Journal of Integrative Plant Biology 2006, **48** (10): 1233–1243

Quantitative Trait Loci Mapping of Maize Yield and Its Components Under Different Water Treatments at Flowering Time

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Abstract

Drought or water stress is a serious agronomic problem resulting in maize (*Zea mays* L.) yield loss throughout the world. Breeding hybrids with drought tolerance is one important approach for solving this problem. However, lower efficiency and a longer period of breeding hybrids are disadvantages of traditional breeding programs. It is generally recognized that applying molecular marker techniques to traditional breeding programs could improve the efficiency of the breeding of drought-tolerant maize. To provide useful information for use in studies of maize drought tolerance, the mapping and tagging of quantitative trait loci (QTL) for yield and its components were performed in the present study on the basis of the principle of a mixed linear model. Two hundred and twenty-one recombinant inbred lines (RIL) of Yuyu 22 were grown under both well-watered and water-stressed conditions. In the former treatment group, plants were well irrigated, whereas those in the latter treatment group were stressed at flowering time. Ten plants of each genotype were grown in a row that was 3.00 m × 0.67 m (length × width). The results show that a few of the QTL were the same (one additive QTL for ear length, two additive QTL and one pair of epistatic QTL for kernel number per row, one additive QTL for kernel weight per plant), whereas most of other QTL were different between the two different water treatment groups. It may be that genetic expression differs under the two different water conditions. Furthermore, differences in the additive and epistatic QTL among the traits under water-stressed conditions indicate that genetic expression also differs from trait to trait. Major and minor QTL were detected for the traits, except for kernel number per row, under water-stressed conditions. Thus, the genetic mechanism of drought tolerance in maize is complex because the additive and epistatic QTL exist at the same time and the major and minor QTL all contribute to phenotype under water-stressed conditions. In particular, epidemic QTL under water-stressed conditions suggest that it is important to investigate the drought tolerance of maize from a genetic viewpoint.

Key words: flowering time; maize (*Zea mays*); quantitative trait loci mapping; water treatment; yield and its components.

Lu GH, Tang JH, Yan JB, Ma XQ, Li JS, Chen SJ, Ma JC, Liu ZX, E LZ, Zhang YR, Dai JR (2006). Quantitative trait loci mapping of maize yield and its components under different water treatments at flowering time. *J Integr Plant Biol* **48**(10), 1233–1243.

doi: 10.1111/j.1672-9072.2006.00289.x; available online at www.blackwell-synergy.com, www.jipb.net

Received 3 Oct. 2005 Accepted 11 Mar. 2006

Supported by the State Key Basic Research and Development Plan of China (2003CB114300).

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Drought or water stress is one of the main environmental factors affecting maize (*Zea mays* L.) yield throughout the world (Edmeades et al. 1992; Dai 1998). Two-thirds of the maize produced in China is grown on farmland suffering from water stress. The drought that occurred in 1997 decreased the maize yield by an average of 15.7% in China's maize belt (Li et al. 2004) and has been the primary factor affecting maize production and yield improvement in China. Breeding hybrids with

drought tolerance has been confirmed as an important method to improve maize production in water-stressed areas. However, traditional breeding has a longer period of breeding hybrids and lower efficiency owing to a shortage of particularly effective techniques. Nowadays, it is generally recognized that applying molecular marker techniques to traditional breeding selection could improve the efficiency of the breeding of drought-tolerant maize.

The mapping of quantitative trait loci (QTL) is the basis of marker-assisted selection (MAS). The mapping and tagging of QTL for maize drought tolerance has been reported in many studies (Agrama et al. 1996; Ribaut et al. 1996, 1997; Frova et al. 1999; Sanguineti et al. 1999; Sari-Gorla et al. 1999; Gao et al. 2003; Li et al. 2003, 2004; Zhang et al. 2004). Agrama and Moussa (1996) reported that there were five QTLs related to grain yield on chromosomes 1, 3, 5, and 8 under drought conditions that could explain 49.6% of the phenotypic variance. Ribaut et al. (1997) studied the QTL of grain yield, ear number, kernel number, and 100-kernel weight under well-watered conditions and two other water-stress regimens and identified one to seven QTL for each trait in the different environments. The meaningful finding was that four genomic regions were identified for the expression of both grain yield and the anthesis-silking interval (ASI). In three of these regions, the allelic contributions were for short ASI and an increase in grain yield. Frova et al. (1999) detected genomic segments responsible for the expression of drought tolerance of yield components (ear length, ear weight, kernel weight, kernel number, and 50-kernel weight) and found that 50% of the QTLs were the same over the two water regimens. The QTL controlling ear length were found on chromosomes 3, 6, 7, and 8, those for ear weight were on chromosomes 4, 5, and 8, those for kernel weight were on chromosomes 3 and 5, and those for kernel number were on chromosomes 2, 4, 5 and 6. Li et al. (2003) performed QTL identification under well-watered and drought-stressed regimens and showed that, under the well-watered regimen, two QTLs for ear setting were found on chromosomes 3 and 6 that explained 19.9% of the phenotypic variance and showed additive and partial dominant effects, respectively. Five QTLs for grain yield were on chromosomes 3, 6, and 7. Four QTLs for ear setting were on chromosomes 3, 7, and 10, explaining 60.4% of the phenotypic variance and displaying dominant and partial dominant effects, respectively. Four QTLs for grain yield were detected on chromosomes 1, 2, 4, and 8 under the drought-stressed regimen.

Quantitative trait loci analysis of maize drought tolerance involves many aspects, including morphological traits (such as plant height, ear position, branches per tassel, days from emergence to pollinating, days from emergence to silk advancing, days from florescence to silk advancing), parameters regarding root, physiological, and biochemical traits (e.g. abscisic acid (ABA), degree of leaf senescence, photosynthesis parameters, water content, carbon metabolism, protein content, stomatal conductance, chlorophyl content, osmotic potential, osmotic adjustment), and yield traits (e.g. ear length, 100-kernal weight, ears per plant, and grain yield) among others (Li et al. 2004). The identification of QTL for grain yield and its components is essential for all QTL studies because increasing yield production under water-stressed conditions is the first target of all breeding programs. Maize is sensitive to water stress and drought results in a delay in ASI and severe yield losses at flowering time (Bolanos et al. 1996; Edmeades et al. 1999; Li et al. 2002; Zhang et al. 2002). However, only a few reports have dealt with QTL analysis of the drought tolerance of maize under conditions of drought or different water levels compared with the number of QTL identified for yield and its components (Gao et al. 2003). Moreover, most of the previous studies were based on the additive-dominant genetic model on the assumption that there was no interactive effect among alleles. In fact, an interactive action does exist, especially for complex quantitative traits, such as drought tolerance. Hence, it is necessary to detect epistatic QTL and to analyze the epistatic effect (Zhu 1997, 1998; Wang et al. 1999b). Thus, in the present study, an analysis of the additive and epistatic QTL of yield and yield components was performed based on a simple sequence repeat (SSR) linkage map using 221 recombinant inbred lines (RIL) as the test material under both well-watered and water-stressed conditions. The aim of the present study was to provide useful information for further theoretical studies and the MAS breeding of drought-tolerant maize.

Results

Yield traits of the Yuyu 22 RIL population and its parents under two different water treatments

The yield traits of the Yuyu 22 RIL population and its parents under two different water treatments are given in Table 1. The average value of yield and its components decreased under water-stressed conditions compared with well-watered conditions. Water stress had a negative effect on the RIL population and its parents. Values obtained for ear length, kernels per row, 100-kernel weight, and the kernel weight per plant were higher in the male parent 87-1 than in the female parent Zong 3 under water-stressed conditions. Thus, the drought tolerance of 87-1 was stronger than that of Zong 3. The average value of each trait of the RIL population was between that of the two parents and showed the characteristics of quantitative traits. The distribution of these traits under different water treatments indicated that the absolute value of skewness and kurtosis for yield and its components was lower than 1 and accorded to normal distribution. Thus, this population was suitable for drought tolerance QTL analysis.

Table 1. Yield traits of the Yuyu 22 recombinant inbred line population and its parents in the two water treatment groups

	Well-watered treatment group				Water-stressed treatment group			
	EL	KR	WK	KWP	EL	KR	WK	KWP
87-1	17.593	25.233	28.472	84.334	15.147	15.367	29.872	58.551
Zong 3	13.708	21.957	26.304	80.127	10.625	10.250	25.336	36.580
RIL mean	15.997	21.134	26.642	81.815	12.618	13.305	26.219	45.779
Kurtosis	0.153	–0.287	–0.561	–0.676	0.584	–0.483	0.319	–0.356
Skewness	0.220	0.028	–0.123	0.001	0.068	0.408	–0.091	0.448
Maximum	22.120	39.467	34.950	141.257	21.427	28.933	38.000	112.336
Minimum	10.580	7.500	17.867	16.447	5.351	2.917	12.400	1.000
SD	2.134	5.465	3.642	27.436	2.350	5.722	4.338	23.213

EL, ear length; KR, kernal number per row; KWP, kernal weight per plant; WK, 100-kernal weight.

The SSR genetic linkage map

Two-hundred and sixty-one polymorphic markers covering the entire genome fell into 10 linkage groups (logarithm of odds (LOD) >3.0; Figure 1). The linkage map covered a 2 740.2 cM length of the maize genome and the average length between two markers was 10.499 cM.

Mapping of QTL

The QTL of yield and its components were mapped under two different water treatments on the basis of the principle of a mixed linear model. Seventeen additive QTLs and 30 pairs of epistatic QTLs under water-stressed conditions were identified, whereas 14 additive QTLs and 31 pairs of epistatic QTLs were identified under well-watered conditions. The interval, effect, and contribution of the additive QTL for yield and its components in the two water treatment groups are given in Table 2 and Figure 1 and the epistatic QTLs are given in Table 3 and Figure 1.

Ear length

The additive and epistatic QTL identified for ear length were different in the two water treatment groups (Tables 2,3). Seven additive QTLs and 13 pairs of epistatic QTLs were detected under well-watered conditions. Seven additive QTLs were found on chromosomes 1, 2, 3, 4, and 10 and showed a positive effect. Values of the additive effect for ear length derived from 87-1 ranged from 0.349 9 to 0.487 7 cm and the contribution was 4.75%–9.23%. Thirteen pairs of epistatic QTLs were detected on chromosomes 1, 3, 4, 5, 7, 8, and 10. Seven pairs showed a negative effect, whereas six pairs exhibited a positive effect. Six additive QTLs and five pairs of epistatic QTLs were detected under water-stressed conditions. Six additive QTLs were found on chromosomes 1, 3, 8, and 10; five showed a positive effect and one had a negative effect in the interval bnlg1452–umc1504 on chromosome 3. The effect of the QTL in the interval phi080–umc1069 on chromosome 8 was the highest and increased ear length by 0.828 6 cm and contributed 11.91%. Five pairs of epistatic QTLs were detected on chromosomes 2, 3, 4, 5, and 10 and their effects were all negative.

Kernel number per row

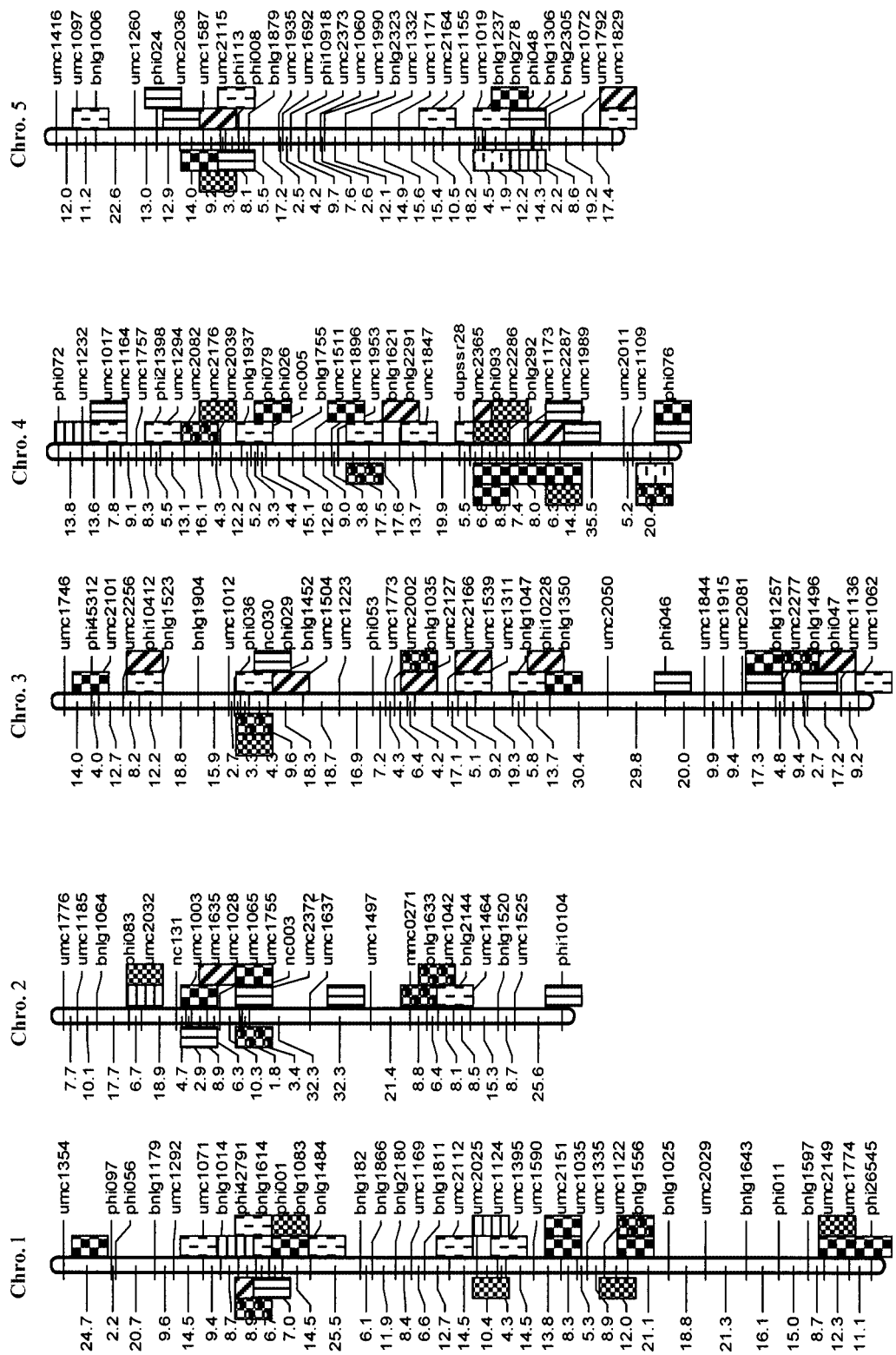
The location, effect and contribution of the additive and epistatic QTL detected are given in Tables 2,3. Two additive QTLs and 13 pairs of epistatic QTLs were identified under well-watered conditions. Two additive QTLs were on chromosomes 2 and 7 and the effect from Zong 3 decreased 1.013 3 and 1.272 7 kernel numbers per row and the contribution was 7.460% and 11.778%, respectively. Thirteen pairs of epistatic QTLs covered all chromosomes, seven pairs of them showing a negative effect and six positive. Five additive QTLs and 11 pairs of epistatic QTLs were identified under water-stressed conditions. Five additive QTLs were chromosomes 2, 4, 5, 7, and 8, three negative and two positive.

100-kernal weight

Two additive QTLs and eight pairs of epistatic QTLs were identified under water-stressed conditions. Two additive QTLs were found on chromosomes 4 and 7 and showed a positive effect. The QTL in the interval phi057–umc1016 increased 100-kernal weight by 1.139 g and contributed highly to 10.61% of the variation. Eight pairs of epistatic QTLs were distributed on chromosomes 1, 2, 3, 4, 7, 8, and 10, Only three additive QTLs identified under water-stressed conditions were on chromosome 4, 5, and 6, one positive and two negative.

Kernel weight per plant

Two additive QTLs and five pairs of epistatic QTLs were detected under well-watered conditions, four additive QTLs and


Chro. 1
umc1354 phi097 phi056 bnlg1179 umc1292 umc1071 bnlg1014 phi42791 bnlg1614 phi001 bnlg1083 bnlg1484 bnlg182 bnlg1866 bnlg2180 umc1169 bnlg1811 umc2112 umc2025 umc1124 umc1395 umc1590 umc2151 umc1035 umc1335 umc1122 bnlg1556 bnlg1025 umc2029 bnlg1643 phi011 bnlg1597 umc2149 umc1774 phi26545
Chro. 2
umc1776 umc1185 bnlg1064 phi083 umc2032 nc131 umc1003 umc1635 umc1028 umc1065 umc1755 nc003 umc2372 umc1637 umc1497 mmc0271 bnlg1633 umc1042 bnlg2144 umc1464 bnlg1520 umc1525 phi10104
Chro. 3
umc1746 phi45312 umc2101 umc2256 phi10412 bnlg1523 bnlg1904 umc1012 phi036 nc030 phi029 bnlg1452 umc1504 umc1223 phi053 umc1773 umc2002 bnlg1035 umc2127 umc2166 umc1539 umc1311 bnlg1047 phi10228 bnlg1350 umc2050 phi046 umc1844 umc1915 umc2081 bnlg1257 umc2277 bnlg1496 phi047 umc1136 umc1062
Chro. 4
phi072 umc1232 umc1017 umc1164 umc1757 phi21398 umc1294 umc2082 umc2176 umc2039 bnlg1937 phi079 phi026 nc005 bnlg1755 umc1511 umc1896 umc1953 bnlg1621 bnlg2291 umc1847 dupssr28 umc2365 phi093 umc2286 bnlg292 umc1173 umc2287 umc1989 umc2011 umc1109 phi076
Chro. 5
umc1416 umc1097 bnlg1006 umc1260 phi024 umc2036 umc1587 umc2115 phi113 phi008 bnlg1879 umc1935 umc1692 phi10918 umc2373 umc1060 umc1990 bnlg2323 umc1332 umc1171 umc2164 umc1155 umc1019 bnlg1237 bnlg278 phi048 bnlg1306 bnlg2305 umc1072 umc1792 umc1829

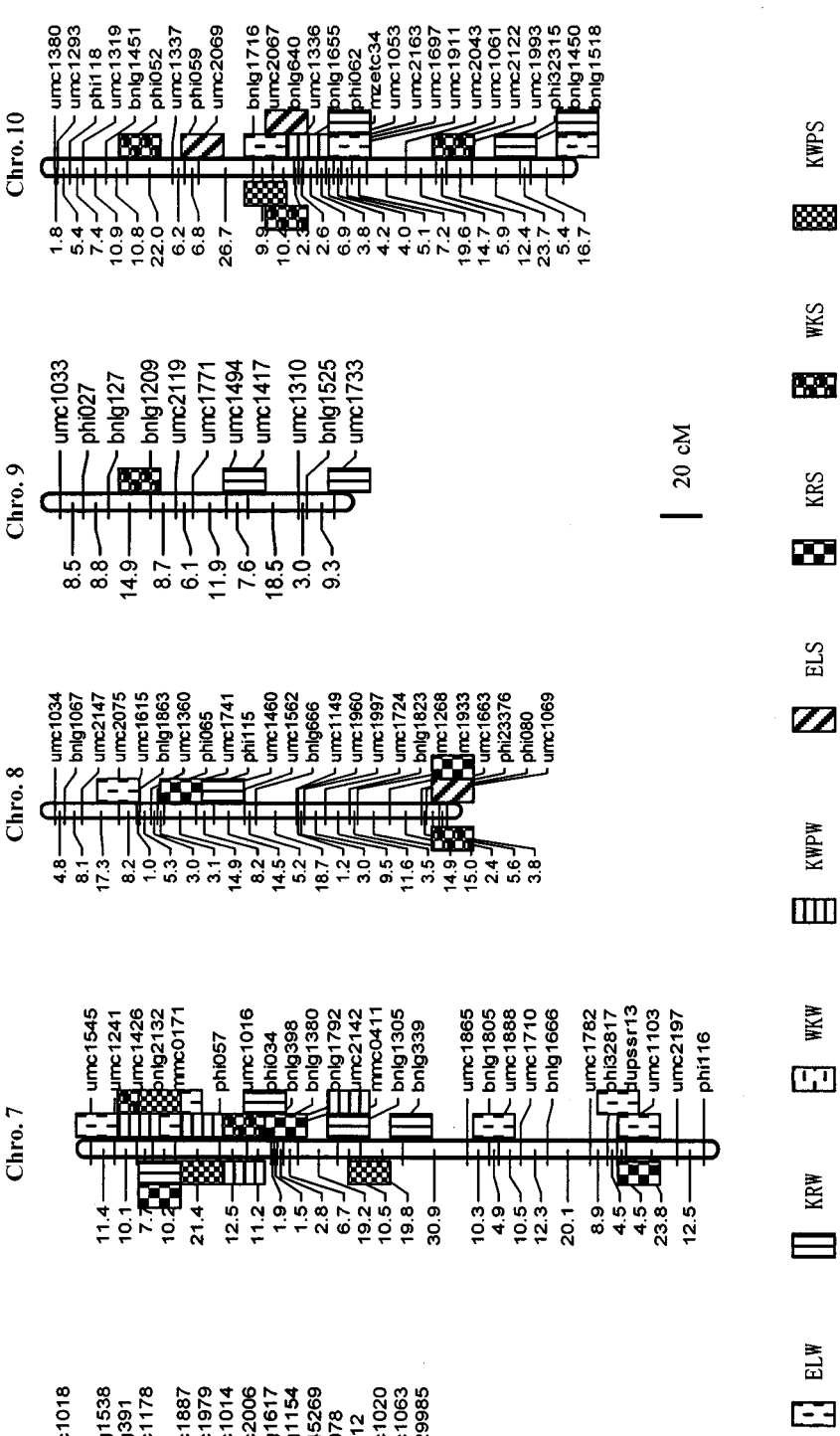


Figure 1. The quantitative trait loci (QTL) on the linkage map with 261 simple sequence repeat markers based on the 221 individuals of the Yuyu 22 recombinant inbred line population.

Quantitative trait loci were named using the trait abbreviations (EL, ear length; KR, kernal number per row; WK, 100-kernal weight; KWP, kernal weight per plant) plus water treatment (w, well-watered; s, water-stressed). Chro., chromosome.

Table 2. Interval, effect, and contribution of the additive quantitative trait loci (QTL) in the two water treatment groups

Trait	Well-watered treatment group						Water-stressed treatment group					
	QTL[a]	Interval	cM[b]	LOD	A	H^2_A[c]	QTL[a]	Interval	cM[b]	LOD	A	H^2_A[c]
EL	ELW1	bnlg1014–phi42791	8	7.51	0.4877	9.23	ELS1	bnlg1014–phi42791	6	6.83	0.7394	9.52
	ELW1	umc1124–umc1395	0	6.45	0.4379	7.44	ELS3	umc2256–phi10412	0	4.84	0.5616	5.49
	ELW2	umc1042–bnlg2144	0	8.08	0.4694	8.55	ELS3	bnlg1452–umc1504	2	5.92	–0.6687	7.79
	ELW3	umc2256–phi10412	0	5.02	0.3869	5.81	ELS5	umc2036–umc1587	6	3.25	0.5119	4.56
	ELW4	bnlg2291–umc1847	0	7.44	0.4725	8.66	ELS8	phi080–umc1069	2	6.45	0.8268	11.91
	ELW10	umc2069–bnlg1716	26	3.51	0.3499	4.75	ELS10	bnlg1716–umc2067	8	4.13	0.5614	5.49
	ELW10	mzetc34–umc1053	0	4.13	0.3989	6.17						
KR	KRW2	umc1003–umc1635	0	4.36	–1.0133	7.46	KRS2	umc1003–umc1635	0	6.65	–1.3816	8.81
	KRW7	umc1426–bnlg2132	0	6.09	–1.2727	11.77	KRS4	umc2365–phi093	6	4.9	1.2459	7.17
							KRS5	umc2036–umc1587	10	5.86	1.4115	9.2
							KRS7	umc1426–bnlg2132	0	4.77	–1.2456	7.16
							KRS8	phi080–umc1069	2	3.51	1.0476	5.07
WK	WKW4	umc1109–phi076	6	3.12	0.9729	6.47	WKS4	umc1109–phi076	0	3.39	0.8469	5.86
	WKW5	umc1019–bnlg1237	0	3.93	–0.9394	6.03	WKS7	phi057–umc1016	12	4.72	1.139	10.61
	WKW6	umc1020–umc1063	24	3.54	–1.0395	7.39						
KWP	KWPW2	phi083–umc2032	6	3.18	–5.959	6.1	KWPS3	nc030–phi029	4	5.22	–6.1782	7.98
	KWPW7	umc1426–bnlg2132	2	4.59	–7.5464	9.78	KWPS4	umc2365–phi093	4	4.68	6.0972	7.77
							KWPS5	umc2036–umc1587	14	3.83	5.1445	5.53
							KWPS7	umc1426–bnlg2132	2	5.88	–7.1534	10.7

[a]QTLs were named using the trait abbreviations plus water treatment (w, well-watered; s, water-stressed) plus chromosome number.

[b]The distance given is from the left marker to the putative QTL.

[c]H^2_A is the contribution from the additive QTL.

EL, ear length; KR, kernal number per row; WK, 100-kernal weight; KWP, kernal weight per plant; LOD, logarithm of odds; A, additive effect.

six pairs of epistatic QTLs under water-stressed conditions. Two additive QTLs were on chromosomes 2 and 7, the effect derived from Zong 3 on kernel weight per plant decreased 5.959 and 7.546 g, respectively, with respective contributions to variation of 6.1% and 9.78% under well-watered conditions. Five pairs of epistatic QTLs were found on chromosomes 1, 4, 5, 6, 7, and 10, two negative and three positive. The interactive positive effect between the QTL in the interval bnlg1014–phi42791 on chromosome 1 and the QTL in the interval bnlg640–umc1336 on chromosome 10 was the highest and added 7.588 g to kernel weight per plant, contributing 7.24%. The negative interactive effect between the QTL in the interval umc1018–bnlg1538 on chromosome 6 and the QTL in the interval phi057–umc1016 on chromosome 7 was the highest, decreasing kernel weight per plant by 8.447 2 g and contributing 8.97%. Four additive QTLs and six pairs of epistatic QTLs were detected under water-stressed conditions. Four additive QTLs were on chromosomes 3, 4, 5, and 7, two positive and two negative. The QTL in the interval umc1426–bnlg2132 on chromosome 7 decreased kernel weight per plant by 7.153 4 g and contributed 10.7% to the variation. Six pairs of epistatic QTLs were on chromosomes 1, 2, 4, 6, 7, and 10, three pairs positive and three pairs negative. The interactive positive effect between the QTL in the interval bnlg1614–phi001 on chromosome 1 and the QTL in the interval bnlg1716–umc2067 on chromosome 10 was the highest, increasing kernel weight per plant by 7.080 9 g and contributing 7.49% to the variation. The negative interactive effect between the QTL in the interval umc1335–umc1122 on chromosome 1 and the QTL in the interval umc2082–umc2176 on chromosome 4 was the highest, decreasing kernel weight per plant by 7.017 7 g and contributing 7.35% to the variation.

Comparison of QTL between well-watered and water-stressed conditions

Both additive and epistatic QTL played an important role in yield and its components in the two different water treatment groups. Seventeen additive QTLs and 30 pairs of epistatic QTLs were identified under water-stressed conditions, 14 additive QTLs and 31 pairs of epistatic QTLs under well-watered conditions on the basis of the principle of a mixed linear model. Furthermore, additive and epistatic QTL were detected in yield traits, except epistatic QTL for the 100-kernal weight under water-stressed conditions. The pervasive epistatic QTL indicated that the

Table 3. Interval, effect, and contribution of the epistatic quantitative trait loci (QTL) in the two water treatment groups

Treatment	Trait	QTL[a]	Interval	cM[b]	QTL	Interval	cM	LOD	AA	H^2_{AA}[c]
WW	EL	ELW1	umc1292–umc1071	0	ELW10	bnlg1450–bnlg1518	12	5.47	0.3482	2.08
		ELW1	bnlg1014–phi42791	8	ELW7	bnlg2132–mmc0171	10	14.46	–0.5386	4.98
		ELW1	bnlg1484–bnlg182	0	ELW3	umc1136–umc1062	8	8.22	–0.4441	3.38
		ELW1	bnlg1811–umc2112	12	ELW5	umc1792–umc1829	16	4.44	–0.3552	2.17
		ELW3	phi036–nc030	0	ELW3	umc2166–umc1539	0	4.46	0.3697	2.35
		ELW3	bnlg1047–phi10228	0	ELW4	bnlg1937–phi079	0	6.51	–0.4764	3.89
		ELW4	umc1232–umc1017	0	ELW5	umc2115–phi113	2	4.2	0.3358	1.94
		ELW4	umc1757–phi21398	8	ELW7	umc1545–umc1241	0	3.64	0.3391	1.97
		ELW4	umc1953–bnlg1621	6	ELW7	bnlg1805–umc1888	0	6.44	0.7161	8.8
		ELW4	dupssr28–umc2365	4	ELW5	umc1171–umc2164	12	5.25	–0.4292	3.16
		ELW5	umc1097–bnlg1006	0	ELW7	dupssr13–umc1103	0	4.79	0.327	1.83
		ELW5	umc1155–umc1019	18	ELW8	umc2147–umc2075	16	3.89	–0.3168	1.72
		ELW7	umc1426–bnlg2132	4	ELW7	umc1103–umc2197	2	6.53	–0.5485	5.16
	KR	KRW1	bnlg1614–phi001	0	KRW10	mzetc34–umc1053	0	3.19	0.9286	2.48
		KRW2	nc003–umc2372	0	KRW3	umc2081–bnlg1257	0	7.89	1.2488	5.13
		KRW2	umc1637–umc1497	4	KRW7	umc1016–phi034	0	5.01	1.4746	7.15
		KRW2	umc1525–phi10104	24	KRW3	phi029–bnlg1452	6	4.67	1.1253	4.17
		KRW3	umc2050–phi046	28	KRW9	bnlg1525–umc1733	4	3.64	1.0725	3.78
		KRW3	umc2277–bnlg1496	6	KRW4	umc1109–phi076	20	4.38	1.049	3.62
		KRW4	umc1232–umc1017	10	KRW7	mmc0411–bnlg1305	0	3.8	–1.0792	3.83
		KRW4	umc2287–umc1989	14	KRW5	phi024–umc2036	12	3.22	–0.8172	2.2
		KRW4	umc1989–umc2011	0	KRW10	bnlg1450–bnlg1518	16	3.5	–0.9093	2.72
		KRW5	umc1260–phi024	10	KRW5	umc2115–phi113	0	5.49	–1.3557	6.05
		KRW5	phi048–bnlg1306	4	KRW10	umc1993–phi32315	4	8.05	–1.7711	10.32
		KRW6	phi45269–phi078	4	KRW8	phi115–umc1460	4	4.79	–1.2082	4.8
		KRW7	bnlg339–umc1865	0	KRW9	umc1771–umc1494	10	4.4	–1.0295	3.49
	KWP	KWPW1	bnlg1014–phi42791	2	KWPW10	bnlg640–umc1336	2	4.13	7.588	7.24
		KWPW1	umc2025–umc1124	0	KWPW4	phi072–umc1232	0	3.67	–6.1387	4.74
		KWPW5	phi048–bnlg1306	0	KWPW7	mmc0411–bnlg1305	0	5.88	7.5877	7.24
		KWPW6	umc1018–bnlg1538	6	KWPW7	phi057–umc1016	8	4.74	–8.4472	8.97
		KWPW6	bnlg1154–phi45269	2	KWPW7	mmc0171–phi057	2	4.72	6.5756	5.43
WS	EL	ELS2	umc1635–umc1028	8	ELS3	umc2166–umc1539	4	3.35	–0.5112	3.08
		ELS3	umc2002–bnlg1035	2	ELS5	umc1792–umc1829	12	3.06	–0.4907	2.83
		ELS3	bnlg1047–phi10228	4	ELS3	phi047–umc1136	0	4.72	–0.5765	3.91
		ELS4	bnlg1621–bnlg2291	12	ELS10	phi059–umc2069	0	6.09	–0.795	7.44
		ELS4	umc2365–phi093	6	ELS4	umc1173–umc2287	2	4.72	–0.4689	2.59
	KR	KRS1	umc1354–phi097	0	KRS1	umc1590–umc2151	2	5.77	–1.4742	5.5
		KRS1	phi001–bnlg1083	6	KRS5	bnlg278–phi048	0	4.73	1.1217	3.19
		KRS1	umc1590–umc2151	2	KRS1	umc1774–phi26545	10	5.07	–1.2642	4.05
		KRS1	umc1122–bnlg1556	10	KRS4	phi079–phi026	0	4.06	–1.1151	3.15
		KRS1	bnlg1597–umc2149	0	KRS3	phi10228–bnlg1350	8	5.83	–1.4032	4.98
		KRS2	nc003–umc2372	2	KRS3	umc2081–bnlg1257	2	4.87	1.3421	4.56
		KRS3	umc1746–phi45312	0	KRS4	bnlg292–umc1173	2	5.29	–1.2973	4.26
		KRS4	umc1511–umc1896	0	KRS8	phi065–umc1741	0	3.79	–0.9657	2.36
		KRS4	umc2365–phi093	6	KRS4	umc2287–umc1989	6	6.67	–0.9817	2.44
		KRS4	umc1109–phi076	20	KRS7	bnlg1380–bnlg1792	0	3.76	1.034	2.71
		KRS6	bnlg1538–bnlg391	8	KRS7	umc1103–umc2197	0	5.33	–1.4445	5.28
	WK	WKS1	phi42791–bnlg1614	0	WKS10	umc2067–bnlg640	0	7.02	1.2733	7.99
		WKS1	umc1122–bnlg1556	10	WKS3	umc2002–bnlg1035	6	3.67	–0.9101	4.08

Table 3. (continued)

Treatment	Trait	QTL[a]	Interval	cM[b]	QTL	Interval	cM	LOD	AA	H^2_{AA}[c]
		WKS2	nc003–umc2372	2	WKS9	bnlg127–bnlg1209	0	4.33	–0.9462	4.41
		WKS2	mmc0271–bnlg1633	0	WKS3	umc2277–bnlg1496	2	5.18	–1.1301	6.30
		WKS2	mmc0271–bnlg1633	8	WKS3	phi036–nc030	0	5.21	–1.1111	6.09
		WKS4	umc1294–umc2082	12	WKS10	umc1061–umc2122	0	4.25	0.9858	4.79
		WKS4	umc1953–bnlg1621	0	WKS10	bnlg1451–phi052	0	3.13	–0.8508	3.57
		WKS7	umc1241–umc1426	10	WKS8	phi080–umc1069	0	7.36	1.3327	8.76
	KWP	KWPS1	bnlg1614–phi001	4	KWPS10	bnlg1716–umc2067	0	5.95	7.0809	7.49
		KWPS1	umc2112–umc2025	12	KWPS2	phi083–umc2032	4	4.71	4.9759	3.70
		KWPS1	umc1335–umc1122	8	KWPS4	umc2082–umc2176	16	5.23	–7.0177	7.35
		KWPS1	bnlg1597–umc2149	0	KWPS6	bnlg391–umc1178	6	4.31	5.5194	4.55
		KWPS4	umc2365–phi093	6	KWPS4	umc2287–umc1989	12	7.66	–5.6047	4.69
		KWPS7	mmc0171–phi057	20	KWPS7	bnlg1305–bnlg339	0	4.78	–5.3137	4.22

[a]QTLs were named using the trait abbreviations plus water treatment (w, well-watered; s, water-stressed) plus chromosome number.

[b]The distance given is from the left marker to the putative QTL.

[c]H^2_{AA} is the contribution from the epistatic QTL.

EL, ear length; KR, kernal number per row; WK, 100-kernal weight; KWP, kernal weight per plant; LOD, logarithm of odds; AA, epistatic effect.

genetic mechanism of the drought tolerance of maize is complex.

Some additive and epistatic QTL identified were similar under the two water conditions, one QTL in the interval umc2256–phi10412 for ear length, two additive QTLs in the interval sumc1003–umc1635 and umc1426–bnlg2132 and one pair of epistatic QTL between the interval nc003–umc2372 and umc2081–bnlg1257 for kernel number per row, and one additive QTL for kernel weight per plant. However, most of QTL under the different water treatments showed different locations and effects. It may be that genetic expression differs under the two water regimens. Moreover, the contribution of the QTL to phenotype values differed in the two water regimens. The maximum contribution of additive QTL to ear length was 9.23% under well-watered conditions and 11.91% under water-stressed conditions. The highest contribution of additive and epistatic QTL to kernel number per row was 11.778% and 10.320% under well-watered conditions, respectively, and 9.2% and 5.5% under water-stressed conditions, respectively. The highest contribution of additive and epistatic QTL to 100-kernal weight was 10.61% and 8.76%, respectively, under water-stressed conditions, whereas the contribution of additive QTL under well-watered conditions was 7.38%.

Interval and effect of the additive and epistatic QTL differ from trait to trait under water-stressed conditions. Both additive and epistatic QTL existed for kernel number per row, but only epistatic QTL existed for 100-kernal weight and ear length and only additive QTL existed for kernel weight per plant. The contribution of additive and epistatic QTL differed among traits. The maximum and minimum contributions of additive QTL were 11.91% and 4.56%, respectively, for ear length, 9.20% and 5.07%, respectively, for kernel number per row, 10.61% and 5.86%, respectively, for 100-kernal weight, and 53.0% and 10.7%, respectively, for kernel weight per plant. The contribution of epistatic QTL to ear length, kernel number per row, 100-kernal weight and kernel weight per plant was 2.59%–7.44%, 2.36%–5.50%, 3.57%–8.76%, and 3.70%–7.49%, respectively. These results indicate that the genetic mechanisms may be different among traits and that studies of the drought tolerance of maize should distinguish different traits.

Discussion

Mapping of QTL and genetic effect of yield components in the two water environments

Drought, or water stress, is one important ecological factor influencing maize production. Studies of maize drought tolerance are the focus of considerable attention. The mapping and tagging of QTL form the basis of MAS. The identification of QTL for maize drought tolerance has been investigated in many studies and some progress has been made (Agrama et al. 1996; Ribaut et al. 1996, 1997; Frova et al. 1999; Sanguineti et al. 1999; Sari-Gorla et al. 1999; Gao et al. 2003; Li et al. 2003, 2004; Zhang et al. 2004). However, most of these previous studies have been based on the additive-dominant genetic model on the assumption that there is no interactive effect among the alleles. In fact, an interactive action does exist, especially for complex quantitative traits, such as drought tolerance. Hence, it is necessary to detect epistatic QTL and analyze the epistatic effect (Zhu 1997, 1998; Wang et al. 1999b).

Yuyu 22 is planted in large areas throughout China and the

Table 4. Rainfall, temperature, and evaporation during the period of maize growth and development

Months	Rainfall (mm)	Compared with past years (%)	Temperature (°C)	Compared with past years (%)	Evaporation (mm)
5	15	24	16	0	176.0
6	10	–61	21	1	198.9
7	16	–47	23	2	219.9
8	23	–23	20	0	177.1
9	2	–86	16	1	144.7

drought tolerance of the male parent 87-1 was stronger than that of the female parent Zong 3. The linkage map constructed by 261 polymorphic markers has higher density and the average length between two markers was found to be 10.499 cM. The mixed linear model is an effective method to analyze complex quantitative traits. The QTL results of maize yield and its components in the two water treatment groups at flowering time showed that the interval, effect, and contribution of the additive and epistatic QTL under water-stressed conditions were different from those under well-watered conditions. The primary reason for this may be that the expression of genes differs under the two water regimens. Some genes expressed under water-stressed conditions are not expressed under well-watered conditions and *vice versa*. The interval, effect, and contribution of the additive and epistatic QTL for each trait under water-stressed conditions also differed from trait to trait. This is in accord with the breeding practice of maize drought tolerance. Comparisons with other studies indicate that one QTL at marker phi093 on chromosome 7 for kernel number per row and kernel weight per plant, respectively, is close to the QTL reported by Frova (1999).

Genetic mechanism and complexity of drought tolerance of maize yield components

Improving maize yield under drought conditions is a chief target for maize breeding. The QTL results showed that most of QTL under different water treatments differed, except for one additive QTL for ear length, two additive QTLs and one pair of epistatic QTLs for kernel number per row, and one additive QTL for kernel weight per plant. It is concluded that the genetic expression differs under the two water regimens. This is consistent with results of other studies (Rebaut et al. 1997; Li et al. 2003) indicating that yield and drought tolerance are controlled by different sets of genes (Johnson and Geadelmann 1989; Atlin and Frey 1990; Li et al. 2004). Differences in the additive and epistatic QTL between traits under water-stressed conditions indicate that the genetic expression also differs from trait to trait. These results could be confirmed by the different drought tolerance of different traits in breeding programs of drought-tolerant maize. Furthermore, major and minor QTL were detected for all traits, except kernel number per row with a 10% contribution as the standard for major and minor QTL. The result that additive and epistatic QTL exist and major and minor QTL contribute to phenotype indicates that the genetic basis of maize drought tolerance is special and complicated. The popular of epidemic QTLs implied it required new idea to study maize drought tolerance from gene viewpoint.

Molecular marker and evaluation of maize drought tolerance

Whether the molecular marker technique could be applied to maize breeding depends mostly on the development of molecular marker techniques and QTL mapping. In recent years, the QTL mapping of maize drought tolerance has been finished for some different populations by different researchers. These studies have laid a foundation for MAS breeding of drought-tolerant maize. However, the drought tolerance of maize is a complicated quantitative trait that is influenced by many factors (e.g. the timing of the drought, the severity of the drought, the duration of the drought and the type of drought). Thus, evaluation of drought tolerance is crucial to confirm the accuracy of the molecular markers used, in addition to the density of the markers and the scale of the population and mapping methods. In the meantime, the relationship between water consumption and crop growth should be further investigated and irrigation methods should also be improved. The maturity and height of the family should be considered in future studies. It is conceivable that MAS will play an important role in drought tolerance breeding, along with the development of molecular marker techniques and the improvement of the identification and evaluation of the drought tolerance of maize.

Materials and Methods

Plant materials

Two hundred and twenty-one RIL randomly selected from a population consisting of 294 F_{10} families derived from the maize (*Zea mays* L.) hybrid Yuyu 22 (Zong 3 × 87-1) were used in the present study. The drought tolerance of the female parent Zong 3 was weaker than that of the male parent 87-1 according to

experiments performed over the past few years by our laboratory. Therefore, the population constructed by our laboratory was chosen for QTL mapping.

DNA preparation and SSR analysis

Fresh leaves from individual plants of each family were obtained from seedlings and preserved at –70 °C in a refrigerator after being frozen in liquid nitrogen. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) procedure (Saghai-Maroof et al. 1984) and 552 pairs of primer equally covering the entire genome were selected from online at http://www.maizegdb.org. Two hundred and sixty-one primers with different fragments for the parents were used to amplify the RIL of Yuyu 22 by polymerase chain reaction. The amplification products were separated by electrophoresis on a 6% undenatured polyacrylamide gel and the gel was silver stained.

Design of field experiments and evaluation of the drought tolerance of maize

Field trials were performed at Zhangye Maize Seed Farm, Gansu Province, China. The climate was drought, warm, and windy. Rainfall is limited and mostly ineffective for crop production because of the high evaporation. It is a region typically suitable for the evaluation of the drought tolerance of maize. The rainfall, temperature, and evaporation for this region in 2004 are given in Table 4.

The experiment was designed using two water treatments: well watered and water stressed. Each treatment was arranged in a complete random block design with three replications. Ten plants of each genotype were grown in rows that were 3.00 m × 0.67 m (length × width). The two treatments were managed using the same farming procedures, except for the irrigation. Both treatment groups were irrigated once before sowing. In order to provide sufficient water, the well-watered treatment group was irrigated six times at an interval of 15–20 d depending on the soil water content and plant growth conditions, maintaining soil moisture at 28.76% on a dry weight basis. The amount of irrigation for the well-watered treatment group was set at 70 m^3/666.7 m^2, which was controlled by a water meter, on the basis of the results of a study of the water requirements of crops using different plant models in a desert oasis (Su 2002). The water-stressed treatment group was stressed at flowering time by adjusting the irrigation before flowering and postponing irrigation after flowering, soil moisture at 0–10, 10–20, 20–30, and 30–40 cm being 3.73%, 7.50%, 8.14%, and 8.15% on a dry weight basis, respectively. The water management of this group during the other growth periods was the same that for the well-watered group.

Treatment measurements and data analysis

Eight plants per row were harvested after reaching maturity. Ear length, row number, kernels per row, and 100-kernel weight on standard individual plants were determined and means calculated. Kernel weight per plant was determined when the water content was below 13%.

Phenotypic data for the RIL population was processed using SAS (SAS Institute, Cary, NC, USA) programs. For the SSR data, fragments amplified from the male parent Zong 3 and the female 87-1 was scored as 1 and 2, respectively, whereas hyterozygosity and missing fragments were scored as 3 and 0, respectively. The genetic linkage map was constructed using MAPMAKER/EXP3.0. The QTLs were detected using QTLMAPPER1.6 (Zhejiang University, Hangzhou, China) according to the mixed linear model and the test standard is an LOD > 3.0 and $P < 0.005$ (Zhu 1997, 1998; Wang et al. 1999a).

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(Managing editor: Wei Wang)