition, for each trait, the correlation between well-watered, water-stressed and drought tolerance was calculated (Table 3). In all cases, the correlation coefficients between the characters under control and stress conditions were very high ($0.78<r<0.88$), between treated and tolerance were moderate ($0.36<r<0.65$, except for 50 KW, for which $r = 0.22$), while they were very low between the control and tolerance. In order to ascertain if tolerance is dependent upon the plant performance in well-watered or in stressed conditions, linear regressions were performed. The results for all traits, not reported here, indicated a highly significant regression of drought tolerance on the trait value under water stress ($P<0.0001$), while only for KWE and KN tolerance was also dependent upon the values under well-watered conditions ($P<0.04$ and 0.001 respectively).

## QTL mapping

The location and the main characteristics of the QTLs identified in the five yield components under two water regimes and for tolerance are reported in Table 4. Markers are indicated as m1, m2, ....m153, according to their position on the map, from chromosome 1 to 10 (see

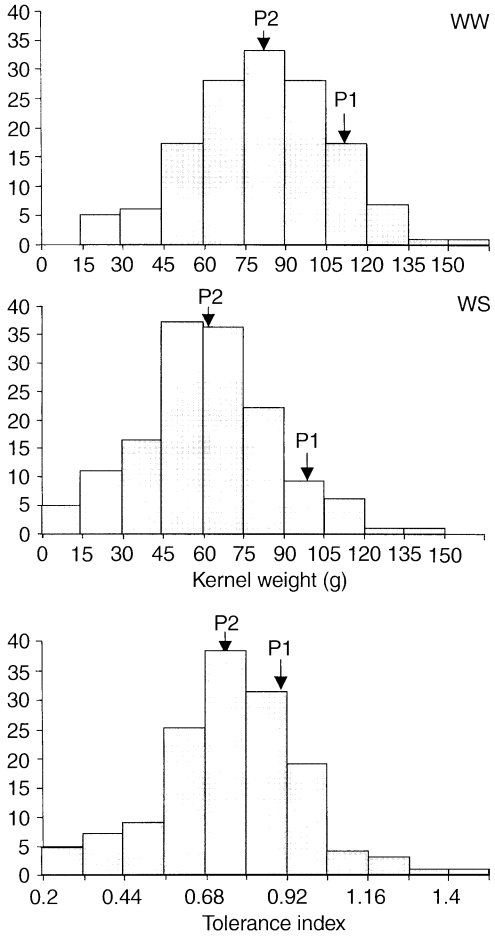


**Fig. 2** Distribution of the adjusted means of kernel weight per ear in the RILs population under two water regimes, well-watered (*WW*) and water-stressed (*WS*), and of the relative tolerance index. Mean values of P1 (B73) and P2 (H99) are indicated by arrows

**Table 2** Correlation coefficients between the five traits under well-watered (WW) and water-stressed (WS) conditions and between the drought tolerance index (TI): EL, ear length; EW, ear weight; KWE, kernel weight per ear; KN, kernel number; 50 KW, 50-kernel weight. N = 142

| Item | | EW | | KWE | | KN | | 50 KW |
|---|---|---|---|---|---|---|---|---|
| EL | WW | 0.60** | | 0.50** | | 0.38** | | 0.19* |
| | WS | 0.61** | | 0.54** | | 0.43** | | 0.18* |
| | TI | 0.64** | | 0. 53** | | 0.50** | | 0.24** |
| EW | | | WW | 0.94** | | 0.79** | | 0.25** |
| | | | WS | 0.97** | | 0.87** | | 0.14 |
| | | | TI | 0.96** | | 0.91** | | 0.36** |
| KWE | | | | | WW | 0.85** | | 0.23* |
| | | | | | WS | 0.91** | | 0.11 |
| | | | | | TI | 0.96** | | 0.37** |
| KN | | | | | | | WW | –0.28** |
| | | | | | | | WS | –0.27** |
| | | | | | | | TI | 0.26** |

*$P$<0.05
**$P$<0.01

**Table 3** Correlation coefficients for all traits between well-watered (WW), water-stressed (WS) conditions and for the drought tolerance index (TI): EL, ear length; EW, ear weight; KWE, kernel weight per ear; KN, kernel number; 50 KW, 50-kernel weight. N = 142

| Item | WS | TI | | WS | TI |
|---|---|---|---|---|---|
| EL WW | 0.83** | –0.22* | EW WW | 0.80** | –0.07 |
| EL WS | | 0.36** | EW WS | | 0.51** |
| KWE WW | 0.78** | 0.04 | KN WW | 0.83** | 0.16 |
| KWE WS | | 0.62** | KN WS | | 0.65** |
| 50 KW WW | 0.88** | –0.25** | | | |
| 50 KW WS | | 0.22* | | | |

*$P$<0.05
** $P$<0.01

Fig. 1). In addition to the QTLs position, the sign of the effect and the coefficient of determination ($R^2$) are indicated. "Shift in cM" indicates the most-likely position of the QTLs relative to the nearest marker: "–" and "+" mean a shift towards the previous or the following marker, respectively; no shift means shift < 1.0 cM. The sign of the effect indicates which of the parental alleles increases the trait value (positive if H99; negative if B73) while $R^2$ estimates the proportion of phenotypic variation of the trait explained by allelic substitution at that particular marker locus. Total $R^2$ measures the cumulative contribution of the QTLs detected in the analysis to the phenotypic variation of each trait. The data were analysed by two methods, interval mapping and one-at-a-time simple linear regression. Since the first procedure is very conservative (see Materials and methods), a relatively small number of QTLs was detected for each trait. By contrast, simple regression identified many more significant markers, often grouped in clusters (data not shown). This discrepancy can be explained considering the large number of markers and traits involved which, with the second procedure, can lead to Type-I errors (i.e. the identification of dubious QTLs). In fact, adopting more selective criteria in the regression analysis, i.e. considering only markers with a relatively high $R^2$ values as indicative of the presence of a QTL, the two procedures essentially gave the same results. In a few cases, markers significant by regression analysis were also selected in the forward selection procedure, but failed to pass the second step in interval mapping. We considered these markers as indicative of "weak" QTLs.

The number of QTLs identified by interval mapping for the different traits was 4 each in well-watered conditions, between 4 and 6 under water stress and less, between 0 and 4, for tolerance. Single marker $R^2$ values were on average not very high, in particular for tolerance, but a few explained more than 10% of the phenotypic variability of the traits. Two such markers were detected for EL, one for KWE, and two each for KN and 50 KW. Total $R^2$ values, however, were fairly high, in particular when compared to the individual values for the QTLs detected. This can be explained taking into account the diverse procedures adopted for estimating total $R^2$ and QTL number. In the first case, the model was fitted with all markers selected by the forward selection procedure, while a second selection step was performed for QTL identification. In one case (EL tolerance) this led to a total $R^2$ of 0.098 even though no QTLs conforming to our critera were detected. Total $R^2$ values ranged from 0.42 to 0.60 for the basic traits, and, for each of them, were comparable between the two water regimes. For tolerance they were significantly lower, between 0.10 and 0.33, reflecting both the limited number of QTLs detected and their single $R^2$ values. In a few cases, regression analysis detected markers, belonging to the A linkage group or else unlinked, that were both significant and with high $R^2$ value. Since these markers cannot be analysed with the interval mapping procedure, they were not presented in this work, but nonetheless they indicate the putative presence of additional QTLs with appreciable effect.

A comparison between QTLs per water regime and for tolerance revealed several chromosomal regions in-

**Table 4** QTLs involved in the expression of ear length (EL), ear weight (EW), kernel weight per ear (KWE), kernel number (KN) and 50-kernel weight (50 KW) under well-watered (WW) or water-stressed (WS) conditions, and of drought tolerance (TI)

| Trait | Chr | WW | | | WS | | | TI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Marker (shift in cM) | Sign | $R^2$ % | Marker (Shift in cM) | Sign | $R^2$ % | Marker (shift in cM) | Sign | $R^2$ % |
| EL | 1 | | | | m16 (+1.4) | + | 3.5 | | | |
| | 2 | | | | m28 | + | 8.0 | | | |
| | 3 | m47 (–2.4) | + | 13.3 | | | | | | |
| | 5 | | | | m86 (-4.3) | – | 5.0 | | | |
| | 6 | m98 | - | 11.9 | m98 (-1.4) | – | 10.1 | | | |
| | 7 | | | | m105 | + | 4.9 | | | |
| | | m108 | + | 9.3 | | | | | | |
| | 8 | m126 (–2.3) | - | 8.2 | m126 (-5.0) | – | 4.2 | | | |
| Total | | | | 57.1 | | | 60.1 | | | 9.8 |
| EW | 2 | | | | m24 (-2.6) | – | 5.2 | | | |
| | | | | | m28 (-1.3) | + | 6.1 | | | |
| | 3 | m44 (–2.6) | + | 5.3 | | | | | | |
| | 4 | m63 (+2.4) | + | 6.2 | | | | | | |
| | | | | | m66 (+3.8) | – | 7.0 | m66 | - | 6.5 |
| | 5 | m78 | - | 5.7 | | | | | | |
| | 8 | m126 (–8.3) | – | 5.2 | | | | | | |
| | 9 | | | | m141 | – | 5.2 | | | |
| Total | | | | 41.6 | | | 47.8 | | | 21.5 |
| KWE | 1 | | | | | | | m2 (–1.4) | + | 4.2 |
| | 2 | | | | m28 (–2.3) | + | 6.6 | | | |
| | 3 | m47 (+3.6) | + | 7.5 | | | | | | |
| | 4 | | | | m66 (+5.8) | – | 10.5 | m66 | – | 7.0 |
| | 5 | | | | m78 | – | 6.5 | | | |
| | | m81 | – | 5.5 | | | | | | |
| | | m83 (–7.5) | + | 5.1 | | | | | | |
| | | m86 (–1.3) | – | 9.3 | | | | | | |
| | 9 | | | | | | | m136 | + | 6.6 |
| | | | | | m140 | – | 9.0 | | | |
| Total | | | | 46.9 | | | 53.5 | | | 29.7 |
| KN | 1 | | | | | | | m2 (–2.4) | + | 7.1 |
| | | | | | | | | m21 | + | 7.4 |
| | 2 | m28 (+5.6) | + | 6.1 | m28 (–4.3) | + | 5.4 | | | |
| | 4 | m66 (+6.8) | – | 6.1 | m66 (–1.2) | – | 11.1 | m66 | – | 12.5 |
| | 5 | m86 | – | 11.4 | m86 | – | 8.3 | | | |
| | 6 | m101 | – | 5.8 | | | | | | |
| | 9 | | | | m140 (+6.0) | – | 8.1 | | | |
| Total | | | | 48.6 | | | 47.3 | | | 29.3 |
| 50KW | 1 | m8 (+7.5) | – | 5.7 | m8 (+6.5) | – | 5.9 | | | |
| | 2 | | | | | | | m23 (–2.7) | – | 6.5 |
| | 3 | m47 | + | 4.2 | m47 | + | 4.8 | | | |
| | 4 | | | | | | | m57 (+3.0) | – | 6.0 |
| | 5 | m82 | – | 11.4 | m82 (–3.0) | – | 12.3 | | | |
| | 7 | | | | | | | m108 | – | 6.9 |
| | 8 | m123(–6.1) | – | 7.6 | m123 (–2.1) | – | 11.5 | | | |
| | 9 | | | | | | | m135 (–8.6) | + | 6.0 |
| | 10 | | | | m150 | – | 4.8 | | | |
| Total | | | | 57.7 | | | 48.7 | | | 32.6 |

volved in the expression of more than one trait. They were even more than those reported in Table 4 if "weak" QTLs, hereafter indicated in brackets, were also considered. In particular, three (five) such regions, on chromosomes (2-m28), 3-m47, (4-m66), 5-m86, 8-m126 and were detected under well-watered conditions, four under water stress on chromosomes 2-m28, 4-m66, 5-m86 and 9-m140, and two (three) for tolerance on chromosomes 1-m2, (2-m22), and 4-m66. These findings reflect the high correlation values observed between the traits in either water regime and for tolerance. The estimated position of the relative QTLs are not always coincident (see for instance, m126 for EL and EW in well-watered conditions, or m66 for KWE and KN under water stress),

which could be interpreted in terms of the presence of different QTLs for the different traits. However, within each common region, the direction of the allelic contribution was consistent for all traits, suggesting the possibility that the same QTL may be involved in the control of different yield-components.

A similar comparison was made for QTLs across water regimes, in order to ascertain their consistency over different environments. In contrast to what was reported by other authors (Ribaut et al. 1997), at least for three traits (EL, KN, 50 KW) around 50% of the QTLs were found to be expressed under both levels of water supply (Table 4). If weak QTLs are also considered, this proportion increased. Again, this is in agreement with the highly significant level of linear correlation observed for these traits across environments. The direction of the allelic contribution was always consistent and, in general, the allele increasing the trait value was that of inbred B73. For the same reasons as given above, even though in some cases the position of the QTLs was not exactly the same in well-watered conditions and under stress (see, for instance, m66 for KN or m123 for 50 KW), it is not unlikely that the same genetic factors control the variability of the traits in the two environmental conditions. When considering tolerance, however, a different picture emerged: although for three traits, EW, KWE and KN, there seemed to be a QTL (defined by marker m66 on chromosome 4) expressed also under water stress, all the other appeared to be specific for tolerance. Even taking into account weak QTLs, the vast majority of tolerance QTLs mapped to very different regions than those controlling the basic traits.

## Discussion

In this study, the identification of QTLs for yield components expressed in two water regimes and for drought tolerance was determined by linkage analysis with molecular markers.

The material used, a RILs population, is particularly useful for this kind of study, in that, for each genotype, an unlimited number of genetically identical individuals is available, allowing a very accurate measurement of each trait as well as a direct comparison of the data regarding different traits or different years/environments. The parental lines, B73 and H99, were quite different for all traits: in the control condition (well-watered), B73 was characterized by shorter but heavier ears, more and heavier kernels and, as expected, a larger kernel weight per ear compared to H99. B73 also performed better under water stress. This result is in agreement with the correlation of the phenotypic data observed in the population across the two environments and with the QTL data. In fact, a large proportion of the QTLs detected cumulatively for the five traits were expressed in both environments, and, for the majority of these, the allele increasing the trait value was that of B73. Thus a fairly good stability of yield QTLs across environments was observed. Similar results were obtained in a study considering growth under normal and excess rainfall conditions (Veldboom and Lee 1996), but not in the case of drought stress (Ribaut et al. 1997): the discrepancy can be explained by differences in both the material used and in the environment tested. The drought-stress trials of Ribaut et al. (1997) were performed in Mexico, using tropical maize specifically adapted to cool and dry environments, while our experiment and that of Veldboom and Lee (1996) were performed on populations derived from crosses (B73 x H99 and Mo17 x H99, respectively) between lines grown in, and adapted to, temperate climates.

Heritability values for all five traits, estimated over the two water regimes, were quite high. This probably reflects the procedure adopted and the large number of observations from which $h^2_B$ was calculated (the observation unit was the single plant for an average of 36 observations for each trait). Indeed our results are in good agreement with those of other authors. For instance, Veldboom and Lee (1996), taking into account a normal and a stressful environment, found $h^2_B$ values of 0.85, 0.83 and 0.83 for EL, KW and 300 KW respectively. Slightly lower values were reported by Ribaut et al. (1997) for KW, KN and 100 KW (0.66, 0.66 and 0.73, respectively), but it has to be taken into account that they were calculated over two drought conditions, one (intermediate) similar to our stress and one (severe) more severe, and it is known that for these kinds of traits stress generally causes an increase of environmental variance relative to genetic variance, which decreases heritability estimates (Blum 1988; Bolanos and Edmeades 1996; Ribaut et al. 1996).

Across-trait analysis indicated a highly significant correlation among the phenotypic data; in theory, this can be explained by either pleiotropy, linkage or environmental effects (Aastveit and Aastveit 1993). Since, in several cases, the same genomic position of QTLs controlling two or more traits was revealed, and this was true in both environments, environmental effects appear unlikely. Whether the same QTLs control more traits (pleiotropy) or whether different QTLs, each specific for one trait, are tightly linked cannot be established with the kind of methodology, univariate analysis, adopted here. However, in both cases, the chromosomal regions identified are good candidates for marker-assisted selection, also considering that, with no exception, it is always the same parental allele (mostly that of B73) that increases the trait value.

A comparison with other studies (Veldboom and Lee 1996; Ribaut et al. 1997), indicates a similar number of QTLs detected, as well as similar individual $R^2$ values, for KWE, KN, 50 KW and EL. As for QTL position, since only few markers are common to the three linkage maps, a comparison was possible for a limited number of chromosomal regions only. In general, however, it appears that the three studies detected mostly different QTLs. In particular, only for EL in well-watered condition, and for KWE under stress, the QTLs defined by

markers m98, m126 (EL) and m78 (KWE) in our study mapped in the same chromosomal regions as the three QTLs detected by Veldboom and Lee (1996), whereas no common regions were found with the study of Ribaut et al. (1997). It is possible that these negative results are due to the very different genetic material used in their drought study by Ribaut et al. (maize tropical lines), although for other traits correlated to crop productivity, such as plant height, female flowering time and the anthesis-silking interval, QTLs were detected in the same chromosomal regions as those identified by the other studies. The possible meaning of these putatively common factors is discussed in an accompanying paper (Sari-Gorla et al. 1999).

In addition to QTLs expressed in both environments, interesting regions were detected by the dissection of tolerance. Also for this parameter two, m2 and m66 (three, m22), QTLs putatively common to more components were identified, and one of these, defined by marker 66, appeared to be expressed also under drought conditions. However, the majority of the tolerance QTLs were unrelated to those controlling the basic traits, as can be deduced from their largely different positions in the genome. Also, while for the basic traits the allele conferring a higher value was predominantly that of line B73, for tolerance a fair amount of transgressive segregation was found, since many favourable alleles were contributed by the less-tolerant line H99. On the whole, these data suggest that tolerance for yield components is mostly based on genetic and physiological characteristics not associated with those determining the traits *per se*. These tolerance QTLs too can be exploited in a selection program, since the "good" alleles could be introduced in a well-performing genotype (i.e. B73) without breaking the existing favourable genetic combinations that control the basic traits.

In conclusion, the key points revealed by our results are: (1) the stability of some QTLs across water regimes for each of the yield components, and (2) the existence of genetic factors specific for tolerance. This information can be useful in a breeding program aimed at improving yield stability, a major goal especially in temperate zones characterized by unpredictable periods of water shortage.

**Acknowledgments** This work was supported by Ministero delle Politiche Agricole (Italian Ministry of Agriculture), Piano Nazionale Biotecnologie Vegetali. The authors thank Zygmunt Kaczmarek and Tadeusz Calinski for helpful discussion of the data, Raffaele Banfi and Michele De Ninno for skilful technical assistance, and Luca Mizzi for the preparation of Fig. 1.

## References

Atlin GN, Frey KJ (1990) Selecting oat lines for yield in low-productivity environments. Crop Sci 30: 556–561

Aastveit AH, Aastveit K (1993) Effects of genotype-environment interactions on genetic correlations. Theor Appl Genet 86: 1007–1013

Blum A (1988) Plant breeding for stress environments. CRC Press, Boca Raton, FL

Blum A (1996) Crop responses to drought and the interpretation of adaptation. In: Belhassen E (ed) Drought tolerance in higher plants. Genetical, physiological and molecular biological analysis. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 57–71

Bolanos J, Edmeades GO (1996) The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Res 48: 65–80

Frova C, Sari-Gorla M (1994) Quantitative trait loci (QTLs) for pollen thermotolerance detected in maize. Mol Gen Genet 245: 424–430

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa

Johnson SS, Geadelmann JL (1989) Influence of water stress on grain response to recurrent selection in maize. Crop Sci 29: 558–564

Lander ES, Green P, Albertson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER, an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1: 174–181

Passioura JB (1996) Drought and drought tolerance. In: Belhassen E (ed) Drought tolerance in higher plants. Genetical, physiological and molecular biological analysis. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 1–7

Ribaut J-M, Hoisington DA, Deutsch JA, Jiang C, Gonzales-de-Leon D (1996) Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor Appl Genet 92: 905–914

Ribaut J-M, Jiang C, Gonzales-de-Leon D, Edmeades GO, Hoisington DA (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor Appl Gent 94: 887–896

Sari-Gorla M, Krajewski P, Binelli G, Frova C, Taramino G, Villa M (1997a) Genetic dissection of herbicide tolerance in maize by molecular markers. Mol Breed 3: 481–493

Sari-Gorla M, Calinski T, Kaczmarek S, Krajewski P (1997b) Detection of QTL × environment interaction in maize by a least squares interval mapping method. Heredity 78: 146–157

Sari-Gorla M, Krajewski P, Di Fonzo N, Villa M, Frova C (1999) Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. Theor Appl Genet 99: 289–295

SAS Institute Inc (1998) SAS language guide for personal computers. Edition 6.03, Cary, North carolina, USA

Veldboom LR, Lee M (1996) Genetic mapping of quantitative trait loci in maize in stress and nonstress environments. I. Grain yield and yield components. Crop Sci 36: 1310–1319

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407–4414