Research Paper

Comparative QTL analysis for yield components and morphological traits in maize (*Zea mays* L.) under water-stressed and well-watered conditions

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Drought significantly influences maize morphology and yield potential. The elucidation of the genetic mechanisms of yield components and morphological traits, and tightly linked molecular markers under drought stress are thus of great importance in marker assisted selection (MAS) breeding. Here, we identified 32 QTLs for grain weight per ear, kernel ratio, and ear height-to-plant height ratio across two F_{2:3} populations under both drought and non-drought conditions by single-environment mapping with composite interval mapping (CIM), of which 21 QTLs were mapped under water-stressed conditions. We identified 29 QTLs by joint analysis of all environments with mixed-linear-model-based composite interval mapping (MCIM), 14 QTLs involved in QTL-by-environment interactions, and 11 epistatic interactions. Further analysis simultaneously identified 20 stable QTLs (sQTLs) by CIM and MCIM could be useful for genetic improvement of these traits via QTL pyramiding. Remarkably, bin 1.07-1.10/6.05/8.03/8.06 exhibited four pleiotropic sQTLs that were consistent with phenotypic correlations among traits, supporting the pleiotropy of QTLs and playing important roles in conferring growth and yield advantages under contrasting watering conditions. These findings provide information on the genetic mechanisms responsible for yield components and morphological traits that are affected by different watering conditions. Furthermore, these alleles provide useful targets for MAS.

Key Words: maize, QTL, yield components, morphological traits, drought.

Introduction

Maize (*Zea mays* L.) is one of the most important crops worldwide that is utilized as food, animal feed, and raw material of bioenergy. Drought is considered one of the most important abiotic stresses that influence global maize production. Drought stress can adversely affect various aspects of maize physiological metabolism and growth, including photosynthesis, plant growth, tassel development, dry matter production, and grain yield formation (Ge *et al.* 2012), even causing a 39.3% reduction in maize yield from 1980 to 2015 using global estimates of drought effects (Daryanto *et al.* 2016). Maize undergoes various morphological, biochemical and physiological changes to respond and adapt to drought stress (Almeida *et al.* 2014). Moreover, the genetic improvement for drought tolerance can ensure sustainable and long-term benefits (Duvick 2005). Therefore, drought-tolerant maize varieties are urgently needed to

maintain sufficient global food production.

Maize grain yield (GY) is the primary objective of breeding for drought tolerance, however, the low heritability of GY, as it decreases under drought stress conditions, makes the direct selection for this trait inefficient (Su *et al.* 2017). However, multiple morphological traits and yield components, namely, plant height (PH), ear height (EH), leaf area (LA), anthesis-silking interval (ASI), tassel branch number (TBN), ear weight (EW), cob weight (CW), 100-kernel weight (KW), and ear length (EL), are correlated with GY and drought tolerance can experience increased genetic variance and heritability under drought environments (Almeida *et al.* 2014, Zhao *et al.* 2017, 2018b, Ziyomo and Bernardo 2012). Moreover, drought stress inhibits growth of vegetative organs, limits photosynthesis, reduces flux of assimilates to the developing ears, slows down ear and silk growth and delays silk emergence (Bänziger *et al.* 2000, Messmer *et al.* 2009, Zhao *et al.* 2017). Therefore, these traits may be more suitable for identifying and improving maize selection responses to drought.

Genetic studies on maize PH, EH, ASI, TBN, EW, CW, KW, and EL have been extensively conducted using quantitative trait locus (QTL) mapping in biparental populations

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during drought (Almeida *et al.* 2014, Lu *et al.* 2010, Messmer *et al.* 2009, Peng *et al.* 2011, Semagn *et al.* 2013, Tan *et al.* 2011, Vargas *et al.* 2006, Zhao *et al.* 2017). QTLs/genes have been identified for morphological traits and yield components in maize using map-based cloning strategies, such as *Brachytic 2* (*Br2*) (Xing *et al.* 2015), *gln1-4* (Martin *et al.* 2006), *Opaque-2* (*o2*) (Schmidt *et al.* 1992), *unbranched2* (*ub2*) (Chuck *et al.* 2014), and *ZmCLA4* (Zhang *et al.* 2014), which can be useful targets for marker assisted selection (MAS) in modern maize breeding programs. In addition, there is growing evidence suggesting that ear height-to-plant height ratio (EHPH), grain weight per ear (GW), and kernel ratio (KR) are closely associated with drought tolerance and yield, EHPH (phenotypic correlation coefficient (r_p) = 0.318~0.578), GW (r_p = 0.979~0.999), and KR (r_p = 0.629~0.688) showed significantly positive correlation to GY, and EHPH had significantly positive correlation to GW (r_p = 0.308~0.3111) and KR (r_p = 0.241~0.262) under both water regimens (Begna *et al.* 2000, Du *et al.* 2011, 2012, He *et al.* 2016, Khush 1999, Meng *et al.* 2016, Zhao 2018). Therefore, higher GW and KR can be achieved by maintaining a suitable EHPH under drought stress. However, a limited number of QTLs for EHPH, GW, and KR have been identified under different levels of water availability (Chang *et al.* 2017, Dong *et al.* 2015, Ku *et al.* 2015, Zhang *et al.* 2013), and the genetic mechanisms underlying these three traits remains poorly understood, thus warranting in-depth investigations. Furthermore, a better understanding of the genotype-by-environment ($G \times E$) interaction will provide a foundation for the genetic improvement and optimization of genotypes across different environments (EI-Soda *et al.* 2014).

The overall objective of the present study was to identify QTLs responsible for EHPH, GW, and KR in two maize $F_{2:3}$ populations subjected to both water regimens at Wuwei in 2014, Zhangye in 2014, Gulang in 2015, and Jingtai in 2015 by single-environment mapping with composite interval mapping (CIM), respectively. Then, joint QTLs, epistatic interactions, and QTL-by-environment (QTL $\times$ E) interactions were identified across all environments using mixed-linear-model based composite interval mapping (MCIM). By combining these experiments, we hope to identify hot spots and areas of co-localization across experiments, which may further contribute to mapping QTLs, revealing genetic mechanisms for EHPH, GW, and KR in drought-stressed and unstressed conditions, and developing MAS breeding for EHPH, GW, and KR to improve drought tolerance and yield in maize.

Materials and Methods

Plant materials

The two sets of study populations comprised 202 (POP1) and 218 (POP2) $F_{2:3}$ families, which were derived from the Langhuang $\times$ TS141 (H1) and Chang7-2 $\times$ TS141 (H2) crosses, respectively, and were used to identify QTLs for

EHPH, GW, and KR under both water-stressed and well-watered conditions, respectively. The parents of the two $F_{2:3}$ populations were selected based on their distinct maize germplasm groups and drought tolerance. The drought-tolerant inbred lines Langhuang and Chang7-2 originated from the Tangsipingtuo (TSPT) germplasm and used as female parents, and the drought-sensitive inbred line TS141 was derived from the Reid yellow dent (Reid) germplasm and used as the common male parent (Peng *et al.* 2014, Zhao *et al.* 2017, 2018a, 2018b).

Field trials and trait evaluation

The two parents (Langhuang and TS141), corresponding to the F_1 hybrid (H1) and 202 $F_{2:3}$ families (POP1), were planted at two sites in Gansu Province, China, namely Wuwei (37.97° N, 102.63° E; 1,508 m altitude; sand-loam) and Zhangye (38.83° N, 106.93° E; 1,536 m altitude; sand-loam) in 2014. The other two parents (Chang7-2 and TS141), corresponding to the F_1 hybrid (H2) and 218 $F_{2:3}$ families (POP2), were evaluated at two other sites in Gansu Province, China, namely Gulang (37.67° N, 102.85° E; 1,785 m altitude; sand-loam) and Jingtai (37.18° N, 104.03° E; 1,640 m altitude; sand-loam) in 2015. We recorded the weather data at Wuwei in 2014, Zhangye in 2014, Gulang in 2015, and Jingtai in 2015, respectively (**Supplemental Fig. 1**). Well-watered (WW, WZ, WG, and WJ) and water-stressed (SW, SZ, SG, and SJ) experiments were conducted at Wuwei, Zhangye, Gulang, and Jingtai following a randomized block design that included three replicates. Plastic film (0.08 mm thick and 120 cm wide) was manually laid out to cover the soil at the four sites. The plants were managed by drip irrigation. In each plot, a single $F_{2:3}$ family, parent, or F_1 hybrid was planted in a row that was 6 m long and 0.6 m wide, with a total of 20 plants at a density of 55,580 plants ha^{-1} . The Langhuang, Chang7-2, TS141, H1, H2, POP1, and POP2 plants under four well-watered conditions (WW, WZ, WG, and WJ) were irrigated when rainfall was insufficient during all developmental stages. Water-stressed Langhuang, Chang7-2, TS141, H1, H2, POP1, and POP2 plants under four water-stressed conditions (SW, SZ, SG, and SJ) were not irrigated from the beginning of the expected V18 stage to the end of the expected R1 stage, although these plants were irrigated at 20-d intervals at other developmental stages. The V18 stage is where the silks from the basal ear ovules elongate first, and brace roots (aerial nodal roots) grow from the nodes above the soil surface, whereas at the R1 stage, silks are visible outside the husks (Ritchie *et al.* 1997). Insufficient moisture from the V18 to R1 stages can cause poor pollination and kernel set.

For the Langhuang, Chang7-2, TS141, H1, H2, POP1, and POP2 plants under each watering condition, ASI (number of days between 50% pollen shedding to 50% silking, d) was assessed in each plot. Five days after pollen shedding, 10 consecutive plants were selected from the middle of each plot to evaluate PH (height from the ground surface to the tassel tip, cm) and EH (height from the ground surface to

the node bearing the highest ear, cm), which were then used in the calculation for EHPH with the following equation (Dong *et al.* 2015):

$$\text{EHPH} = \text{EH/PH} \quad (1)$$

We then harvested the corresponding plants from each plot, which were subsequently air-dried to determine EW (weight per ear including the cob, g), GW (grain weight per ear on a scale, g), KW (100 kernels randomly selected from the bulked kernels, g), CW (weight per cob on a fine scale, g), EL (length per ear including the bald head, cm). These values were then used in calculating for KR using the following equation (Chang *et al.* 2017):

$$\text{KR} = \text{GW/EW} \times 100\% \quad (2)$$

Relative to the well-watered conditions, we also calculated the average rate of change (RC) of each trait under water-stressed conditions using the following equation (Zhao *et al.* 2018b):

$$\text{RC} = (1 - T_s/T_w) \times 100\% \quad (3)$$

where T_s is the average value of a trait under water-stressed conditions, and T_w is the average value of the corresponding trait under well-watered conditions.

Phenotypic data analysis

The collected phenotypic data from each watering condition were statistically analyzed. The general linear model for univariate (GLM-Univariate) tests was used for conducting an ANOVA (Zhao *et al.* 2017), which was employed to evaluate the total and residual variances among $F_{2,3}$ populations for each trait. Pearson correlation analyses and dendrogram construction were performed using the IBM-SPSS Statistics 19.0 software (SPSS Inc., Chicago, IL, USA). Broad-sense heritability (H^2) was calculated using the following equation (Knapp *et al.* 1985):

$$H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2/n + \sigma^2/nr) \times 100\% \quad (4)$$

where σ_g^2 is the genetic variance, σ_{ge}^2 is the $G \times E$ variance, σ^2 is the error variance, r ($r = 3$) is the replication number, and n ($n = 2$) is the number of environments.

Genetic linkage map construction and QTL analysis

A total of 213/217 genome-wide polymorphic simple sequence repeats (SSRs) were identified between Langhuang/Chang7-2 and TS141 using the MaizeGDB (<http://www.maizegdb.org/>), which were subsequently used to genotype the 202/218 F_2 individuals and establish two corresponding genetic linkage maps (Zhao *et al.* 2017). The two genetic linkage maps were 1,542.5 and 1,648.8 cM in length, with an average interval of 7.8 and 8.0 cM, respectively.

The identification of QTLs for each trait in each watering

environment was performed using Windows QTL Cartographer software version 2.5 with CIM approach (Wang *et al.* 2007). For CIM, model 6 of the Zmapqtl module was used to identify QTLs. Window size was 10 cM, and cofactors were selected through forward and backward regressions with the in and out thresholds at a $P < 0.05$. A genome-wide critical threshold value was estimated for experiment-wise type I error rate of 0.05 with 1,000 random permutations (Trachsel *et al.* 2009). The genetic action of each QTL was estimated by Stuber *et al.* (1987) as: $|d/a| = |\text{dominance/additive}|$; A, additive ($|d/a| = 0.00\sim 0.20$); PD, partial dominance ($|d/a| = 0.21\sim 0.80$); D, dominance ($|d/a| = 0.81\sim 1.20$); OD, over-dominance ($|d/a| > 1.20$).

The joint QTL, epistatic interaction between QTLs for each trait at each watering environment and QTL $\times$ E interaction across all watering environments were identified using QTL Network version 2.0, with MCIM approach (Yang *et al.* 2016). The testing window, walk speed, and filtration window of the genome scan were set at 10, 2, and 10 cM, respectively. A total of 1,000 permutations were performed to determine the threshold logarithm (base 10) of odds ratio (LOD), for each trait for declaring a significant QTL at a $P < 0.05$ probability level. The name of the QTL was assigned according to the modifying nomenclature of McCouch *et al.* (1997). Moreover, the letter 'J' was added into the middle of this QTL name (namely, inserted between trait abbreviation and chromosome number), whereas one QTL was only detected in joint analysis with MCIM but not in a single environment through the CIM program. The confidence interval (CI) of the identified QTL was estimated using the following equation (Darvasi and Soller 1997):

$$\text{CI} = 530/(N \times R^2) \quad (5)$$

where N is the population size, and R^2 is the value of the phenotypic variation contributed by the QTL. QTLs for tested traits were detected within the same marker interval or within overlapping CIs. The corresponding loci were assumed to be common QTLs with pleiotropic effects. QTLs that were repeatedly detected with CIM/MCIM in different environments or different mapping populations were considered stable QTLs (sQTLs).

Results

Phenotypic variations under water-stressed conditions

We analyzed the phenotypic values of the three parents (Langhuang, Chang7-2, and TS141), two F_1 hybrids (H1 and H2), and two $F_{2,3}$ populations (POP1 and POP2) under both well-watered (WW, WZ, WG, and WJ) and water-stressed (SW, SZ, SG, and SJ) conditions, respectively. Under well-watered conditions, except for KR, other nine traits of the common male parent (TS141) were larger than the female parents (Langhuang and Chang7-2) (Supplemental Table 1). This finding shows that these phenotypic traits were highly significantly different between the male

Fig. 1. Extensive phenotypic variations, correlation, and dendrogram in 10 maize traits. (A) Average rate of changes (RC) for 10 tested traits in three parents (Langhuang, Chang7-2, and TS141), two F₁ hybrids (H1 and H2), and two F_{2.3} populations (POP1 and POP2) under water-stressed conditions, respectively. F -value**/* means the significant difference at $P < 0.01$ or $P < 0.05$ via ANOVA. (B) Correlation among 10 maize traits. Red/blue lines designate positive/negative correlations between two traits ($P < 0.01$), and green/gray lines designate positive/negative correlations between two traits ($P < 0.05$), respectively. Circles of different sizes reflect the number of corresponding traits. (C) Dendrogram of all 10 maize traits. The scale indicates Euclidean distance, which was calculated based on the standardized phenotypic data across three parents, two F₁ hybrids, and two F_{2.3} populations, respectively.

and female parents. Under water-stressed conditions, PH, EH, EHPH, EW, CW, GW, KW, and EL significantly decreased in Langhuang, Chang7-2, TS141, H1, H2, POP1, and POP2, and KR exhibited a slight decrease. ASI, however, displayed a significant increase. Further analysis indicated that the drought-sensitive male parent TS141 had more changes (RC) in all 10 tested traits compared to the drought-tolerant female parents Langhuang and Chang7-2 (Fig. 1A). Moreover, wide variations (σ_g^2 , σ_e^2 , and σ_{ge}^2) in the two F_{2.3} populations were observed for the 10 tested traits ($P < 0.01$ or $P < 0.05$) (Supplemental Table 2) and showed a continuous distribution, with absolute values of skewness and kurtosis < 1.0 (Supplemental Table 1). H^2 of the 10 tested traits in POP1 and POP2 were predominantly high at 72.79~90.95% (Supplemental Table 2). The observed extensive phenotypic variations and high heritability suggest that the two F_{2.3} populations have diverse genetic backgrounds and are suitable for identifying the genetic factors underlying various morphological traits and yield components in maize under contrasting watering conditions.

Phenotypic relationships among all tested traits

Analyses showed that the 10 tested traits were significantly correlated to each other, with each trait correlating with seven to nine other traits ($P < 0.01$ or $P < 0.05$). Moreover, a total of 42 correlations were identified among the 10 traits, the majority (31) of which were positive associations,

except for the 11 correlations involving PH/EHPH, ASI/EHPH, ASI/EW, ASI/GW, ASI/KW, ASI/KR, EW/KR, CW/GW, CW/KW, CW/KR, and KW/EL (Fig. 1B). Furthermore, PH, EH, ASI, CW, EL, and EL of the progeny populations (two F₁ hybrids and two F_{2.3} populations) depict a significantly positive correlation to female parents (Langhuang/Chang7-2) and male parent TS141 ($P < 0.01$). EHPH, EW, GW, and KW, however, were significantly positively correlated to the male parent ($P < 0.05$ or $P < 0.01$) and were positively correlated to the female parents ($P > 0.05$) (Supplemental Table 3). This finding shows that the 10 tested traits in the progeny populations are simultaneously controlled by both parents, the degree of effects for these traits in both parents differed, and the degree of the effects of EHPH, EW, GW, and KW in the male parent are evidently larger than the female parent. In addition, dendrogram construction was performed for all 10 tested traits, which demonstrated strong correlations as well as extensive phenotypic variations. This classified the 10 tested traits into two unrooted groups. The biggest cluster included seven tested traits, namely, KW, GW, EW, CW, EL, KR, and ASI, whereas the other three traits (EH, PH, and EHPH) were clustered together (Fig. 1C). This finding suggests that maize corresponding vegetative organs and reproductive organs are clearly separated during the periods from vegetative growth to reproductive growth, then the shoot apical meristem differentiated into inflorescence meristem. Thereby,

Fig. 2. Genetic linkage map and QTL identification in POP1 and POP2 by single-environment mapping with composite interval mapping (CIM) and joint analysis of all environments with mixed-linear-model-based composite interval mapping (MCIM). Red/yellow/green/blue and black/purple/white/brown pentagrams, rectangles, and circles represent QTLs for ear height-to-plant height ratio (EHPH), grain weight per ear (GW), and kernel ratio (KR) under well-watered/water-stressed conditions at Wuwei (WW/SW)/Zhangye (WZ/SZ) in POP1 and Gulang (WG/SG)/Jingtai (WJ/SJ) in POP2 with CIM, respectively. Gray and pink pentagrams, rectangles, and circles represent QTLs for EHPH, GW, and KR in POP1 and POP2 with MCIM, respectively. The black line and red empty oval were combined to a bridge to identify stable QTLs (sQTLs) from both F_{2:3} populations.

PH, EH, and EHPH stop growing, and the inflorescences (tassel and ear) start growing in the following stages.

Identification of QTLs for EHPH, GW, and KR

To investigate the genetic control underlying EHPH, GW, and KW improvement using single-environment mapping with CIM, we identified 32 significant ($P < 0.05$) QTLs (10 for EHPH, 13 for GW, and 9 for KR) across the two F_{2:3} populations (POP1 and POP2) under eight watering conditions (WW, SW, WZ, SZ, WG, SG, WJ, and SJ). The phenotypic variance explained by individual QTLs ranged from 4.07% to 13.64% within each watering condition. In addition, 21 QTLs (65.6%) were identified under water-stressed conditions (Fig. 2, Supplemental Fig. 2, Supplemental Table 4). For these identified QTLs, approximately 26.1, 65.2, and 8.7% of the QTLs for EHPH showed additive (A), partial dominance (PD), and dominance (D) effects, respectively. Approximately 25.0, 35.7, 17.9, and 21.4% of the QTLs for GW showed A, PD, D, and over-dominance (OD) effects, respectively. Approximately 66.7, 19.0, and 14.3% of the QTLs for KR showed A, PD, and D effects, respectively (Fig. 3). Moreover, all alleles for an increase in EHPH and GW and 42.9% alleles for a decrease in KR were contributed by male parent TS141 (Supplemental Table 4).

Fig. 3. Summary of identified QTLs for ear height-to-plant height ratio (EHPH), grain weight per ear (GW), and kernel ratio (KR) in the two F_{2:3} populations (POP1 and POP2) by single-environment mapping with composite interval mapping (CIM), including number of QTLs and QTLs effects (A, additive effect; PD, partial dominance effect; D, dominance effect; OD, over-dominance effect), respectively.

Identification of joint QTLs and QTL × E interactions

Using joint analysis of all watering environments with MCIM, we identified 29 significant ($P < 0.05$) QTLs (9 for EHPH, 11 for GW, and 9 for KR) in the two F_{2:3} populations (POP1 and POP2). Eleven of the 29 QTLs were consistent with those of EHPH, GW, and KR through single watering

Table 1. QTLs for EHPH, GW, and KR were identified in POP1 and POP2 by joint analysis among all environments with MCIM, respectively

Trait	QTL	Chr.	QTL position			A ^b	AE1 ^c	AE2 ^c	AE3 ^c	AE4 ^c	h ² (A) (%) ^d	h ² (AE) (%) ^e
			cM	Mb ^a	Marker interval							
POP1 (No. of POP1 = 202)												
EHPH	qEHPH-J1-1	1	56.4	0.06	umc2025-umc1395	-1.51	-0.38		-0.40	-0.27	9.14	4.30
	qEHPH-Ch.1-2	1	110.7	0.40	mmc0041-phi308707	-1.07					7.52	
	qEHPH-Ch.6-1	6	119.8	0.03	umc2040-bnlgl1174a	-0.94					7.36	
	qEHPH-Ch.8-1	8	45.5	0.01	bnlg1863-umc2075	-1.15					7.89	
GW	qGW-J1-1	1	114.2	5.03	phi308707-umc1847	-1.20	-0.64	-0.37	0.45		8.14	5.01
	qGW-J2-1	2	10.5	0.39	umc2363-umc2403	-0.53					4.85	
	qGW-J2-2	2	101.8	0.24	bnlg1520-umc1736	-1.55		-0.86	-0.40	-0.71	9.31	6.86
	qGW-Ch.4-1	4	181.9	1.32	umc2041-umc2287	-1.31					8.32	
KR	qGW-Ch.8-1	8	44.2	0.01	bnlg1863-umc2075	-0.35		-0.26		-0.17	2.79	1.24
	qKR-Ch.1-1	1	40.7	1.56	bnlg1484-umc1917	1.11					11.69	
	qKR-J1-1	1	95.4	2.30	bnlg1025-mmc0041	0.60			0.37		5.30	2.73
	qKR-Ch.6-1	6	119.8	0.03	umc2040-bnlgl1174a	0.47					3.51	
	qKR-Ch.7-1	7	110.5	1.01	umc1708-umc1768	0.65					5.37	
	qKR-J8-1	8	88.3	0.54	umc2356-umc1607	0.84	0.26	0.43		-0.31	8.14	2.20
POP2 (No. of POP2 = 218)												
EHPH	qEHPH-Ch.1-1	1	153.2	0.40	mmc0041-phi308707	-1.80					10.19	
	qEHPH-J5-1	5	239.4	0.84	umc2216-umc1072	-0.44					4.83	
	qEHPH-J6-1	6	40.5	0.48	phi126-umc1606	-0.63		-0.22		-0.42	5.48	4.15
	qEHPH-J6-2	6	87.8	0.17	umc2141-umc2040	-0.56	-0.39	-0.35			5.12	4.29
GW	qEHPH-Ch.8-1	8	40.3	0.01	bnlg1863-umc2075	-0.71		-0.38		-0.35	5.53	4.26
	qGW-J1-1	1	154.9	0.04	mmc0041-phi308707	-2.03	-0.84	-0.66	-0.41	-0.68	11.07	5.50
	qGW-J2-1	2	38.6	2.01	umc2363-umc1024	-0.88					7.31	
	qGW-J2-2	2	127.1	0.24	bnlg1520-umc1736	-1.63	-0.89	-0.41	-0.64	-0.50	9.95	4.19
KR	qGW-Ch.4-1	4	120.3	0.66	umc2041-umc2188	-1.77					10.14	
	qGW-J6-1	6	97.4	0.03	umc2040-bnlgl1174a	-1.05					8.96	
	qGW-J8-1	8	40.7	0.01	bnlg1863-umc2075	-0.84	-0.36	-0.52			7.20	4.47
	qKR-J1-1	1	139.3	2.30	bnlg1025-mmc0041	0.51	0.26	0.39			4.15	2.93
	qKR-J6-1	6	88.1	0.17	umc2141-umc2040	0.58					4.20	
	qKR-J7-1	7	118.0	1.01	umc1708-umc1768	0.73					6.12	
	qKR-J8-1	8	114.9	1.96	umc2356-phi233376	0.62	0.37	0.18			5.04	2.11

EHPH, ear height-to-plant height ratio; GW, grain weight per ear; KR, kernel ratio.

MCIM, mixed-linear-model based composite interval mapping.

^a Mb denotes the estimated physical distance of marker interval.

^b A denotes the additive effect of corresponding QTL.

^c AE1–4 denote the additive by designated environment (1, well-watered condition at Wuwei in 2014 (WW)/Gulang in 2015 (WG); 2, water-stressed condition at Wuwei in 2014 (SW)/Gulang in 2015 (SG); 3, well-watered condition at Zhangye in 2014 (WZ)/Jingtai in 2015 (WJ); 4, water-stressed condition at Zhangye in 2014 (SZ)/Jingtai in 2015 (SJ) in the POP1/POP2) interaction effects.

^d $h^2(A)$ denotes phenotypic variance explained by the QTL across all environments.

^e $h^2(AE)$ denotes phenotypic variance explained by the additive by designated environment interaction effects.

environment mapping with CIM. The additive effect of each significant QTL ranged from -2.03 to 1.11, and the phenotypic variance contributed by [$h^2(A)$] varied between 2.79% and 11.69% (Table 1, Fig. 2). Moreover, 14 significant ($P < 0.05$) QTLs (4 for EHPH, 6 for GW, and 4 for KR) were involved in QTL $\times$ E interactions in the two $F_{2:3}$ populations, and accounted for 1.24–6.86% of the phenotypic variance explained by [$h^2(AE)$] (Table 1). These QTLs may thus impart stronger effects on EHPH, GW, and KR. For the two $F_{2:3}$ populations, bin 1.08-1.10 (phi308707-umc1847/mmc0041-phi308707), bin 2.09 (bnlg1520-umc1736), and bin 8.03 (bnlg1863-umc2075) exhibited three stable QTL $\times$ E interactions for GW, and bin 1.07-1.08 (bnlg1025-mmc0041) and bin 8.06 (umc2356-umc1607/umc2356-phi233376) exhibited two stable QTL $\times$ E interactions for KR (Table 1). These findings imply that the five bin intervals may be mediated by environmental factors.

Further analysis showed that 20 sQTLs were simultane-

ously identified by single-environment mapping with CIM and joint analysis through MCIM in two $F_{2:3}$ populations and were located on chromosomes 1, 2, 3, 4, 6, 8, and 9. Each sQTL explained 4.15–5.30% (sQTL17) to 8.81–13.64% (sQTL15) of the observed phenotypic variance (Table 2). Approximately 15 (sQTL2, 3, and 5 for EHPH; sQTL6, 7, 8, 10, 11, 12, 13, and 14 for GW; sQTL15, 16, 18, and 20 for KR) of the 20 sQTLs were identified under water-stressed conditions. This finding indicates that these intervals may have several stable alleles that are involved in plant development and grain yield formation under stress environments. In addition, bin 1.08-1.10 (mmc0041-phi308707)/bin 1.07-1.10 (bnlg1025-phi308707) exhibited one common sQTL that imparted a pleiotropic effect on EHPH and GW; bin 6.05 (umc2141-bnlgl1174a)/bin 6.05 (umc2040-bnlgl1174a) included one common sQTL that imparted a pleiotropic effect on EHPH, GW, and KR; bin 8.03 (bnlg1025-umc2075) mapped one common sQTL that

Table 2. Summary of stable QTLs (sQTLs) for all investigated traits in POP1 and POP2

Trait	sQTL	Population	Environment	QTL	Bin	Marker interval	R^2 (%) ^a
EHPH	sQTL1	POP1	WW, WZ	qEHPH-Ch.1-1	1.00-1.01	bnlg149-umc1177	6.94~9.09
	sQTL2	POP1, POP2	WW, SW, WZ, SZ, WG, SG, WJ, SJ, Joint	qEHPH-Ch.1-1, qEHPH-Ch.1-1	1.08-1.10	mmc0041-phi308707	4.82~10.38
GW	sQTL3	POP1, POP2	SW, SZ, SG, WJ, SJ	qEHPH-Ch.3-1	3.08-3.09	umc1320-umc2152	4.14~11.23
	sQTL4	POP1, POP2	WW, Joint	qEHPH-Ch.6-1, qEHPH-J6-2	6.05	umc2141-bnlg1174a	5.12~7.86
	sQTL5	POP1, POP2	WW, WZ, WG, SG, WJ, Joint	qEHPH-Ch.8-1	8.03	bnlg1863-umc2075	5.48~9.89
	sQTL6	POP1	WW, WZ, SZ	qGW-Ch.1-1	1.05	umc2025-umc1395	7.25~9.96
	sQTL7	POP1, POP2	WZ, WG, SG, SJ, Joint	qGW-Ch.1-2, qGW-Ch.1-1, qGW-J1-1	1.07-1.10	bnlg1025-phi308707	5.99~11.22
	sQTL8	POP1, POP2	SW, Joint	qGW-Ch.2-1, qGW-J2-1	2.02-2.04	umc2363-umc1024	4.85~7.31
	sQTL9	POP1, POP2	Joint	qGW-J2-2, qGW-J2-1	2.09	bnlg1520-umc1736	9.31~9.95
	sQTL10	POP1, POP2	WW, SW, SZ, WG, SG, WJ, Joint	qGW-Ch.4-1	4.08-4.09	umc2041-umc2287	4.07~12.90
	sQTL11	POP1, POP2	WW, SW, WJ, Joint	qGW-Ch.6-1, qGW-J6-1	6.05	umc2040-bnlg1174a	6.96~9.23
	sQTL12	POP1, POP2	WZ, SZ, Joint	qGW-Ch.8-1, qGW-J8-1	8.03	bnlg1863-umc2075	2.79~12.42
KR	sQTL13	POP2	WJ, SJ	qGW-Ch.8-1	8.06	umc2218-umc2356	9.79~10.55
	sQTL14	POP1	WW, WZ, SZ	qGW-Ch.9-1	9.04-9.06	umc1120-umc2134	6.34~11.02
	sQTL15	POP1, POP2	WW, SW, SZ, SG, WJ, SJ	qKR-Ch.1-1	1.03	bnlg1484-umc2505	8.81~13.64
	sQTL16	POP1, POP2	WW, SW, WZ, SZ, WG, SG, SJ	qKR-Ch.1-2	1.07	umc1356-umc1278	4.10~12.19
	sQTL17	POP1, POP2	Joint	qKR-J1-1	1.07-1.08	bnlg1025-mmc0041	4.15~5.30
	sQTL18	POP1	WZ, SZ	qKR-Ch.3-1	3.06-3.07	umc1311-umc1286	5.02~5.33
	sQTL19	POP1, POP2	WW, WZ, Joint	qKR-Ch.6-1, qKR-J6-1	6.05	umc2141-bnlg1174a	3.51~11.06
	sQTL20	POP1, POP2	SG, SJ, Joint	qKR-J8-1, qKR-Ch.8-1	8.06	umc2356-phi233376	5.04~10.27

EHPH, ear height-to-plant height ratio; GW, grain weight per ear; KR, kernel ratio.

WW, well-watered condition at Wuwei in 2014; SW, water-stressed condition at Wuwei in 2014; WZ, well-watered condition at Zhangye in 2014; SZ, water-stressed condition at Zhangye in 2014; WG, well-watered condition at Gulang in 2015; SG, water-stressed condition at Gulang in 2015; WJ, well-watered condition at Jingtai in 2015; SJ, water-stressed condition at Jingtai in 2015; Joint, the joint QTLs were identified across all water environments by the QTL Network version 2.

^a R^2 represents the phenotypic variance explained by the QTL.

placed a pleiotropic effect on EHPH and GW; and bin 8.06 (umc2218-umc2356)/bin 8.06 (umc2356-phi233376) identified one common sQTL that had a pleiotropic effect on GW and KR, respectively (Table 2, Fig. 2). These findings indicate that these bin intervals with QTLs control two or more tightly linked traits.

Epistatic interactions among identified QTLs

For the two $F_{2:3}$ populations (POP1 and POP2), 2/1 pair of significant ($P < 0.05$) epistatic interactions for EHPH were identified with additive by additive (AA)/dominance by dominance (DD) effects under multiple environments, 2/1 pairs of significant ($P < 0.05$) epistatic interactions for GW were detected with AA/additive by dominance (AD) effects across multiple environments, and 5 pairs of significant ($P < 0.05$) epistatic interactions for KR were mapped with AA effects among multiple environments, respectively. Each epistatic effect explained 2.24~6.01% of the observed phenotypic variance contributed by [$h^2(AA)$], 3.61~4.93% of the phenotypic variance contributed by [$h^2(AD)$], and 4.08~4.21% of the phenotypic variance contributed by [$h^2(DD)$], respectively (Table 3, Fig. 4). These findings indicate that the main effects of significant QTLs may be stronger on EHPH, GW, and KR.

Discussion

The complex quantitative traits of PH, EH, ASI, EW, CW,

GW, KW, and EL have been successfully used as selection criteria in breeding drought-tolerant maize. Drought stress can significantly inhibit PH and EH growth, delay ASI, and adversely decrease EW, GW, KW, and EL in maize (Almeida *et al.* 2014, Bänziger *et al.* 2000, Ge *et al.* 2012, Messmer *et al.* 2009, Zhao *et al.* 2018b), which agree with our results involving three parents, two F_1 hybrids, and two $F_{2:3}$ populations under water deprivation (Fig. 1A). Moreover, the suitable PH, EH, and EHPH can reduce the plant center of gravity to improve lodging, drought tolerance, and yield potential in crops (Begna *et al.* 2000, He *et al.* 2016, Khush 1999). Our results indicate that EHPH and KR significantly decreased under drought conditions because the rate of decrease in EH/GW (RC, 12.0/9.9%) was significantly greater than that of PH/EW (RC, 8.2/9.6%) under drought conditions (Fig. 1A). Therefore, these findings imply that maize can significantly regulate the growth and development of the entire plant, tassel, and ear in response to drought.

Genetic insights into morphological traits and yield components are important for breeding high-yielding maize varieties under drought stress. Our results indicate that PH, EH, ASI, CW, EL, and KR in F_1 hybrids are highly correlated to both parents; however, other four traits were highly correlated to the male parent (Supplemental Table 3), which agrees with the results of Si *et al.* (2009), Wei *et al.* (2013), and Zhao (2009). Therefore, to cultivate high-yield maize varieties, breeders should carefully select parents with elite traits.

Table 3. Epistatic interactions were identified in POP1 and POP2 with mixed-linear-model based composite interval mapping (MCIM), respectively

Trait	Environ- ment	QTL (i)	Marker interval (i)	Bin (i)	QTL (j)	Marker interval (j)	Bin (j)	AA ^a	AD ^b	DD ^c	$h^2(AA)$ (%) ^d	$h^2(AD)$ (%) ^e	$h^2(DD)$ (%) ^f
POP1 (No. of POP1 = 202)													
EHPH	SZ	qEHPH-J1-1	umc2025-umc1395	1.05	qEHPH-J3-1	umc1780-umc2376	3.00-3.01		-0.51			4.37	
GW	SW	qGW-J2-1	umc2363-umc2403	2.02	qGW-Ch.8-1	bnlg1863-umc2075	8.03		-0.44			3.61	
	SZ	qGW-J2-1	umc2363-umc2403	2.02	qGW-Ch.8-1	bnlg1863-umc2075	8.03		-0.57			4.52	
KR	WW	qKR-J1-1	bnlg1025-mmnc0041	1.07-1.08	qKR-J8-1	umc2356-umc1607	8.06-8.07	0.36			3.28		
	SW	qKR-J1-1	bnlg1025-mmnc0041	1.07-1.08	qKR-J8-1	umc2356-umc1607	8.06-8.07	0.41			3.40		
SZ	WZ	qKR-J1-1	bnlg1025-mmnc0041	1.07-1.08	qKR-J8-1	umc2356-umc1607	8.06-8.07	0.49			4.75		
	WW	qKR-Ch.6-1	umc2040-bnlg1174a	6.05	qKR-Ch.7-1	umc1708-umc1768	7.04	0.58			5.53		
		qKR-Ch.6-1	umc2040-bnlg1174a	6.05	qKR-J8-1	umc2356-umc1607	8.06-8.07	0.62			6.01		
POP2 (No. of POP2 = 218)													
EHPH	WG	qEHPH-Ch.1-1	mmnc0041-phi308707	1.08-1.10	qEHPH-J5-1	umc2216-umc1072	5.06-5.07		-0.67		4.75		
GW	SG	qEHPH-J6-1	phi126-umc1606	6.00-6.01	qEHPH-Ch.8-1	bnlg1863-umc2075	8.03		-0.43			4.08	
	SJ	qEHPH-J6-1	phi126-umc1606	6.00-6.01	qEHPH-Ch.8-1	bnlg1863-umc2075	8.03		-0.49			4.21	
WG	WJ	qGW-J2-1	umc2363-umc1024	2.02-2.04	qGW-J6-1	umc2040-bnlg1174a	6.05	-0.86			5.94		
	SG	qGW-J2-1	umc2363-umc1024	2.02-2.04	qGW-J8-1	bnlg1863-umc2075	8.03		-0.72		4.93		
KR	WG	qGW-J2-1	umc2363-umc1024	2.02-2.04	qGW-J8-1	bnlg1863-umc2075	8.03		-0.54		4.36		
	WG	qKR-J1-1	bnlg1025-mmnc0041	1.07-1.08	qKR-J8-1	umc2356-phi23376	8.06	0.57			4.03		
SG	SG	qKR-J1-1	bnlg1025-mmnc0041	1.07-1.08	qKR-J8-1	umc2356-phi23376	8.06	0.43			3.41		
	SG	qKR-J6-1	umc2141-umc2040	6.05	qKR-J7-1	umc1708-umc1768	7.04	0.45			3.48		
WG	WG	qKR-J6-1	umc2141-umc2040	6.05	qKR-J8-1	umc2356-phi23376	8.06	0.40			3.39		
	SG	qKR-J6-1	umc2141-umc2040	6.05	qKR-J8-1	umc2356-phi23376	8.06	0.32			2.24		

EHPH, ear height-to-plant height ratio; GW, grain weight per ear; KR, kernel ratio.

MCIM, mixed-linear-model based composite interval mapping.

WW, well-watered condition at Wuwei in 2014; SW, water-stressed condition at Wuwei in 2014; WZ, well-watered condition at Zhangye in 2014; SZ, water-stressed condition at Zhangye in 2014;

WG, well-watered condition at Gulang in 2015; SG, water-stressed condition at Gulang in 2015; WJ, well-watered condition at Jingtai in 2015; SJ, water-stressed condition at Jingtai in 2015.

^a AA, the additive by additive epistatic interaction effects.

^b AD, the additive by dominance epistatic interaction effects.

^c DD, the dominance by dominance epistatic interaction effects.

^d $h^2(AA)$, percentage of phenotypic variance explained by the additive by additive epistatic interaction effects.

^e $h^2(AD)$, percentage of phenotypic variance explained by the additive by dominance epistatic interaction effects.

^f $h^2(DD)$, percentage of phenotypic variance explained by the dominance by dominance epistatic interaction effects.

Fig. 4. QTLs with epistatic effects were identified for ear height-to-plant height ratio (EHPH), grain weight per ear (GW), and kernel ratio (KR) in POP1 and POP2 by joint analysis among all environments with mixed-linear-model-based composite interval mapping (MCIM). Red/black/blue dotted lines represent additive by additive epistatic interaction effects (AA)/additive by dominance epistatic interaction effects (AD)/dominance by dominance epistatic interaction effects (DD). (A) Pentagrams, rectangles, and circles represent corresponding QTLs for EHPH, GW, and KR under well-watered/water-stressed conditions at Wuwei (WW/SW, red/yellow) and well-watered/water-stressed conditions at Zhangye (WZ/SZ, green/blue) in POP1, respectively. (B) Pentagrams, rectangles, and circles represent corresponding QTLs for EHPH, GW, and KR under well-watered/water-stressed conditions at Gulang (WG/SG, black/purple) and well-watered/water-stressed conditions at Jingtai (WJ/SJ, white/brown) in POP2, respectively.

Recent genetic improvements in maize grain yield are mostly attributable to increased water stress tolerance of new hybrids, which is achieved by selection for morphological traits and yield components stabilities across target environments, and can be conducted using MAS (Peng *et al.* 2011). However, genetic investigations on yield components and morphological traits by QTL mapping are often influenced by the environment and genetic background, and even the molecular mechanisms of yield components and morphological traits (especially EHPH, GW, and KR) in maize under different environments remain unclear. Thus, in this study, we detected 32 QTLs for EHPH, GW, and KR based on the two $F_{2.3}$ populations under eight watering conditions through single-environment mapping with CIM, which displayed both additive and non-additive effects (Fig. 3). Our findings demonstrate that additive and non-additive effects, as well as general and specific combining abilities improve EHPH, GW, and KR under both drought and non-drought stress. In addition, 21 of the 32 QTLs were mapped under water-stressed conditions (Supplemental Table 4). It has been suggested that these detected QTLs for EHPH, GW, and KR could be changed under both water regimens, some detected QTLs (e.g. bnlgl621a-umc2027 for EHPH, bnlgl018-bnlgl1909 for GW, and mmc0162-bnlgl2160a for KR) under well-watered conditions were inhibited under water-stressed conditions, whereas some undetected QTLs (e.g., umc1320-umc2152 for EHPH, umc1823-umc2363 for GW, and umc2356-phi233376 for KR) under well-watered conditions were activated under drought stress. Therefore, the QTLs were specifically identified under different watering conditions that directly led to differences in EHPH, GW, and KR. Using joint analysis across environments with MCIM, we detected 29 QTLs for EHPH, GW, and KR across POP1 and POP2, and 11 QTLs were involved in QTL $\times$ E interactions, some of these QTL $\times$ E, e.g. qGW-Ch.8-1 in POP1 and qEHPH-Ch.8-1 in

POP2, could also be detected under multiple conditions via single-environment mapping with CIM (Table 1). The observations show that the larger phenotypic variance explained by QTL $\times$ E by joint analysis led to the corresponding QTLs that are easily detected under different environments through single-environment mapping. Further analysis also showed that 20 sQTLs were detected in POP1 and POP2, and 15 of the 20 sQTLs were identified under water-stressed conditions (Table 2). Therefore, these sQTLs can provide reliable information for breeding and future researches on the genetics of EHPH, GW, and KR under different watering environments.

We also noted that the four pleiotropic sQTLs were co-localized with different traits (Table 2, Fig. 2). For example, bin 1.08-1.10 (mmc0041-phi308707, sQTL2)/bin 1.07-1.10 (bnlg1025-phi308707, sQTL17), exhibited one pleiotropic sQTL that simultaneously controlled EHPH and GW. Multiple QTLs for PH/PH/KR were identified in bin 1.07 (umc2151-bnlgl1556) (Tang *et al.* 2007)/bin 1.07 (umc23-umc58) (Lubberstedt *et al.* 1997, Melchinger *et al.* 1998)/bin 1.07 (umc1335-umc2236) (Zhang *et al.* 2013). Using meta-QTL (mQTL) analysis, Dong *et al.* (2015) reported one mQTL for EH and top height to plant height ratio in bin 1.08-1.10 (bnlg1643-umc2189). Li *et al.* (2011) mapped to one mQTL for EL and kernel number per row in bin 1.07-1.08 (bnlg1556-phi039), and mapped one QTL for ear diameter in bin 1.08-1.09 (bnlg1643-umc2047). Wang *et al.* (2016) found two mQTLs (bin 1.07 and 1.10) for PH, EH, and yield. Using map-based cloning, Remington *et al.* (2001) also obtained the *indeterminate 1* (*idl1*; bin 1.08) gene that controls PH growth and development in maize.

In bin 6.05 (umc2141-bnlgl1174a/umc2040-bnlgl1174a, sQTL4/sQTL19), we identified one pleiotropic sQTL simultaneously affected EHPH, GW, and KR. One sQTL/major QTL for EH was identified in bin 6.05-6.06 (bnlg1732-umc2162) (He *et al.* 2016)/bin 6.05 (bnlg2249-umc1341)

(Wu *et al.* 2005). In addition, two mQTLs for yield, ear number per plant, and GW were validated in bin 6.05 (bnlg1732-bnlg8.08c) (Wang *et al.* 2008, 2016). In bin 6.05 (umc2040-bnlg1174a), Zhao *et al.* (2017) also found a mQTL affecting ear number per plant and predicted the *liguleless3* (*Ig3*) gene, which regulates tassel and ear development in maize. In addition, Bauer *et al.* (2004) elucidated that *Ig3* is also a *knox* class-I homeobox gene that possibly functions in shoot apical meristem maintenance.

In bin 8.03 (bnlg1863-umc2075, sQTL5 and sQTL12), we validated one pleiotropic sQTL that was simultaneously associated with EHPH and GW. Multiple QTLs for kernel depth/PH/KW were identified in bin 8.03-8.05 (umc1302-umc1121) (Zhang *et al.* 2013)/bin 8.03 (umc1741-umc1868) (Wu *et al.* 2005)/bin 8.03 (bnlg1352-umc1778) (Peng *et al.* 2011). Two mQTLs for PH, EH, top height, and yield were also detected in bin 8.03 (bnlg1863-phi15) (Dong *et al.* 2015, Wang *et al.* 2016). Additionally, Yang *et al.* (2015) identified one sQTL for ear row number in bin 8.03 (near umc1236), and mapped two mutant genes (*vanishing tassel2*, *vt2* and *Barren inflorescence1*, *bif1*) in the interval. Phillips *et al.* (2011) reported that the *vt2* mutant ear typically has barren patches on one or both sides of the ear. Barazesh and McSteen (2008) reported that the mutant gene *bif1* plays a role in auxin transport and induces the development of an intermediate (two/more ranked) ear (phyllotaxy).

In bin 8.06 (umc2218-umc2356/umc2356-phi233376, sQTL13/sQTL20), we mapped one pleiotropic sQTL that was simultaneously related to GW and KR. Three QTLs for 10-kernel length/KR/EW were identified in bin 8.06 (umc2175-umc2199) (Peng *et al.* 2011)/bin 8.06 (umc1960-umc1997) (Chang *et al.* 2017)/bin 8.06 (umc2395-mm0181) (Tan *et al.* 2011). Moreover, Wang *et al.* (2016) validated one mQTL for PH, EH, and yield within the same interval and predicted the *liguleless1* (*Ig1*) and *liguleless4* (*Ig4*) genes in the interval, which have functions similar to that of *Ig3* (Bauer *et al.* 2004, Zhao *et al.* 2017). Therefore, taken together, the bin 1.07-1.10, 6.05, 8.03, and 8.06 intervals coincided with phenotypic features, thereby supporting pleiotropy of QTLs and playing important roles in conferring growth and yield advantages under contrasting watering conditions.

Epistasis or the interaction effects between a pair of loci may play fundamental roles in broadening our understanding of the genetic mechanism underlying complex quantitative traits (Phillips 2008). In our study, all phenotypic variance was explained by all QTLs for EHPH, GW, and KR, but were always less than the corresponding H^2 , regardless of these traits. This finding implies that the missing proportion of the observed phenotypic variance may be partly explained by epistasis (Zhao *et al.* 2017). Chang *et al.* (2017) showed that 15 epistatic interactions for KR are identified based on AA effects. Lan and Chu (2005) reported that 13/14 epistatic interactions for PH/EH are identified using AA, AD, DA, and dominance by dominance (DD) effects. Lu *et al.* (2007) found that four epistatic interactions for EL

are identified using AA, AD, DA, and DD effects. Yang *et al.* (2015) reported that two/seven epistatic interactions for ear row number/two-ranked versus many-ranked ear are distinguished by AA effects. Yang *et al.* (2016) mapped one epistatic interaction for KW that demonstrated an AA effect. Zhao *et al.* (2017) detected four epistatic interactions for ear number per plant that depicted AA and AD effects. In our study, we identified 11 epistatic interactions with AA, AD, and DD effects among all environments in the two $F_{2:3}$ populations (POP1 and POP2). These findings show that AA, AD, and DD may be the main epistatic interaction effects for EHPH, GW, and KR. Notably, five stable epistatic interactions were detected in our study (Table 3, Fig. 4). Namely, one AD epistatic interaction for GW between qGW-J2-1 and qGW-Ch.8-1/qGW-Ch.8-1 was repeatedly identified in POP1/POP2 under four watering conditions; one AA epistatic interaction for KR between qKR-J1-1 and qKR-J8-1 was repeatedly mapped in POP1/POP2 under five watering conditions; one AA epistatic interaction for KR between qKR-J6-1 and qKR-J7-1 was repeatedly detected in POP1/POP2 under two watering conditions; one AA epistatic interaction for KR between qKR-J6-1 and qKR-J8-1 was repeatedly found in POP1/POP2 under three watering conditions; and one DD epistatic interaction for EHPH between qEHPH-J6-1 and qEHPH-Ch.6-1 was repeatedly identified in POP2 under two watering conditions. These results with the findings of Chang *et al.* (2017), Lan and Chu (2005), Lu *et al.* (2007), Sa *et al.* (2015), Yang *et al.* (2015), and Yang *et al.* (2016). Therefore, these five QTL intervals may be considered as epistatic regulators that influence maize plant and ear development under multiple watering conditions.

Here, the three parents, two F_1 hybrids, and two $F_{2:3}$ populations were planted under eight different watering environments to analyze the phenotypic variations, and QTLs for corresponding morphological traits and yield components. Then the results show that: (1) the four morphological traits (PH, EH, EHPH, and ASI) and six yield components (EW, CW, GW, KW, EL, and KR) may be used as selection criteria in breeding drought-tolerant maize; (2) The identified QTLs for EHPH, GW, and KR by single-environment mapping with CIM and the identified joint QTLs, epistatic interactions, and QTL $\times$ E interactions for the three traits across all environments via MCIM may provide valuable information on the genetic mechanisms of yield components and morphological traits under both drought and non-drought conditions; (3) The identified sQTLs and candidate genes provide reliable information that may be utilized in future studies in identifying quantitative trait genes under contrasting watering conditions. Furthermore, alleles involved in yield components and morphological traits may be used to rapidly generate elite germplasms for MAS, as well as provide a novel strategy for the improvement of maize morphology and yield in arid areas.

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