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Combining meta-QTL with RNA-seq data to identify candidate genes of kernel row number trait in maize

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Abstract

Kernel row number is an important component of grain yield in maize. With development of classical quantitative trait loci (QTL) mapping and modern RNA-seq, numerous QTL and tissue-specific gene expression data were accumulated in previous studies. In this paper, a total of initial 373 QTL for grain yield (GY) and kernel row number (KRN) were collected based on 29 previous literatures. Fifty-four meta-QTL (MQTL) were detected via the meta-analysis method with IBM2 2008 Neighbors as a reference map, including 19 for GY and 35 for KRN. These MQTL were unevenly distributed on all 10 chromosomes. Chromosome 1 harbored the most initial QTL and MQTL, and chromosome 7 contained the least. Three MQTL for KRN have been overlapped with MQTL for GY on chromosomes 1 and 3. A total of 1,588 (46.07%) out of 3,447 genes located in the KRN MQTL regions were identified by gene expression data, and categorized into 101 significant GO terms. Meanwhile, six candidate genes were identified from MQTL regions, which are homologous to three functionally characterized genes found to participate in plant inflorescence development. The identified MQTL could be applied to marker-assisted selection (MAS) to facilitate yield architecture, QTL fine mapping and gene cloning in the maize community. Furthermore, the identified candidate genes could enhance the selection efficiency by MAS directly, and could illuminate molecular mechanisms of grain yield in maize.

Keywords: maize, kernel row number, meta-analysis, meta-QTL, candidate genes

Introduction

Maize (*Zea mays* L) is one of the most important food crops throughout the world. It has not only served as staple for humans and animals, but as source of bioenergy. With the expansion of population and deterioration of the global energy crisis, maize will face greater demands for grain production. Hence, improvement of crop production is of great significance. Grain yield is a quantitative trait, which is influenced by environmental factors and multi-genes with minor effects (Li et al, 2011). The decomposition of the complex yield trait into its elementary component traits may be the best strategy for elucidating the genetic mechanism of yield trait.

Kernel row number (KRN) trait is more accessible and accurate than other complex quantitative traits. KRN was not only an important yield component (one of the major components of grain yield), but also one of the key trait during the domestication of maize. The domestication of maize changed with dramatic differentiation in KRN from its wild ancestor teosinte (2 rows of kernel) to most varieties of modern maize (8-20 rows of kernel). Due to the crucial role of KRN trait in maize genetic and breeding, a considerable number of studies have been conducted on KRN trait and various types of data had been accumulated. In addition, lots of genes related to homology traits have been cloned from closely related model species.

Quantitative trait loci (QTL) mapping is a powerful tool for locating chromosomal regions involved in complex traits (Somers et al, 2007). Based on phenotypic and genotypic data, QTL mapping enables the identification of action, interaction, numbers and chromosomal locations of loci affecting particular traits (Miles and Wayne, 2008). In the past several decades, hundreds of QTL have been detected for KRN, which were then mapped on all ten chromosomes in maize (Cai et al, 2014; Choe and Rocheford, 2012; Upadyayula et al, 2006). Liu et al (2015) identified a main QTL KRN4 mapping on chromosome 4 (bin4.08) through genome-wide association studies (GWAS) and linkage mapping. The published QTL of KRN were derived from the different QTL mapping populations ($F_{2:3}$, BC_1S_1, BC_2F_2, IF_2, and RIL) and molecular markers (RFLP, SSR, INDEL, and SNP) (Austin and Lee, 1996; Calderón et al, 2016; Karen Sabadin et al, 2008; Li et al, 2009; Li et al, 2007; Lu et al, 2011; Veldboom and Lee, 1994). However, the validity of QTL mapping is influenced by many factors including different experiment environments, materials, mapping population, population type and size, number of QTL, density of genetic markers, and heritability of traits, among which the analysis of genetic and statistical models have significantly influenced QTL detection and location (Austin and Lee, 1996; Martinez et al, 2016). These QTL associated with the same trait increased gradually, but the results dramatically dif-

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fered. It is difficult to apply to these QTL to molecular marker-assisted selection (MAS) breeding practice.

Compared with QTL mapping, molecular cloning of genes associated with KRN has lagged behind, especially, the field of positional or map-based cloning. Several genes associated with KRN have been identified and cloned through mutations or map-based cloning, include *zfl2* (Bomblies and Doebley, 2006; Bomblies et al, 2003), *fea2* (Bommert et al, 2013b) and *ub3* (Chuck et al, 2014) and it's enhancing QTL (Liu et al, 2015). *fea2* gene of all these genes resulted in the over-proliferation of ear inflorescence meristem, increased the KRN, and was cloned and mapped into the major QTL regions for KRN on chromosome 4 (bin4.05) (Bommert et al, 2013b; Taguchi-Shiobara et al, 2001). Moreover, the rapid development of next generation sequencing (NGS) technologies and computational methods enables extensive transcriptome-wide analysis of different tissues and organs in maize by RNA-seq, such as the developmental dynamics of the maize leaf transcriptome, regulatory modules of inflorescence architecture, regulatory network in the maize kernel and so on (Eveland et al, 2014; Fu et al, 2013; Li et al, 2010b). Although some genes are found to be associated with KRN, the genetic mechanism is poorly known. Therefore, understanding genetic basis of KRN will not only accelerate the breeding of maize, but also make a further step into the study of maize evolution.

Meta-analysis is a quantitative comprehensive analysis method for synthesizing data from various different independent results (Rosenberg et al, 2004). It could mine the «real» QTL from a host of positioning results, refine confidence interval (CI) and enhance the accuracy and effectiveness of QTL mapping (Li et al, 2013). In recent years, the method has been frequently applied in maize (Xiang et al, 2012), wheat (Hanocq et al, 2007) and rice (Ballini et al, 2008). For instance, Lv et al (2008) identified three meta-QTL (MQTL) for resistance to sugarcane mosaic virus in maize and mined four candidate genes on the MQTL of chromosome 3. One of MQTL for photoperiod sensitivity was identified in maize bin 10.04 where a CCT-domain containing gene was found (Xu et al, 2012). And then this gene was proved to be *ZmCCT* that functioned on controlling photoperiod response in maize (Hung et al, 2012). Therefore, meta-analysis could facilitate to excavate consistency of main gene loci controlling the same trait, and also furnish valuable «clue» for QTL fine-mapping.

In the current study, the published QTL associated with KRN and GY in maize were collected and MQTL were retrieved through meta-analysis pathway with IBM2 2008 Neighbors as a reference map. Meanwhile, the purpose of combining comprehensive meta-analysis with currently available RNA-seq data of critical periods in the KRN morphogenesis is to identify candidate gene responsible for KRN trait. It provided important information for MAS breeding and selecting candidate genes for KRN in maize.

Materials and Methods

Collection of data

Grain yield (GY) of maize is a complex trait consisting of several yield components, including kernel row number, kernel number per row and kernel weight. In this study, GY and KRN were investigated. The key words «grain yield» and «kernel row number» were retrieved in the NCBI (http://www.ncbi.nlm.nih.gov/pubmed/) and MaizeGDB website (http://www.maizegdb.org/). Here, we collected 29 manuscripts published from 1996 to 2015. The information of QTL consisted of parent lines, the types and size of QTL mapping population, and the numbers of QTL per trait was provided in Table 1. Furthermore, we also surveyed flanking molecular markers, QTL position, confidence interval (CI), LOD score and proportion of phenotypic variance explained (PVE or R^2) in terms of each QTL. If two or several QTL for the same trait existed in consensus map positions in one study, they were considered as identical QTL, and thus only one QTL was counted in our study. The gene action and epistatic loci were not considered in this paper.

The genome sequences for B73 (Release ZmB73_RefGen_v2) was downloaded from http://www.maizesequence.org/index.html. The 5'-UTR, coding determining sequences (CDS), 3'-UTR, exon, intron and intergenic regions were determined based on their original annotations (ZmB73_5b_FGS, http://ftp.maizesequence.org/current/filtered-set/). TSS_up_0.5kb region are defined as 0.5kp upstream of the transcription start site and TES_down_0.5kb region are defined as 0.5kp downstream of the transcription end site. The RNA-seq data of inflorescence meristem (IM) period of B73 were downloaded from NCBI (accession number GSE51050) (Eveland et al, 2014).

QTL projection and meta-analysis QTL

The 373 identified QTL were projected onto the reference map (IBM2 2008 Neighbors) for meta-analysis based on the original map positions, LOD score, CI and R^2. The following formulas were used to estimate 95% CI in case the CI of QTL was not demonstrated.

$$CI = 530 / (N \times R^2) \quad (1)$$

$$CI = 163 / (N \times R^2) \quad (2)$$

where N represented the mapping population size and R^2 represented the ratio of phenotypic variation explained by the identified QTL. The formula (1) was applied in backcrossed lines (BC), test-cross lines (TC) and F_2 population (Darvasi and Soller, 1997) and formula (2) was applied in recombinant inbred lines (RIL) (Guo et al, 2006).

The high-density genetic linkage map of IBM2 2008 Neighbors (Intermated B73 × Mo17) played a crucial role in the reference map. This map crossed 8,054.28 centiMorgans (cM) and included 15,991 RFLP, SSR, AFLP, genes, PARD markers etc. This wealthy map shared plentiful common markers with

Table 1 - Detailed information of the QTL used for Meta-analysis.

References	Parents	Population size	Cross type	N° of QTL for GY	N° of QTL for KRN
Barrière et al, 2010	F838 x F286	240	RIL	8	-
Liu et al, 2011	Huang C x Xu178	203	RIL	7	-
Ribaut et al, 2007	Ac7643S5 x Ac7729	240	$F_{2:3}$	8	-
Messmer et al, 2009	CML444 x SC-Malawi	236	RIL	5	-
Liu et al, 2010	Mo17 x Huangzao4	239	RIL	-	6
Guo et al, 2011	Zheng58 x Chang7-2	231	$F_{2:3}$	10	-
Cai et al, 2012	Ye478 x Wu312	218	RIL	18	-
Liu et al, 2014	V671 x Mc	270	$F_{2:3}$	-	-
Yang et al, 2015	B73 x SICAU1212	325	RIL	-	26
Li et al, 2014	Nongxi531 x H21	526	BC4F1	-	17
Cai et al, 2014	MT-6 x B73	266	F_2	-	7
Yu et al, 2014	1132 x 751	342	F_2	-	3
Tian et al, 2014	Y1648 x Y2348	180	$F_{2:3}$	-	7
	Y1648 x Y2348	180	$F_{2:4}$	-	6
		-	$BC_3F_{2:3}$	-	4
Choe and Rocheford	2012BH20 x BH30	264	$F_{2:3}$	-	5
Yang et al, 2012	GY220 x 8984	282	RIL	8	20
	GY220 x 8622	263	RIL	6	13
Li et al, 2011	Dan232 x N04	258	RIL	10	13
Peng et al, 2011	Huangzao4 x Qi319	230	$F_{2:3}$	4	-
	Huangzao4 x Ye478	235	$F_{2:3}$	4	-
Lu et al, 2011	Ye478 x Dan340	397	$F_{2:3}$	12	13
Tang et al, 2010	Zong3 x 871	426 433	IF_2	3	1
Li et al, 2010a	5003 x 178	210	$F_{2:3}$	7	12
Li et al, 2009	GY220 x 8984	284	$F_{2:3}$	1	4
	GY220×8622	265	$F_{2:3}$	2	4
Karen Sabadin et al	2008L-08-05F x L-14-4B	400	$F_{2:3}$	-	10
Li et al, 2007	Dan232 x N04	220	BC_2F_2	4	2
		259	$F_{2:3}$	1	4
Yan et al, 2006	Zong3 x 87-1	266	$F_{2:3}$	5	18
Upadyayula et al, 2006	ILP x B73	150	BC_1F_1	-	4
Ho et al, 2002	RD6501 x RD3013	204	BC_2 as TC	5	-
Huang et al, 2010	F2 x F252	300	Conventional F_3	9	-
	F2 x F252	322	Intermated F_3	12	-
Coque and Gallais, 2006	Io x F2	99	RIL as TC	11	-
Austin and Lee, 1996	Mo17 x H99	186	RIL	6	8
Total				166	207

the original maps recorded in this paper. The projection of QTL was carried out by BioMercator V3.0 software (Sosnowski et al, 2012). The best positions of original QTL and CI were projected on the reference map underlying the homothetic function (Chardon et al, 2004). MQTL are the consensus QTL from the model with lowest Akaike Information Criterion (AIC) value (Goffinet and Gerber, 2000). When some QTL could not be projected or map positions were beyond the scope on the reference map, the QTL would be discarded to ensure accuracy and dependability of projection.

Candidate genes mining and GO analysis

These MQTL involved in KRN were selected as the candidate genomic region for further analysis. RNA-seq data controlling maize inflorescence architecture were downloaded from NCBI website (www.ncbi.nlm.nih.gov/geo/; GSE51050; Eveland et al, 2014). The candidate genes were identified by the gene-level expression values, which are represented by fragments per kilo base exon per million reads mapped (FPKM), and a consensus FPKM was determined for each gene based on its representation across biological replicates. The GO enrichment analysis of the significant genes with FPKM > 1 of MQTL regions was carried out using singular enrichment analysis (SEA) approach by online AgriGO tool (http://bioinfo.cau.edu.cn/agriGO/) with the B73 reference genome (AGPv3.30) as background (Du et al, 2010). The highly significant enriched terms were chosen by default P-value and false discovery rate (FDR). Protein sequence alignment using BLAST by default e-value (e^{-10}), and homologous genes were identified with identity larger than 40% and coverage more than 60% length alignment.

Results

Initial QTL on chromosomes in maize

Information on QTL for GY and KRN was collected from 29 literatures reported over the past 19 years from 1996 to 2015. A total of 373 QTL were collected (Table 1) and 166 special for GY and 207 for KRN,

Table 2 - Numbers of initial QTL and identified MQTL (in brackets) on the chromosomes.

Chr	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	Total
GY	37(6)	11(1)	16(2)	12(1)	13(1)	18(2)	15(2)	13(1)	15(2)	7(1)	157(19)
KRN	32(6)	21(5)	26(5)	27(4)	26(5)	3(0)	5(0)	20(3)	11(1)	29(5)	200(35)
Total	69(12)	33(6)	42(7)	39(5)	39(6)	21(2)	19(2)	33(4)	26(3)	36(6)	357(54)

respectively. Nine original QTL for GY and seven for KRN could not be projected on the reference map. These initial QTL were unevenly distributed on the 10 chromosomes. The amount of initial QTL (Table 2) ranged from 19 (chromosome 7) to 69 (chromosome 1). QTL for GY (37) and KRN (32) was the highest on the chromosome 1. GY QTL was the least (7) on the chromosome 10. The least (3) of KRN QTL were located onto chromosome 6.

Meta-QTL of KRN and GY on chromosomes in maize

Based on the principle of the lowest AIC value, 35 MQTL for KRN and 19 MQTL for GY were detected via meta-analysis pathway, respectively, which were also unevenly distributed on all chromosomes (Tables 2 and 3). The number of MQTL changed from two (chromosomes 6 and 7) to 12 (chromosome 1), with an average of 5.4 MQTL per chromosome. Chromosome1 had the highest frequency of MQTL (12), while chromosomes 6 and 7 had the lowest (2). The numbers of initial QTL in one MQTL ranged from two to nine. The maximum of MQTL for GY and KRN were distributed on chromosome 1, corresponding to the distribution of original QTL. The MQTL for GY were detected on all ten chromosomes, whereas KRN MQTL were located on all chromosomes except chromosomes 6 and 7.

The overlapped MQTL between GY and KRN traits were identified with interval on chromosome. As is shown in table 3, three overlapped MQTL were detected from 54 MQTL, and they were GY5 overlapped with KRN4 on chromosome 1 (bin 1.06), GY4 overlapped with KRN5 on chromosome 1 (bin 1.05), and GY10 overlapped with KRN18 on chromosome 3 (bin 3.05), respectively. The overlapping regions maybe inferred some pleiotropic QTL controlled of KRN and GY traits, which could facilitate to screen candidate genes and could process the MAS breeding.

Mining possible candidate genes in MQTL and GO analysis

A total of 3447 annotated genes were contained in these MQTL regions (Supplementary Table 1). FPKM value of 46.07% (1,588) of these genes ranged from 1.03 to 1,160.35, with an average of 23.39 (Supplementary Table 2). 1,154 genes with FPKM value >1 were annotated and categorized into 101 significant GO terms, including 58 GO terms involved in biological process such as cellular process (GO:0009987), signaling process (GO:0023046), signal transmission (GO:0023060) and 43 GO terms, which were extensively involved in cellular process, intracellular part (GO:0044424), intracellular (GO:0005622) and cell part (GO:0044464) (Figure 1, Supplementary Table 3).

Significant progress has been made in understanding the molecular mechanism of inflorescence development in Arabidopsis, and the classical ABC model had been upgraded to ABCDE model. In order to identify candidate genes involved in inflorescence development in the MQTL regions, 52 genes that were functionally characterized to inflorescence development were collected from Arabidopsis and Rice (Supplementary Table 4). A total of six homologs genes in the MQTL regions were identified by protein sequences alignment. These genes involved in the MADS-box gene family and transcription factor in rice and the CLV pathway in Arabidopsis. Three candidate genes GRMZM2G043584, GRMZM2G017386, and GRMZM2G404207, which are homologous to CLV1 (Stone et al, 1998). *OsMADS7* (homologous gene of the candidate gene *GRMZM2G159397*) and *OsMADS32* (homologous gene of the candidate gene *GRMZM2G001139*) functioned on inflorescence development and impacted the kernel number per ear in rice (Cui et al, 2010; Wang et al, 2015). RFL (homologous gene of the candidate gene *GRMZM2G180190*) controlled of the inflorescence and floral development (Ikeda-Kawakatsu et al, 2012). Among of them, GRMZM2G043584 was mapped in the KRN6 interval and its FPKM value was 86.97. GRMZM2G017386, GRMZM2G404207, and GRMZM2G001139 were located in the KRN34 interval and the FPKM values were 4.68, 7.64, and 129.75, respectively. GRMZM2G159397 was mapped in the KRN4 interval and its FPKM value was 3.9. GRMZM2G180190 was mapped in the KRN12 interval and its FPKM value was 69.17.

Discussion

With the development of molecular marker technology and QTL mapping software, plenty of QTL have been generated on over 10 chromosomes and gene cloning for agronomic traits in maize. By far, thousands of QTL have been generated, that are relevant to phenotype (plant height, leaf angle, and ear diameter, etc), flowering time, biotic stress, abiotic stress, and so on. Extensive studies had been done on yield and yield-related traits, especially for KRN trait. Consequently, the researchers have to face the problems that how to effectively employ these independent study programs. In addition, increase of gene expression data produced by microarray-based and RNA-seq would unlock new area in meta-analy-

Table 3 - Meta-analysis results of GY and KRN in maize.

MQTL	Bin	Map position(cM)	Physical distance (bp)	QTL number	R^2 (%)
GY1	1.01	82.8-83.7	6,954,139-7,860,111	2	6.52
GY2	1.01-1.02	113.8-133.6	12,187,402-15,081,335	7	10.81
GY3	1.03	270.6-284.13	44,227,633-51,364,865	3	6.32
GY4	1.04-1.05	401.3-417	83,525,378-92,380,497	3	7.83
GY5	1.06	548.4-599.89	191,089,609-198,332,129	4	10.12
GY6	1.1	898.7-908.47	273,251,258-275,484,407	3	10.47
GY7	10.04	287.72-295.9	117,897,673-126,625,283	4	9.1
GY8	2.03-2.04	234.37-250.1	22,753,277-29,124,988	3	6.9
GY9	3.04	150-160.74	190,889,172-15,281,332	2	2.81
GY10	3.04-3.05	280.4-305.88	119,647,741-129,046,658	3	9.64
GY11	4.03-4.04	208.85-218.5	32,240,789-25,233,582	6	8.32
GY12	5.04	312.6-318.9	135,817,962-139,233,160	3	7.39
GY13	6.01-6.02	107.32-121.09	70,934,148 -71,079,235	5	5.62
GY14	6.02	145.7-148.7	91,687,346-92,109,561	2	8.3
GY15	7.04	410.5-430.5	156,589,630-159,146,113	3	10.77
GY16	7.02	187.91-188.1	21,437,175-83,038,512	3	6.07
GY17	8.07	483.4-494.7	167,143,057-168,933,171	2	7.35
GY18	9.06	500.1-519.02	145,303,921-147,424,091	2	4.86
GY19	9.04	340.38-344.8	131,066,648-133,267,815	3	5.99
KRN1	1.02	133.6-143.5	15,080,522-16,243,042	5	9.67
KRN2	1.05	471.7-473.8	164,555,621-166,770,972	2	5.06
KRN3	1.11	1010.2-1019.1	287,892,994-288,334,071	4	5.71
KRN4	1.06-1.07	541.3-658.6	187,975,047-209,868,319	2	13
KRN5	1.05	410.76-432.4	87,363,085-103,311,831	2	3.08
KRN6	1.02-1.03	190.53-219	27,030,719-34,917,215	2	4.11
KRN7	10.03	196.14-198.28	65,096,249-75,723,023	2	9.85
KRN8	10.03	217.8-228.3	82,081,161-86,418,123	7	16.26
KRN9	10.07	473.04-483.61	148,996,063-149,073,556	9	8.13
KRN10	10.06	380.5-383.12	138,475,535-138,981,043	6	9.02
KRN11	10.04	344.8-352.94	133,215,331-135,382,131	2	10.33
KRN12	2.02	148.1-151.03	12,874,870-13,827,670	5	5.3
KRN13	2.02	135.59-147.12	12,025,692-12,644,804	3	6.21
KRN14	2.03	191.5-197.76	18,133,383-19,461,962	5	11.22
KRN15	2.09	596.78-599.13	223,833,611-224,605,071	2	3.26
KRN16	2.07	425.14-435.82	193,207,119-195,782,417	4	7.91
KRN17	3.03	129.4-131.7	9,963,297-10,076,403	3	5.01
KRN18	3.05	299.2-312.8	126,509,015-136,874,092	4	6.19
KRN19	3.08	633.8-652.4	213,547,173-213,644,376	6	9.83
KRN20	3.06	436.31-439.72	178,144,821-179,875,893	2	6.48
KRN21	3.08	592.61-608.17	208,900,189-209,849,913	4	6.89
KRN22	4.01-4.02	81-94.7	4,746,444-5,327,212	4	7.92
KRN23	4.05	254.9-268.4	36,124,099-43,215,480	3	4.84
KRN24	4.09	581.14-587.83	224,240,449-225,801,290	5	6.87
KRN25	4.07	424.08-428	173,915,604-177,558,650	8	11.52
KRN26	4.09	602.1-619.4	228,653,486-231,903,992	3	6.57
KRN27	5.01	114.17-116.84	5,996,253-6,721,366	4	6
KRN28	5.01	172.26-181.11	11,733,612-13,003,041	5	8.97
KRN29	5.05	440.34-449.93	185,561,749-189,463,674	6	8.27
KRN30	5.05	404.9-410.8	175,676,893-180,186,824	6	11.82
KRN31	5.03-5.04	297.5-305.17	78,362,776-84,252,685	4	9.98
KRN32	8.02	153.3-157.57	16,951,357-18,202,247	6	12.14
KRN33	8.03	293.77-295.91	104,279,060-104,774,930	5	8.7
KRN34	8.06	409.62-439.67	138,853,275-164,088,415	2	9.4
KRN35	9.03	240.5-257.6	89,923,716-102,441,499	7	8.42

sis linked to QTL and expression-based information.

Meta-QTL of KRN trait

In this study, we collected and reallocated 207 QTL of KRN from myriads of individuals studies via meta-analysis pathway, and a total of 35 KRN MQTL were identified. Generally, the more initial QTL in the MQTL, the higher reliability is. Twenty-two (62.8%) MQTL were integrated more than 3 initial QTL, and 9 (25.7%) MQTL integrated more than 5 initial QTL, with an average of 3.5 initial QTL. Among these KRN MQTL, the MQTL of KRN9 possessing the most initial QTL (9 QTL) were mapped on chromosome

10 (bin10.07). The MQTL of KRN8 possessing the highest R^2 (16.26%) were mapped on chromosome 10 (bin10.03). Furthermore, 3 (8.5%) out of all KRN MQTL were overlapped with MQTL for GY, which were some common and overlapped intervals in previous research relevant to GY trait (Martinez et al, 2016; Wang et al, 2016).

Compared to the previously published genes associated with KRN, three reported genes were mapped in these MQTL intervals, including *ub2* (bin 1.06) involved in KRN (Chuck et al, 2014), *ct2* (bin 1.01) resulted in fasciated ear (Bommert et al, 2013a) and *zfl2* (bin 2.02) controlling KRN (Bomblies et al, 2003). Meanwhile, the cloned genes *td1* (Bommert et al, 2005), *fea2* (Taguchi-Shiobara et al, 2001), *fea4* (Je et al, 2016), and *ub3* (Chuck et al, 2014) associated with KRN were not able to be mapped in the MQTL intervals in this study. It may be because the clone's genetic materials coming from specific genetic background. Generally, the genetic R^2 of the consistency of QTL under various genetic backgrounds were not high, while those QTL exhibiting high R^2 values were parent-of-origin specific QTL.

Candidate genes mining of KRN Meta-QTL

QTL mapping and cloning of the corresponding genes were an effective way to elucidate the molecular mechanism. Although a lot of QTL were identified in previous experiments, the genes underlying QTL had only been cloned in a few cases, particularly for minor QTL. In many cases, the major QTL region typically contained minor QTL clusters, and the phenotype of traits is controlled by polygenes. The traditional way was difficult to perform in-depth case-study research. RNA-seq technology provided a solution. For example, Eveland et al (2014) revealed regulatory modules controlling maize inflorescence architecture by RNA-seq technologies, and provided comprehensive insight into the developmental dynamics of gene expression for inner ear morphogenesis. Interestingly, the number of known genes, including *ub2*, *ub3* and *zfl2*, controlling KRN trait that had expression of FPKM between > 6.62 and < 65.37 in 1-mm ear stage. The genes related to abnormal ear morphology, including *ra1*, *ra2*, *ra3*, *ct2*, *fea2*, *fea3*, and *fea4*, had expression of FPKM between > 2.30 and < 67.66. FPKM value was far less than that of highest value (GRMZM2G153292; 4,006.72). The results show that the FPKM value of key genes response for significant morphological changes was not the higher the better and might be around the average of 13.45.

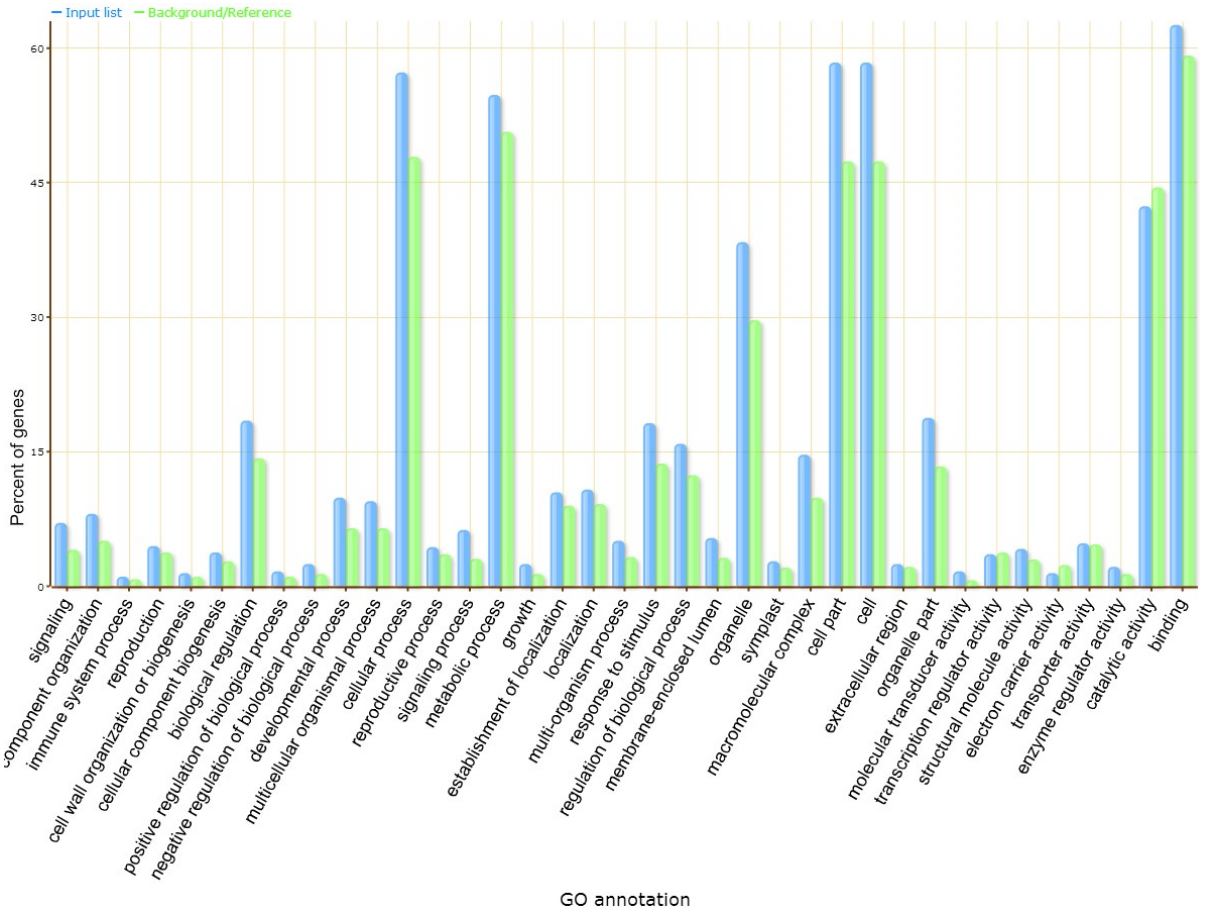

Figure 1 - Summary of the functional analysis of GO terms.

Applications of meta-QTL in MAS

MAS technologies were significantly contributing in increasing accuracy in selection and breeding work to improve the efficiency of breeding practice, which was the trend of molecular breeding strategies. In recent years, the success of MAS for genetic improvement of yield related traits had been reported in maize and rice (Liang et al, 2004; Stuber et al, 1987). Meta-QTL originated from different genetic backgrounds, growth environments and types of molecular markers contained clear indication of QTL location and the interactions of environment-environment and gene-environment could improve efficiency in MAS. Particularly, the QTL-rich regions related to various traits had higher efficiency of selection. There were also three overlapping intervals in our study. In summary, these «hot bins» may be the pleiotropic regions character of yield and yield components, which could take a shortcut for the MAS breeding.

In comparison to QTL mapping of important agronomic traits, the gene cloning could be more useful to understand the molecular mechanism and genetic improvement of a trait, which is also the foundation of breeding by design. All identified candidate genes for KRN in maize, especially, those genes with conserved ortholog in closely related species, might have a potential influence on elucidating the molecular mechanisms of morphogenesis and increasing the breeding efficiency by marker-assisted selection based on the functional marker.

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References

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

Ballini E, Morel JB, Droc G, Price A, Courtois B, Notteghem JL, Tharreau D, 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Mol Plant Microbe In 21: 859-868

Barrière Y, Méchin V, Denoue D, Bauland C, Laborde J, 2010. QTL for yield, earliness, and cell wall quality traits in topcross experiments of the F838 × F286 early maize RIL progeny. Crop Sci 50: 1761-1772

Bomblies K, Doebley JF, 2006. Pleiotropic effects of the duplicate maize FLORICAULA/LEAFY genes zfl1 and zfl2 on traits under selection during maize domestication. Genetics 172: 519-531

Bomblies K, Wang RL, Ambrose BA, Schmidt RJ, Meeley RB, Doebley J, 2003. Duplicate FLORICAULA/LEAFY homologs zfl1 and zfl2 control inflorescence architecture and flower patterning in maize. Development 130: 2385-2395

Bommert P, Je BI, Goldshmidt A, Jackson D, 2013a. The maize Galpha gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. Nature 502: 555-558

Bommert P, Nagasawa NS, Jackson D, 2013b. Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus. Nat Genet 45: 334-337

Bommert P, Nardmann J, Vollbrecht E, Running M, Jackson D, Hake S, Werr W, 2005. Thick tassel dwarf1 encodes a putative maize ortholog of the Arabidopsis CLAVATA1 leucine-rich repeat receptor-like kinase. Development 132: 1235-1245

Cai HG, Chu Q, Gu RL, Yuan LX, Liu JC, Zhang XZ, Chen FJ, Mi GH, Zhang FS, 2012. Identification of QTLs for plant height, ear height and grain yield in maize (*Zea mays* L.) in response to nitrogen and phosphorus supply. Plant Breeding 131: 502-510

Cai LC, Li K, Yang XH, Li JS, 2014. Identification of large-effect QTL for kernel row number has potential for maize yield improvement. Mol Breed 34: 1087-1096

Calderón Claudia I, Yandell Brian S, Doebley John F, 2016. Fine Mapping of a QTL Associated with Kernel Row Number on Chromosome 1 of Maize. PloS One 11: e0150276

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A, 2004. Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168: 2169-2185

Choe E, Rocheford TR, 2012. Genetic and QTL analysis of pericarp thickness and ear architecture traits of Korean waxy corn germplasm. Euphytica 183: 243-260

Chuck GS, Brown PJ, Meeley R, Hake S, 2014. Maize SBP-box transcription factors unbranched2 and unbranched3 affect yield traits by regulating the rate of lateral primordia initiation. P Natl Acad Sci 111: 18775-18780

Coque M, Gallais A, 2006. Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. Theor Appl Genet 112: 1205-1220

Cui RF, Han JK, Zhao SZ, Su KM, Wu F, Du XQ, Xu QJ, Chong K, Theißen G, Meng Z, 2010. Functional conservation and diversification of class E floral homeotic genes in rice (*Oryza sativa*). Plant J 61: 767-781

Darvasi A, Soller M, 1997. A simple method to calculate resolving power and confidence interval of QTL map location. Behav Genet 27: 125-132

Du Z, Zhou X, Ling Y, Zhang ZH, Su Z, 2010. agriGO:

a GO analysis toolkit for the agricultural community. Nucleic Acids Res: gkq310
Eveland AL, Goldshmidt A, Pautler M, Morohashi K, Liseron-Monfils C, Lewis MW, Kumari S, Hiraga S, Yang F, Unger-Wallace E, 2014. Regulatory modules controlling maize inflorescence architecture. Genome Res 24: 431-443
Fu JJ, Cheng YB, Linghu JJ, Yang XH, Kang L, Zhang ZX, Zhang J, He C, Du XM, Peng ZY, 2013. RNA sequencing reveals the complex regulatory network in the maize kernel. Nat Commun 4
Goffinet B, Gerber S, 2000. Quantitative trait loci: a meta-analysis. Genetics 155: 463-473
Guo B, Sleper DA, Lu P, Shannon JG, Nguyen HT, Arelli PR, 2006. QTLs associated with resistance to soybean cyst nematode in soybean: meta-analysis of QTL locations. Crop Sci 46: 595-602
Guo JJ, Chen ZL, Liu ZP, Wang BB, Song WB, Li W, Chen J, Dai JR, Lai JS, 2011. Identification of genetic factors affecting plant density response through QTL mapping of yield component traits in maize (*Zea mays* L.). Euphytica 182: 409-422
Hanocq E, Laperche A, Jaminon O, Lainé AL, Le Gouis J, 2007. Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. Theor Appl Genet 114: 569-584
Ho J, McCouch S, Smith M, 2002. Improvement of hybrid yield by advanced backcross QTL analysis in elite maize. Theor Appl Genet 105: 440-448
Huang YF, Madur D, Combes V, Ky CL, Coubriche D, Jamin P, Jouanne S, Dumas F, Bouty E, Bertin P, 2010. The genetic architecture of grain yield and related traits in *Zea mays* L. revealed by comparing intermated and conventional populations. Genetics 186: 395-404
Hung HY, Shannon LM, Tian F, Bradbury PJ, Chen C, Flint-Garcia SA, McMullen MD, Ware D, Buckler ES, Doebley JF, 2012. ZmCCT and the genetic basis of day-length adaptation underlying the postdomestication spread of maize. Proc Natl Acad Sci USA 109: E1913-E1921
Ikeda-Kawakatsu K, Maekawa M, Izawa T, Itoh JI, Nagato Y, 2012. ABERRANT PANICLE ORGANIZATION 2/RFL, the rice ortholog of Arabidopsis LEAFY, suppresses the transition from inflorescence meristem to floral meristem through interaction with APO1. Plant J 69: 168-180
Je BI, Gruel J, Lee YK, Bommert P, Arevalo ED, Eveland AL, Wu QY, Goldshmidt A, Meeley R, Bartlett M, 2016. Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. Nat Genet 48(7): 785-791
Karen Sabadin P, Lopes de Souza Júnior C, Pereira de Souza A, Augusto Franco Garcia A, 2008. QTL mapping for yield components in a tropical maize population using microsatellite markers. Hereditas 145: 194-203
Li F, Jia H, Liu L, Zhang C, Liu Z, Zhang Z, 2014. Quantitative trait loci mapping for kernel row number using chromosome segment substitution lines in maize. Genet Mol Res 13: 1707-1716
Li JZ, Zhang ZW, Li YL, Wang QL, Zhou YG, 2011. QTL consistency and meta-analysis for grain yield components in three generations in maize. Theor App Genet 122: 771-782
Li M, Guo XH, Zhang M, Wang XP, Zhang GD, Tian YC, Wang ZL, 2010a. Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (*Zea mays* L.). Plant Sci 178: 454-462
Li PH, Ponnala L, Gandotra N, Wang L, Si YQ, Tausta SL, Kebrom TH, Provart N, Patel R, Myers CR, 2010b. The developmental dynamics of the maize leaf transcriptome. Nat Genet 42: 1060-1067
Li WT, Liu CJ, Liu YX, Pu ZE, Dai SF, Wang JR, Lan XJ, Zheng YL, Wei YM, 2013. Meta-analysis of QTL associated with tolerance to abiotic stresses in barley. Euphytica 189: 31-49
Li YL, Li XH, Li JZ, Fu JF, Wang YZ, Wei MG, 2009. Dent corn genetic background influences QTL detection for grain yield and yield components in high-oil maize. Euphytica 169: 273-284
Li YL, Niu SZ, Dong YB, Cui DQ, Wang YZ, Liu YY, Wei MG, 2007. Identification of trait-improving quantitative trait loci for grain yield components from a dent corn inbred line in an advanced backcross BC2F2 population and comparison with its F2: 3 population in popcorn. Theor Appl Genet 115: 129-140
Liang FS, Deng QY, Wang YG, Xiong YD, Jin DM, Li JM, Wang B, 2004. Molecular marker-assisted selection for yield-enhancing genes in the progeny of «9311 × O. rufipogon» using SSR. Euphytica 139: 159-165
Liu L, Du YF, Shen XM, Li MF, Sun W, Huang J, Liu ZJ, Tao YS, Zheng YL, Yan JB, 2015. KRN4 Controls Quantitative Variation in Maize Kernel Row Number. PLoS Genet 11: e1005670
Liu XH, He SL, Zheng ZP, Huang YB, Tan ZB, Wu X, 2010. QTL identification for row number per ear and grain number per row in maize. Maydica 55: 127-133
Liu Y, Wang LW, Sun CL, Zhang ZX, Zheng YL, Qiu FZ, 2014. Genetic analysis and major QTL detection for maize kernel size and weight in multi-environments. Theor Appl Genet 127: 1019-1037
Liu ZH, Ji HQ, Cui ZT, Wu X, Duan LJ, Feng XX, Tang JH, 2011. QTL detected for grain-filling rate in maize using a RIL population. Mol Breed 27: 25-36
Lu M, Xie CX, Li XH, Hao ZF, Li MS, Weng JF, Zhang DG, Bai L, Zhang SH, 2011. Mapping of quantitative trait loci for kernel row number in maize across seven environments. Mol Breed 28: 143-152
Lv XL, Li XH, Xie CX, Hao ZF, Ji HL, Shi LY, Zhang SH, 2008. Comparative QTL mapping of resis-

tance to sugarcane mosaic virus in maize based on bioinformatics. Hereditas 30: 101-108

Martinez AK, Soriano JM, Tuberosa R, Koumproglou R, Jahrmann T, Salvi S, 2016. Yield QTLome distribution correlates with gene density in maize. Plant Sci 242: 300-309

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

Miles C, Wayne M, 2008. Quantitative trait locus (QTL) analysis. Nature Education 1: 208

Peng B, Li YX, Wang Y, Liu C, Liu ZZ, Tan WW, Zhang Y, Wang D, Shi YS, Sun BC, 2011. QTL analysis for yield components and kernel-related traits in maize across multi-environments. Theor Appl Genet 122: 1305-1320

Ribaut JM, Fracheboud Y, Monneveux P, Banziger M, Vargas M, Jiang CJ, 2007. Quantitative trait loci for yield and correlated traits under high and low soil nitrogen conditions in tropical maize. Mol Breed 20: 15-29

Rosenberg MS, Garrett KA, Su Z, Bowden RL, 2004. Meta-analysis in plant pathology: synthesizing research results. Phytopathology 94: 1013-1017

Somers D, Gyenis L, Yun SJ, Smith KP, Steffenson BJ, Bossolini E, Sanguineti MC, Muehlbauer GJ, 2007. Genetic architecture of quantitative trait loci associated with morphological and agronomic trait differences in a wild by cultivated barley cross. Genome 50: 714-723

Sosnowski O, Charcosset A, Joets J, 2012. BioMercator V3: an upgrade of genetic map compilation and quantitative trait loci meta-analysis algorithms. Bioinformatics 28: 2082-2083

Stone JM, Trotochaud AE, Walker JC, Clark SE, 1998. Control of meristem development by CLAVATA1 receptor kinase and kinase-associated protein phosphatase interactions. Plant Physiol 117: 1217-1225

Stuber CW, Edwards MD, Wendel JF, 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. II: Factors influencing yield and its component traits. Crop Sci 27: 639-648

Taguchi-Shiobara F, Yuan Z, Hake S, Jackson D, 2001. The *fasciated ear2* gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. Genes Dev 15: 2755-2766

Tang JH, Yan JB, Ma XQ, Teng WT, Wu WR, Dai JR, Dhillon BS, Melchinger AE, Li JS, 2010. Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F2 population. Theor Appl Genet 120: 333-340

Tian BH, Wang JH, Wang GY, 2014. Confirmation of a major QTL on chromosome 10 for maize kernel row number in different environments. Plant Breeding 133: 184-188

Upadyayula N, Da Silva HS, Bohn MO, Rocheford TR, 2006. Genetic and QTL analysis of maize tassel and ear inflorescence architecture. Theor App Genet 112: 592-606

Veldboom LR, Lee M1, 1994. Molecular-marker-facilitated studies of morphological traits in maize. II: Determination of QTLs for grain yield and yield components. Theor Appl Genet 89: 451-458

Wang HH, Zhang L, Cai Q, Hu Y, Jin ZM, Zhao XX, Fan W, Huang QM, Luo ZJ, Chen MJ, 2015. OsMADS32 interacts with PI-like proteins and regulates rice flower development. J Integr Plant Biol 57: 504-513

Wang YJ, Xu J, Deng DX, Ding HD, Bian YL, Yin ZT, Wu YR, Zhou B, Zhao Y, 2016. A comprehensive meta-analysis of plant morphology, yield, stay-green, and virus disease resistance QTL in maize (*Zea mays* L.). Planta 243: 459-471

Xiang K, Reid LM, Zhang ZM, Zhu XY, Pan GT, 2012. Characterization of correlation between grain moisture and ear rot resistance in maize by QTL meta-analysis. Euphytica 183: 185-195

Xu J, Liu YX, Liu J, Cao MJ, Wang J, Lan H, Xu YB, Lu YL, Pan GT, Rong TZ, 2012. The genetic architecture of flowering time and photoperiod sensitivity in maize as revealed by QTL review and meta analysis. J Integr Plant Biol 54: 358-373

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

Yang C, Tang DG, Zhang L, Liu J, Rong TZ, 2015. Identification of QTL for ear row number and two-ranked versus many-ranked ear in maize across four environments. Euphytica 206: 33-47

Yang GH, Li YL, Wang QL, Zhou YG, Zhou Q, Shen BT, Zhang FF, Liang XJ, 2012. Detection and integration of quantitative trait loci for grain yield components and oil content in two connected recombinant inbred line populations of high-oil maize. Mol Breed 29: 313-333

Yu Y, Li G, Yang Z, Hu J, Zheng J, Qi X, 2014. Identification of a major quantitative trait locus for ear size induced by space flight in sweet corn. Genet Mol Res 13: 3069-3078