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Combining Three Mapping Strategies to Reveal Quantitative Trait Loci and Candidate Genes for Maize Ear Length

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ABSTRACT Ear length (EL) is an important trait in maize (*Zea mays* L.) because it is positively correlated with grain yield. To understand the genetic basis of natural EL variation, a $F_{2:3}$, a four-way cross and a genome-wide association study (GWAS) population were used to identify the quantitative trait loci (QTLs) and candidate EL genes. Linkage mapping identified 14 QTLs in two types of populations from multiple environments. Six of them were located in three common genomic regions considered "stable QTLs". Candidate genes for the three stable QTLs were identified by the GWAS results. These were related to auxin transport, cell proliferation, and developmental regulation. These results confirm that maize EL is under strong genetic control by many small-effect genes. They also improve our understanding of the genetic basis of maize EL.

CORE IDEAS

- Ear length (EL) is significantly positively correlated with grain yield in maize.
- Both linkage quantitative trait loci and genome-wide association study mapping were used to identify the genes regulating EL.
- Linkage mapping identified three stable QTL in the $F_{2:3}$ and four-way-cross populations; GWAS identified the genes associated with them.
- Maize EL is under strong genetic control by many small-effect genes.
- Ear length candidate genes code for auxin transport, cell proliferation, and developmental regulation.

Maize is an important crop providing food, feed, and raw materials for various industrial products. Its versatility and wide adaptability make it one of the most cultivated crops worldwide. In maize, EL is an important trait that directly affects the number of kernels per ear, which is the major component of grain yield. Previous reports have shown it is significantly correlated with grain yield (Upadyayula et al., 2006). Previous studies have indicated limited variation in many of the elite

Abbreviations: 7-TM, seven transmembrane receptors; ALOG, *Arabidopsis* LIGHT_DEPENDENT SHORT HYPOCOTYLS1 and *Oryza* G1; BLUP, best linear unbiased prediction; EL, ear length; GWAS, genome-wide association study; MAGIC, multiparent advanced generation intercross; NDR, N-myc Down Regulated; PCA, principal component analysis; PVE, phenotypic variation explained; QTL, quantitative trait locus; SNP, single nucleotide polymorphism.

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materials for maize EL (Seebauer et al., 2004; Halvorson and Johnson, 2009). Understanding the genetic architecture of EL could be useful for breeders developing varieties with high grain yield.

Quantitative trait locus mapping is based on linkage populations and is used to detect genetic variation in many crop traits (Agrama and Moussa, 1996; Buckler et al., 2009; Zwonitzer et al., 2010; Chen et al., 2012, 2014a). Maize EL has already been extensively mapped by QTL. Through the use of $F_{2:3}$ populations, the QTLs for EL detected in various studies were found to be located on chromosomes 1, 3, 4, 5, 6, 7, and 10 (Sabadin et al., 2008; Li et al., 2009, 2010; Park et al., 2014). In permanent recombinant inbred line and intermated B73 × Mo17 populations, the QTLs for EL were detected on chromosomes 1, 5, 6, 8, and 9 (Zhang et al., 2010; Jansen et al., 2013; Sa et al., 2015). A bin-map strategy based on high-throughput sequencing technology was used to detect the QTLs for maize EL. In 2014, two QTLs on chromosomes 4 and 5 were identified (Chen et al., 2014b). Although many QTLs for EL were found, very few of them were common to different populations (Jansen and Lübberstedt, 2012).

In recent years, a multiparent advanced generation inter-cross (MAGIC) strategy was proposed as an alternative to QTL mapping in plants. The population is usually founded by a few very diverse germplasms, followed by a few rounds of intercrossing to increase the recombination and self-crossing to fix the genome (Cavanagh et al., 2008). Several MAGIC populations were developed to determine the genetic architecture and the causative factors in *Arabidopsis thaliana* (L.) Heynh. (Kover et al., 2009; Huang et al., 2011; Gnan et al., 2014), tomato (*Solanum lycopersicum* L.) (Pascual et al., 2015), wheat (*Triticum aestivum* L.) (Huang et al., 2012; Rebetzke et al., 2014), rice (*Oryza sativa* L.) (Bandillo et al., 2013), and barley (*Hordeum vulgare* L.) (Sannemann et al., 2015). Compared with traditional biparental populations, MAGIC populations interrogate multiple alleles that could potentially detect more QTLs and could increase the mapping resolution caused by increased recombination (Cavanagh et al., 2008). The four-way-cross population used in the study is similar to a MAGIC population, except with limited recombination and high heterozygosity; it has been used to identify the QTLs for maize leaf angle and kernel traits (Ding et al., 2015; Chen et al., 2016c).

Many single nucleotide polymorphisms (SNPs) and detection methods have already been developed (Elshire et al., 2011; Chia et al., 2012; Chen et al., 2016b). Genome-wide association studies have been widely used to discover causal loci for many maize traits, including various agronomic ones (Tian et al., 2011; Yang et al., 2014; Li et al., 2016b) and those conferring resistance to abiotic and biotic stress (Zila et al., 2014; Samayoa et al., 2015; Li et al., 2016a; Ju et al., 2017). Genome-wide association study has multiple advantages over linkage and traditional QTL mapping such as high resolution and the ability to target multiple traits within the same population (Yu and Buckler, 2006; Breseghello and Sorrells, 2006; Yan et al., 2011). Nevertheless, association mapping has its own shortcomings. For example, the population structure causes a high rate of false positives. This limitation continues to affect GWAS (Andersen et al., 2005; Yu et al., 2006). Traditional QTL mapping in biparental populations powerfully compares allele pairs at a low resolution, whereas association analysis evaluates numerous alleles at a high resolution but with uneven statistical power (Wilson et al., 2004). The combination of linkage and GWAS mapping is an even more powerful approach to identifying candidate genes for complex traits (Chen et al., 2016a; Mahuku et al., 2016).

In this study, both GWAS and two types of linkage populations were used to identify the QTLs related to EL. This method exploited the complementary strengths and weaknesses of both approaches to identify causal loci and candidate genes across the genome.

MATERIALS AND METHODS

Germplasm and Phenotype Evaluation

One biparental population was used and consisted of 225 $F_{2:3}$ families. It was produced from a cross between Zheng58 and Chang7–2, which are the parental lines of the most popular maize hybrid in China, Zhengdan958. The 225 $F_{2:3}$ families were evaluated at Zhengzhou (34°52′N 113°37′E), Jiyuan (35°34′N 112°5′E), and Xichang (27°32′N 102°10′E) in 2007. They were re-evaluated in 2008 at Zhengzhou and Jiyuan.

The four-way-cross population consisted of 277 families and was constructed with four inbred maize lines, D276, D72, A188, and Jiao51. These were presented in an earlier report (Ding et al., 2015). The 277 four-way-cross families were evaluated at Zhengzhou and Jiyuan in 2010.

The GWAS population consisted of 298 maize inbred lines. It mainly contained inbred lines from the Tangsipingtou heterotic group from China, inbred lines from CIMMYT and other non-Tangsipingtou lines from China (Li et al., 2016b). These materials were evaluated for ear length at Zhengzhou (34°52′N 113°37′E), Wenxian (34°95′N 113°6′E), and Hainan (18°21′N 109°10′E) in 2015.

Each location included three replications with 16 plants per plot in 4-m rows with 0.67-m spacing between rows. Treatments were arranged according to a completely randomized block design. "Ear length" is the length from the bottom to the tip of the ear for the top ear on the plant; all the normal plants in the plot were measured. The phenotype for each family was the mean value of all normal plants in the plot.

Phenotypic Data Analysis

ANOVA was performed on the phenotype data with R version 3.2.2 software (R Core Team, 2015). The variance components were estimated using a completely random effects model. Broad-sense heritability was calculated according to Knapp et al. (1985). Best linear unbiased predictions (BLUPs) of the combined phenotypes were calculated with a mixed linear model (lmer) in R version 3.2.2 (R Core Team, 2015) with replication, environment

(the combination of location and year), and entries as random effects. The BLUP value for each line was used in the QTL analysis.

Linkage Mapping

Two linkage populations were genotyped with simple sequence repeat markers. The linkage map of the four-way-cross population contained 219 polymorphic simple sequence repeat markers. Its total map length was 1766.92 cM, and the average distance between markers was 8.45 cM. These findings were published in our previous study (Ding et al., 2015). The $F_{2:3}$ population was genotyped with 180 simple sequence repeat markers. The total length of its linkage map was 1987.7 cM and the average linkage distance was 11.0 cM across the genome (Ding et al., 2011). The threshold value was set by using 1000 random permutations (Churchill and Doerge, 1994).

Linkage mapping analyses were conducted in QTL IciMapping version 4.0 and in GACD version 1.1.7 (http://www.isbreeding.net, assessed 28 May 2018). The BLUP values for each family were used as phenotypic data in multilocation QTL analysis. The scanning step was 1 cM. Only one-dimensional QTL scanning was conducted. Stepwise regression was used to select significant marker variables and the two probabilities for entering and removing variables were set at 0.001 and 0.002 (Ding et al., 2015). The QTL region was determined by the flanking marker that closed to the QTL peak.

Genome-Wide Association Study and Candidate Gene Annotation

The GWAS population consisting of 298 inbred lines was genotyped via the genotyping-by-sequencing method (Elshire et al., 2011). A total of 955,650 SNPs were obtained and after missing values of >0.25 were filtered out and minor allele frequencies of <0.05, 189,545 SNPs remained for future analysis. The population structure was conducted in our previous study (Li et al., 2016b). Briefly, principal component analysis (PCA), kinship matrices (**K**), and linkage disequilibrium between each pair of SNPs were determined with Tassel version 5.0 (Bradbury et al., 2007). The population structure was determined with an admixture ancestry model and correlated allele frequency in STRUCTURE v. 2.3.3 (Pritchard et al., 2000). STRUCTURE was run with four replicates, *k* (number of subpopulations) = 1–8, and a run length of 100,000 Markov chain Monte Carlo repetitions after a burn-in period of 10,000 iterations. Genome-wide association studywas conducted with a mixed linear model that included BLUPs, markers, kinship matrices (**K**), and PCA in TASSEL version 5.0 (Bradbury et al., 2007).

Candidate genes were obtained from the MaizeGDB genome browser (http://www.maizegdb.org/, accessed 28 May 2018) based on the physical positions of the significant SNPs. The genome annotations and gene models used B73 as the reference genome (AGPv3 at the MaizeGDB genome browser).

Meta-analysis

All of the QTLs detected by linkage population appeared on the chromosome according to their physical positions. The figure was generated in R and the source code was derived from our previous study (Li et al., 2016b). The QTLs detected from different mapping populations located in common genomic regions were considered to be "stable QTL".

RESULTS

Phenotype Analysis

Descriptive statistics for the EL in the three different maize populations are presented in Table 1. The EL of the parents in both linkage populations was consistently different in all environments. In the $F_{2:3}$ population, Zheng58 (14.13 cm) had longer ears than Chang7–2 (10.94 cm) across all environments. In the four-way-cross population, parent D276 had shorter ears than other parents, with an average ear length of 6.36 cm, whereas parent A188 had longer EL with an average of 7.37 cm, followed by D72 and Jiao51, with average ELs of 10.39 cm and 10.63 cm, respectively. There were wide variations in each population for all individual and combined environments. For example, the EL ranges were 8.84 to 17.5 cm for the combined environments in the four-way-cross population, 7.01 to 18.30 cm in the $F_{2:3}$ population, and 5.88 to 20.78 cm in the GWAS population. The frequencies of the phenotypic values in the three populations all approximated a normal distribution. The genotypic components of the variance in EL for the combined environments were significant in all three populations. The genotype × environment variances were also significant in the three populations. The heritability was 0.93 for the $F_{2:3}$ population. High repeatability and heritability indicated that most of the phenotypic variance was genetically controlled in the $F_{2:3}$ population. Nevertheless, the heritabilities were slightly lower in the other two populations (0.62 for the four-way-cross population and 0.77 for the GWAS population).

Quantitative Trait Locus Analysis

The logarithm of odds ratio threshold was set at 2.3, which was generated from 1000 random permutations. With this threshold, five QTLs were identified from the combined EL analysis in the $F_{2:3}$ population (Table 2). They were located on chromosomes 1 (umc1703–umc1590), 2 (umc2372–umc2019), 3 (bnlg1325–umc2369), 4 (umc1511–umc1702), and 5 (phi085–bnlg118). The phenotypic variation explained (PVE) of the QTLs on chromosomes 3 and 4 was >10%, so they had major effects on the EL in the $F_{2:3}$ population.

In the four-way-cross population, nine QTLs were detected for EL with the threshold set to 2.5. They were located on chromosomes 1 (bnlg1484–phi109275 and umc1590–bnlg1556), 2 (bnlg1338–umc1823, umc1946–umc2372, and umc1736–umc2214), 3 (umc2265–umc1539), and 5 (umc1587–phi113, umc1394–umc1482, and umc2201–bnlg1346) (Table 2).

To identify the consistent EL loci, a meta-analysis of both linkage mapping results was conducted. Of the

Table 1. Means, variances, and heritabilities of ear length in three maize populations.

Population	Environment	Mean ± SD	Range	σ^2_g‡	σ^2_e	σ^2_{ge}	H^2†
$F_{2:3}$	2007 Zhengzhou	12.36 ± 1.46	7.01–15.98	1.37	0.78	–	0.85
	2007 Jiyuan	13.22 ± 1.37	8.00–16.77	1.46	0.42	–	0.91
	2007 Xichang	14.02 ± 1.44	10.06–18.07	1.48	0.63	–	0.87
	2008 Zhengzhou	12.56 ± 1.34	8.33–16.00	1.11	0.71	–	0.82
	2008 Jiyuan	13.97 ± 1.44	8.25–18.30	1.69	0.41	–	0.93
	Combined	13.21 ± 1.57	7.01–18.30	1.21	1.17	0.26	0.93
Four-way cross	2010 Zhengzhou	13.72 ± 1.38	9.16–17.49	1.08	0.83	–	0.79
	2010 Jiyuan	13.67 ± 1.73	8.84–17.50	1.35	2.70	–	0.60
	Combined	13.70 ± 1.56	8.84–17.50	0.66	1.67	0.26	0.62
Genome-wide association study	2015 Zhengzhou	12.82 ± 2.45	5.88–20.78	3.91	2.22	–	0.79
	2015 Wenxian	12.85 ± 1.94	7.7–19.2	2.97	0.80	–	0.88
	2015 Hainan	12.08 ± 1.59	7.7–16.3	2.34	0.21	–	0.96
	Combined	12.59 ± 2.06	5.88–20.78	2.14	1.27	1.39	0.77

† Repeatability of individual locations and heritability in combined environments.

‡ σ^2_g, genotypic components of the variance; σ^2_e, environmental components of the variance; σ^2_{ge}, genotype × environment variance.

Table 2. Quantitative trait loci for maize ear length identified in two linkage populations.

Population	Chr#	Marker interval†	Left position‡	Right position§	LOD¶	PVE
			bp			%
$F_{2:3}$	1	umc1703–umc1590	154,952,735	183,904,444	2.36	3.51
	2	umc2372–umc2019	177,849,512	187,894,598	3.93	6.11
	3	bnlg1325–umc2369	4,775,329	8,939,314	6.97	12.17
	4	umc1511–umc1702	75,390,036	142,343,300	7.22	11.78
	5	phi085–bnlg118	203,156,715	217,012,402	2.73	5.56
Four-way cross	1	bnlg1484–phi109275	34,652,377	51,946,864	4.65	5.16
	1	umc1590–bnlg1556	182,926,541	209,868,319	4.25	4.16
	2	bnlg1338–umc1823	4,051,998	8,984,891	4.09	3.82
	2	umc1946–umc2372	177,849,521	187,894,598	7.64	7.56
	2	umc1736–umc2214	228,594,342	235,463,024	2.52	2.22
	3	umc2265–umc1539	157,094,188	168,492,776	3.15	4.08
	5	umc1587–phi113	9,373,020	–	4.81	4.51
	5	umc1394–umc1482	–	172,104,088	5.44	5.08
	5	umc2201–bnlg1346	205,440,973	217,012,402	8.88	9.12

† The marker interval was defined by the flanking markers close to the QTL peak.

‡ Physical location of the left marker of the marker interval.

§ Physical location of the right marker of the marker interval.

¶ The log-likelihood value was calculated by composite interval mapping.

Chr., chromosome; PVE, phenotypic variation explained; LOD, logarithm of odds ratio.

QTLs detected for EL in the $F_{2:3}$ and four-way-cross populations, three common loci were identified on chromosomes 1, 2, and 5 and they overlap on the marker interval (Fig. 1). We named these stable QTLs *qEL1*, *qEL2*, and *qEL5*, respectively. The QTLs *qEL1*, *qEL2*, and *qEL5* were located at chromosome 1 (154,952,735–209,868,319), chromosome 2 (177,849,512–187,894,598), and chromosome 5 (203,156,715–217,012,402), respectively. These determinations were based on the B73 reference genome (AGPv3 at http://maizegdb.org, accessed 28 May 2018).

Genome-wide Association Studies for EL

The population structure and PCA were presented in our previous study (Li et al., 2016b). Briefly, the GWAS population contained three major subgroups. Subgroup 1 derived mainly from the material from CIMMYT. Subgroup 2 came primarily from the Tangsipingtou heterotic group of Chinese materials. Most of the lines in Subgroup 3 originated in Chinese Reid and other non-Tangsipingtou heterotic groups. Using the preceding population structure information, the first three principal components were included in the mixed model for GWAS analysis.

Single marker-based GWAS was performed with a mixed linear model, incorporating both population structure (first three principal components) and kinship (**K**). In the combined GWAS, four significant SNPs associated with maize EL were identified from the mixed linear model ($P < 1.0 \times 10^{-4}$) in a genome-wide scan (Fig. 2a, Table 3). They explained 8.0 to 8.7% of the phenotypic variation in EL. These SNPs were located at chromosome

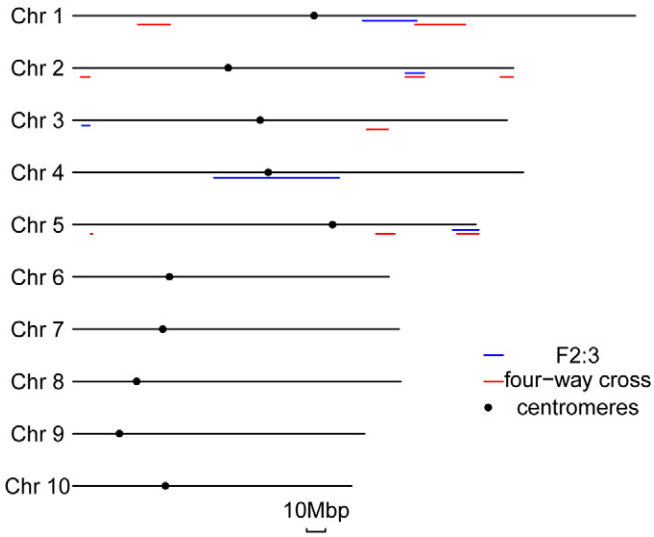

Fig. 1. Meta-analysis of quantitative trait loci (QTLs) showing QTLs detected by two linkage populations of maize. Colored lines represent the different QTLs that were detected.

2 34007544, chromosome 7 165471917 and 165471933, and chromosome 1 294133952. The quantile–quantile plot showed that the most frequently observed *P*-value (statistical significance of detection for each SNP) was in agreement with the expected *P*-value. At the lowest *P*-value, however, the observed *P*-value was greater than the expected *P*-value after $-\log(p) = 3$ (Fig. 2b). Therefore, the population structure was well controlled by the first three principal components. Certain loci remained undetected but their absence should not have affected the identification of the loci significantly associated with EL.

Candidate Genes of the Stable QTLs for EL Based on the GWAS Output

The Manhattan plot of the GWAS finding in the QTL region was enlarged to find the most significant SNPs for EL. Three contiguous markers (S1_193519950, S1_193520199, and S1_193520212) with the lowest *P*-values in the region of *qEL1* were the most significant SNPs for *qEL1* and were located in the same gene, *GRMZM2G383817* (Fig. 3a). A protein encoded by the candidate gene *GRMZM2G383817* had a major predicted N-myc Down Regulated (NDR) domain (E-value = 2×10^{-43}) (Fig. 3d). It has been reported that the NDR protein alters auxin transport, local auxin gradients, and the expression levels of auxin transport proteins (Mudgil and Jones, 2010).

The most significant marker (S2_184757471) in the region of *qEL2* was located upstream from *GRMZM2G161291* (–736 bp) (Fig. 3b). *GRMZM2G161291* encodes a seven transmembrane receptor (7-TM) protein, which participates in cell proliferation, pollen hydration, and defense (Fig. 3e). *GRMZM2G114667* has a high expression level in the prepollen cob and may be involved in its development.

Two markers (S5_208350728 and S5_214950631) with the lowest *P*-values were found in the region of *qEL5* (Fig. 3c). No coding gene was identified near S5_208350728.

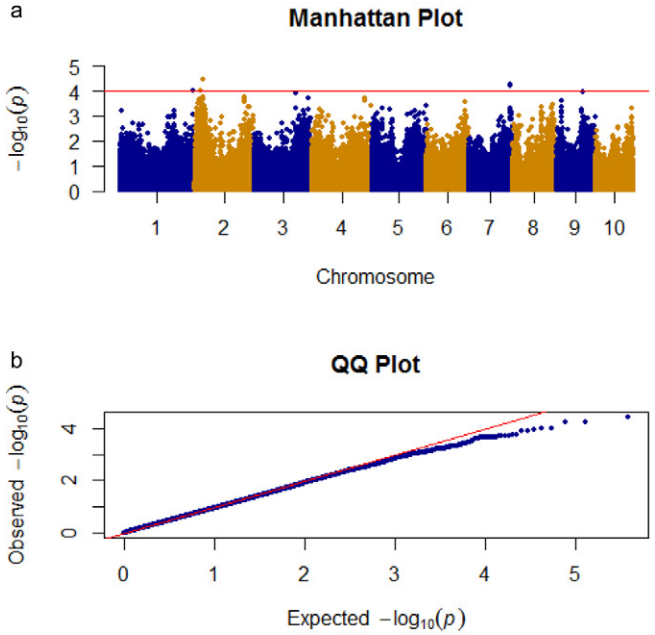

Fig. 2. Manhattan plots (a) and quantile–quantile (Q–Q) plots (b) for the combined genome-wide association study with a mixed linear model of maize ear length.

Nevertheless, S5_214950631 and two other markers near to it (S5_214950577 and S5_214950576), also with low *P*-values, were located upstream from *GRMZM2G168371*. Normalized nonmodified protein abundance indices showed that GRMZM2G168371 had the highest abundance in the ear tip. Therefore, *GRMZM2G168371*, which encodes a protein with a Duf640 domain, was identified as the candidate gene of *qEL5* (Fig. 3f).

DISCUSSION

Ear Length Heredity in Maize

EL is a complex, quantitatively inherited trait. It is easily influenced by environmental conditions. We tested the effects of multiple environments on EL, performing each treatment in triplicate. We measured the EL of all plants in each plot. About 90 plants were measured for each material. The heritabilities were 0.93, 0.62, and 0.77 for the $F_{2:3}$, four-way cross, and GWAS populations, respectively. These high heritability values indicate that most of the phenotypic variance in EL was genetically controlled and the data were reliable enough to be used in accurate mapping. Only two QTLs with a PVE of >10% were detected in the $F_{2:3}$ population. In the four-way-cross and GWAS populations, the QTLs were of small effect and had a PVE of <10%. The aforementioned results confirmed that EL is a highly polygenic trait controlled by multiple small-effect genes.

Stable QTLs for EL

In a previous study, different QTLs for EL were identified in various populations (Sabadin et al., 2008; Li et al., 2009, 2010; Park et al., 2014; Zhang et al., 2010; Jansen et al., 2013; Sa et al., 2015; Chen et al., 2014b; Jansen and Lübberstedt,

Table 3. The top significant markers and their candidate genes associated with maize ear length (EL) identified in the genome-wide association study population.

Marker	Chr†	Position	*P*-value	PVE	Candidate gene	Annotation
		bp				
S1_294133952	1	294,133,952	9.55×10^{-5}	0.080	*GRMZM2G343741*	PPR repeat domain containing protein
S2_34007544	2	34,007,544	3.65×10^{-5}	0.088	*GRMZM2G080176*	DNA-directed RNA polymerase
S7_165471917	7	165,471,917	5.53×10^{-5}	0.087	*GRMZM2G089736*	TIFY domain or divergent
S7_165471933	7	165,471,933	6.03×10^{-5}	0.087		CCT motif family protein

† Chr, chromosome number; PVE, the percentage of total genetic variance explained by the markers; PPR, pentatricopeptide repeat; CCT, CONSTANS, CO-like, and TOC1.

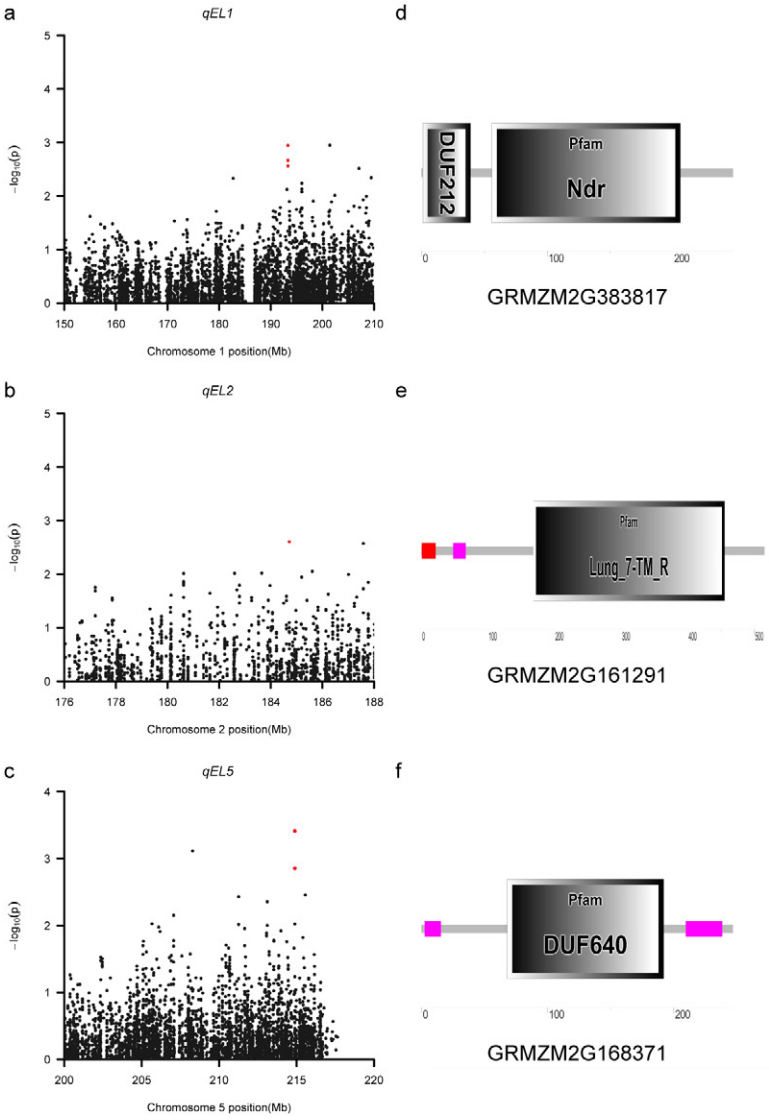

Fig. 3. Results of the genome-wide association study (GWAS) and the candidate genes of the three stable quantitative trait loci (QTLs) for maize ear length (EL). (a) Manhattan plot of the *qEL1* region. (b) Manhattan plot of the *qEL2* region. (c) Manhattan plot of the *qEL5* region. The single nucleotide polymorphisms (SNPs) for the candidate genes are in red. (d) Protein structure of GRMZM2G383817. (e) Protein structure of GRMZM2G161291. (f) Protein structure of GRMZM2G168371.

2012). To detect stable QTLs for EL, we performed a linkage analysis with two linkage populations formed from six different parents. Five QTLs were identified for EL in the $F_{2:3}$ population from the combined analysis. They were located on chromosomes 1, 2, 3, 4, and 5. The PVE of the QTLs on chromosomes 3 and 4 was >10%, indicating that they have major effects on EL. Nevertheless, neither of these QTLs was identified in the four-way-cross population. The other three QTLs with comparatively small effects were found among the nine QTLs found in the four-way-cross population. The effects of EL QTLs were significantly influenced by the genetic background. For this reason, the QTLs reported for EL differ among various studies. Linkage analysis of the four-way-cross population identified more small-effect QTL for EL than it did for the biparental population. Therefore, the complex genetic background increases the probability of detecting QTLs while masking the genetic effects of independent QTLs.

Three stable QTLs were identified for EL in both the $F_{2:3}$ and four-way-cross populations. The stable QTL region on chromosome 1 has been reported in previous studies such as Li et al. (2009) and Li et al. (2010). The *qEL5* locus was also reported by Li et al. (2009). Therefore, those loci are stable QTLs that control EL in modern inbred maize lines. In addition, *qEL2* was not reported in any other study, so it may in fact be an important newly discovered locus for EL breeding.

Combined Linkage and Association Mapping Reveal Maize EL Candidate Genes

Considering the number of SNPs available and the state-of-the-art detection methods (Elshire et al., 2011; Chia et al., 2012), GWAS has been widely used in causal locus discovery for many maize traits. Compared to traditional QTL and linkage mapping, GWAS has multiple advantages, including high resolution and its applicability to multiple traits within the same population (Yu and Buckler, 2006; Breseghello and Sorrells, 2006; Yan et al., 2011). On the other hand, association mapping has limitations as well. Its population structure causes a high rate of false positives. Several novel association mapping methods were developed to reduce the false positive rates but this deficiency remains a challenge for GWAS (Andersen et al., 2005; Yu et al., 2006). For example, in the present study, GWAS failed to find any gene near the most significant marker for EL.

Although traditional QTL mapping of a linkage population powerfully compares allele pairs with low

resolution, association analysis provides high-resolution allele evaluation but with uneven statistical power (Wilson et al., 2004). In this study, linkage analysis was used for identification of stable QTLs for EL and GWAS for prediction of candidate genes for QTLs.

By applying the aforementioned strategy, we identified *GRMZM2G383817* as the candidate gene for *qEL1*, which encodes a protein with a major NDR domain resembling a typical animal protein. It has been reported that NDR alters auxin transport, local auxin gradients, and the expression levels of auxin transport proteins in *A. thaliana* (Mudgil and Jones, 2010; Mudgil et al., 2009). Therefore, we speculate that *qEL1* affects EL by regulating the transport of auxin toward the maize ear.

GRMZM2G161291, the candidate gene for *qEL2*, encodes a 7-TM protein, which also resembles a typical animal protein. It has been reported that the 7-TM protein is involved in cell proliferation, pollen hydration, and defense (Plakidoudymock-Dymock et al., 1998; Chen et al., 2003; Yi et al., 2014; Lorek et al., 2010). *GRMZM2G114667* also has a high expression level in the prepollen cob of B73 and may participate in its development. We therefore propose that *qEL2* affects EL by regulating cell proliferation in the maize ear.

GRMZM2G168371, the candidate gene for *qEL5*, encodes a protein with the Duf640 domain [*Arabidopsis* LIGHT_DEPENDENT SHORT HYPOCOTYLS1and *Oryza* G1 (ALOG) domains]. This domain is rich in basic amino acids, especially arginine, and is highly conserved among land plants. Members of the ALOG protein family are key developmental regulators (Yoshida et al., 2009; Iyer and Aravind, 2012). The following are proteins known to contain an ALOG domain: *A. thaliana* LIGHT_DEPENDENT SHORT HYPOCOTYLS1, which is involved in phytochrome-dependent light signaling, and *O. sativa* G1, which specifies the identity of sterile lemmata. Normalized nonmodified protein abundance assays showed that *GRMZM2G168371* has the highest abundance in the ear tip of B73. We speculate that *qEL5* affects EL by acting as an ear development regulator.

The candidate genes must be functionally verified by genetic transformation to clarify their roles in the maize ear lengthening pathway. This information may prove to be useful for molecular maize breeding in the future.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest.

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