Genetics and Molecular Research

Comparative quantitative trait locus mapping of maize flowering-related traits in an $F_{2:3}$ and recombinant inbred line population

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Genet. Mol. Res. 15 (2): gmr.15028465
Received January 20, 2016
Accepted March 21, 2016
Published June 30, 2016
DOI http://dx.doi.org/10.4238/gmr.15028465

ABSTRACT. Flowering-related traits in maize are affected by complex factors and are important for the improvement of cropping systems in the maize zone. Quantitative trait loci (QTLs) detected using different materials and methods usually vary. In the present study, 266 maize (*Zea mays*) $F_{2:3}$ families and 301 recombinant inbred lines (RIL) derived from a cross between 08-641 (founding parent from southeast China) and Ye478 (founding parent from China) were evaluated for four flowering-related traits, including days to tasseling (DTT), days to pollen shedding (DPS), days to silking (DTS), and anthesis-silking interval. Sixty-six QTLs controlling the target traits were detected in the $F_{2:3}$ and RIL populations via single environment analysis and joint analysis across all environments (JAAE). The

QTLs explained 0.8-13.47% of the phenotypic variation, with 12 QTLs explaining more than 10%. The results of meta-QTL (MQTL) analysis indicated that 41 QTLs could be integrated into 14 MQTLs. One MQTL included 2.9 QTLs, ranging from two to ten QTLs for one to three traits. QTLs, including MQTL1-1 and MQTL9-1, were detected across the $F_{2:3}$ and RIL populations via SAE and JAAE. Among the MQTLs, nine QTLs were integrated into MQTL9-1 and affected DTT, DPS, and DTS, with the favored allele being derived from 08-641. MQTL3-2 showed high phenotypic variation and was suitable for fine mapping to determine the genetic mechanisms of flowering. MQTL3-2 could be applied to improve inbred lines using marker-assisted selection.

Key words: Maize; Flowering-related traits; $F_{2:3}$, RIL; QTL; MQTL

INTRODUCTION

Flowering-related quantitative traits are important features in maize (*Zea mays*) and are affected by complex factors such as light, temperature, latitude, agronomic measures, and stress (Bonhomme et al., 1994; Otegui et al., 1995; Dowswell et al., 1996; Traore et al., 2000; Li et al., 2003). Flowering-related quantitative traits are very important for the improvement of cropping systems within the maize zone, which is subjected to annual multi-cropping rotation with other crops. Flowering-related traits of maize have been extensively studied in China, and in other countries, using different populations and methods (Wang et al., 2010; Yang, 2012; Zheng et al., 2011, 2012; Wei et al., 2014). $F_{2:3}$ populations and recombinant inbred lines (RILs) are useful in the study of flowering traits, plant types, yield, and resistance (Lima et al., 2006; Salgado et al., 2008; Ku et al., 2012; Yang et al., 2014a). Genetic mapping studies on flowering traits of maize are influenced by the genetic background, environment, population, marker numbers, and mapping methods used (Austin and Lee, 1996; Li et al., 2007, 2011). In this study, 266 $F_{2:3}$ family lines and a population of 301 RILs were derived from a cross between the founding parents 08-641 and Ye478. A high-density single nucleotide polymorphism (SNP)-based genetic linkage map was constructed to carry out quantitative trait locus (QTL) mapping analysis for maize flowering traits in different years and environments. The objectives of this research were as follows: 1) to identify uniform QTLs for flowering-related traits in maize in the ecological area of southwestern China in different mapping populations; and 2) to perform map-based cloning and marker-assisted selection (MAS) of flowering-related traits.

MATERIAL AND METHODS

Plant materials

The F_1 hybrid population was derived from a cross between the founding parents 08-641 (southeast China, PB) and Ye478 (China, PA). An $F_{2:3}$ population, with a total of 266 family lines, was derived at the end of 2011, and a RIL population comprising 301 family lines was derived in 2014.

Field experimental design and trait investigation

The two parents and the $F_{2:3}$ population were cultivated at Jinghong, Yunnan (Jinghong, JH, 100°76'E, 21°95'N) and Nanning, Guangxi (Nanning, NN, 108°19'E, 22°48'N) in 2012 and 2013, respectively (both were cultivated in spring). Tests were performed in triplicate using a randomized block design, with seeds planted in single rows, 3 m length, and 0.8 m between rows, with 14 plants per row. The RIL population was cultivated in March 2014, March 2015, and April 2015 at JH using the same planting method as described above. Field management was performed as described by Hou et al. (2015). Four flowering-related traits [days to tasseling (DTT), days to pollen shedding (DPS), days to silking (DTS), and anthesis-silking interval (ASI)] were evaluated based on the standards provided by Shi et al. (2006).

Data analysis of phenotype traits

Analysis of variance (ANOVA) was carried out using PROC GLM with the SPSS software (http://www.spss.com). Broad-sense heritability (*H2 B*) and its confidence intervals were computed as described by Hallauer et al. (2010) as:

$$H2\ B = s2\ g / (s2\ g + s2\ ge / n + s2 / nb) \qquad \text{(Equation 1)}$$

where *s2 g* is the genetic variance, *s2 ge* is the genotype x environment interaction variance, *s2* is the error variance, *n* is the number of environments, and *b* is the number of replications in each experiment. Pearson's phenotypic correlations were determined using SPSS PROC CORR (http://www.spss.com).

Molecular linkage map construction

Genomic DNA was extracted from young leaves of $F_{2:3}$ plants and their parents (at least 10 plants per $F_{2:3}$ line as a bulk) and F_7 lines (five plants per line as a bulk) using a modiðed cetyltrimethyl ammonium bromide procedure, according to the method described by Chen and Ronald (1999). The quality and quantity of DNA were reviewed carefully before genotyping. The oligonucleotide pool assay used in this study was developed by the National Maize Improvement Center of China using IlluminaGoldenGate technology (Hou et al., 2015). Genotyping was carried out using an IlluminaBeadStation 500 G (Illumina, San Diego, CA, USA) at the National Maize Improvement Center of China using the protocol described by Fan et al. (2006a). The genetic map was developed using the MapDisto 1.7.5 software (http://mapdisto.free.fr/DL/) and Joinmap 4.0 (https://www.kyazma.nl/index.php/mc.JoinMap/). The genetic map was drawn using the Mapchart 2.2 software (http://www.wageningenur.nl/en/show/Mapchart.htm) (Voorrips, 2002).

QTL mapping

Analyses of the QTL locations, origin of positive alleles, effects of QTLs on each trait for each environment (SEA), and joint analysis across all environments (JAAE) were performed using the QTLNetwork software version 2.1 (Yang et al., 2008). The genome

scan configuration was set to a 10-cM testing window with a 1-cM walk speed to identify QTLs associated with each trait. A 10-cM filtration window was set to distinguish between two adjacent test statistical peaks (i.e., whether they are two QTLs or not). The threshold for declaring the presence of a QTL was deðned by 1000 permutations at a significance level of P = 0.05. QTLs detected in different environments for the same trait were considered to be the same if their confidence intervals overlapped. Each mapped QTL was denominated in accordance with the following: q + abbreviated name of the trait + population type abbreviation ($F_{2:3}$ population, F, RIL population, R) + environment and detection method (SEA or JAAE) abbreviation + serial number on chromosome.

RESULTS

Phenotype analysis

Phenotypic analysis of the $F_{2:3}$ and RIL populations (Table 1) revealed significant variation for all four flowering-related traits. The mean value of each flowering trait fell between and outside the values of the two parents. All traits showed transgressive segregation, and the absolute values of skewness and kurtosis for most traits were less than 1. The traits were distributed normally, and these data were deemed suitable for QTL mapping and analysis. The generalized heritage rate of each trait varied from 61.92 to 85.75%, indicating that heritable factors play an important role in those traits.

Table 1. Phenotypic performance for flowering-related traits in the $F_{2:3}$ population, the RIL population, and their parents.

Trait	Pop.	Env.	08-641	Ye478	Mean	Minimum	Maximum	Standard deviation	Skewness	Kurtosis	Hb^2 (%)
DTT	$F_{2:3}$	12JH	67.5	65.5	63.6	56.0	70.0	2.31	-0.38	-0.15	68.55
	$F_{2:3}$	13NN	56.0	54.0	54.9	45.0	62.0	2.63	0.22	0.39	71.01
DPS	$F_{2:3}$	12JH	68.5	66.5	65.9	60.0	72.0	1.72	-0.02	0.67	61.92
	$F_{2:3}$	13NN	57.0	56.0	57.2	45.0	63.0	2.55	-0.82	3.16	69.24
	RIL	14JH	64.5	63.0	63.5	59.0	71.0	2.39	0.16	-0.22	80.86
	RIL	15JH3	65.0	63.3	63.0	55.0	72.0	2.60	-0.34	0.10	71.58
	RIL	15JH4	65.0	63.5	66.6	60.0	75.0	2.24	0.26	0.74	74.81
DTS	$F_{2:3}$	12JH	73.5	68.5	68.8	61.0	80.0	2.78	0.71	0.92	74.20
	$F_{2:3}$	13NN	61.0	58.0	58.4	45.0	64.0	2.53	-1.17	5.32	70.80
	RIL	14JH	67.5	63.5	66.0	59.0	73.0	2.68	-0.13	0.06	85.87
	RIL	15JH3	69.0	63.3	65.4	56.0	75.0	3.00	-0.2	0.53	72.74
	RIL	15JH4	70.5	66.0	70.0	58.0	79.0	3.48	0.41	0.30	76.97
ASI	$F_{2:3}$	12JH	5.0	2.0	3.0	0	13.0	2.30	1.30	2.35	73.87
	$F_{2:3}$	13NN	4.0	2.0	1.5	0	6.0	1.27	0.75	0.14	68.81
	RIL	14JH	3.0	0.5	2.6	0	10.0	1.93	0.51	-0.14	75.85
	RIL	15JH3	4.0	1.3	2.6	0	8.0	1.94	0.44	-0.61	65.54
	RIL	15JH4	5.5	2.5	3.5	0	14.0	2.45	0.80	0.63	71.61

RIL population represent recombinant inbred lines population. 12JH, 13NN, 14JH, 15JH3, and 15JH4 represent the five environments: Jinghong in 2012, Nanning in 2013, Jinghong in 2014, Jinghong in March 2015, Jinghong in April 2015.

ANOVA

ANOVA indicated that there were significant differences between the family lines (Table 2). In addition, a significant difference was found between the individual environments, and the interaction between the individual environments and the family lines

had a highly significant effect on the four traits (Table 2). Thus, the interaction between these two factors is associated with flowering traits. The environmental effects detected in the RIL population were less than those detected in the $F_{2:3}$ family lines. Moreover, there was a significant increase in the difference between the lines. This indicated that the RIL population is more effective than $F_{2:3}$ family lines at detecting flowering-related traits in multiple environments.

Table 2. ANOVA for flowering-related traits of the $F_{2:3}$ families and the RIL population.

Source of variation	$F_{2:3}$ population				RIL population		
	DTT	DPS	DTS	AIS	DPS	DTS	ASI
Environment	552.65*	420.54*	653.91**	115.86	64.57	112.45*	1.47
Families	9.72**	7.37**	12.55**	5.93**	14.94**	20.86**	9.02**
Environment x families	2.61**	1.84**	2.89**	1.39	4.30**	5.29**	2.46**
Error	1.24	1.01	1.56	1.17	2.33	2.77	1.68

RIL population represent recombinant inbred lines population. *Significant at $P< 0.05$, **significant at $P < 0.01$.

Molecular linkage map construction

In this study, 3072 SNPs were used for genotyping. To construct the linkage map, 471 SNPs with good polymorphism between lines were selected from the $F_{2:3}$ population. The full length of the linkage map was 2007.91 cM, the average space between markers was 4.26 cM, and there were 26-86 markers in each linkage group, with an average of 47 markers per group. Chromosome 1 had the most markers and the largest map distance (86 markers and 325.52 cM, respectively) (Hou et al., 2015). To construct the genetic linkage map for the RIL population, 683 SNP markers with good polymorphism were screened. The RIL map had a full length of 1786.1 cM, and an average of 2.61 cM between markers. There were 44-115 makers in each linkage group, with 68 SNP markers per group on average. Finally, a joint map for the $F_{2:3}$ and RIL populations was constructed, and QTLs obtained from the individual maps are indicated on the joint map (Figure 1). The total length of the joint map was 1690.48 cM, in which the map of chromosome 1 had the longest distance at 231.44 cM.

QTL mapping of flowering-related traits based on the $F_{2:3}$ and RIL populations

QTLs for the four flowering-related traits were detected in the $F_{2:3}$ and RIL populations by composite interval mapping. Sixty-six QTLs were mapped in the $F_{2:3}$ and RIL populations (Table 3) by SEA (single-environment analysis) and JAAE. Thirty-five QTLs were obtained through SEA and 31 QTLs were obtained through JAAE. Among them, nine QTLs were detected in Jinghong in 2012 (12JH), four QTLs were detected in Nanning in 2013 (13NN), 10 QTLs were detected in Jinghong in 2014 (14JH), seven QTLs were detected in Jinghong in March 2015 (15JH3), and five QTLs were detected in Jinghong in April 2015 (15JH4). QTLs were detected on chromosomes 1, 3, 4, 5, 6, 7, 8, 9, and 10. In total, 13, 9, 7, 7, 4, 13, and 7 QTLs were located on chromosomes 1, 3, 5, 7, 8, 9, and 10, respectively. Each QTL contributed 0.56-13.47% of the phenotypic variance, and 12 QTLs contributed more than 10% of the phenotypic variance.

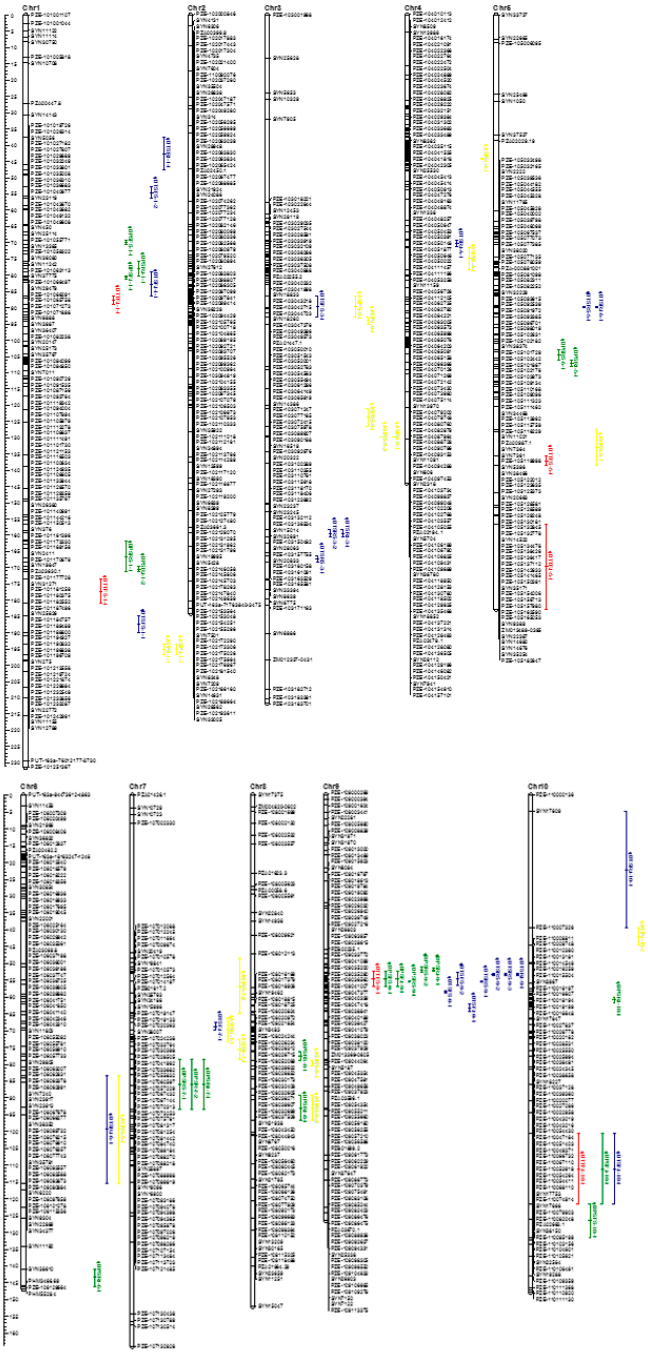

Figure 1. Distribution of identified QTLs (quantitative trait loci) for flowering-related traits on the joint map produced in this study. Vertical lines on the right of each chromosome indicate the confidence interval, DTT is in red, DPS is in green, DTS is in blue, and AIS is in yellow.

Table 3. Main features of the QTLs for flowering-related traits of the $F_{2:3}$ and RIL populations based on SEA and JAAE.

Env.	QTL	Flanking marker	Peak positions (cm)	Range (cm)	Bin loci	A	D	Gene action	R^2 (%)	F
DTT										
JH2012	qDTTFS-1-1	PZE-101196709/SYN275	260.1	255.3-269.7	1.08-1.09	0.1852	-0.7078**	OD	3.41	9.84
	qDTTFS-5-1	PZE-105163590/PZE-105165053	153.2	144.5-161.4	5.07	-0.6900**	-0.8760**	PD	10.42	9.58
NN2013	qDTTFS-9-1	PZE-109028615/PZE-109041079	62.3	55.9-67.3	9.03	-1.3291**	0.3867	A	11.79	16.7
Joint	qDTTFJ-1-1	PZE-101071273/SYN6888	121	116.6-124.0	1.04	-0.7945**	-0.1625	A	7.68	7.39
	qDTTFJ-5-1	SYN35254/PZE-105182647	182.9	168.7-192.9	5.08	-0.6432**	-0.5246*	PD	3.82	5.81
	qDTTFJ-10-1	PZE-110095199/PZE-110103156	131	122.0-144.3	10.06-10.07	-0.8602**	0.3266	A	7.69	8.89
DPS										
JH2012	qDPSFS-1-1	PZE-101046132/PZE-101049608	89.6	85.9-99.7	1.03	-0.6340**	0.1765	A	11.66	14.94
	qDPSFS-10-1	PZE-110103156/PZE-110105621	139.3	125.0-144.3	10.07	-0.7161**	0.0482	A	12.95	19.04
NN2013	qDPSFS-9-1	PZE-109028615/PZE-109041079	62.3	57.9-66.3	9.03	-1.3473**	0.5330	A	12.86	18.8
Joint	qDPSFJ-1-1	SYN29479/SYN37775	111.1	106.8-112.9	1.03	-0.5681**	-0.2193	A	8.36	7.13
	qDPSFJ-9-1	PZE-109028615/PZE-109041079	62.3	58.9-66.3	9.03	-0.6160**	0.2258	A	9.24	11.35
	qDPSFJ-10-1	PZE-110095199/PZE-110103156	132	124.0-142.3	10.06-10.07	-0.8301**	-0.0005	A	8.67	9.55
JH2014	qDPSRS-1-1	PZE-101187496/PZE-101196838	178.6	174.6-179.5	1.08	0.6119**	\	\	6.84	28.4
	qDPSRS-5-1	SYN5396/PZE-105125373	131.3	129.3-131.7	5.05	-0.4600**	\	\	4.63	21.57
	qDPSRS-7-1	PZE-107057229/PZE-107081317	77	69.0-83.9	7.02-7.03	-0.5802**	\	\	6.00	16.93
	qDPSRS-9-1	PZE-109038841/PZE-109047418	73	72.0-73.1	9.03	-0.7022**	\	\	12.95	46.29
JH2015-03	qDPSRS-8-1	SYN9237/PZE-108056460	92.6	90.6-94.6	8.03	0.5048**	\	\	4.49	18.12
JH2015-04	qDPSRS-7-2	PZE-107057229/PZE-107081317	73	66.0-81.0	7.02-7.03	-0.5210**	\	\	5.20	14.23
	qDPSRS-9-2	SYN26803/PZE-109028615	61.8	60.8-61.9	9.03	-0.3701**	\	\	4.42	14.46
Joint	qDPSRJ-1-1	SYN13385/SYN37775	65.4	61.4-65.5	1.03	-0.3813**	\	\	2.58	8.67
	qDPSRJ-1-2	PZE-101196838/PZE-101194927	180.5	179.5-181.0	1.08	0.4586	\	\	2.90	15.75
	qDPSRJ-5-1	PZE-105125373/PZE-105128589	132.7	131.7-134.6	5.05	-0.3285**	\	\	1.49	10.05
	qDPSRJ-6-1	SYN38610/PZE-106129664	152.4	148.4-153.2	6.07-6.08	0.3187	\	\	0.56	7.58
	qDPSRJ-7-1	PZE-107057229/PZE-107081317	75	70.0-80.0	7.02-7.03	-0.6177**	\	\	5.29	15.78
	qDPSRJ-8-1	PZE-108074750/PZE-108092173	109.8	107.8-114.8	8.05-8.06	0.3802	\	\	1.98	8.95
	qDPSRJ-9-1	PZE-109028615/PZE-109063957	62.9	61.9-63.7	9.03-9.04	-0.5270**	\	\	7.13	7.95
	qDPSRJ-10-1	PZE-110019199/PZE-110020162	53.9	52.9-54.4	10.03	-0.3413**	\	\	1.95	8.44
DTS										
JH2012	qDTSFS-1-1	PZE-101213558/PZE-101219724	274.1	268.7-278.8	1.09-1.10	-0.9697**	-0.7421*	PD	6.47	16.57
	qDTSFS-3-1	PZE-103089927/SYN20322	149.2	143.2-150.8	3.05	-0.6312**	0.2441	A	4.12	12.4
	qDTSFS-9-1	PZE-109045354/PZE-109049656	75.3	71.6-77.2	9.03	-1.0747**	0.2591	A	9.40	9.37
NN2013	qDTSFS-1-2	PZE-101029689/PZE-101033801	60.6	49.0-75.4	1.02	-0.8413**	-0.2470	A	6.92	9
	qDTSFS-9-2	PZE-109028615/PZE-109041079	63.3	58.9-73.5	9.03	-1.156**	0.4109	A	11.06	15.82
Joint	qDTSFJ-1-1	PZE-101063113/PZE-101071273	120.6	114.6-124.9	1.03-1.04	-0.7481**	-0.3846	A	5.92	6.53
	qDTSFJ-7-1	PZE-107020363/SYN38007	89.3	78.4-91.9	7.02	0.7951**	-0.5154*	PD	4.88	7.25
	qDTSFJ-9-1	PZE-109056255/PZB01899.2	83.6	83.2-85.6	9.03-9.04	-0.9257**	0.1882	A	10.61	11.59
	qDTSFJ-10-1	PZE-110095199/PZE-110103156	131	122.0-142.3	10.06-10.07	-0.8241**	-0.2874	A	6.57	7.22
JH2014	qDTSRS-3-1	PZE-103161091/PZE-103163529	177	176.0-178.0	3.08	-0.6217**	\	\	7.41	22.75
	qDTSRS-9-1	PZE-109038841/PZE-109047418	73	72.0-73.1	9.03	-0.8729**	\	\	13.47	41.89
JH2015-03	qDTSRS-3-2	SYN28063/PZE-103157755	166	165.0-168.2	3.08	-0.6739**	\	\	6.96	25.21
	qDTSRS-9-2	PZE-109033772/PZE-109041099	66.7	65.7-66.7	9.03	-0.6084**	\	\	6.16	15.6
JH2015-04	qDTSRS-5-1	PZE-105109096/PZE-105110168	108.8	107.8-109.7	5.04	-0.8068**	\	\	7.14	23.42
	qDTSRS-9-3	PZE-109033772/PZE-109041099	66.7	65.7-66.7	9.03	-0.6807**	\	\	5.47	16.29
Joint	qDTSRJ-1-1	PZE-101019726/SYN5056	11.3	9.3-17.2	1.01-1.05	-0.3605**	\	\	3.02	7.28
	qDTSRJ-3-1	SYN28063/PZE-103157755	166	165.0-168.0	3.08	-0.4746**	\	\	3.24	8.89
	qDTSRJ-4-1	SYN11091/PZE-104094288	108.6	106.6-109.1	4.06-4.07	0.5245	\	\	2.08	13

RIL represent recombinant inbred lines. SEA represent single-environment analysis and JAAE represent joint analysis across all environments. Positive and negative values of additive effects indicate that the positive alleles are from Ye478 and 08-641 in the $F_{2:3}$ and RIL populations; *significant at $P < 0.05$, **significant at $P < 0.01$; A, D, PD, and OD represent additive, dominance, partial dominance, and over-dominance effect, respectively.

The DTT phenotype was only measured in the $F_{2:3}$ population. Three QTLs were detected via SEA and three via JAAE for DTT, which were located on chromosomes 1, 5, 9, and 10. The contributions of QTLs detected through JAAE were less than those detected through SEA. Each QTL contributed 3.41-11.79% of the phenotypic variance. In this study, two main-effect QTLs, namely qDTTFS-5-1 and qDTTFS-9-1, which contributed more than 10% of the phenotypic variance, were detected through SEA, and the alleles that increased the phenotype were all derived from the 08-641 parent. The additive effects of three QTLs detected via JAAE were negative, which showed that the alleles were derived from parent 08-641 and delayed DTT. The QTL qDTTFJ-5-1 showed partially dominance, while other QTLs showed additive effects.

For DPS, 10 and 11 QTLs were detected via SEA and JAAE, respectively, which were located on chromosomes 1, 5, 6, 7, 8, 9, and 10. Six QTLs were detected in the $F_{2:3}$ population (three by SEA and three by JAAE), while seven and eight QTLs were detected in

the RIL population via SEA and JAAE, respectively. Each QTL accounted for 0.8-12.95% of the phenotypic variation. Four QTLs, namely qDPSFS-1-1, qDPSFS-9-1, qDPSFS-10-1, and qDPSRS-9-1, contributed more than 10% of the phenotypic variance. QTLs qDPSRS-1-1/qDPSRJ-1-2, qDPSRS-5-1/qDPSRJ-5-1, qDPSRS-7-1/qDPSRJ-7-1, and qDPSFS-9-1/qDPSFJ-9-1 were detected via SEA and JAAE. In two populations, QTLs qDPSFS-9-1, qDPSFJ-9-1, qDPSRS-9-1, and qDPSRJ-9-1 were located within the same marker interval, which was PZE-109028615-PZE-109063957. The gene action modes of all DPS QTLs were additive. Sixteen and five QTL alleles were derived from 08-641 and Ye478, respectively, which increased the phenotype.

For DTS, 22 QTLs were detected using SEA or JAAE, which were located on chromosomes 1, 3, 4, 5, 6, 7, 9, and 10. Five and four QTLs were detected via SEA and JAAE in the $F_{2:3}$ population, and six and seven were detected via SEA and JAAE in the RIL population. Each QTL accounted for 1.77-13.47% of the phenotypic variation. Three QTLs accounted for more than 10% of the phenotypic variation. The QTL qDTSRS-9-1, which was detected in the RIL population, contributed 13.47% of the phenotypic variance. QTLs qDTSFS-9-1, qDTSFS-9-2, qDTSRS-9-1, qDTSRS-9-2, qDTSRS-9-3, and qDTSRJ-9-1 were located within the same marker interval, which was PZE-109028615-PZE-109049656. QTLs qDTSRS-3-1 and qDTSRJ-3-1were both located within the marker interval PZE-103161091-PZE-103163529. QTLs qDTSFS-1-1 and qDTSFJ-7-1 showed partial dominance, and other QTLs showed additive effects. The additive effects of qDTSFJ-7-1, qDTSRJ-4-1, and qDTSRJ-6-1 had positive values, whereas the additive effects of the other QTLs had negative values.

For ASI, 11 and six QTLs were detected via SEA and JAAE, respectively, which were located on chromosomes 1, 3, 4, 5, 6, 7, 8, and 10. Each QTL accounted for 0.38-13% of the phenotypic variation. Two QTLs accounted for more than 10% of the phenotypic variation in the two populations. qASIRS-3-1, which was detected in the RIL population, accounted for 13% of the phenotypic variation. QTLs qASIFS-1-1 and qASIFJ-1-1 were located in the marker interval PZE-101229884-PZE-101232549, and QTLs qASIRS-3-2 and qASIRJ-3-1 were located in the interval SYN23245-PZE-103132112. QTLs qASIFS-1-1 and qASIFS-3-1 showed partial dominance, while the other QTLs showed additive effects. The additive effect of 10 QTLs that had negative values and whose alleles prolonged ASI were derived from parent 08-641; the additive effect of the other seven QTLs that had positive values and whose alleles prolonged ASI were derived from parent Ye478.

Epistatic interactions

In this study, three and five pairs of QTLs with significant ($P < 0.05$) epistatic effects were detected in the $F_{2:3}$ and RIL populations, respectively (Table 4). One pair of QTLs was detected each for DPS, DTS, and ASI in the $F_{2:3}$ population. In addition, two pairs of QTLs affecting DPS, and three pairs of QTLs affecting DTS, were detected in the RIL population. Six pairs of loci with additive x additive epistatic effects were significant, and their individual variance ranged from 0.3 to 1.13%. Two pairs of loci with additive x dominant or dominant x additive epistatic effects were significant, and one pair of loci with dominant x dominant epistatic effects was significant. There were no significant effects of the interaction between epistasis and the environment.

Table 4. Epistatic effects of QTLs for flowering-related traits identified in the $F_{2:3}$ and RIL populations.

Pop.	Trait	QTL_i	QTL_j	AA	h^2 (aa) (%)	AD	h^2 (ad) (%)	DA	h^2 (da) (%)	DD	h^2 (dd) (%)
$F_{2:3}$	DPS	qDPSFJ-1-1	qDPSFJ-10-1			-0.7928**	0.95				
	DTS	qDTSFJ-9-1	qDTSFJ-10-1					-0.9523**	1.7		
	AIS	qASIFJ-1-1	qASIFJ-10-1	0.3344*	0.39					-0.9733**	1.92
RIL	DPS	qDPSRJ-1-1	qDPSRJ-6-1	-0.2135**	0.83						
		qDPSRJ-6-1	qDPSRJ-7-1	-0.1999**	0.44						
	DTS	qDTSRJ-3-1	qDTSRJ-4-1	-0.3427**	1.13						
		qDTSRJ-3-1	qDTSRJ-10-1	0.2211**	0.58						
		qDTSRJ-9-1	qDTSRJ-10-1	0.2717**	0.90						

RIL represent recombinant inbred lines. *Significant at $P < 0.05$, **significant at $P < 0.01$; AA, AD, DA, and DD represent additive x additive, additive x dominance, dominance x additive, and dominance x dominance, respectively; h^2 represents the contribution rate of one interaction effect.

Meta-analysis of $F_{2:3}$ and RIL populations

Fourteen meta-QTLs (MQTL, a collection of QTLs with overlapping confidence intervals), containing 41 QTLs, were detected in this study, based on genetic mapping of $F_{2:3}$ and RIL populations by SEA and JAAE (Table 5). These MQTLs are located on chromosomes 1, 3, 5, 6, 7, 8, 9, and 10. Each MQTL contains 2.9 QTLs on average, including 2-10 QTLs and affecting one to three traits. MQTL1-3, MQTL3-1, and MQTL10-1 were detected in the $F_{2:3}$ population; MQTL3-2, MQTL3-3, MQTL5-1, MQTL6-1, MQTL7-2, and MQTL8-1 were detected in the RIL population. Nine alleles of the 41 QTLs that had positive additive effect were derived from Ye478, while the other 32 alleles were derived from 08-641. Among the 14 MQTLs, nine and three were derived from 08-641 and Ye478, respectively. The other two, namely MQTL5-2 and MQTL8-1, were conferred by both Ye478 and 08-641. MQTL1-3 comprised two QTLs that affected ASI. MQTL6-1 contained two QTLs that affected DTS and ASI. MQTL9-1 contained 10 QTLs that affected DTT, DPS, and DTS.

Table 5. Meta-QTL identification for traits related to flowering across two different populations.

No.	Physical interval (bp)	Flanking marker	QTL No.	Traits (population)	Integrated QTLs	Positive allele derived from
MQTL1-1	43974129/53663576	SYN13385/PZE-101071273	3	DPS ($F_{2:3}$, RIL), DTS ($F_{2:3}$)	qDPSRJ-1-1, qDTSFJ-1-1, qDPSFJ-1-1	08-641(3)
MQTL1-2	232527769/260149117	PZE-101187496/SYN275	3	DPS (RIL), DTS ($F_{2:3}$)	qDPSRS-1-1, qDTTFS-1-1, qDPSRJ-1-2	Ye478(3)
MQTL1-3	279410741/281068865	PZE-101229884/PZE-101232549	2	ASI ($F_{2:3}$)	qASIFS-1-1, qASIFJ-1-1	08-641(2)
MQTL3-1	148540478/160901885	PZE-103089927/SYN20322	2	DTS ($F_{2:3}$), AIS ($F_{2:3}$)	qDTSFS-3-1, qASIFS-3-1	08-641(2)
MQTL3-2	184674522/188091257	SYN23245/PZE-103132112	2	ASI (RIL)	qASIRS-3-2, qASIRJ-3-1	08-641(2)
MQTL3-3	208785867/209798825	SYN28063/PZE-103157755	2	DTS (RIL)	qDTSRS-3-2, qDTSRJ-3-1	08-641(2)
MQTL5-1	166332322/167276024	PZE-105109096/PZE-105110168	2	DTS (RIL)	qDTSRS-5-1, qDTSRJ-5-1	08-641(2)
MQTL5-2	205552836/208935009	PZE-105156713/PZE-105165053	2	AIS (RIL), DTT ($F_{2:3}$)	qASIRS-5-1, qDTTFS-5-1	08-641(1)+Ye478(1)
MQTL6-1	141080410/161454721	PZE-106083873/PZE-106115356	2	DTS (RIL), AIS (RIL)	qDTSRJ-6-1, qASIRS-6-1	Ye478(2)
MQTL7-1	17478189/46213540	PZE-107019133/PZE-107033682	2	ASI (RIL), DTS ($F_{2:3}$)	qASIRS-7-1, qDTSFJ-7-1	Ye478(2)
MQTL7-2	109535093/136261616	PZE-107057229/PZE-107081317	3	DPS (RIL)	qDPSRS-7-1, qDPSRS-7-2, qDPSRJ-7-1	08-641(3)
MQTL8-1	130213045/149193811	PZE-108074750/PZE-108092173	2	DPS (RIL), ASI (RIL)	qDPSRJ-8-1, qASIRS-8-2	08-641(1)+Ye478(1)
MQTL9-1	30646914/106788007	PZE-109028615/PZE-109063957	10	DTT ($F_{2:3}$), DPS ($F_{2:3}$, RIL), DTS ($F_{2:3}$, RIL)	qDTTFS-9-1, qDPSFS-9-1, qDPSFJ-9-1, qDPSRJ-9-1, qDTSFS-9-2, qDTSRS-9-2, qDTSRS-9-3, qDTSRJ-9-1, qDPSRS-9-1, qDTSRS-9-1	08-641(10)
MQTL10-1	142189873/146124494	PZE-110095199/PZE-110103156	3	DTT ($F_{2:3}$), DPS ($F_{2:3}$), DTS ($F_{2:3}$)	qDTTFJ-10-1, qDPSFJ-10-1, qDTSFJ-10-1	08-641(3)

DISCUSSION

Comparative QTL mapping between the $F_{2:3}$ and RIL populations

Maize flowering time is a complex trait, which is affected by the genetic background, the environment, and by other factors. To date, numerous genetic studies have been conducted for maize flowering traits (Sari-Gorla et al., 1999; Chardon et al., 2004; Buckler et al., 2009; Xu et al., 2012). Some related genes have been identified, including *VGT1* (vegetative to

generative transition 1) (Salvi et al., 2002; Salvi et al., 2007). QTL mapping results have been obtained from many different genetic backgrounds and are useful for understanding flowering-related traits (Beavis et al., 1994; Li et al., 2007; Buckler et al., 2009; Steinhoff et al., 2012; Mace et al., 2013).

In this study, four flowering-related traits were analyzed using QTL mapping in the $F_{2:3}$ and RIL populations across multiple environments. In total, 35 and 31 QTLs were detected via SEA and JAAE, respectively; 26 and 40 QTLs were detected in the $F_{2:3}$ and RIL populations, respectively. The total phenotypic variance explained by all QTLs detected via JAAE for DTT, DPS, DTS, and ASI in the $F_{2:3}$ population was 19.19, 26.27, 27.98, and 10.76%, respectively. The total phenotypic variance explained by all QTLs detected via JAAE for DPS, DTS, and ASI in the RIL population was 23.88, 25.88, and 14.85%, respectively. This suggests that the $F_{2:3}$ and RIL populations have an approximate mapping effect (Austin and Lee, 1996; Li et al., 2007). The QTL mapping results obtained from SEA and JAAE were comparatively uniform, which suggests that JAAE could replace SEA to map QTLs in multi-environment studies (Hou et al., 2015). However, some differences in the mapping results also exist between different populations, although they were derived from the same genetic background, and these differences might be influenced by heterozygosity or by environmental effects (Austin and Lee, 1996; Li et al., 2007, 2011). Austin and Lee (1996) used an $F_{2:3}$ and a RIL population, both of which were derived from a cross between Mo17 and H99, to detect the QTL mapping efficiency between different populations. Their results showed that the RIL population had a higher QTL mapping resolution and detected more QTLs than the $F_{2:3}$ population. Li et al. (2007) used 259 $F_{2:3}$ lines and 220 BC_2S_1 lines, which were derived from a cross between Dan 232 and N04, to detect QTLs for maize growth stages. They found significant differences in QTL information (total number, positions, and effects) between the two populations. This might have been caused by the $F_{2:3}$ and BC_2S_1 populations having different population structures. Li et al. (2011) used a RIL population derived from a cross between Dan 232 and N04 to analyze yield-related traits. However, they only identified adjacent QTLs, which were thought to result from the genetic background and from environmental effects. Therefore, the results of the present study showed that QTL mapping results obtained from populations derived from founding parents at different generations are reliable. Furthermore, the results of this study lay the foundation for MAS and for other further studies.

Meta-analysis of maize-flowering time traits and relevant studies

In this study, QTLs were located on all chromosomes except for chromosome 2. In terms of QTL information, our results are consistent with those from some previous studies (**S1 Table**). The qDTTFS-1-1 locus in bin 1.08, which is close to PZE-101196709, was located in the same region as the QTL associated with DTT detected by Wei et al. (2014). qDTTFS-1-1 has a negative dominant effect and showed partial dominance. The qDPSRS-1-1 locus in bin 1.08 was located on the same region as the QTL associated with DPS obtained by Austin and Lee (1996), Veldboom et al. (1994), and Veldboom and Lee (1996). The qAISFS-3-1 locus in bin 3.05 was the same as the QTL mapped by Veldboom et al. (1994) and Veldboom and Lee (1996), indicating that this region might be a QTL hotspot (Xu et al., 2012; Wang et al., 2013). Loci qDPSRJ-8-1 and qASIRS-8-2, both in bin 8.05-8.06, were located on the same region as the *RAP2* and *VGT1* genes, which affect DPS (Salvi et al., 2002, 2007).

Compared with the results obtained in maize-nested association mapping populations

and the enlarged maize association panel, we found many MQTLs that could be considered significant chromosome regions for flowering-related traits (Buckler et al., 2009; Yang et al., 2014b). The MQTL1-1 locus in bin 1.03/1.04 and MQTL9-1 locus in bin 9.03 affect both DPS and DTS, and seem to be located in the same regions as the QTLs that are close to PZA03742.1 and PZB00959.1, respectively (Buckler et al., 2009). The MQTL1-3 locus in bin 1.10 and the MQTL3-2 locus in bin 3.06 affect ASI, and seem to be located in the same regions as the QTLs that are close to PZB00063.1 and PZA01228.2, respectively (Buckler et al., 2009). The MQTL1-2 locus in bin 1.09 affects DTT, and seems to be located in the same region as the QTL that is close to chr1.S_260516920 (Yang et al., 2014b). The MQTL7-2 locus in bin 7.03 affects DPS, and seems to be located in the same region as the QTL that is close to PZE-105088747 (Yang et al., 2014b).

In this study, two different populations derived from maize parents 08-641 and Ye478 were used to detect QTLs for flowering-related traits. Sixty-six QTLs were detected via SEA or JAAE, and 41 QTLs were integrated into 14 MQTLs. Nine alleles of the 41 QTLs that had positive additive effects were derived from Ye478, and the other 32 alleles were derived from the parent 08-641. MQTL9-1, which included 10 QTLs, was detected in both the $F_{2:3}$ and RIL populations, and affected DPS and DTS. Among the 14 MQTLs, nine and three were derived from 08-641 and Ye478, respectively. The other two, MQTL5-2 and MQTL8-1, were conferred by both Ye478 and 08-641. MQTL3-2 (two integrated QTLs, both explaining more than 10% of the phenotypic variance; positive alleles derived from 08-641) is a main-effect QTL hotspot that affects pollen shedding and silking plastochron. Furthermore, the MQTL information will be useful for MAS, to construct near-isogenic lines, for forward map-based cloning, and to analyze the genetic mechanisms of maize flowering-related traits. This study further verifies the existence of clustering, pleiotropic effects, and multigenic effects of QTLs (Fan et al., 2006b; Upadyayula et al., 2006).

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Project of National Major Basic Dairy Research “973” Plan (#2014CB138202 and #2011CB100106).

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Supplementary material

S1 Table. Analysis of QTLs reported in the present study and those reported in previous studies.