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Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits

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ORIGINAL PAPER

Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits

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Abstract A recombinant inbred line (RIL) population was evaluated in seven field experiments representing four environments: water stress at flowering (WS) and well-watered (WW) conditions in Mexico and Zimbabwe. The QTLs were identified for each trait in each individual experiment (single-experiment analysis) as well as per environment, per water regime across locations and across all experiments (joint analyses). For the six target traits (male flowering, anthesis-to-silking interval, grain yield, kernel number, 100-kernel fresh weight and plant height) 81, 57, 51 and 34 QTLs were identified in the four stepwise analyses, respectively. Despite high values of heritability, the phenotypic variance explained by QTLs was reduced, indicating epistatic interactions. About 80, 60 and 6% of the QTLs did not present significant QTL-by-environment interactions (QTL × E) in the joint analyses per environment, per water regime and across all experiments. The expression of QTLs was quite stable across years at a given location and across locations under the same water regime. However, the stability of QTLs decreased drastically when data were combined across water regimes, reflecting a different genetic basis of the target traits in the drought and well-watered trials. Several clusters of QTLs for different traits were identified by the joint analyses of the WW (chromosomes 1 and 8) and WS (chromosomes 1, 3 and 5) treatments and across water regimes (chromosome 1). Those regions are clear targets for future marker-assisted breeding, and our results confirm that the best approach to breeding for drought tolerance includes selection under water stress.

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Introduction

There is evidence that global mean temperatures are increasing and the climate is becoming increasingly erratic, with increased drought in some areas and more and stronger storms (IPCC 2007). The future challenges of crop production in the tropics, especially in certain arid and semi-arid areas of Africa, will be related to higher temperatures and less rainfall (Sivakumar et al. 2005). Drought and heat stress often occur simultaneously in the field, which affects crops more severely than drought or heat stress alone (Mittler 2006). Crop improvement cannot mitigate all the economic losses under water-limited conditions but will probably play a key role in maintaining and increasing cereal production in drought-prone areas (Heisey and Morris 2006). In order to gain the knowledge required to improve the drought tolerance of crops, it is important to perform molecular studies under actual field conditions.

Water stress at flowering, when pollination, fertilization and grain initiation take place, has a stronger negative effect on cereal production than at other developmental stages (Salter and Goode 1967; Saini and Westgate 2000). Maize in particular is highly susceptible to water stress at flowering (Claassen and Shaw 1970; Westgate and Boyer 1985), because it is an open pollinating crop, the male and female flowers of which are spatially separated on the plant. Extensive research into the tolerance of maize to drought stress at flowering identified key secondary traits of grain yield, such as the anthesis-to-silking interval (ASI), improved ear fertility, stay-green and, to a lesser extent, leaf rolling (Bänziger et al. 2000; Bruce et al. 2002). Drought stress limits photosynthesis and reduces the flux of assimilates to the developing ears (Schussler and Westgate 1995; Zinselmeier et al. 1995), slowing down ear and silk growth and delaying silk emergence. Since tassel growth is less affected by drought than ear growth, the characteristic widening of the ASI is observed under water-limited conditions (Heisey and Edmeades 1999; Bänziger et al. 2000). As a consequence of the time lag between pollen release and silk emergence, pollination and kernel set are affected. Pollen viability and silk receptivity can also be reduced (Saini and Westgate 2000). Conventional selection for grain yield and secondary traits considerably improved the tolerance of maize to water-limited conditions (Campos et al. 2004; Monneveux et al. 2006; Ribaut et al. 2008), but remains a slow and challenging task.

The molecular-marker techniques evolved fast during the past two decades. As a result, almost 1,000 QTL studies of Arabidopsis, soybean, rice, sorghum, maize, barley and wheat were published from 2000 to 2004 (Salvi and Tuberosa 2005). About 2,200 QTLs for maize (as of December 2008) are deposited in the Maize Genetics and Genomics Database (MGDB, Lawrence et al. 2008). Although these data probably cover only a small part of the information about QTLs, they illustrate the efforts made to identify associations between the phenotype and the corresponding marker genotype in segregating populations. Nevertheless, it will take some time to identify the genes underlying quantitative traits, especially when the QTL × E interaction is significant, as it is often the case for drought tolerance.

The interactions of QTLs with the environment (Beavis and Keim 1996), the lack of stable QTLs for grain yield (Moreau et al. 2004), the sensitivity of the QTLs to the genetic background (Campos et al. 2004) and the low heritability of yield components as well as the complex interactions among genes involved in drought tolerance (Ribaut et al. 2008) are, at present, the main constraints of marker-assisted breeding (MAB) under water-limited conditions. Current ways of estimating genotypic effects are no longer purely biometrical but include various assays of variations in the DNA sequence (Cooper et al. 2006). In particular, the genotype-by-environment interaction (G × E) has been broken down into its constituent QTL × E interactions. This allowed for the development of models, by which characteristics of a complex phenotype, expressed in a stressed environment, are described in relation to molecular mechanisms. Factorial regression and mixed QTL models are particularly useful for this type of analysis, especially when the phenotypic data are derived from multi-environment experiments (including both stressed and non-stressed conditions) (Malosetti et al. 2004; Vargas et al. 2006). Multi-trait multi-environment QTL models serve to define the genomic regions associated with genetic correlations and to determine whether they are the outcome of pleiotropy or genetic linkage. Moreover, they can illustrate the dependence of genetic correlations on environmental conditions. Based on multi-trait multi-environment data, several QTLs for the adaptation of tropical maize to drought stress have been identified (Malosetti et al. 2008). A better understanding of the contribution of the G × E and QTL × E components to the phenotypic variance may lead to a breakthrough in breeding under drought conditions, as QTL stability across environments plays a crucial role in developing a successful MAB strategy. A multidisciplinary approach, combining phenotypic selection and molecular markers to pyramid favourable alleles at key regulatory loci, represents the most efficient strategy for breeding maize adapted to marginal environments (Ribaut and Ragot 2007).

The overall objective of this study was to identify the genomic segments responsible for the expression of drought-related traits in a segregating maize population under different water regimes at different locations and to better understand the stability of QTLs and their interactions across environments. The results of this study will contribute to the development of the most efficient approach to breeding for drought tolerance.

Materials and methods

Plant material

The two subtropical white dent maize lines CML444 and SC-Malawi were crossed, and a segregating population of 236 recombinant inbred lines (RIL, F_7:S_6) was developed by single-seed descent. CML444 was developed from CIMMYT's Population 43 by nine cycles of recurrent selection in the 1990s. It has a compact phenotype with strong, erectophile, dark green leaves and is considered to be very tolerant to water-limited conditions at flowering, as shown by its higher yield under drought stress. SC-Malawi was developed in Zimbabwe (formerly Rhodesia) in the

1960s. It is light green in colour, has long, horizontal leaves, short internodes at higher positions on the stem and a relatively low yield under stress.

Experimental evaluation

The seven field experiments in 2003 and 2004 correspond to four environments: water-stress conditions in Mexico (WSM, two experiments) and Zimbabwe (WSZ, two experiments) and well-watered conditions in Mexico (WWM, two experiments) and Zimbabwe (WWZ, one experiment). The experiments in Mexico were conducted in Tlaltizapán (18°N, 99°W, 940 m a.s.l.); the stress experiments in Zimbabwe were conducted in Chiredzi (21°S, 31°E, 392 m a.s.l.), the well-watered experiment near Harare (17°S, 31°E, 1,468 m a.s.l.). The soil in Tlaltizapán is classified as a Vertisol, that at Chiredzi and Harare as an Alfisol (USDA taxonomy).

All the experiments were designed as alpha (0, 1) lattices with one-row plots and two replications. The rows were 0.75 m apart and 2.5 (WSM and WWM), 3 (WSZ) or 4 m (WWZ) long. The plant density was 6.4 m^{-1} in Mexico and 5.3 m^{-1} in Zimbabwe. Fertilizers, insecticides and herbicides were applied as required and in accordance with local practices. All the fields were sprinkler-irrigated twice after sowing. The water-stress experiments in Mexico were then furrow-irrigated at 10-day intervals until 3 weeks before the expected average date of anthesis. The plants were not irrigated until the end of flowering, but two further furrow irrigations were carried out during the grain-filling period to ensure adequate development of the fertilized ovaries. The drought-stress experiments in Zimbabwe were irrigated with sprinklers once a week for 7 weeks. There was no further irrigation during the rest of the growing cycle. Different types of management were necessary in the WSM and WSZ environments, depending on the capacity of the soil to retain water. The well-watered experiments at both locations were irrigated when there was insufficient rainfall.

To evaluate the level of water stress, the predawn leaf water potential of CML444 and SC-Malawi was measured under water stress in Mexico in 2005, when approximately 50% of the RILs had reached flowering. The experiment was conducted at the same site, using a similar irrigation management as in the WSM experiments. The second leaf from the tassel of at least 45 plants per genotype was cut, put in sealed plastic bags and processed immediately. The leaf water potential was measured with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) in which the pressure was slowly increased until a drop of leaf sap appeared in the middle vein of the leaf.

The time of male flowering (MFLW), i.e. the number of days from sowing to pollen release (anthesis), was recorded either as the average value of ten plants per plot, the MFLW of which was determined individually (Mexico), or as the number of days from sowing to the day on which 50% of the plants per plot had extruded anthers (Zimbabwe). The anthesis-to-silking interval (ASI) (d) was calculated according to Ribaut et al. (1996) for the experiments in Mexico and as the plot-wise difference between MFLW and the day on which the first silks were visible on 50% of the plants per plot in Zimbabwe. The mature ears were harvested manually, bagged, air-dried and shelled using an electric shelling device. The total grain yield of each plot was weighed on electronic scales. The grain yield (GY) (g m^{-2}) was calculated by dividing the total grain weight per plot by the area of the plot without taking into account location-specific changes in plant density. For plots that produced a sufficient number of kernels, the fresh weight of 100 kernels (HKFW) (g) was determined. The number of kernels per square meter (KNO) was calculated as 100 × GY/HKFW. Plant height (PHT) (cm) was recorded as the average height of five plants per plot, i.e. the distance from the soil surface to the first tassel branch.

It was impossible to fully standardize the water management and measurements, and because of this some environmental effects are confounded with the effects of local protocols.

Data analysis

The plot raw data of each experiment were adjusted for local and global variation in ASReml (Gilmour et al. 2002). Replications were considered fixed; incomplete blocks and genotypes were random factors. MFLW was included as a covariate in the statistical model of the spatial analysis of ASI in one of the two experiments in WSM due to the strong and highly significant correlation between both traits. The phenotypic correlation coefficients (Pearson's) and significance levels were determined by linear regressions in R (R Development Core Team 2007) based on adjusted and standardized (0 mean, 1 standard deviation) phenotypic data. The genetic correlations among traits corresponded to the ratio between the genetic covariance for each pair of traits and the product of the respective standard deviations. These variance components were estimated for the standardized (0, 1) plot raw data in a linear mixed model (ProcMixed) in SAS (The SAS Institute, Cary, NC, USA) with experiments, replications, incomplete blocks, genotypes and the experiment:genotype interactions as random factors. The heritability of traits (h^2) was also calculated on an plot basis as the ratio between the genetic variance and the sum of the genetic variance, the variance of the experiment:genotype interaction divided by the number of

experiments and the variance of the residuals divided by twice the number of experiments.

Construction of linkage map and QTL identification

The linkage map was constructed with 160 publicly available RFLP (79) and SSR (81) markers, tested primarily for polymorphism between the parental lines, using the Mapmaker v3.0 software (Lander et al. 1987) and the Haldane's mapping function (Haldane 1919) to transform the recombination frequencies into centiMorgans (cM).

QTLs were identified for the adjusted data by composite interval mapping (Zeng 1994; Jiang and Zeng 1995), with the QTLMMAP software (CIMMYT) as follows: (1) for each individual experiment (single-experiment QTLs), (2) for each of the four environments ("joint QTLs" combining data of two experiments, except for WWZ), (3) for both treatments ("joint QTLs" combining data of three WW or four WS experiments) and (4) across all experiments ("joint QTLs" combining data of seven experiments). The co-factors, defined as the markers closest to the peaks in the LOD profile above the significance threshold, were identified by precursory simple and composite interval mapping with a window size larger than the longest chromosome. The size of the genetic window was then reduced to 30 cM. A QTL was considered to be significant (comparison-wise Type-I error rate $\alpha_c = 0.001$, experiment-wise error rate $\alpha_e = 0.02$) when the LOD exceeded the appropriate threshold: 3.0 (single-experiment QTL), 3.53 (joint QTL, two experiments), 4.01 (joint QTL, three experiments), 4.45 (joint QTL, four experiments) or 5.67 (joint QTL, seven experiments). A joint QTL was considered to be stable ($\alpha_i = 0.01$) when the LOD of the QTL × E interaction at the QTL position was below 2.0 (two experiments), 2.46 (three experiments) 2.88 (four experiments) or 4.01 (seven experiments). The support interval of a QTL was defined as the segment of the chromosome, in which the LOD at the peak decreased by half.

The presence of binary epistatic interactions between pairs of QTLs identified by the single-experiment mapping procedure was tested with linear models. The allelic information of marker genotypes close to significant QTL peaks was transformed to numeric regressors with values 1 (CML444 allele), −1 (SC-Malawi allele), or 0 (both alleles). Additive-by-additive epistatic regressors were calculated by multiplying the marker regressors in pairs. For each of the phenotypic traits (the response), $0.5 \times n \times (n - 1)$ linear models were fitted including the n regressors of the markers closest to the significant single-experiment QTLs, which accounted for the additive main effects of these QTLs, and one of the corresponding epistatic regressors. The binary additive-by-additive epistatic interactions were considered significant when the P value of the t statistic for the epistatic regressor was below 0.05.

Results

Environments

Drought stress was not alleviated by unexpected rainfall in any of the experiments under water-limited conditions. The lower average minimum temperature (5–9°C) before flowering in the drought cycles, in combination with other climatic factors such as photoperiod, irradiation and drought stress, delayed anthesis by an average of 40 days (Table 1). Moreover, the maximum temperature at flowering was higher in the WS than in the WW experiments (data not shown).

The average water potential of the second leaf from the tassel of the parental lines measured under stress was −190 kPa. The differences between the two genotypes were highly significant ($P < 0.001$); the leaf water potential of CML444 (−260 kPa) was considerably lower than that of SC-Malawi (−110 kPa).

Phenotypic results

Table 1 lists the average phenotypic data per environment and the heritability of the six target traits. SC-Malawi reached anthesis earlier than CML444, especially under stress conditions in Zimbabwe. Nevertheless, the segregation of the entire population was within 10 (WSM) to 15 (WSZ) days, which more or less corresponded to the situation in WWM, where both lines had a similar MFLW. The heritability of MFLW was high, except in WWZ where the segregation of the RIL was very narrow.

The average ASI was larger under WS than under WW conditions at both locations; this was expected, as a large ASI indicates susceptibility to drought. Independent of the water regime, the ASI was notably shorter in Zimbabwe than in Mexico. Both the segregation of the RIL and the difference between the ASI of CML444 and SC-Malawi were also smaller. This is probably the result not only of how ASI was calculated at both locations, but also of the different level of adaptability of the parental lines. The heritability of ASI in WWZ was close to zero, mainly because of the lack of segregation for that trait.

The average GY varied considerably across environments. GY was 80% lower under stress conditions in Mexico (WSM) compared to the highest-yielding environment (WWZ). The surprisingly low GY in WWM was due to a reduction in yield of 50% compared to WWZ in one of the two experiments in Mexico, during which a thunderstorm caused extensive root logging shortly after

Table 1 Average, minimum and maximum values for the parental lines and the RILs for days to anthesis (MFLW), anthesis-to-silking interval (ASI) (d), grain yield (GY) (g m^{-2}), kernel number (KNO) (m^{-2}), fresh weight of hundred kernels (HKFW) (g) and plant height (PHT) (cm) in four environments under water stress (WS) or well-watered conditions (WW) in Mexico (M) and Zimbabwe (Z)

		Parental lines		RILs			h^2		
Trait	Env	CML444	SC-Malawi	Mean	Min	Max	Env	Treat	All
MFLW	WSM	104.2	101.1	101.1	96.5	106.2	0.74	0.85	0.87
	WSZ	121.1	114.1	117.3	110.2	124.9	0.85		
	WWM	65.4	64.5	64.1	59.6	70.8	0.76	0.68	
	WWZ	75.6	74.9	75.5	73.2	79.8	0.24		
ASI	WSM	5.1	9.8	7.5	2.2	12.0	0.68	0.75	0.79
	WSZ	2.2	3.8	2.8	0.8	6.3	0.66		
	WWM	−0.4	4.8	1.7	−1.3	7.5	0.69	0.52	
	WWZ	0.9	1.1	1.0	0.4	1.6	0.09		
GY	WSM	37.2	15.0	39.4	9.4	161.2	0.57	0.70	0.65
	WSZ	117.1	79.9	103.6	35.7	254.1	0.63		
	WWM	193.2	50.4	120.2	30.2	291.0	0.60	0.31	
	WWZ	323.2	155.1	200.4	69.3	460.6	0.61		
KNO	WSM	148	61	164	34	624	0.62	0.74	0.67
	WSZ	420	305	384	134	892	0.66		
	WWM	893	211	538	157	1355	0.59	0.35	
	WWZ	1322	766	871	381	1606	0.52		
HKFW	WSM	22.5	21.5	21.9	18.9	25.8	0.64	0.76	0.81
	WSZ	24.5	24.1	25.3	19.5	32.5	0.71		
	WWM	21.9	20.0	22.1	15.0	30.6	0.64	0.67	
	WWZ	23.5	21.3	23.0	16.9	32.2	0.56		
PHT	WSM	128.3	158.2	149.2	107.6	192.9	0.87	0.84	0.90
	WSZ	126.6	135.6	133.6	117.5	155.5	0.65		
	WWM	167.9	172.6	167.7	116.7	213.9	0.84	0.79	
	WWZ	162.0	166.0	164.5	132.0	208.0	0.51		

Trait heritability was calculated for each environment as well as per water regime and across all environments

flowering. The average GY in the other WWM experiment was actually only 8% lower than in WWZ. For both WS and WW conditions, the average GY was higher in Zimbabwe than in Mexico, because the parental lines of the RIL population are better adapted to this location. The elite line CML444 performed about twice and four times as well as SC-Malawi in WWZ and WWM, respectively. The regression lines in Fig. 1 show that CML444 has a very high potential for yield but reacts strongly to drought and the percentage of reduction in yield is large when the overall yield level is low. Growing conditions that reduced the average GY of the population by 10% reduced the GY of CML444 by 17%, whereas the GY of SC-Malawi was reduced by only 8%. The heritability of GY was close to 0.6 in all four environments. However, the much lower h^2 when the data of the WW environments were combined indicated strong interactions between genotypes and locations. The results of KNO are analogous to those of GY, which is a consequence of the strong and highly significant phenotypic and genetic correlations between both traits in all environments (Table 2).

The segregation of the population for HKFW was relatively consistent across environments. Since the HKFW of both parental lines was almost the same in all environments, the differences in GY were principally due to differences in KNO, not to differences in kernel size. The heritability of HKFW, calculated across all seven experiments, was higher than the respective heritability of GY and KNO.

Plant height was lower under stress. The lower average PHT in WSZ compared to WSM might be due to the fact that irrigation was stopped earlier in Zimbabwe, and drought stress was applied before the plants were fully developed. However, even under WW conditions, the PHT values tended to be higher in Mexico than in Zimbabwe, which does not correlate with the large decrease in GY in Mexico. Therefore, the regulation of carbon partitioning within the plants clearly differs between the two locations.

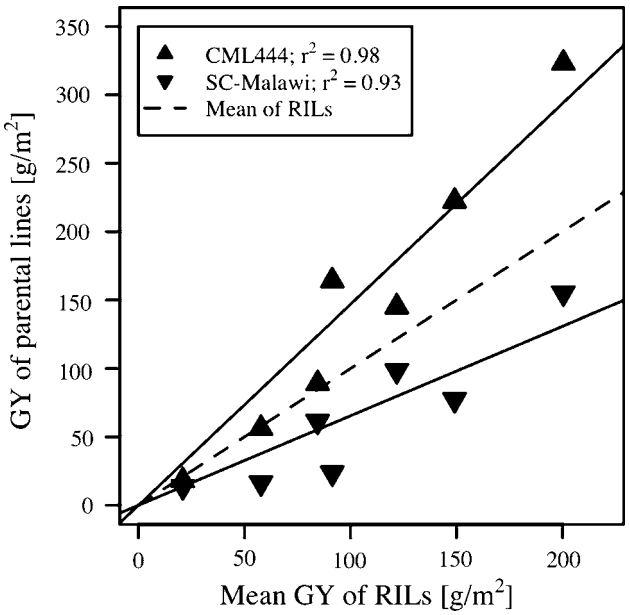

Fig. 1 Linear regression of average grain yield (GY) per experiment for the parental lines on the corresponding average GY of the RIL population

CML444 reached the same height in both stress environments, whereas SC-Malawi was more than 20 cm taller in WSM than in WSZ. Moreover, the segregation of the RIL population for PHT was larger and the heritability higher in Mexico than in Zimbabwe, irrespective of the water management. Together with the location-specific responses of ASI, GY, KNO and HKFW, these results are proof of large genotype-by-location interactions.

Correlations

The weak phenotypic correlations between MFLW and ASI (Table 2) indicated that a large ASI under water stress was due to delayed silking rather than to differences in precocity. Nevertheless, the genetic correlation in WSM ($r_g = 0.54$) and WWZ ($r_g = 0.57$) suggested that MFLW and ASI were regulated by common genes under certain environmental conditions.

According to the negative phenotypic and genetic correlations between MFLW and GY and between MFLW and KNO under WS conditions (Table 2), late anthesis was unfavourable for kernel set and grain yield when water was limited. The negative genetic correlations between ASI and GY were highly significant in all four environments (Table 2), with an extremely high value for WSM, where the average ASI was largest and the average GY lowest (Table 1). Beyond the negative phenotypic and genetic correlations in each environment, the results of the present study are exceptional insofar as a non-linear relationship between ASI and GY, and a very large variation in both traits was observed in a bi-parental segregating population at three locations on two continents with very different climates (Fig. 2). The relation between ASI and GY (Fig. 2) also shows considerable differences between experiments within locations, especially in WSM. This is due to different stress intensities in the two experiments despite comparable stress managements. The two WSM experiments were conducted in subsequent years, one of which was extraordinarily dry at the time of flowering because of a constant warm and dry wind. Thus, Fig. 2 demonstrates indirectly the challenge of breeding for drought tolerance under field conditions.

The correlations confirmed that variations in GY among genotypes resulted primarily from differences in KNO, not HKFW, because the correlations between GY and KNO were much stronger than those between GY and HKFW (Table 2). At the same time, the genetic and phenotypic correlations between PHT and GY and between PHT and HKFW (with the exception of the genetic correlation in WSZ) were higher under WW than under WS conditions. Tall plants apparently had a greater capacity for grain filling than short plants, probably because of a larger photosynthetically active leaf area and more stem reserves.

Linkage map

The genetic linkage map was constructed with 160 publicly available markers (81 SSRs and 79 RFLPs). It was 2105.6 cM long and the average marker distance was 12.2 cM. The longest interval (58.9 cM) was located on chromosome 3 between markers umc1307 and bnl10.24a. Most of the markers (146) were co-dominant. The percentage of heterozygous bands per co-dominant marker ranged from 0 to 8.5%, with an average of 4.2%. This is somewhat higher than the expected $(0.5)^5 \approx 3.1\%$ of heterozygous bands for a co-dominant locus after five generations of inbreeding (from the F_2), assuming the simplified single-locus model and with a constant generation transition probability (Liu 1998, p. 567). Only 1.4% of the allelic information was missing. The linkage map will be deposited in the Maize Genetics and Genomics Database (http://www.maizegdb.org) together with the phenotypic and QTL data.

QTL results

The single-experiment mapping revealed 81 significant QTLs for all six traits (Table 3). Overall, a larger number of QTLs were detected for the secondary traits than for yield components. Chromosomes 1, 8 and 3 presented the largest number of QTLs in both water regimes (Fig. 3); most of the QTLs on chromosome 3 were detected under stress conditions. Two genomic regions on chromosomes 1 (at about

Table 2 Genetic (*right*) and phenotypic (*left*) correlations among traits in the RIL population grown under water stress (WS) or well-watered (WW) conditions in Mexico (M) (*upper value*) and Zimbabwe (Z) (*lower value*)

Env.	Trait	MFLW	ASI	GY	KNO	HKFW	PHT
WSM	MFLW	1	0.54***	−0.71***	−0.70***	0.04NS	0.11NS
WSZ			0.19**	−0.46***	−0.50***	0.07NS	0.17**
	ASI	0.24***	1	−0.99***	−0.97***	−0.18*	0.04NS
		0.13*		−0.47***	−0.52***	0.18**	0.55***
	GY	−0.50***	−0.45***	1	0.99***	0.32***	−0.08NS
		−0.38***	−0.34***		0.91***	0.23***	0.23***
	KNO	−0.51***	−0.47***	0.98***	1	0.06NS	−0.10NS
		−0.42***	−0.37***	0.91***		−0.20**	0.07NS
	HFKW	0.01NS	−0.06NS	0.19***	0.06NS	1	0.09NS
		0.08NS	0.04NS	0.16***	−0.04NS		0.57***
	PHT	−0.03NS	−0.02NS	0.04NS	0.01NS	0.14**	1
		−0.01NS	0.06NS	0.23***	0.14**	0.13**	
WWM	MFLW	1	0.23***	−0.21***	−0.26***	0.24***	0.32***
WWZ			0.57***	−0.15*	−0.13*	−0.09NS	0.07NS
	ASI	0.21***	1	−0.64***	−0.65***	0.00NS	−0.11NS
		0.00NS		−0.51***	−0.49***	−0.15*	0.07NS
	GY	−0.27***	−0.48***	1	0.87***	0.22***	0.46***
		−0.14*	−0.19**		0.93***	0.47***	0.51***
	KNO	−0.28***	−0.47***	0.92***	1	−0.17**	0.28***
		−0.12NS	−0.17**	0.93***		0.13*	0.43***
	HFKW	0.08NS	−0.06NS	0.29***	−0.04NS	1	0.54***
		−0.05NS	−0.04NS	0.38***	0.05NS		0.34***
	PHT	0.21***	−0.17***	0.37***	0.26***	0.41***	1
		0.04NS	−0.01NS	0.41***	0.35***	0.24***	

Correlation coefficients were calculated for combinations of two experiments per environment, except for WWZ (one experiment), and were significant at $P < 0.05$ (*), 0.01 (**) and 0.001 (***) or not significant (NS). Abbreviations of traits are given in Table 1

135 cM, bin 1.04–05) and 8 (at about 130 cM, bin 8.06) accumulated several significant QTLs for different traits (Fig. 3). Although the profile of the LODs can differ considerably under WW and WS conditions, the total number of single-experiment QTLs detected in the two water regimes was similar in Mexico and in Zimbabwe (Fig. 3; Table 3). However, the nature of the identified QTLs differed considerably. In Zimbabwe, for instance, QTLs for secondary traits were mainly detected under WS, while QTLs for yield components were mainly identified under WW conditions. About twice as many single-experiment QTLs were detected in Mexico as in Zimbabwe. The lower number of QTLs detected in Zimbabwe under WW conditions is related, at least in parts, to the smaller segregation of the population and the lower heritability of most of the target traits in that environment (Table 1). For example, there was no QTL identified for MFLW in WWZ, and the heritability of this trait was quite low.

The QTL joint analyses including data from two trials in each environment revealed 50 QTLs for three environments (WWZ had only one trial). A larger number of joint QTLs were identified for secondary traits and HKFW, while for KNO and particularly for GY only a few QTLs were detected. Similar to the analysis of single-experiment QTLs, the number of joint QTLs per environment was larger in Mexico than in Zimbabwe, particularly under WW conditions (Table 3). That is why more than 60% of the treatment-specific joint QTLs were detected under WW conditions.

Of the 50 QTLs revealed by the joint analysis per environment, 40 did not show significant QTL × E interactions. Hence, the genetic effects were mostly stable over years in a single environment. Overall, CML444 contributed to a delay in male flowering and a reduction of plant height at most loci (Table 4). CML444 also contributed to a higher HKFW at all loci detected under WS but only at half the loci under WW conditions. It is interesting that the inverse was found for KNO. For both GY and ASI the favourable allelic contribution at significant loci was from both parental lines.

The joint analysis of the four WS and the three WW trials revealed 19 and 32 QTLs, respectively (Table 3).

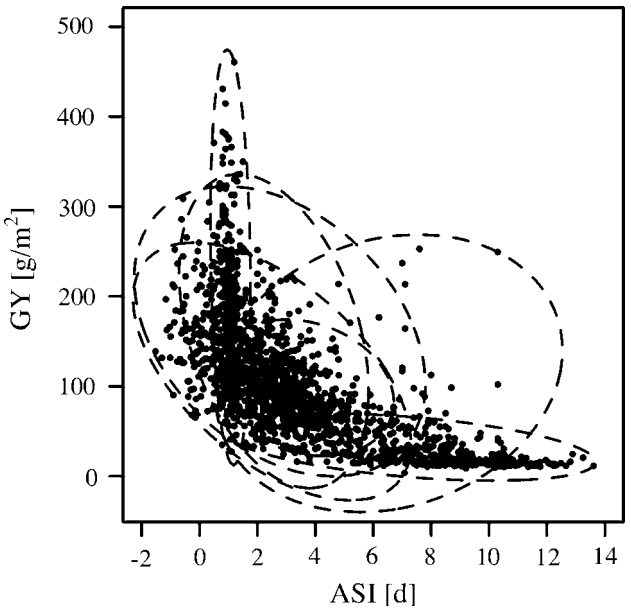

Fig. 2 Relationship between the anthesis-to-silking interval (ASI) and grain yield (GY) of the RIL population in seven field experiments. The ellipses contain all data points of individual experiments

Half the QTLs under WS and two-thirds of the QTLs under WW conditions did not show significant QTL × E interactions. Only one of the six detected QTLs for GY was stable, whereas several stable QTLs were detected for the yield components under WW and even under WS conditions. For ASI, a typical secondary trait for drought tolerance, four of five QTLs under WS were stable. In contrast, all three QTLs identified for ASI under WW showed significant QTL × E interactions.

Running a joint analysis of all seven trials revealed 34 QTLs, most of which showed a significant QTL × E interaction. Only two QTLs were stable across all trials (Table 3): one QTL for MFLW in bin 4.09 and one for HKFW in bin 7.04. The joint analysis of all the trials revealed genomic regions, which were not detected in the analysis of individual trials. This was the case for the genomic region on chromosome 7 (bin 7.04), controlling GY, KNO and HKFW (Fig. 4c). Joint mapping per treatment revealed a weaker but significant effect for GY and KNO at the same position (Fig. 4b), but neither joint mapping per environment (Fig. 4a), nor singe-trait mapping (Fig. 3) revealed a significant effect at this position,

Table 3 Number of QTLs detected by four different mapping procedures

	MFLW	ASI	GY	KNO	HKFW	PHT	Total
Single-experiment QTLs							
Total (7 exp.)	18	14	6	9	13	21	81
WSM (av.)	4	2	1.5	2.5	1	4	15
WSZ (av.)	2.5	2.5	0	0	2	1	8
WWM (av.)	2.5	2	0.5	1	3	5	14
WWZ	0	1	2	2	1	1	7
WS (av.)	3.3	2.3	0.8	1.3	1.5	2.5	11.5
WW (av.)	1.7	1.7	1	1.3	2.3	3.7	11.7
Joint QTLs per environment							
Total (4 env.)	12 (10/12)	8 (7/7)	5 (2/3)	7 (3/5)	11 (8/10)	14 (10/13)	57 (40/50)
WSM	5 (4)	2 (2)	2 (2)	2 (1)	1 (1)	4 (3)	16 (13)
WSZ	3 (2)	1 (1)	0 (−)	2 (1)	3 (2)	1 (1)	10 (7)
WWM	4 (4)	4 (4)	1 (0)	1 (1)	6 (5)	8 (6)	24 (20)
WWZ (1 exp.)	0 (−)	1 (−)	2 (−)	2 (−)	1 (−)	1 (−)	7 (−)
WS (av.)	4	1.5	1	2	2	2.5	13
WW (av.)	2	2.5	1.5	1.5	3.5	4.5	15.5
Joint QTLs per treatment							
Total (2 treat.)	7 (5)	8 (4)	6 (1)	7 (5)	9 (5)	14 (10)	51 (30)
WS	3 (3)	5 (4)	4 (0)	2 (1)	2 (1)	3 (1)	19 (10)
WW	4 (2)	3 (0)	2 (1)	5 (4)	7 (4)	11 (9)	32 (20)
Joint QTLs, all exp.							
Total	4 (1)	4 (0)	6 (0)	5 (0)	8 (1)	7 (0)	34 (2)

Single values in parentheses show the number of stable QTLs with non-significant QTL × E interaction. Ratios in parentheses represent the number of stable joint QTLs over the total number of QTLs detected in WSM, WSZ and WWM (no information on QTL × E in WWZ). Abbreviations of traits are given in Table 1

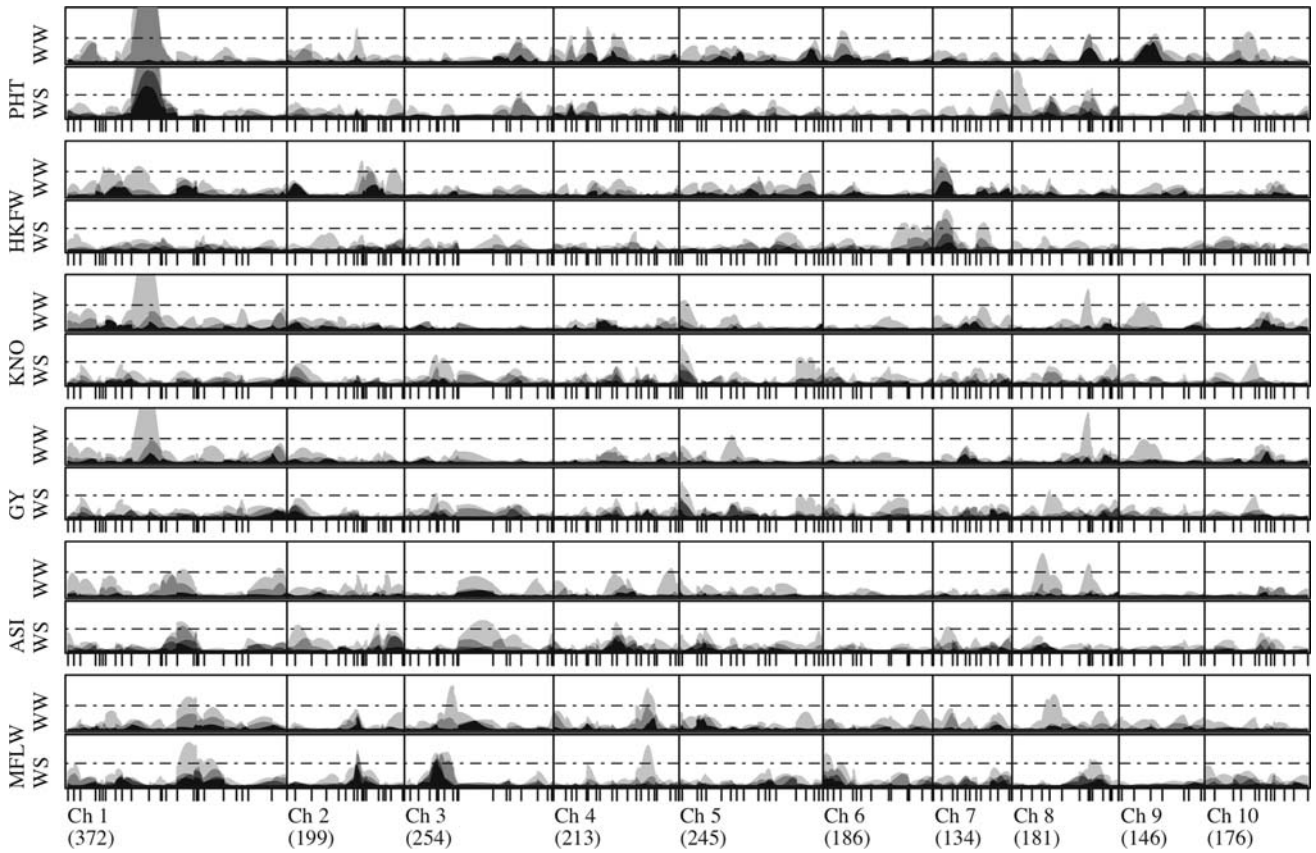

Fig. 3 LOD profiles ($0 < \text{LOD} \leq 6.5$) along the genome resulting from the single QTL analyses per experiment. For each trait, profiles of four water-stress (WS) and three well-watered (WW) experiments are plotted separately. The shading increases as overlap increases. The *dashed-dotted lines* indicate the significance threshold (LOD = 3.0). The length (in cM) of each chromosome is given in parentheses. Abbreviations of traits are given in Table 1

with the exception of HKFW in one stress experiment. Several regions were consistently identified by joint mapping in the different sequential analyses. This is the case of the QTL for HKFW on chromosome 7 (bin 7.01–02, Fig. 4c), which was identified in the single-experiment analysis (Fig. 3), in the joint analysis per environment (Fig. 4a; Table 4) and in the joint analysis per treatment (Fig. 4b). Finally, some QTLs identified by the joint analysis of all the trials resulted mainly from large genetic effects in one environment. This is the case for the genetic region on chromosome 8 (bin 8.06) controlling ASI, GY, KNO and PHT. Figure 4a clearly shows that the overall effect of the QTL was due mainly to the WWM environment, with all the favourable alleles coming from CML444. The QTL analysis across the seven trials revealed six genomic regions involved in the expression of two or more traits (Fig. 4c).

The different analyses revealed several clusters of QTLs for different traits, suggesting a common genetic control of the traits through close linkage or pleiotropy. Besides the clusters of QTLs on chromosomes 7 (bin 7.04) and 8 (bin 8.06) mentioned above, clusters with a large number of QTLs were detected on chromosomes 1 (two regions, bins 1.04 and 1.07–08) and 3 (bin 3.04–05). All the clusters of QTLs, with the exception of that in bin 1.07–08, and two additional loci on chromosome 5 showed linked QTLs for GY and KNO (bins 1.04 and 8.06 under WW, bins 3.04, 5.01 and 5.07 under WS, bin 7.04 under both WW and WS), as expected from the high correlation between these two traits. The favourable alleles for GY and KNO came from CML444 at all loci detected under WW, according to the high-yield potential of this line, but only at one locus (bin 5.01) under WS. The first cluster on chromosome 1 comprised highly significant QTLs for GY and KNO in the highest-yielding environment WWZ, QTLs for PHT in the other three environments and a QTL for HKFW in WWM (Fig. 4a; Table 4). The mutually exclusive expression of the QTLs for GY and PHT in each environment suggests the presence of one gene or a few major genes regulating carbon-partitioning mechanisms and the use of assimilates for either vegetative or reproductive growth. The QTLs for PHT and HKFW were stable within each environment but showed significant QTL × E interactions as soon as data from WWZ was included in the analysis (Fig. 4b, c). The

Table 4 Genetic characteristics of QTLs identified by joint mapping per environment combining data from two experiments, with the exception of WWZ (one experiment)

				Distance (cM)		LOD				Add	R^2 (%)	
Trait	Env	Bin	Mark	Peak	Interval	E1	E2	Joint	QTL × E	Joint	E1	E2
MFLW	WSM	1.08	umc128	218	193–230	4.8	3.0	6.1	0.4	0.55	9.7	2.7
		2.06	csu54a	120	105–127	2.3	3.7	4.6	0.0	−0.52	1.6	3.7
		3.04	bnlg1019a	72	49–83	4.0	2.7	5.2	0.3	0.57	8.9	7.7
		4.09	csu11b	162	144–172	1.1	3.9	4.1	0.5	0.43	1.4	9.1
		10.02	npi285a	12	2–36	0.5	2.7	4.1	3.6	0.19	2.6	1.1
	WSZ	2.06	csu54a	120	115–125	2.5	4.4	4.5	0.4	−0.70	1.6	4.4
		3.04	umc154	54	43–64	4.0	4.4	5.1	0.0	0.77	10.8	9.1
		6.03	umc1887	52	19–56	1.9	0.0	3.7	3.3	0.20	0.8	0.7
	WWM	1.08	umc128	217	186–222	4.4	1.5	4.5	0.3	0.50	5.8	2.9
		3.05	phi053	81	69–89	3.6	2.0	3.9	0.0	0.50	6.3	4.7
		4.09	csu11b	161	151–173	5.1	2.4	5.3	0.1	0.56	9.6	4.9
		8.03	bnlg669	62	53–81	3.8	0.8	3.8	0.5	−0.49	8.8	3.6
ASI	WSM	1.07	umc1122	192	169–212	2.1	5.0	5.5	0.1	0.83	1.8	3.5
		4.07	umc19	109	100–122	3.0	3.6	4.7	0.1	−0.81	5.0	5.0
	WSZ	1.07	umc1122	186	168–213	3.8	1.5	4.0	0.3	0.21	4.9	2.2
	WWM	1.02	bnlg1627	66	55–82	3.7	0.6	3.7	1.4	−0.28	5.2	0.1
		1.07	umc1128	200	167–215	2.4	4.0	4.4	0.2	0.40	1.8	4.1
		8.02	umc103a	51	40–62	5.6	1.8	5.8	1.3	−0.39	10.3	3.9
		8.06	umc48a	131	120–140	1.1	4.7	4.7	0.9	−0.36	2.8	7.2
	WWZ	4.10	bnlg1337	200	183–208	3.4	NA	NA	NA	0.06	6.2	NA
GY	WSM	5.01	npi409	5	0–14	2.9	2.7	4.1	1.9	2.32	8.2	6.4
		5.07	bnlg1346	205	200–230	3.8	0.3	3.8	0.0	−3.38	5.7	0.3
	WWM	8.06	umc48a	130	121–133	0.9	6.1	6.1	2.0	10.93	1.9	10.2
	WWZ	1.04	bnlg2086	138	117–151	10.0	NA	NA	NA	31.06	15.6	NA
		5.03	umc166a	90	78–130	3.3	NA	NA	NA	−0.58	5.7	NA
KNO	WSM	5.01	npi409	5	1–19	3.1	3.3	4.6	2.1	13.06	7.8	6.8
		5.07	bnlg1346	205	199–231	4.7	0.7	4.7	0.1	−19.41	7.2	0.6
	WSZ	1.10	umc106a	293	270–303	2.0	0.2	3.6	3.5	1.66	5.0	0.1
		2.02	bnlg1297	19	5–42	3.8	2.1	4.1	1.5	−26.03	8.3	4.8
	WWM	8.06	umc48a	130	121–134	0.7	4.9	4.9	0.6	49.00	1.1	7.6
	WWZ	1.04	bnlg2086	135	115–155	11.4	NA	NA	NA	123.10	17.9	NA
		9.02	umc105a	41	24–65	3.2	NA	NA	NA	72.13	4.4	NA
HKFW	WSM	7.02	bnlg1094	24	4–35	2.2	4.9	5.4	0.8	0.37	5.6	8.0
	WSZ	4.08	umc133a	135	117–141	3.5	0.1	3.6	0.3	0.39	2.6	0.2
		6.06	umc39	156	129–184	2.0	3.3	5.2	1.3	0.39	4.3	8.7
		7.02	bnlg1094	21	1–34	1.0	4.6	5.6	2.5	0.34	2.5	9.3
	WWM	1.03	umc11a	75	64–93	1.4	4.4	4.9	1.2	−0.59	1.6	4.7
		1.04	bnlg2238	119	93–139	4.8	1.4	5.2	0.4	−0.72	7.2	3.1
		1.07	umc1122	198	185–219	2.8	3.4	4.7	0.2	0.70	2.5	2.2
		2.07	umc14b	140	122–152	2.9	2.6	4.4	0.0	−0.69	5.6	5.7
		7.02	bnlg1094	8	0–34	0.7	3.5	3.6	1.1	0.49	2.8	7.3
		10.07	bnl7.49a	131	108–142	1.1	1.8	3.8	3.8	0.01	1.7	2.1
	WWZ	7.02	bnlg1094	15	2–34	3.5	NA	NA	NA	0.77	9.7	NA

Table 4 continued

Trait	Env	Bin	Mark	Distance (cM)		LOD				Add	R^2 (%)	
				Peak	Interval	E1	E2	Joint	QTL × E	Joint	E1	E2
PHT	WSM	1.04	bnlg2086	143	117–158	12.0	13.2	15.6	1.0	−6.97	23.7	24.1
		8.01	umc1327	11	0–25	5.6	0.7	6.2	2.3	−3.87	13.8	2.9
		8.03	bnlg669	65	58–75	2.5	3.6	4.0	0.5	−3.42	7.4	5.2
		9.05	umc1231	118	96–129	3.9	1.0	3.9	0.7	−3.09	2.3	0.6
	WSZ	1.04	bnlg2086	136	117–155	4.3	7.6	9.1	0.4	−2.58	7.5	12.7
	WWM	1.04	bnlg2086	135	114–153	12.9	12.0	15.5	0.2	−8.19	18.3	16.2
		2.06	csu54a	119	115–127	0.9	4.1	4.2	2.0	−2.96	0.2	3.0
		4.02	phi021	31	23–35	3.8	0.4	4.2	1.5	−3.26	5.3	1.2
		4.06	bnlg2291	103	97–126	2.1	3.6	3.7	0.6	3.83	1.7	3.7
		6.02	bnlg2151	31	18–46	4.0	0.6	4.2	1.3	−3.18	3.4	1.2
		8.06	umc48a	131	121–144	3.4	3.1	4.1	0.0	3.86	5.5	5.9
		9.02	umc105a	61	38–74	3.9	2.0	4.1	0.1	4.48	8.0	4.0
		10.04	umc1115	87	69–93	1.8	0.6	5.1	4.8	−1.05	3.8	0.1
	WWZ	4.04	bnlg490	58	50– 68	4.3	NA	NA	NA	−3.59	5.8	NA

Bin: Location of the QTL with respect to chromosome segments flanked by two fixed core markers on the maize reference map. Mark: Closest marker to the QTL position. Peak: Position of the LOD peak on the genetic linkage map in centiMorgans. Interval: Support interval on the linkage map in which the LOD decreases by half. LOD: LOD of the joint analysis (Joint) combining data from two experiments (E1 and E2). QTL × E: LOD of the QTL-by-environment interaction. Add Joint: Overall additive genetic effect of the CML444 allele on trait expression [in (d) for MFLW and ASI, in (g m^{-2}) for GY, in (g) for HKFW, and in (cm) for PHT]. R^2: Percentage of phenotypic variance explained by the QTL in two experiments. Abbreviations of traits are given in Table 1

QTLs for GY, KNO and HKFW, although expressed only in one environment, were significant enough to be identified by the joint analysis across all experiments (Fig. 4c). Several QTLs in this cluster on chromosome 1 expressed a large percentage of the phenotypic variance (Table 4) such as 24% (WSM) and 18% (WWM) for PHT, 7% (WWM) for HKFW, 18% (WWZ) for KNO and 16% (WWZ) for GY. This is undoubtedly the most remarkable cluster identified in this study.

QTLs for ASI in three environments were identified in a second cluster on the same chromosome in bin 1.07. The CML444 allele was associated with a larger ASI (Table 4), which is unfavourable for grain yield under drought. Considering the linked QTLs for MFLW in WSM and WWM (bin 1.08, Fig. 4a; Table 4), this segment of chromosome 1 apparently carries important flowering-related genes. The QTLs for both flowering traits did not show significant QTL × E interactions within environments (Fig. 4a), the QTL for MFLW under WS was even stable across locations (Fig. 4b). The position of the corresponding QTL for ASI, revealed by the overall joint analysis, moved by about 25 cM towards the end of the short arm of chromosome 1 compared to the average position in the environment-specific and treatment-specific analyses. Consequently, it was located approximately half way between the QTL for MFLW and the QTL for GY (Fig. 4c).

The cluster on chromosome 3 (bin 3.04–05) comprised stable QTLs for MFLW in three environments (Fig. 4a). The treatment-specific analyses (Fig. 4b) also revealed a significant and stable effect under drought stress but not under well-watered conditions, probably because of the low LOD of the QTL in WWM and strong interactions between genotypes and the two WW locations. The treatment-specific analysis also revealed linked QTLs for GY and KNO under drought. The additive genetic effects on MFLW and GY in the drought environments were in agreement with the corresponding, relatively strong negative genetic correlations between these traits (Table 2).

Binary epistatic interactions

Ten significant binary additive-by-additive epistatic interactions (of which three at $P < 0.01$) between pairs of single-experiment QTLs were identified. The majority (i.e. six) of them were detected between loci with coupled additive main effects and showed a positive estimate for the epistatic regressors. Taking into account only the 69 QTLs controlling those traits, for which at least two QTLs were identified, this number corresponds to about one-third of QTLs presenting binary epistatic interactions. Three of the ten significant interactions were detected for PHT, two for both MFLW and GY, and one for ASI, KNO, and HKFW when looking at all experiments simultaneously.

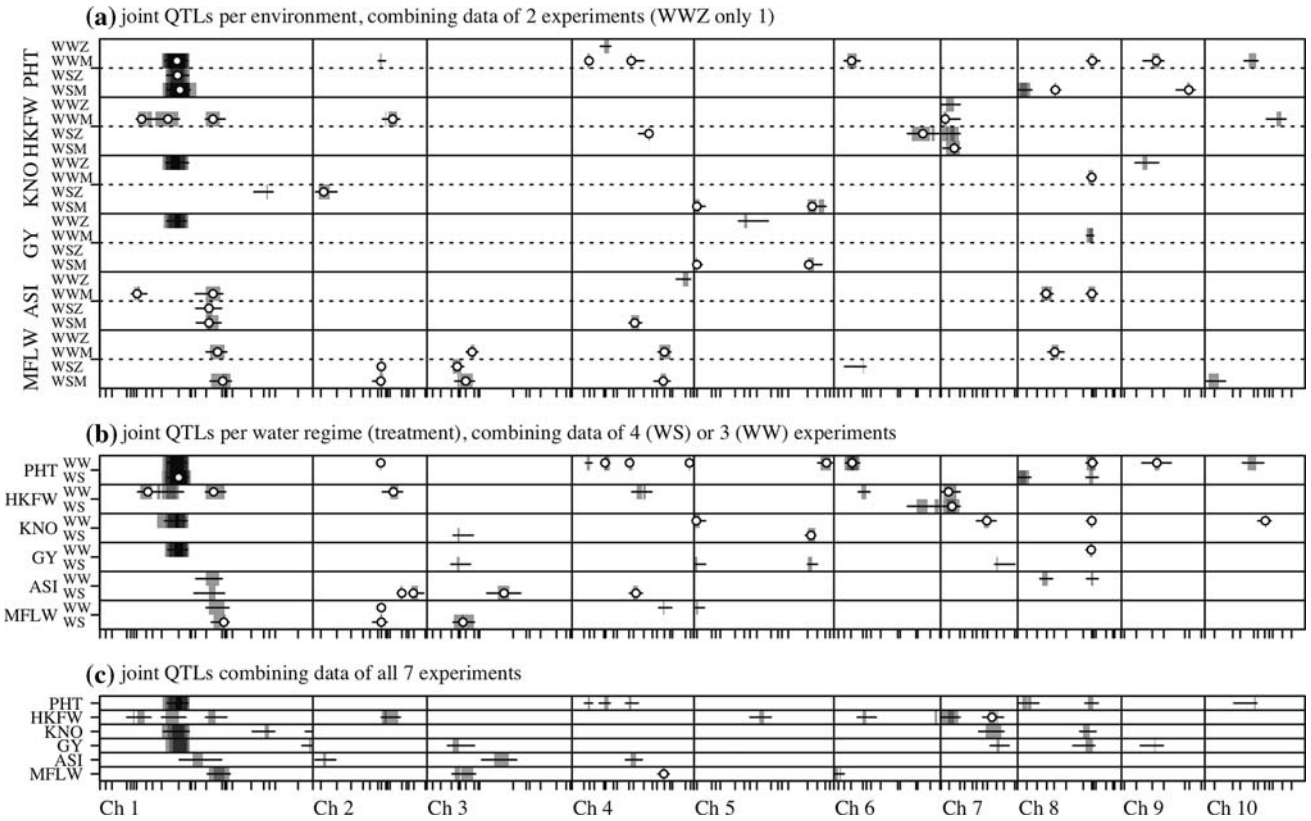

Fig. 4 Segments of the genome where the LOD surpasses the significance threshold for different mapping procedures: joint QTLs (**a**) per environment, (**b**) per treatment and (**c**) across all experiments. The higher the LOD the darker the area. Horizontal lines indicate the support intervals where the LOD at the peak position decreases by half. The dots indicate stable QTLs with a non-significant QTL × E interaction. Abbreviations of traits are given in Table 1

The consistency across experiments of epistatic interactions was low. The interaction between the markers bnlg2086 on chromosome 1 (bin 1.04) and umc1115 on chromosome 10 (bin 10.04), both of which had a significant negative additive main effect on PHT in one of the WWM experiments and in one of the WSM experiments, was significant in the former ($P < 0.01$), but not in the latter ($P < 0.1$) experiment. The concurrence of the same parent's alleles at both loci explained a reduction in plant height by not more than half the average of the corresponding additive main effects.

Furthermore, some evidence for epistatic interactions between the neighbouring markers umc1122 and umc1128 on chromosome 1 (bin 1.07) on the one hand and the neighbouring markers umc48a and asg52a on chromosome 8 (bin 8.06) on the other hand was observed for MFLW and ASI in two different experiments. The presence of the same parent's alleles at both loci delayed MFLW (in WSM) but shortened ASI (in WWM) by about half the corresponding additive main effects. Although not significant ($P < 0.1$), these interactions are worthwhile to be mentioned, because they occurred between markers that are located in genomic regions of particular importance in this study, as shown by the results of the different joint QTL analyses.

Discussion

Effects of drought stress on leaf water potential

The water status of maize leaves decreases quite slowly in response to decreasing soil water content. Xylem nitrate concentration, xylem ABA and stomatal conductance respond faster to progressing drought stress than leaf water status (Bahrun et al. 2002). Bahrun et al. (2002) reported that the relative leaf water potential (i.e. leaf water potential under WS/leaf water potential under WW) is correlated with relative soil moisture deficit (i.e. the difference between soil moisture deficit under WW and under WS). Up to relative soil moisture deficits of about 60%, the former remains close to 1, but increases drastically at relative soil moisture deficits below 70%. The leaf water potential under WW remained relatively constant at a value of −100 kPa in that study. Comparing the average leaf

water potential of both parental lines in the present study with the results by Bahrun et al. (2002), one can deduce that the relative soil water deficit had reached about 80% at the time of the measurement. Although CML444 had constitutively higher relative contents of chlorophyll in the leaves, lower levels of senescence under stress (R. Messmer et al., in preparation) and a higher yield potential, its leaf water potential was significantly lower under stress, compared to SC-Malawi. This is well in line with the observation that CML444 is lacking physiological drought-tolerance mechanisms (see below). However, it cannot be taken for granted that the plants of SC-Malawi were completely unstressed. The water potential of leaves at lower positions on the stem might have been considerably lower than that of the second leaf from the tassel. Cochard (2002) reported that water potentials within maize plants tend to equilibrate at a value close to −1.6 MPa, which is far below the values observed for CML444 and SC-Malawi.

Clusters of QTLs

The four regions in bins 1.08, 2.06, 3.04–05 and 4.09, with stable QTLs controlling MFLW in two or three environments, together with the high heritability of MFLW suggested the presence of genes, which influence the time to flowering in response to various environmental conditions. The position of these QTLs coincided with consensus loci for the time to flowering identified by Chardon et al. (2004), as far as this can be deduced from aligning both maps with the IBM2 2005 Neighbors Map available on MaizeGDB (Lawrence et al. 2008) by means of common molecular markers. The coincidence suggested that the position of the flowering-related genes underlying these QTLs is consistent across different genetic backgrounds. The ASI depended more on the time to female flowering than on the time to male flowering (data not shown), as is usually the case (Edmeades et al. 2000). Several authors reported QTLs for ASI under drought (Agrama and Moussa 1996; Ribaut et al. 1996; Hao et al. 2008) or low nitrogen (Agrama et al. 1999; Ribaut et al. 2007). Ribaut et al. (1996) identified a major QTL for ASI in a region on chromosome 6 (bin 6.05) involved in the expression of ASI in other studies as well (e.g. Veldboom and Lee 1996). In contrast, Hao et al. (2008) reported two clusters of QTLs for ASI on chromosomes 1 (bins 1.03–05) and 9 (bins 9.02–04). Our major QTL for ASI was located in bin 1.07 and did not coincide with any of those clusters. It seems, therefore, that the position of QTLs controlling ASI is less consistent across genetic backgrounds than the position of QTLs controlling MFLW. However, this comparison of the QTLs for ASI across studies is less detailed than the comparison of QTLs for flowering time by Chardon et al. (2004).

As demonstrated by previous studies (Bolaños and Edmeades 1996; Ribaut et al. 1997) we also found that a short ASI is genetically linked to high grain productivity under WS conditions and that ASI is an important secondary trait for grain yield under drought and other stresses (Edmeades et al. 2000). Co-locating QTLs for both traits are likely due to close genetic linkage or pleiotropy. The QTL for ASI on chromosome 8 (bin 8.06) co-located with a QTL for GY as well as for PHT under well-watered conditions in Mexico (WWM). However, there was no co-location between the major QTL for ASI (bin 1.07) and QTLs for GY. Vargas et al. (2006) also noticed a lack of closely linked or pleiotropic QTLs for grain yield and ASI on chromosome 1, but detected a close coincidence of QTLs for both traits on chromosomes 8 and 10. The QTL for ASI on chromosome 8, identified by Vargas et al. (2006), showed significant QTL × E interactions, which were explained by differences in precipitation during flowering between experiments, as revealed by a factorial regression analysis, which included several environmental co-variables.

Bin 8.06 harboured relevant QTLs in several other studies as well, for example, QTLs for grain yield and kernel weight in temperate maize under cool and wet conditions (Austin and Lee 1998) as well as QTLs for grain yield under water-limited conditions (Tuberosa et al. 2002b). Sibov et al. (2003) identified a QTL for GY in tropical maize based on data from five experiments. Ribaut et al. (1997) reported a QTL for GY under normal irrigation in a tropical maize population segregating for drought tolerance. When the same population was evaluated under low-nitrogen conditions (at a different location), bin 8.06 also harboured a QTL for GY as well as for ASI (Ribaut et al. 2007). Therefore, bin 8.06 seems to be important for the genetic control of grain yield of distinct genetic material.

The two QTL clusters on chromosome 1, however, were more dominant than the other clusters, suggesting the presence of flowering-related genes in bins 1.07–08 and genes, which substantially control the distribution of assimilates in the plant in bin 1.04. The *ID1* gene is an attractive positional candidate gene for the QTL for MFLW in bin 1.08. The phenotype resulting from the *id1* mutation described by Colasanti et al. (1998) was not found, but a rearrangement, similar to id1-N2286A (http://www.maizegdb.org, Lawrence et al. 2008), might occur in the RIL population. This rearrangement requires short-day conditions for the initiation of flowering and is associated with extended growth and short internodes on the upper part of the stem. The rearrangement might, therefore, be responsible for the phenotype of SC-Malawi, which is also characterized by more leaves and shorter internodes on the upper part of the plant, in contrast to CML444. The lack of significant genetic effects in bin 1.08 on MFLW in

Zimbabwe suggested that location-specific environmental factors, such as photoperiod or irradiance, activated the gene in Mexico.

QTLs for PHT and/or grain yield near the centromere on chromosome 1 are common in maize (Agrama and Moussa 1996; Ribaut et al. 1997; Austin and Lee 1998; Sari-Gorla et al. 1999; Sibov et al. 2003; Moreau et al. 2004; Lima et al. 2006; Hao et al. 2008). However, to the best of our knowledge, a mutually exclusive expression of QTLs for both traits within environments has never been observed. Moreover, there is evidence that the adjacent bins 1.03 and 1.06 deserve special attention in drought-stressed maize, as they also play a role in the metabolism of abscisic acid and in the control of root growth (Tuberosa et al. 2002a).

Stability of QTLs across trials

Through stepwise joint mapping we successfully identified the major QTLs with additive effects on the target traits in the RIL population in response to various environmental conditions. The results of the single-experiment QTL analysis did not always provide a good prediction of the positions, effects and stability of QTLs controlling the target traits, as was also found by Malosetti et al. (2008).

The genetic effects across years within environments were quite stable; 80% of the environment-specific QTLs did not show significant QTL × E interactions. However, the proportion of QTLs with significant QTL × E interactions was higher in the treatment-specific analyses combining data of Mexico and Zimbabwe and highest in the global analysis across all experiments. The joint analysis per environment revealed the co-location of QTLs across environments for MFLW (four positions) and for ASI, HKFW and PHT (one position each) but not for GY and KNO. Similarly, the joint analysis per treatment revealed the co-location of QTLs in the WS and WW treatments for MFLW and PHT (two positions each) and for ASI and HKFW (one position each) but again not for GY and KNO.

The joint analysis per treatment showed an increase in the QTL × E interactions at significant QTLs as well as a decrease in the number of co-locating QTLs for a given trait in both water regimes in relation to the total number of QTLs identified. It is concluded that, in contrast to the QTLs for GY and KNO, those for MFLW, ASI and PHT were fairly stable across years under the same water regime at a given location, with co-locating QTLs in two, three and sometimes four environments. However, the stability of QTLs was considerably lower, when data were combined across water regimes. These findings are supported by several QTL studies of tropical and temperate maize. Ribaut et al. (1996) found that almost all QTLs for ASI were consistent across the drought trials (same location, different years). The co-location of QTLs for flowering parameters and ASI across water regimes was also identified, but to a lesser degree. In contrast, the QTLs for grain yield and yield components were not stable across water regimes (Ribaut et al. 1997). Austin and Lee (1998) found inconsistency in the position of QTLs for yield when comparing a favourable cropping season with a cool and wet cropping season, whereas the QTLs for morphological traits were more consistent in both years. Lima et al. (2006) found similar results: The expression of most QTLs for grain yield and about 50% of the QTLs for plant height changed across environments, whereby the environments, defined as combinations of locations and cropping seasons, did not impose a pre-defined stress. Furthermore, most of the QTLs for grain yield and yield components, which were identified by Lu et al. (2006), differed in the water-stress and well-watered treatments. Vargas et al. (2006) showed the possibilities of factorial regression for mapping QTLs and for dissecting QTL × E interactions in terms of environmental co-variables by using data, some of which were analyzed by Ribaut et al. (1996, 1997). Both environment-specific and stable QTLs for grain yield were detected, but they were less stable than the QTLs for ASI, which were usually consistent across the eight environments.

The QTLs for ASI in the present study differed from those reported in other studies; almost all the QTLs per environment as well as in the WS treatment were stable, but there was little co-location of QTLs across environments and treatments (with the exception of bin 1.07). Vargas et al. (2006) concluded that pyramiding favourable alleles for ASI at significant loci could improve the grain yield of maize in a broad set of environments, including optimal and water-limited as well as low nitrogen conditions. Similarly, it is advisable to address the major QTL for ASI on chromosome 1 in a marker-assisted breeding programme with CML444 and SC-Malawi; however, because of the distinct morphology of both lines (cf. below) this must be complemented by additional clusters of QTLs, as mentioned above.

Epistasis

The reduced proportion of phenotypic variance accounted for by the detected QTLs, compared to the heritability of the respective traits, suggests significant levels of digenic epistasis (Li et al. 2008). This is in agreement with other reports, according to which epistasis makes a substantial contribution to the genetic control of quantitative traits (Frankel and Schork 1996; Zeng et al. 2005). Here, only 30% of the single-experiment QTLs presented significant binary epistatic interactions. They do not explain the entire difference between the proportion of phenotypic variance accounted for by the QTLs and the heritability of the

different traits. Following the approach developed by Li et al. (2007), a genome-wide scan for digenic epistasis will be conducted on the same data set to better understand epistatic effects in this population.

Morphology of drought tolerance

As mentioned in "Materials and methods", the plant architecture of both parental lines differs considerably. The strong vigour of CML444, a largely improved line with high yields under optimal conditions, masked the lack of physiological mechanisms conferring drought tolerance. Due to these mechanisms, CML444 maintained a shorter ASI and produced higher yields than SC-Malawi in almost all the environments, despite the positive (unfavourable) additive effect of its allele at the major QTL for ASI in bin 1.07. However, CML444 contributed favourable alleles to four of the six clusters of QTLs described above (bin 1.04, 5.01, 7.04 and 8.06).

The plants were less vigorous in the environments in Mexico, as indicated by the greater reduction in yield in WSM compared to WWM as well as by the lower average yield under both water regimes, in contrast to Zimbabwe. Consequently, secondary traits such as the ASI were more important in Mexico. This was revealed by larger segregations, a higher number of detected QTLs as well as by higher correlations between ASI and GY. At the same time, the limited adaptation of SC-Malawi to the Mexican environments affected its vegetative growth. The segregation of PHT was greater and the number of detected QTLs for PHT increased drastically. Even the genetic control of HKFW, the most consistent trait across environments at the phenotypic level, was affected. The number of QTLs for HKFW was much higher in WSM than in the other three environments. In addition, the allele of CML444 was responsible for a decrease in HKFW at 50% of the QTLs detected for this trait in WSM, whereas negative additivity was not observed in any other environment. It is concluded that the evaluation of plants in environments, to which they are not fully adapted, accentuates genetic differences among lines, increases phenotypic segregation and enhances the power of the detection of QTLs for secondary traits because the plants are less vigorous.

Root system

Although there is reason to expect that the lines that perform best in drought environments have an extensive and deep root system, recurrent selection in tropical maize populations has actually led to a reduction of root biomass. Inbred lines with poor early root development have higher yields under drought than inbred lines with vigorous early development of roots (Bruce et al. 2002). The relationship between root traits and drought tolerance of maize is still unclear. This is largely due to the fact that more research has been devoted to improving the redirection of scarce assimilates to the ear (Edmeades et al. 1999). However, although selection has decreased root biomass, the root system may have reached deeper soil layers, while the lateral branching of roots in the topsoil was reduced. Consistent with this hypothesis is the finding that the QTL *root-ABA1* on the maize chromosome 2 affects both the extent of root branching and grain yield under water stress (Landi et al. 2007).

The vigour of CML444 might be related to an efficient root system. To test this hypothesis some experiments were conducted under controlled conditions. The roots of CML444 were deeper than those of SC-Malawi when the plants were grown in 80-cm-long sand columns until the 5-leaf stage. Moreover, CML444 produced significantly more roots between 50 and 80 cm and extracted more water below 40 cm than SC-Malawi, irrespective of water availability (Hund et al. 2008). CML444 also had longer axile roots, essential for the wide vertical (and horizontal) distribution of the root system, than SC-Malawi at the 8-leaf stage in containers with 1 m of soil (Hund et al. 2008). These apparently constitutive differences in the root system may also be expressed in the field and may be of advantage for CML444, a modern improved cultivar. It is assumed that phenotypic selection for better performance under drought stress and well-watered conditions has modified the root morphology compared to older lines like SC-Malawi. Therefore, as indicated above, the plant vigour of CML444 seems to be related more to changes in plant morphology than to changes in physiology. The constitutive ability to avoid dehydration under stress, such as by a better-adapted root system, which enables the extraction of water from deep soil layers, and shorter plants with upright leaves explain the better performance of CML444 under drought conditions (in particular in Zimbabwe). At the same time, however, this line does not possess strong mechanisms for drought tolerance, as demonstrated by the large reductions in grain yield under drought, compared to WW conditions. Ribaut et al. (2008) proposed that sustained progress in breeding for drought tolerance in tropical maize will probably entail the selection of plants with a smaller leaf area (especially on the upper part of the plant), short and thick stems, small tassels, erect leaves and delayed senescence. Less important traits or traits, for which selection is impractical, include a smaller root biomass and a deep root system with little branching of lateral roots in the upper part of the soil.

Breeding for drought tolerance

The genetic structure of populations, from which inbred lines are derived, determines the extent of heterosis that

can be achieved in inter-inbred hybrids. The probability of obtaining a hybrid of tropical maize, which yields 30–50% more than the mean of all hybrids under drought stress, was three to six times higher when the inbred lines were selected from stress-tolerant source populations rather than from conventionally selected populations (Betrán et al. 2003). Therefore, it is very important for breeders to select drought-tolerant inbred lines per se and to understand the genetic mechanisms underlying the performance of fixed material, as in this study.

Because of limited resources or limited access to field facilities for the screening of plants under drought stress, selection for drought tolerance has often been conducted in rain-fed nurseries, which are occasionally prone to drought. Under these circumstances, large populations were grown at high planting densities to simulate drought stress, and inbred lines with stable yields were recycled (Bruce et al. 2002). Our results clearly demonstrate the limitations of that approach and indicate that efficient selection must be conducted under water-limited conditions because the genetic control of key traits in this population under WS differs from that under WW conditions. This is confirmed by the large decrease in the stability of QTLs when combining phenotypic data across water regimes, whereby the QTL × E interaction was significant for 94% of the QTLs. Correspondingly, Agrama et al. (1999) concluded from the relatively inconsistent position of QTLs for yield under high- and low-nitrogen availabilities that improving tolerance to low nitrogen by marker-assisted selection will be most efficient when QTLs for grain yield are identified under low nitrogen. Moreover, Ribaut et al. (2007) found a similar genetic basis of ASI (and to a lesser extent for the number of ears per plant) under drought and low nitrogen. Their results explain the increased tolerance to low nitrogen stress of tropical maize selected for drought tolerance and emphasize the relevance of selecting under stress conditions.

Conclusions

The QTLs identified in this population of RILs were quite stable across years and locations under a given water regime. Clusters of QTLs for different traits were identified, with the favourable alleles mostly coming from CML444. CML444 performed better than SC-Malawi because of improved constitutive traits, conferring high plant vigour across water regimes. The combination of favourable alleles for dehydration avoidance (CML444) and some favourable alleles for dehydration tolerance (SC-Malawi) mean that the most tolerant RILs in this population are attractive for use as new breeding material. A marker-assisted selection experiment has been initiated with this population and will focus mainly on the clusters of QTLs identified through the joint analysis per water regime (on chromosomes 1, 3 and 5 for WS; on chromosomes 1 and 8 for WW) and the joint analysis across water regimes (on chromosome 1). Finally, the instability of QTLs across water regimes confirmed the importance of selection under drought conditions to achieve significant gains in drought tolerance and emphasizes the limited output of QTL analyses when phenotypic data from different water regimes are combined. However, QTLs identified in joint analyses across treatments (e.g. on chromosome 1 in this study) are also very important, because they contribute to the broad adaptation of plants.

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