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QTL analysis for yield-related traits under different water regimes in maize

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KeyWords *Maize, QTL, ear-related trait, kernel-related trait, drought*

Abstract

Drought is one of the most essential factors influencing maize yield. Improving maize varieties with drought tolerance by using marker-assisted or genomic selection requires more understanding of the genetic basis of yield-related traits under different water regimes. In the present study, 213 $F_{2:3}$ families of the cross of H082183 (drought-tolerant) × Lv28 (drought susceptible) were phenotyped with five yield-related traits under four well-watered and six drought environments for two years. Quantitative trait loci analysis identified 133 significant QTLs (94 QTLs for ear traits and 39 QTLs for kernel traits) based on single environment analysis. The joint-environment analysis detected 25 QTLs under well-watered environments (eight QTLs for ear length, eight for ear diameter, one for ear weight, two for kernel weight per ear, and six for 100-kernel weight), and nine QTLs under water-stressed environments (two QTLs for ear length, three for ear diameter, one for ear weight, one for kernel weight, and two for 100-kernel weight). Among these joint-environment QTLs, one common QTL (*qEL5*) was stably identified at both of the water regimes. Meanwhile, two main-effect QTLs were detected in the well-watered environments, i.e. *qEL10* for ear length and *qHKW2* for 100-kernel weight. Also, *qED8*, *qEW8* and *qKW8* were found to be located in the same interval of Chr. 8. Similarly, *qEL4s* and *qKW4s* were found to be located in the same interval under water-stressed environments. These genomic regions could be candidate targets for further fine mapping and marker-assisted breeding in maize.

Introduction

Drought is one of the main constraints to crop production throughout the world (Boyer, 1982). Daryanto et al (2016) found that maize had a yield reduction of 39.3% at approximately 40% water reduction, indicating that it was very sensitive to drought, particularly during the reproductive phase. Due to the global climate change, the frequency of drought disaster occurrence will increase in the world, resulting in the big fluctuation of maize yield and thus instability of food security (Li et al, 2009).

Development of improved maize genotypes with drought tolerance is one important approach to ameliorating the yield reduction under drought because genetic improvement can probably close 20-25% of the yield gaps between drought-affected and optimal conditions (Edmeades, 2013). In the past, much breeding research was conducted to improve performance under drought conditions, with some achievements (Campos et al, 2004). Conventional selection by CIMMYT specifically for drought tolerance by focusing on yield and associated secondary traits has resulted in a gain of around 100 kg/ha/yr, in tropical maize populations (Edmeades, 2013). However, the breeding progress of drought tolerance improvement has been slow as the decreasing heritability of phenotypes under drought stress (Messmer et al, 2009). Marker-assisted selection (MAS) is now having a significant impact, and when well executed could double gains from conventional drought tolerance selection (Edmeades, 2013). Thus, more understanding of the genetic basis of yield-related traits under different water regimes is necessary for molecular breeding for drought tolerance (Mir et al, 2012).

Because of the extremely complicated genetic basis of yield, the stability of crop performance under drought-stressed conditions is low (Tuberosa et al, 2002). Genetic dissection of yield-related traits such as ear traits and kernel traits may be helpful to breed for drought-tolerant maize hybrids due to the high correlations between the traits of kernel structure and yield components (Li et al, 2009). Actually, a lot of papers regarding quantitative trait loci (QTLs) of those yield-related traits in maize have been published since the advent of molecular markers (Ajnone-Marsan et al, 1995; Austin and Lee, 1996; Berke and Rocheford, 1995; Chen et al, 2016; Chen et al, 2017; Frova et al, 1999; Jiang et al, 2015; Karen Sabadin et al, 2008; Li et al, 2013; Li et al, 2017; Liu et al, 2014; Messmer et al, 2009; Peng et al, 2011; Raihan et al, 2016; Stange et al, 2013; Veldboom and Lee, 1994; Veldboom and Lee, 1996; Yan et al, 2006; Yang et al, 2016; Zhang et al, 2014) . Especially, a number of QTLs conferring ear- and kernel-traits have been detected by using phenotypic data obtained under different water regimes, which provide valuable information to MAS and genomic selection (Guo et al, 2011; Nikolić et al, 2011; Nikolić et al, 2013; Prasanna et al, 2009; Ribaut et al, 1997; Tan et al, 2011). For example, Almeida et al (2013) identified a total of 83 QTLs through the single environment analyses and seven meta-QTL (mQTL) for grain yield by using three tropical populations, among which six mQTLs on Chr. 1, 4, 5 and 10 for GY were constitutively expressed across water stressed (WS) and well-watered (WW) environments. Semagn et al (2013) identified 59 meta-QTLs for grain yield (GY) across 18 bi-parental maize populations evaluated in the same conditions across 2-4 managed water stressed and 3-4 well-watered environments, among which the four mQTLs (mQTL2.2, mQTL6.1, mQTL7.5 and mQTL9.2) could be considered for fine mapping. But they found that few QTL were detected under both environmental treatments and/or multiple (> 4 populations) genetic backgrounds. Zhao et al (2018) identified 52 QTLs under water-stressed conditions, among which 21 were validated to be stable under multiple water-stressed conditions. They also identified 36 meta-QTLs across 26 populations under 52 well-watered and 38 water-stressed conditions by using a meta-analysis.

However, the genetic and molecular mechanisms of yield-related traits have still been poorly understood in maize until now. Because the majority of detected QTLs for yield-related traits could explain only a small percentage of phenotypic variation and could not be refined in different environments and populations (Bernier et al, 2008), more investigations on genetic dissection of yield-related traits under different water regimes are required to provide valuable information to geneticists and breeders. Therefore, the main aims of this study were to (1) detect and map QTLs controlling yield-related traits under normal and water-stressed conditions using an $F_{2:3}$ population across multiple environments and evaluate their effects, and (2) dissect QTL by QTL interaction (epistasis) for these traits. The results obtained in this research could contribute to the development of effective approaches for fine mapping and breeding in maize in the future.

Materials and Methods

Field experiments and trait valuations

Totally, 213 $F_{2:3}$ families resulted from the cross of H082183 (a drought-tolerant inbred) and Lv28 (a drought-sensitive inbred) were planted at four locations in 2017, i.e. Changping of Beijing (N39°54', E116°23'), Zhangye of Gansu (N38°56', E100°27'), Bayannur of Inner Mongolia (N40°43', E107°24') and Xinxiang of Henan (N35°18', E113°55'), and four locations in 2018, i.e. Changping, Hohhot of Inner Mongolia (N40°83', E111°73'), Dingxiang of Shanxi (N38°50', E112°95'), and Zhangjiakou of Hebei (N40°82', E114°88'). At the locations of Zhangye and Bayannur in 2017, there were two irrigation schemes, i.e. normal irrigation and half-amount irrigation that half of the water amount for normal irrigation was applied at the time point of irrigation. At the locations of Changping, Zhangjiakou, Huhehot and Dingxiang in 2018, no irrigation was applied except for the sowing irrigation to simulate the rain-fed cultivation. Thus, a total of six drought environments (Zhangye-2017S, Bayannur-2017S, Changping-2018, Hohhot-2018, Dingxiang-2018, and Zhangjiakou-2018) and four well-watered environments (Changping-2017, Zhangye-2017W, Bayannur-2017W, and Xinxiang-2017) were set, with two replicates. The randomized block design with two-row plots was used in all the experiments. The rows were set to 3 m in length and 0.6 m apart, including 11 plants in each row.

Five yield-related traits were investigated in this study: ear length (EL), ear diameter (ED), ear weight (EW), kernel weight per ear (KW), and hundred-kernel weight (HKW). Five ears in each row were selected for traits measurements. EL, ED, EW and KW were measured manually. The method of HKW measurement is referred to as Li et al (2013).

Statistical analysis

The variance and ANOVA analysis of phenotypic data were performed using SAS v9.1. The broad-sense heritability (h^2) for each trait was calculated based on the formula: $h^2 = \sigma_g^2/(\sigma_g^2+\sigma_{ge}^2/e + \sigma_e^2/re)$, where σ_g^2 is the genotypic variance, σ_{ge}^2 is the interaction between genotype and environment, σe is the variance of experimental error, e is the environment's number and r is the replications number per environment (Hallauer

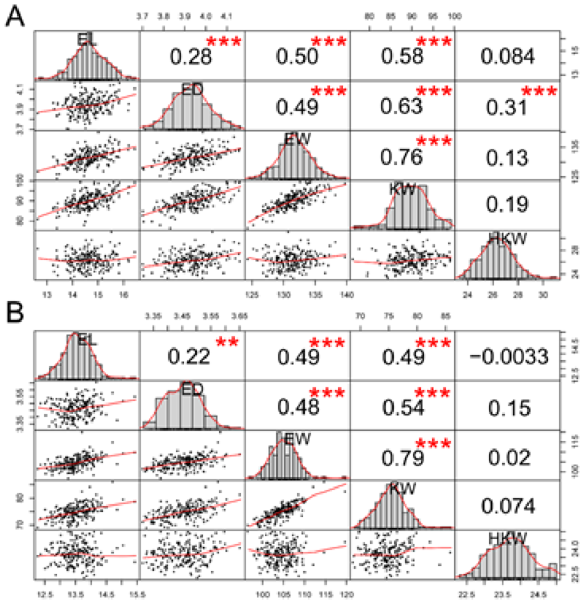

Fig. 1 - The correlations between different traits. (A) The correlations between different traits across the four well-watered environments. (B) The correlations between different traits across the six water-stressed environments. The numbers in the upper right panel refer to the correlation coefficients between the five traits. **: *P* < 0.01, *: *P* < 0.001.**

and Miranda, 1988). Pearson's correlation coefficient between different traits was calculated using R v3.5.1.

Genotyping and bin-map construction

The genotyping and bin-map construction of H082183 × Lv28 $F_{2:3}$ population was published in our previous study (Liu et al, 2019). Briefly, the DNA of each family was isolated using CTAB. The SNPs were genotyped by Axiom Maize 55K SNP Array (Xu et al, 2017). After filtering out the monomorphic SNPs, low-quality SNPs and segregation distortion SNPs, a modified hidden Markov model (HMM) was used for bin-map construction. The genetic map was constructed using IciMapping v4.1 (Meng et al, 2015).

QTL analysis

QTL mapping was conducted using the inclusive composite interval mapping (ICIM) method of IciMapping v4.1 (Meng et al, 2015). BLUP values of each trait under drought and well-watered treatments were used for QTL analysis, separately, using SAS v9.1 (Henderson, 1975). The logarithm of odds (LOD) threshold (LOD > 3.24) was determined by 1000 permutations test at $P < 0.05$. The confidence interval of QTL was confirmed by 2.5-LOD drop method. The epistatic QTL detection was performed using the model of EPI in IciMappingv4.1 (Meng et al, 2015), with a threshold of LOD > 5.

Results

Phenotypic variation

The ear length (EL), ear diameter (ED), ear weight (EW), kernel weight per ear (KW), and hundred-kernel weight (HKW) of the H082183 × Lv28 $F_{2:3}$ population were measured under four well-watered environments and six drought environments. ANOVA analysis showed that the genotypic effects were significant (P < 0.05)

Table 1 - The distribution and heritability of different yield-related traits.

Treatment	Trait [a]	Parent		Population							
		H082183	Lv28	Mean	SD[b]	Range	Skew	SE[c]	G[d]	G×E[e]	H^2[f]
Well-watered	EL (cm)	13.69	12.16	14.69	1.43	9.40-19.68	-0.11	0.03	5.6**	1.7**	0.72
	ED (cm)	3.68	3.72	3.94	0.25	3.00-5.80	0.03	0.01	0.14**	0.06**	0.64
	EW (g)	125.93	104.01	131.62	58.91	43.40-444	1.67	1.44	1327.81ns	976.18ns	0.28
	KW (g)	69.28	59.68	90	20.16	25.33-161.31	0.05	0.49	521.03**	290.46**	0.51
	HKW (g)	28.76	27.32	26.28	4.35	13.27-41.26	0.22	0.09	26.85**	9.72**	0.69
Water-stressed	EL (cm)	13.34	11.66	13.57	1.93	4.00-19.44	-0.29	0.04	6.34**	2.39**	0.65
	ED (cm)	3.46	3.33	3.46	0.49	1.07-5.60	-0.13	0.01	0.28**	0.10ns	0.54
	EW (cm)	107.86	83.97	104.7	52.81	23.33-372.00	1.93	1.05	1415.52**	898.79ns	0.39
	KW (g)	76.23	60.26	75.26	21.8	13.80-188.80	0.26	0.43	559.29**	314.32ns	0.46
	HKW (g)	26.28	25.44	23.68	4.38	11.87-47.10	1.2	0.09	25.15**	16.47**	0.47

[a] EL: ear length, ED: ear diameter, EW: ear weight, KW: kernel weight per ear, HKW: hundred kernel weight.

[b] Standard deviation.

[c] Standard error

[d] Genotype. **: *P* < 0.01, ns: not significant

[e] Genotype×environment interaction. **: *P* < 0.01, ns: not significant

[f] Broad-sense heritability.

Table 2 - QTLs identified by the joint-environment analysis

Treatment	Trait [a]	QTL	Chr	Interval (cM)	Physical position (Mb) [b]	LOD [c]	PVE (%) [d]	Add [e]	Dom [f]
Well-watered	EL	*qEL1-1*	1	32.01-32.72	17.90-18.63	5.97	4.88	0.2	0.08
	EL	*qEL1-2*	1	212.99-213.23	298.59-299.17	5.11	4.17	-0.21	0.01
	EL	*qEL3*	3	116.88-117.82	180.73-181.9	4.3	3.47	-0.18	0.1
	EL	*qEL5*	5	50.98-51.93	22.89-23.46	4.42	3.59	0.2	0.03
	EL	*qEL7-1*	7	67.07-68.96	125.06-125.32	6.08	5.05	0.21	0.05
	EL	*qEL7-2*	7	125.63-125.86	162.66-162.88	10.15	8.76	-0.29	0.01
	EL	*qEL8*	8	91.76-92.47	160.13-160.22	9.48	8.15	0.29	0.01
	EL	*qEL10*	10	47.91-48.14	113.16-114.42	19.28	18.11	0.43	0.09
	ED	*qED1-1*	1	42.01-43.67	25.49-27.35	5.9	7.93	0.04	0.01
	ED	*qED1-2*	1	91.95-92.42	150.40-157.92	6.21	6.57	0.04	0.01
	ED	*qED1-3*	1	174.95-176.13	273.88-274.03	3.83	3.97	-0.03	0
	ED	*qED3*	3	142.1-143.76	236.09-237.12	8.89	12.26	-0.04	0.01
	ED	*qED4-1*	4	34.68-35.39	10.45-10.75	4.02	5.25	-0.03	0.01
	ED	*qED4-2*	4	56.65-57.35	99.96-100.07	5.19	6.87	0.03	-0.01
	ED	*qED5*	5	0.71-1.18	1.53-1.55	5.38	5.7	-0.03	-0.01
	ED	*qED8*	8	73.59-74.54	144.12-144.77	7.48	7.98	0.04	0.01
	EW	*qEW8*	8	73.59-74.54	144.12-144.77	5.37	12.08	6.17	-1.65
	KW	*qKW8*	8	73.59-74.54	144.12-144.77	4.11	8.26	7.87	4.09
	KW	*qKW10*	10	0-2.14	1.92-2.14	3.5	6.55	4.82	9.27
	HKW	*qHKW1-1*	1	19.4-20.35	9.57-10.06	3.8	4.34	0.37	-0.27
	HKW	*qHKW1-2*	1	69.21-70.86	52.65-54.39	3.92	4.62	0.42	0.02
	HKW	*qHKW2*	2	63.39-64.81	39.78-40.48	19.34	26.89	-1.05	-0.07
	HKW	*qHKW4*	4	137.86-138.33	205.83-217.94	3.64	4.23	0.38	0
	HKW	*qHKW7*	7	103.85-104.8	153.08-153.27	7.07	8.5	0.57	0.06
	HKW	*qHKW9*	9	36.66-39.28	20.38-20.90	5.69	6.74	0.46	0.21
Water-stressed	EL	*qEL4s*	4	145.17-145.64	237.35-237.63	3.86	6.32	-0.16	-0.05
	EL	*qEL5*	5	50.98-51.93	22.89-23.46	4.26	7.02	0.2	0.02
	ED	*qED3s*	3	129.12-129.35	199.50-199.74	4.62	7.63	0.02	0.01
	ED	*qED4s*	4	135.74-136.69	197.64-197.96	5.77	9.82	-0.03	0
	ED	*qED8s*	8	65.35-66.06	119.22-121.62	3.72	6.26	0.02	0
	EW	*qEW1s*	1	76.04-76.75	66.62-68.75	5.37	10.35	12.3	9.62
	KW	*qKW4s*	4	145.17-145.64	237.35-237.63	4.1	8.05	-0.99	0.26
	HKW	*qHKW2s*	2	61.51-62.45	37.17-38.76	3.49	6.45	-0.23	-0.03
	HKW	*qHKW7s*	7	88.55-89.02	137.83-138.42	6.41	12.14	0.3	0.04

[a] EL: ear length, ED: ear diameter, EW: ear weight, KW: kernel weight per ear, HKW: hundred kernel weight.

[b] The physical posotion referred to B73_RefGen_v3.

[c] Logarithm of odds for each QTL.

[d] Phenotypic variation explained.

[e] Positive value indicated the additive effect was derived from H082183.

[f] Positive value indicated the dominant effect was derived from H082183

for all traits under both well-watered and water stress environments (Table 1). EL, ED, KW, HKW under well-watered environments and EL and HKW under drought environments were significantly affected by the interaction of genotype and environment. Under well-watered environments, the broad-sense heritability (H^2) of EL was the highest (72%), followed by HKW (69 %), ED (64%), KW (51%), and the H^2 of EW was the lowest (28%). Under drought environments, the *H2* of EL was also the highest (65%), followed by ED (54 %), HKW (47%), KW (46%) and EW (39%). EL, ED, EW and KW showed a significant correlation ($P < 0.01$) under both well-watered and drought treatments (Figure 1). KW and EW showed a strongest significant correlation under well-watered ($r = 0.76$) and drought ($r = 0.79$) conditions ($P < 0.001$).

QTL detection for the five yield-related traits based on single environment analysis

For the five yield-related traits, a total of 133 QTLs were detected based on the analysis of single environment, including 35 QTLs for EL, 41 QTLs for ED, 18 QTLs for EW, 24 QTLs for HKW, and 15 QTLs for KW, with the total explained phenotypic variation varying from 0.94 % to 26.64 % (Table S1). The 133 QTLs were located on 10 maize chromosomes.

With regard to EL, thirty-five QTLs were found on the ten chromosomes across the ten environments. The interval length for these QTLs varied from 0.03 Mb on Chr. 1 to 6.66 Mb on Chr. 9, whereas the ranges of LOD and PVE were 3.31-15.98, and 2.50-17.66%, respectively.

For ED, forty-one QTLs were found out on the all chromosomes except Chr. 6 and Chr. 7 in all environments except for Dingxiang of Shanxi. The interval length of these QTLs ranged from 0.02 Mb on Chr. 5 to 5.35 Mb on Chr. 8, while the LOD range was 3.43-13.55 and PVE range was 2.83-17.99%.

Concerning to EW, eighteen QTLs were found on five chromosomes (1, 3, 4, 8 and 10). The interval length of these QTLs varied from 0.06 Mb to 5.85 Mb on Chr. 4 and Chr. 1, respectively, whilst the range of LOD was 3.44-6.37 and the range of PVE was 1.04-12.3 %.

Respecting KW, fifteen QTLs were found on chromosomes 1, 3, 4, 8 and 10 (Table S1). The range of the interval length was 0.09-2.71 Mb, whereas the ranges of LOD and PVE were 3.26-5.25 and 0.94-9.61%, respectively.

As for HKW, twenty-four QTLs were found on all the 10 chromosomes except for Chr. 4 and Chr. 6. The range of the interval length was 0.12-4.15 Mb, whereas the LOD and PVE ranges were 3.28-49.01 and 1.77-26.64%, respectively.

QTL detection for the five yield-related traits based on joint environment analysis

Based on the joint QTL analysis across the four well-watered environments, eight significant EL QTLs were identified on six chromosomes (1, 3, 5, 7, 8 and 10) (Table 2). The LOD range was 4.3-19.28 and the range of PVE was 3.47-18.11 %. Eight ED QTLs were identified on five chromosomes (1, 3, 4, 5 and 8) where the range of LOD was 3.83-8.89 and the PVE range was from 3.97 to 12.26 %. One QTL for EW was identified on Chr. 8. Two KW QTLs were identified on chromosomes 8 and 10. The LOD and PVE ranges were 3.5-4.11% and 6.55-8.26%, respectively. Six QTLs for HKW were found on five chromosomes (1, 2, 4, 7 and 9). LOD and PVE ranges were 3.64-19.34 and 4.23-26.89%, respectively.

The joint QTL analysis across the six water-stressed environments was also conducted (Table 2). Three EL QTLs were identified on three chromosomes (4, 5 and 10), and the LOD range was 3.86-7.42 and the PVE range was 6.32-12.71%. Whereas four ED QTLs were identified on four chromosomes (1, 3, 4 and 8) and the LOD and PVE ranges were 3.72-7.51 and 6.26-12.78%, respectively. One QTL for EW was identified on Chr. 1. Also, one QTL was identified for KW on Chr. 4. Two QTLs for HKW were detected on Chr. 2 and Chr. 7.

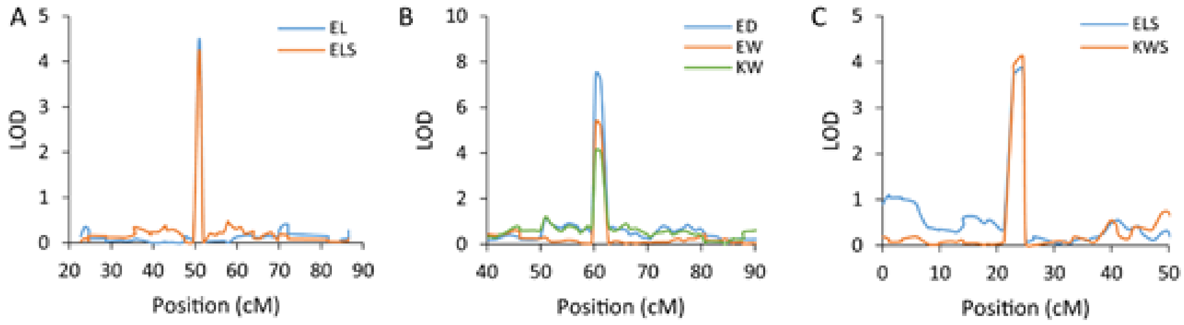

Fig. 2 - The consensus and pleiotropic QTLs. (A) The consensus QTL on Chr. 5 controlled EL in both well-watered and water-stressed conditions. (B) The pleiotropic QTL on Chr. 8 controlled ED, EW and KW in well-watered conditions. (C) The pleiotropic QTL on Chr. 4 controlled EL and KW in water-stressed conditions.

There was one QTL (*qEL5*) (Figure 2A) that could stably be detected under well-watered and water-stressed environments. *qEL5* had LOD of 4.42 and explained 3.59% of phenotypic variation under well-watered environments, and had LOD of 4.26 and explained 7.02% of phenotypic variation under water-stressed environments.

Based on joint QTL analysis across the four well-watered environments and the six water-stressed environments, two pleiotropic loci were identified under both well-watered and water-stressed conditions, respectively. One genomic region harboring *qED8*, *qEW8* and *qKW8*, which were located in bin 8.05 and detected under well-watered environments, had a pleiotropic effect on ED, EW and KW (Figure 2B). The other genomic region harboring *qEL4s* and *qKW4s* (Figure 2C) which were located in bin 4.1 and detected under water-stressed environments, had a pleiotropic effect on EL and KW.

Detection of epistatic effects for the traits

A total of 281 pairs of epistatic interaction loci were found based on single environment analysis (Table S2). Moreover, 18 and 18 pairs of epistatic interaction loci were found in the joint analysis across the four well water environments and six water stress environments, respectively (Table S3).

Discussion

QTL for ear traits

Ear length, ear diameter and ear weight are determined as yield-related traits and essential maize breeding targets (Hallauer and Miranda, 1988). Therefore, the genetic dissection of these traits is important for high yield breeding in maize. In this study, for ear traits, 94 QTLs were identified based on single environment analysis including 35 QTLs for EL, 41 QTLs for ED and 18 QTLs for EW (Table S1), while QTLs were identified based on joint environment analysis including nine QTLs for EL, eleven QTLs for ED and two QTLs for EW (Table 2).

Among the nine QTLs for ear length identified in the present study (Table 2), qEL3 and qEL8 are falling into the intervals of MQTL-22 for kernel-related traits and grain yield and MQTL-58 for ear-, kernel-related traits and yield, respectively, described by Chen et al (2017), while qEL1-1 and qEL7-1 are falling into the intervals of mQTL1-3 for ear length and grain yield and mQTL7-2 for ear length, respectively, described by Zhao et al (2018). The QTL on Chr. 10, qEL10, could explain 18.11% of phenotypic variation with the LOD of 19.28 and might be considered as a main-effect QTL. In fact, the QTL is overlapped with the intervals of qEL1-10-1 for ear length detected by Li et al (2009), qnkw10 for kernel weight and qnkn10 for kernel number by Cai et al (2012), qcEWP2-10-1 for ear weight and qcGWP2-10-1 for grain yield by Yang et al (2012). Thus, qEL10 might be worth fine mapping further.

Among the eleven QTLs for ear diameter identified in this study (Table 2), qED4-2 and qEd8s are falling into the intervals of MQTL-27 and MQTL-56 for ear-, kernel-related traits and yield, respectively, described by Chen et al (2017). qED1-2 and qED4s are falling into the intervals of mQTL1-4 and mQTL4-3 for ear weight and kernel weight, respectively, described by Zhao et al (2018). More detailed investigations are needed to understand the relationship between the ear size and the ear/kernel weight.

In the present study, the kernel weight had high correlation with ear traits, but low correlation with 100-kernel weight (Figure 1). This indicated that the decrease of total kernel weight (grain yield per plant) under drought might be caused by extend of anthesis-silking interval (ASI), inhibition of ear development and reduction of kernel number per plant in this biparental population by the cross of H082183 and Lv28, suggesting these traits are primary selection targets in maize drought tolerance improvement.

QTL for kernel traits

Similarly, kernel weight per ear (KW) and 100-kernel weight (HKW) are very essential yield-related traits in maize. From the single environment analysis, 39 QTLs including15 QTLs for KW and 24 QTLs for HKW were identified (Table S1). Based on the joint environment analysis, two QTLs for KW and six QTLs for HKW were detected across the well-watered environments while one QTL for KW and two QTLs for HKW were detected across the water-stressed environments (Table 2).

qHKW7, *qHKW9* and *qHKW7s* for 100-kernel weight are falling into the intervals of *MQTL-50* for ear-, kernel-related traits and yield, *MQTL-64* for ear-related and kernel-related traits and *MQTL49* for kernel-related traits, respectively, described by Chen et al (2017). *qHKW1-1* and *qHKW2* are falling into the interval of *mQTL1-2* for anthesis-silking interval and *mQTL2-2* for kernel weight, ear length and grain yield, respectively, described by Zhao et al (2018). Particularly, *qHKW2* could explain 26.89% of phenotypic variation with the LOD of 19.34 and might be taken as a main-effect QTL. *qHKW2* is overlapping with the intervals of *Xew2-1* for ear weight and *Xkw2-2* for kernel weight detected by Xiao et al (2005), *kpr2a* for kernel number by Yan et al (2006), *qkw2-1* for kernel width by Yang et al (2016), *qpkw2* for kernel weight by Cai et al (2012), and *kgy2* for grain yield by Lu et al (2006). Therefore, *qHKW2* might also be considered as an important target for fine mapping and maize improvement in the future.

QTLs among yield-related traits

Pleiotropy is the well-established phenomenon of a single gene affecting multiple traits, and QTL analysis has been used to estimate genome-wide pleiotropy

(Paaby and Rockman, 2013). In maize, the pleiotropy is also widespread. For example, Zhou et al (2015) identified a pleiotropic QTL (*qEL7.2*) on Chr. 7 for kernel number per row, ear length and ear weight by using an $F_{2:3}$ population of Ye478 x SL17-1. The same group also found another pleiotropic QTL (*qKNPR6*) on Chr. 6 for kernel number per row, ear length, kernel weight and grain yield (Liu et al, 2012).

In the present study, two pleiotropic QTLs were identified. The first one is the QTL on Chr. 8 where *qED8*, *qEW8* and *qKW8* were co-located in the region of 144.12-144.77 Mb, thus probably simultaneously affecting ear diameter, ear weight and kernel weight per ear (Table 2). Li et al (2011) also found a QTL (*qed8-1*) in the region of 129 - 148 Mb conferring ear diameter. In a larger interval than this region of the pleiotropic QTL obtained in the present study, *qkwid8* for kernel width (Li et al, 2013), *qGPR1-8-1* for kernel number (Li et al, 2009), and *qKR2-8-1* for kernel ration (Yang et al, 2012) were also identified. The second pleiotropic QTL was the one on Chr. 4 where *qEL4s* and *qKW4s* were detected across the water-stressed environments, affecting both of ear length and kernel weight per ear (Table 2). This pleiotropic QTL has overlapped the intervals of *kw4* for kernel weight (Yan et al, 2006), *kgr4* for grain yield and *krs4* for kernel number (Lu et al, 2006). Therefore, it was supposed that these two pleiotropic QTLs could be important candidate targets for maize improvement and are needed to be investigated further.

QTLs for well-watered and water-stressed environments

In the present study, it was found that fewer QTLs for the five yield-related traits were identified in the joint environment analyses across the six water-stressed environments than those across the four well-watered environments (Table 2). For example, six QTLs were detected for EL on 5 chromosomes (1, 3, 7, 8 and 10) across the four well-watered environments, but only two QTLs on Chr. 4 and Chr. 5 across the water-stressed environments. The phenomenon was also evidenced by previous investigations (Almeida et al, 2013; Frova et al, 1999; Messmer et al, 2009; Nikolić et al, 2011; Nikolić et al, 2013; Prasanna et al, 2009; Ribaut et al, 1997; Tan et al, 2011; Zhao et al, 2018). The possible explanation is that water stress affects growth and development of maize plants and results in the decrease of phenotypic variance which is not helpful in QTL identification.

It was notable that *qEL5* on Chr. 5 conferring ear length was detected across both of the water-stressed environments and the well-watered environments. The QTL was overlapped with *wqgy5* for grain yield found by Wei et al (2016). Interestingly, *qEL5* was also overlapped with *qED2-5-1* for ear diameter found by Li et al (2009). This QTL may be an important candidate target for detailed analysis because it seems to be an environment-insensitive QTL.

Epistasis

Epistasis plays a paramount role in the genetic basis of the heterosis, grain yield, and kernel-related traits in maize (Ma et al, 2007; Tang et al, 2010; Yan et al, 2006). A greater understanding of QTL by QTL interaction (epistasis) is decisive for MAS (Mohan et al, 1997). In this study, 256 QTL pairs of the yield-related traits were detected based on individual environment analysis, while 36 QTL pairs were detected based on joint environment analysis, with multiple genetic modes including additive-by-additive, additive-by-dominant and dominant-by-dominant interactions. Peng et al (2011) and Yang et al (2016) also found similar results in their QTL studies for yield-related traits. However, because the epistatic analysis would be more powerful when larger populations are used (Carlborg and Haley, 2004), considerable size of mapping populations in combination with high-density mapping markers are desired to clarify the epistasis of QTLs for quantitative traits such as yield-related traits.

Conclusions

Grain yield-related traits have an extremely complicated genetic mechanism in maize due to their complex genetic networks and strong genotype by environment interactions. In the present study, the joint-environment analysis identified 25 yield-related QTLs under well-watered environment and nine QTLs under water-stressed environment, but only one common QTL for ear length was stably identified at both water regimes. Fortunately, two main-effect QTLs, one for ear length and one for 100-kernel weight were detected in the well-watered environments. Additionally, one QTL on Chr. 8 is supposed to be a pleiotropic QTL conferring ear diameter, ear weight and kernel weight per ear under well-watered environments, while one QTL on Chr. 4 is also probably one pleiotropic QTL conferring ear length and kernel weight per ear under water-stressed environments. These genomic regions could be candidate targets for further fine mapping and marker-assisted breeding in maize.

Acknowledgments

This work was partly supported by the Innovation Program of the Chinese Academy of Agricultural Sciences and the Modern Agricultural Talents Program of the Ministry of Agriculture of P. R. China. Thanks go to Prof. Jiuran Zhao from Maize Research Center of

Beijing Academy of Agricultural and Forestry Sciences for providing seeds of H082183

References

Ajnone-Marsan P, Monfredini G, Ludwig WF, Melchinger AE, Franceschini P, Pagnotto G, et al. 1995. In an elite cross of maize, a major quantitative trait locus controls one-fourth of the genetic variation for grain yield. Theor Appl Genet 90:415–424

Almeida GD, Makumbi D, Magorokosho C, Nair S, Borém A, Ribaut JM, et al. 2013. QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance. Theor Appl Genet 126:583–600

Austin DF, Lee M. 1996. Comparative mapping in F2:3 and F6:7 generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 92:817–826

Berke TG, Rocheford TR. 1995. Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35:1542–1549

Bernier J, Atlin GN, Serraj R, Kumar A, Spaner D. 2008. Breeding upland rice for drought resistance. J Sci Food Agric 88:927–939

Boyer JS. 1982. Plant productivity and environment. Science 218:443–448

Cai H, Chu Q, Gu R, Yuan L, Liu J, Zhang X, et al. 2012. Identification of QTLs for plant height, ear height and grain yield in maize (Zea mays L.) in response to nitrogen and phosphorus supply. Plant Breed 131:502–510

Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR. 2004. Improving drought tolerance in maize: a view from industry. F Crop Res 90:19–34

Carlborg Ö, Haley CS. 2004. Epistasis: too often neglected in complex trait studies? Nat Rev Genet 5:618

Chen L, An Y, Li YX, Li C, Shi Y, Song Y, et al. 2017. Candidate loci for yield-related traits in maize revealed by a combination of metaQTL analysis and regional association mapping. Front Plant Sci 8:2190

Chen L, Li C, Li Y, Song Y, Zhang D, Wang T, et al. 2016. Quantitative trait loci mapping of yield and related traits using a high-density genetic map of maize. Mol Breed 36

Daryanto S, Wang L, Jacinthe PA. 2016. Global synthesis of drought effects on maize and wheat production. PLoS ONE 11:e0156362

Edmeades GO. 2013. Progress in Achieving and Delivering Drought Tolerance in Maize-An Update. ISAAA Ithaca, NY

Frova C, Krajewski P, Di Fonzo N, Villa M, Sari-Gorla M. 1999. Genetic analysis of drought tolerance in maize by molecular markers I. Yield components. Theor Appl Genet 99:280–288

Guo J, Chen Z, Liu Z, Wang B, Song W, Li W, et al. 2011. Identification of genetic factors affecting plant density response through QTL mapping of yield component traits in maize (Zea mays L.). Euphytica 182:409

Hallauer AR, Miranda JB. 1988. Quantitative Genetics in Maize Breeding, 2nd edn, Iowa St. Univ Press Ames, IA

Henderson CR. 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics:423–447

Jiang L, Ge M, Zhao H, Zhang T. 2015. Analysis of heterosis and quantitative trait loci for kernel shape related traits using triple testcross population in maize. PLoS ONE 10:e0124779

Karen Sabadin P, Lopes de Souza Júnior C, Pereira de Souza A, Augusto Franco Garcia A. 2008. QTL mapping for yield components in a tropical maize population using microsatellite markers. Hereditas 145:194–203

Li C, Li Y, Sun B, Peng B, Liu C, Liu Z, et al. 2013. Quantitative trait loci mapping for yield components and kernel-related traits in multiple connected RIL populations in maize. Euphytica 193:303–316

Li H, Yang Q, Gao L, Zhang M, Ni Z, Zhang Y. 2017. Identification of heterosis-associated stable QTLs for ear-weight-related traits in an elite maize hybrid zhengdan 958 by design III. Front Plant Sci 8:561

Li Y, Ma X, Wang T, Li Y, Liu C, Liu Z, et al. 2011. Increasing maize productivity in China by planting hybrids with germplasm that responds favorably to higher planting densities. Crop Sci 51:2391–2400

Li Y, Wang Y, Shi Y, Song Y, Wang T, Li Y. 2009. Correlation analysis and QTL mapping for traits of kernel structure and yield components in maize. Sci Agric Sin 42:408–418

Li Y, Ye W, Wang M, Yan X. 2009. Climate change and drought: a risk assessment of crop-yield impacts. Clim Res 39:31–46

Li YL, Li XH, Li JZ, Fu JF, Wang YZ, Wei MG. 2009. Dent corn genetic background influences QTL detection for grain yield and yield components in high-oil maize. Euphytica 169:273–284

Liu R, Jia H, Cao X, Huang J, Li F, Tao Y, et al. 2012. Fine mapping and candidate gene prediction of a pleiotropic quantitative trait locus for yield-related trait in Zea mays. PLoS ONE 7:e49836

Liu X, Hao L, Kou S, Su E, Zhou Y, Wang R, et al. 2019. High-density quantitative trait locus mapping revealed genetic architecture of leaf angle and tassel size in maize. Mol Breed 39:7

Liu Y, Wang L, Sun C, Zhang Z, Zheng Y, Qiu F. 2014. Genetic analysis and major QTL detection for maize kernel size and weight in multi-environments. Theor Appl Genet 127:1019–1037

Lu G, Tang J, Yan J, Ma X, Li J, Chen S, et al. 2006. Quantitative trait loci mapping of maize yield and its components under different water treatments at flowering time. J Integr Plant Biol 48:1233–1243

Ma XQ, Tang JH, Teng WT, Yan JB, Meng YJ, Li JS. 2007. Epistatic interaction is an important genetic basis of grain yield and its components in maize. Mol Breed 20:41–51

Meng L, Li H, Zhang L, Wang J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Messmer R, Fracheboud Y, Bänziger M, Vargas M, Stamp P, Ribaut JM. 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theor Appl Genet 119:913–930

Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theor Appl Genet 125:625–645

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, et al. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Nikolić A, Anđelković V, Dodig D, Ignjatović-Micić D. 2011. Quantitative trait loci for yield and morphological traits in maize under drought stress. Genetika 43:263–276

Nikolić A, Anđelković V, Dodig D, Mladenović-Drinić S, Kravić N, Ignjatović-Micić D. 2013. Identification of QTLs for drought tolerance in maize, II: yield and yield components. Genetika 45:341–350

Paaby AB, Rockman MV. 2013. The many faces of pleiotropy. Trends Genet 29:66–73

Peng B, Li Y, Wang Y, Liu C, Liu Z, Tan W, et al. 2011. QTL analysis for yield components and kernel-related traits in maize across multi-environments. Theor Appl Genet 122:1305–1320

Prasanna BM, Beiki AH, Sekhar JC, Srinivas A, Ribaut JM. 2009. Mapping QTLs for component traits influencing drought stress tolerance of maize (Zea mays L.) in India. J Plant Biochem Biotechnol 18:151–160

Raihan MS, Liu J, Huang J, Guo H, Pan Q, Yan J. 2016. Multi-environment QTL analysis of grain morphology traits and fine mapping of a kernel-width QTL in Zheng58 × SK maize population. Theor Appl Genet 129:1465–1477

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

Semagn K, Beyene Y, Warburton ML, Tarekegne A, Mugo S, Meisel B, et al. 2013. Meta-analyses of QTL for grain yield and anthesis-silking interval in 18 maize populations evaluated under water-stressed and well-watered environments. BMC Genomics 14:313

Stange M, Schrag TA, Utz HF, Riedelsheimer C, Bauer E, Melchinger AE. 2013. High-density linkage mapping of yield components and epistatic interactions in maize with doubled haploid lines from four crosses. Mol Breed 32:533–546

Tan WW, Wang Y, Li YX, Liu C, Liu ZZ, Peng B, et al. 2011. QTL mapping of ear traits of maize under different water regimes. Acta Agron Sin 37:235–248

Tang J, Yan J, Ma X, Teng W, Wu W, Dai J, et al. 2010. Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F2 population. Theor Appl Genet 120:333–340

Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S. 2002. Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. Ann Bot 89:941–963

Veldboom LR, Lee M. 1994. Molecular-marker-facilitated studies of morphological traits in maize. II: Determination of QTLs for grain yield and yield components. Theor Appl Genet 89:451–458

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

Wei X, Lu X, Zhang Z, Xu M, Mao K, Li W, et al.

2016. Genetic analysis of heterosis for maize grain yield and its components in a set of SSSL testcross populations. Euphytica 210:181–193

Xiao YN, Li XH, George ML, Li MS, Zhang SH, Zheng YL. 2005. Quantitative trait locus analysis of drought tolerance and yield in maize in China. Plant Mol Biol Report 23:155–165

Xu C, Ren Y, Jian Y, Guo Z, Zhang Y, Xie C, et al. 2017. Development of a maize 55 K SNP array with improved genome coverage for molecular breeding. Mol Breed 37:20

Yan J, Tang H, Huang Y, Zheng Y, Li J. 2006. Quantitative trait loci mapping and epistatic analysis for grain yield and yield components using molecular markers with an elite maize hybrid. Euphytica 149:121–131

Yan J, Tang H, Huang Y, Zheng Y, Subhash C, Li J. 2006. A genome scan for quantitative trait loci affecting grain yield and its components of maize both in single-and two-locus levels. Chinese Sci Bull 51:1452–1461

Yang C, Zhang L, Jia A, Rong T. 2016. Identification of QTL for maize grain yield and kernel-related traits. J Genet 95:239–247

Yang G, Li Y, Wang Q, Zhou Y, Zhou Q, Shen B, et al. 2012. Detection and integration of quantitative trait loci for grain yield components and oil content in two connected recombinant inbred line populations of high-oil maize. Mol Breed 29:313–333

Zhang Z, Liu Z, Hu Y, Li W, Fu Z, Ding D, et al. 2014. QTL analysis of kernel-related traits in maize using an immortalized F2 population. PLoS ONE 9:e89645

Zhao X, Peng Y, Zhang J, Fang P, Wu B. 2018. Identification of QTLs and meta-QTLs for seven agronomic traits in multiple maize populations under well-watered and water-stressed conditions. Crop Sci 58:507–520

Zhou G, Zhu Q, Yang G, Huang J, Cheng S, Yue B, et al. 2015. qEL7.2 is a pleiotropic QTL for kernel number per row, ear length and ear weight in maize (Zea mays L.). Euphytica 203:429–436