

# Comprehensive Meta-Analysis of Maize QTLs Associated With Grain Yield, Flowering Date and Plant Height Under Drought Conditions

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## Abstract

Drought remains the primary abiotic constraint to maize (*Zea mays* L.) productivity globally. Maize drought response involves several regulatory quantitative traits and complex gene networks. Therefore, precise location of drought-related quantitative trait loci (QTL) is imperative for drought tolerance breeding. Despite numerous studies identifying several drought-related maize QTLs, some QTL from particular genetic backgrounds showed smaller effects or could not be identified at all in different backgrounds, affected by marker sets, experimental design, mapping populations and statistical methods. Herein, therefore; using 457 published maize QTLs conferring for 18 traits, we have performed meta-analysis of data from various experiments to obtain meta-QTL (MQTL), integrate these fruitful QTL and to mine candidate genes related to drought. Resultantly, 24 MQTL with confidence interval (CI) < 5 cm were identified to be hot regions. Additionally, 47 drought related gene loci were observed and several candidate genes of the hot MQTL were reorganized by bioinformatics techniques. Thirteen gene (*sod4*, *tafl*, *rps1*, *nthr3*, *oc13*, *bas*, *apx1*, *asn4*, *pck2*, *nac1*, *gst2*, *ao1* and *kch4*) loci of hot MQTL regions were homologous to their corresponding gene sequences from the PlantGDB database (<http://www.plantgdb.org/search/>). Further, we used a comparative genomics approach to identify the homologous regions of MQTL in rice (*Oryza sativa* Japonica) database (<http://www.gramene.org>) and observed that drought-related rice gene ATG6 was homologous to maize candidate genes *GRMZM2G027857\_T01* and *GRMZM2G027857\_T02*. Conclusively, our identified MQTLs with narrowed CI could be useful for marker-assisted selection and the candidate genes harnessed for maize drought tolerance breeding.

**Keywords:** *Zea mays* L., drought stress, marker-assisted selection, meta-analysis, QTL, candidate genes

## 1. Introduction

Maize (*Zea mays* L.) is the third most important cereal in the world after wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L., Golam et al., 2011). The continued rise in world human population and consequent food demands against the backdrop of worsening global climate change has prompted greater need for the crop (Xu et al., 2014; Maazou et al., 2016). However, as a result of the continuing climate change phenomenon, drought stress will remain the primary environmental limitation to maize productivity (Rao et al., 2016). The maize crop is most susceptible to drought stress at the reproductive stage (Tuberosa, 2012). Therefore, maize breeders have the burden of developing drought resilient crop varieties in order to sustain higher yields and global food security under the ravaging climate change conditions.

Drought tolerance breeding has remained a very tedious task because of the high variability of drought stress and insufficient understanding of its complexity (Hao et al., 2010). Maize drought response involves several regulatory quantitative traits and complex gene and metabolic networks (Li et al., 2010; Adebayo et al., 2014). Quantitative trait loci (QTL) analysis of maize drought tolerance involves various aspects, including morphological traits (such as plant height, ear position, and branches per tassel etc.), root, physiological, and biochemical related traits (ABA, degree of leaf senescence, photosynthesis parameters, protein content, stomatal conductance, osmotic adjustment etc.), and yield traits (ear length, 100-kernel weight, ears per plant, and grain yield etc.) among others (Lu et al., 2006; Edmeades, 2013).

Since increasing yield potential under water-stressed conditions is the first target of all breeding programs, the identification of QTL for grain yield and its components becomes crucial for all QTL studies (Luo et al., 2006; Adebayo et al., 2014). The main indicators for identification of yield traits include number of ears per plant, ear weight, number of grains per ear, 100-grain weight and grain yield (Shin et al., 2015). Amongst all these, grain yield can best reflect the effect of drought stress (Edmeades et al., 1999). In addition, morphological characters have a close relationship with plant water absorption and loss. These include a well-developed root system, leaf morphology, plant type and anthesis-silking interval (Hall et al., 1982; Ribaut et al., 2009).

Whereas drought stress might occur throughout the maize growing period whenever the crop is grown without irrigation, flowering period is the most susceptible to drought stress (Tuberosa et al., 2002). Under water-limited conditions, ear growth slows, eventually retarding silk emergence. Consequently, the length of the ASI increases significantly, which may lead to complete abortion of ears and the plant becomes barren (Maazou et al., 2016). Thus, maize drought tolerance is a consequence of coordination amongst morphological, physiological and biochemical characters, and will also depend on genotypes (Li et al., 2010). Additionally, maize drought tolerance related agronomic traits are not only influenced by external environment, but also controlled by a vast array of genes with minor effects. To this end, precise quantitative trait loci (QTL) identification becomes the focal point when researchers apply molecular biology to study the trait (Arcade et al., 2014).

Over the previous two decades, a mounting body of literature underlying yield-related, plant type, root-related and physiological traits have been published; the number of identified QTL on all maize chromosomes (Chr) related to water deficit tolerance has increased significantly (Hao et al., 2010). However, there is an obvious issue that few identical QTL were identified even in the same environment in the same experimental population by different researchers or different years (Li et al., 2010). Moreover, most data showed that partly or wholly non-overlapping QTL are affected by different combinations and/or experiments conducted in different environments (Orf et al., 1999; Rong et al., 2007). Since maize drought tolerance is under highly complex genetic control, it is difficult to manipulate, making it hard to predict the usefulness of QTL for marker-assisted selection (MAS). The integration of QTL data from diverse resources to compare their locational position consistence and specifying co-locations with candidate genes is therefore imperative for selecting QTL for breeding applications (Hanocq et al., 2007). Despite numerous studies identifying several drought-related maize QTLs, some QTL from particular genetic backgrounds showed smaller effects or could not be identified at all in different backgrounds, affected by marker sets, experimental design, mapping populations and statistical methods (Li et al., 2010). The lack of repeatability of QTL effects across different populations and across environments limits the use of this kind of QTL for MAS by plant breeders. Further, it is very difficult to identify the functional drought-tolerant genes and then develop effective molecular markers inside them (Hao et al., 2010). Therefore, integrating these 'initial' QTL, developing a consensus map, and exploiting consensus QTL and their underlying genes becomes a crucial short cut to assist breeding activities.

Meta-analysis is a statistical analysis that combines data from different sources in a single study, and can overcome the limits of individual studies by analysing generally all practical data to achieve a more informative and truthful conclusion (Wang et al., 2006). It combines data from independent researches to identify consensus QTL across studies, validate QTL effects from across environments or genetic backgrounds and to refine QTL positions on the consensus map (Rong et al., 2007; Swamy et al., 2011). This method has been proved an efficient way for use in maize (Goffinet & Gerber, 2000), wheat (Wang et al., 2016), soybean (Wang et al., 2017), grasses (Swamy et al., 2011), potato (Danan et al., 2011) and rice (Courtois et al., 2009). Although QTL and other loci gathered from multiple maps remain a manual and tedious task, this is a critical step to reveal co-locations between genes and QTL. To take advantage of QTL meta-analysis method in maize breeding, several researchers are aiming at diverse agronomic characters by integrating the results from different experiments. For instance, Xiang and co-workers (2012) integrated 241 QTL for correlations of ear rot (ER) and grain moisture content (GM) from 29 studies in maize and observed 29 MQTL and 44 MQTL for ER and GM, respectively chromosomally located differently. Thus, meta-analysis has been widely used in map integration in

crops such as wheat (Hanocq et al., 2007), maize (Truntzler et al., 2010), grasses (Swammy et al., 2011) and cotton (Lacape et al., 2010).

Discovering genes involved in the quantitative traits is the first step in understanding biological processes underlying these traits. QTLs often have long confidence intervals (CIs), therefore, it is difficult to map genes related to agronomic traits (Semagn et al., 2013). Fortunately, meta-analysis could narrow down the CI of integrated QTLs to increase about twofold precision in estimation of QTL position compared to the initial QTL position of the corresponding region (Goffinet & Gerber, 2000).

In the present study; therefore, we used QTL meta-analysis strategy to integrate and reanalyze QTL for three agronomically important traits in maize; including yield, plant height and flowering time, under drought stressed conditions. Additionally, we developed a consensus map and identified consensus QTL for these traits, and have provided MQTL markers with high effects and narrowed confidence intervals (< 5 cm) for possible employment in marker assisted selection or fine-mapping QTL for gene mining. Moreover, we used some bioinformatics tools to dig-out some candidate genes responding to drought stress that were harboured in diverse chromosomal regions. Further, we used a comparative genomics approach to identify the homologous regions of MQTL in rice (*Oryza sativa Japonica*). We hope that several promising MQTL and candidate genes identified herein could be useful for MAS and maize drought tolerance breeding.

## 2. Method

### 2.1 Maize Quantitative Trait Loci (QTL) Data Collection

In this study, we surveyed the QTLs detected under water-deficit condition or under both water-sufficient and water-deficit conditions. We used twenty-two studies on maize yield and yield-related traits (yield, kernel weight, kernel row number, kernel number per ear, kernel number per row, ear diameter, ear length, ear weight, kernel length, kernel width; published from 1994 to 2017), seventeen reports on flowering-date-related traits (male flowering time, female flowering time and anthesis-silking interval; published from 1994 to 2017), and fourteen researches on maize plant-type-related traits (plant height, ear height; published from 1994 to 2012) (Table 1). The English and Chinese literature were retrieved from the PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), and the China Knowledge Resource Integrated Database (CNKI; <http://www.cnki.net>) and Baidu Scholar (<http://xueshu.baidu.com>), respectively. The information including QTL names, map names, traits, likelihood of odd (LOD) score, proportion of variance explained ( $R^2$ ), marker and linkage group, QTL position, “QTL from” and “QTL to” was then reorganized.

### 2.2 Refinement of Maize QTLs

In QTL position refinement procedure, the most important parameter is each QTL map position (most likely position and confidence interval) and the proportion of phenotype. When a QTL position was not available in the paper, the 95% confidence interval was calculated according to Darvasi and Soller (1997) using the following formulae:

$$CI = 530/(NR^2) \quad (1)$$

$$CI = 163/(NR^2) \quad (2)$$

where, Formular 1 was suitable for both backcross population and F<sub>2</sub> population, and formular 2 was suitable for recombinant inbred line (RIL) population. CI is the confidence interval of a QTL, N is the population size, and R<sub>2</sub> is the phenotypic contribution of a QTL.

### 2.3 Consensus Map Construction

It is vital to verify connectivity between input maps before performing the construction of the consensus map (Hanocq et al., 2007). In order to list markers which were not consistent in the different individual maps, in the current study, we used the command “InfoMap” of the BioMercator 4.2 software (<http://www.mybiosoftware.com/biomercator-2-1-genetic-maps-qt1-integration.html>) (URGI, Versailles, France). For each linkage group, InfoMap display some descriptive statistics about the marker maps to determine if the input data can be used for creating a consensus map. Then, we used the “ConsMap” command of the MetaQTL software BioMercator V4.2 (URGI, Versailles, France) to create a consensus marker map. The method implemented in MetaQTL is based on a Weighted Least Square (WLS) strategy (Arcade et al., 2014). For this analysis, the population size and the type are necessary (in the input data) to determine the accuracy. We used the high-density genetic linkage map IBM2 2008 Neighbors (Intermated B73 × Mo17 Map) as reference map, which covered 8054.28 cm and consisted of

15 991 markers. The original maps of QTL collected from different researches (which used different backgrounds, populations and methods) were compared with the reference map to generate a consensus map.

Table 1. Bibliography of Quantitative Trait Loci (QTL) used in this study

Parent lines	Population size	Population type	Environment	Marker type	Analytical method	Traits	Reference
Mo17×H99	150	F2:3	2	RFLP	CIM	GY KW EN EL ED CD KD KR	Veldboom & Lee (1996)
SD34×SD35	120	F3	2	RFLP	IM	GY EN ASI	Agrama & Moussa (1996)
Ac7643S5×Ac7729/TZSRWS5	234	F3	2	AFLP	IM and CIM	MF FF ASI	Ribaut et al.(1996)
Ac7643S5×Ac7729/TZSRWS5	234	F3	2	AFLP	IM and CIM	GY KN EN	Ribaut et al. (1997)
Ki3×CML139	472	F2	2	RFLP	CIM	ASI FF FH	Khairallah et al. (1998)
B73×H99	142	RIL	2	RFLP, SSR and AFLP	IM	MF FF ASI FH	Sari-Gorla et al. (1999)
B73×H99	142	RIL	2	RFLP, SSR and AFLP	IM	EN EL KN EW KW	Frova et al. (1999)
Lo964×Lo1016	171	F3	2	RFLP	IM	GY	Tuberosa et al. (2002)
Huangzao4×Ye107	184	F2:3	2	SSR	CIM	ASI GY ES	Li et al. (2003)
N87-1×9526	183	F2:3	2	SSR	CIM	FH ASI GY EH MF FF	Gao (2004)
A118×91huang15	139	F2:3	2	SSR	IM	FH EH MF FF ASI GY	Wu (2005)
X178×B73	234	F2	2	SSR	CIM	MF FF ASI KN EW EN CW GY KW FH EH	Xiao (2005)
N87-1×9526	183	F2:3	2	SSR	CIM	GY	Gao et al. (2005a)
N87-1×9526	183	F2	2	SSR	CIM	ASI FF MF	Gao et al. (2005b)
Zong3×87-1	221	RIL	2	SSR	CIM	EL KN KW	Lu et al. (2006)
5003×p138	450	RIL	2	SSR	CIM	FH GY	Jiang (2006)
D5×7924	180	F5:6	4	SSR	CIM	EH FH GY ES ASI	Zhu (2008)
Huangzao4×Ye478	235	F2:3	2	SSR	CIM	FH	Wang (2008)
N87-1×9526	183	F2:4	2	SSR	CIM	GY ASI FH	Fu et al. (2008)
B73×H99	142	RIL	2	RFLP, SSR and AFLP	IM	MF FF ASI FH EL EW KW KN	Marino et al. (2009)
CML444×SC-Malawi	236	RIL	4	RFLP and SSR	CIM	KW GY KN MF ASI FH	Messmer et al. (2009)
Huangzao4×Qi319	230	F2:3	4	SSR	CIM	GY KN KW KR EL ED CD EW	Peng et al. (2010)
D5×7924	180	F2:3	2	SSR	CIM	EH FH GY ES ASI	Zhu et al. (2011)
D5×9381	215	F3:4	2	SSR	CIM	GY ASI EL ED FH KN	Wang (2011)
Lin1×Xiang97-7	160	F2:3	4	SSR	CIM	ASI KW GY FH	Chen et al. (2012)
Chang7-2×TS141	218	F2:3	4	SSR	CIM	EN	Zhao et al. (2017)
Langhuang×TS141	202	F2:3	4	SSR	CIM	EN	Zhao et al. (2017)
X178×B73	234	F2:3	6	SSR and AFLP	IM and CIM	ES GY ASI	Hao et al. (2017)

*Note.* (Population type: RIL = recombinant inbred line; F2:3, F2:4, F3:4, F5:6 = segregating populations at different stages); Environment: number of geographical locations; (Marker type: SSR = simple sequence repeat, RFLP = restriction fragment length polymorphism, AFLP = amplified fragment length polymorphism); (Analytical method: IM = interval mapping, CIM = composite interval mapping); (Traits: FH = plant height; EH = ear height; ASI = anthesis-silking interval; FF = female flowering; GY = grain yield; KW = kernel weight; EN = ear number; EL = ear length; ED = ear diameter; CD = cob diameter; KD = kernel diameter; KR = kernel row number; ES = ear-setting; BIO = biomass; CW = cob weight).

#### 2.4 QTL Projection and Meta-Analysis

We created a consensus map and QTL of individual experiments were projected based on the common marker between the original map and reference map by means of a homothetic function described by Chardon et al. (2004). Some controversial markers between original and reference maps were deleted to make sure the analysis was accurate. When the consensus map was built, meta-analysis was used to determine the existence of the consensus QTL and locate the confidence interval (CI). Map projection and QTL meta-analysis were performed using the BioMercator 4.2 software. Although the software has been given five models, the lowest Akaike Information Criterion (AIC) value is believed to be optimum. In the model, the position of each QTL in LG decided the consensus QTL position. The formular for meta-analysis was according to Goffinet and Gerber (2000) as follows:

$$\text{var}(QTL) = 1/\sum 1/\sigma_i^2 \quad (3)$$

where,  $\sigma_i$  is the position of the variance for each of the QTLs on the LG. A 95% CI of the consensus QTL was calculated from the var (QTL):

$$CI = 3.92 \times \text{var}(QTL)^{1/2} \quad (4)$$

The AIC value depends on the simulation of each model. The smallest AIC model is close to the consensus QTL. The mean R<sup>2</sup> values of the original QTLs in the region were the explained variance of this consensus QTL. In this study, the MQTL with less than three original QTLs were discarded.

### *2.5 Meta-QTL (MQTL) Interval Mapping of Candidate Genes Under Drought Stress*

The physical locations of meta-QTL (MQTL) were estimated with the help of MaizeGDB (<https://maizegdb.org>) and GRAMENE (<http://www.gramene.org>) web servers. The B73 RefGen\_v2 sequence information of maize physical map was integrated with the marker information of IBM2 2008 linkage map which could detect the approximate location of given markers or genes on the B73 RefGen\_v3. Then, we downloaded the candidate gene sequences of the MQTL interval through online batch download tool “Download Region Data” in PlantGDB database (<http://plantgdb.org>). In this study, we only identified annotated transcripts in MQTL with < 5 Mb interval.

### *2.6 Discovery of Drought-Related Gene Loci (and Candidate Genes) in Maize MQTL*

Firstly, we reorganized the MQTL interval and adjacent area of the drought related gene loci of the reference map IBM2 2008 Neighbors, retrieved from the NCBI (<http://www.ncbi.nlm.nih.gov>) web server according to the related gene loci for obtaining sequence information. We downloaded the protein sequences of the MQTL interval through online batch download tool “Download Region Data” in PlantGDB database. Then, we analyzed the conserved domains of all protein sequences using online batch ‘Search tool’ in website Pfam (<http://pfam.sanger.ac.uk>). Lastly, the results of these analyses were compared and integrated with the genetic annotation information provided by the NCBI website to dig out drought related genes of MQTL interval and adjacent area.

### *2.7 Extraction of Drought Related Genes in Maize Based on Gene Comparative Mapping*

Drought related genes were searched from the rice genome database Gramene (<http://www.gramene.org>) and mapped to the maize IBM2008 linkage map by the “Cmap” function of the GRAMENE website. Then, we downloaded the protein sequences of the MQTL intervals through online batch download tool “Download Region Data” in PlantGDB database. To dig out drought related genes in maize which were homologous to rice, we analyzed the conserved domains of the downloaded and predicted maize gene/protein sequences by comparing them to the rice drought-related gene/protein sequences through the use of “batch search function” of the Pfam (<http://pfam.xfam.org>) website.

## **3. Results**

### *3.1 Summary of QTL Information Collected*

A total of 457 QTLs for maize yield, plant morphology, and florescence under drought stress condition were compiled from 28 published papers and 20 populations. These QTLs were associated with 18 traits categorized into three types, including grain yield, flowering date, and plant morphology (height). Amongst the 457 QTL, 207 from 22 researches and 18 populations were related to grain yield, 130 from 17 published papers and 12 populations were related to flowering date, whereas 94 from 14 papers and 11 populations were related to plant morphology. Population size ranged from 120 to 472, including F<sub>2</sub>, F<sub>3</sub>, F<sub>2</sub>:3, F<sub>3</sub>:4 and recombinant inbred lines (RILs) populations (Table 1).

### *3.2 QTL Number and Distribution on Maize Chromosomes*

Our results showed that the collected QTLs were non-uniformly distributed in all the ten maize chromosomes; QTL on Chr 1, 2, 3, 5 and 9 were more uniformly distributed amongst all of them. QTL number was highest (up to 83 QTL) on Chr 1 and lowest (29) on Chr 7. Meanwhile, with regards to yield and flowering date related traits, QTL were mostly found on Chr 1, whereas pertaining to plant morphology related traits, the QTL were almost uniformly distributed across all ten maize chromosomes. The trait of anthesis-silking interval, with 70 QTL, was the highest among all the traits (Table 2).

Table 2. Summary of QTLs associated with drought tolerance in maize

Trait	Trait	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	QTL number
Yield-related	Kernel weight	6	4	4	5	3	3	4	2	2	4	37
	Ear diameter	1	1	-	-	2	1	1	1	-	-	7
	Ear length	2	2	2	-	2	1	-	1	1	1	12
	Ear number	6	2	3	-	1	4	1	-	2	1	20
	Ear weight	1	2	-	-	-	-	-	-	2	2	7
	Ear-setting	3	1	3	-	1	1	-	1	3	2	15
	Grain yield	10	1	7	8	9	6	2	4	3	7	57
	Kernel diameter	1	1	1	-	-	1	-	-	-	-	4
	Kernel number	4	2	1	3	3	-	1	2	4	3	23
	Kernel row number	-	1	2	1	2	1	-	-	-	-	7
	Cob diameter	2	2	-	-	2	-	1	1	-	-	8
	Cob weight	1	-	1	-	1	-	1	-	2	-	6
	Biomass	1	-	-	-	-	-	1	2	-	-	4
Flowering date	Male flowering	9	7	4	5	4	3	2	3	4	2	43
	Female flowering	7	6	3	1	2	4	5	6	6	3	43
	Anthesis-silking interval	16	9	13	3	6	4	3	5	7	4	70
Plant type	Plant height	7	9	2	7	3	5	6	10	7	2	58
	Ear height	6	3	4	5	4	6	1	2	1	4	36
	Total	83	53	50	38	45	40	29	40	44	35	457

Note. Chr = chromosome.

### 3.3 Constructed QTL Consensus Map and MQTL Identified by Meta-analysis

The initial map was projected onto the reference map by BioMeccator 4.2 software and the consensus map constructed. The initial markers of the initial map on Chr 4 and 10 failed to connect with other maps and we discarded them in constructing the consensus map [31]. A total of 233 QTL from 22 studies were short-listed for grain yield and its components under drought stress. After deleting the QTL with likelihood of odd (LOD) value < 2 and phenotypic variance < 5, there were 220 QTLs projected onto a consensus map. The chromosomal regions with < 3 original QTLs were not considered for MQTL. Thus, a total of 37 MQTL covering 10 maize chromosomes were identified based on the lowest Akaike Information Criteion (AIC) values criteria (Hao et al., 2010). Each Chr contained an average of 2 to 6 MQTL. There were 6 MQTL on Chr 1; 3 MQTL on Chr 3, 7, 8 and 9; 4 MQTL on Chr 2, 4 and 6; 5 MQTL on Chr 5; and 2 MQTL on Chr 10 (Table 3; Figure S1). Similar to the collected QTL distribution, MQTL on Chr 1 was the highest. Of the identified 37 MQTL, 13 initial QTLs were condensed on Chr 10 with the map genetic position of 117.4 cm, which explained 9.69% phenotypic variance and narrowed the confidence intervals to 2.44 cm. Twelve initial QTLs were condensed on Chr 1 with the map genetic position of 208.46 cm and mean phenotypic variance of more than 10% (reaching up to 12.33%). These MQTLs were designated sequentially from MQTL 1 to MQTL 37 according to their Chr locations (Table 3; Figure S1).

A total of 140 flowering time related traits were collected from 17 studies and were projected onto the reference map; 133 QTL were successfully projected on all the 10 maize chromosomes. Maximum (25) QTLs were on Chr 1. In this part, 28 MQTL related to maize flowering date were identified. The number of MQTL on each chromosome ranged from 1 to 5. Chromosome 1 had the most number of MQTL, similar to the results of meta-analysis on grain yield related traits (Table 3; Figure S1). The phenotypic variance of the MQTL varied from 6% to 34.67%, with the confidence interval ranging from 0.69 cm to 31.33 cm. Meanwhile, MQTL51 on Chr 7 had minimum CI of 0.69 cm and corresponded to 4 initial QTLs, with an average R<sup>2</sup> of 8.75%. MQTL42, MQTL46, MQTL47, MQTL58 and MQTL62 on Chr 1, 3, 3, 8 and 9, respectively corresponded to 3, 6, 3, 6 and 4 initial QTL, with R<sup>2</sup> ranging from 6.5% to 18.75%. The location, confidence interval, phenotypic variance and flanking markers information of each MQTL are provided in Table 3.

A total of 94 QTLs collected from 14 researches were associated with plant height and ear height, and were distributed on all the ten chromosomes. Among these, 4 QTL could not be projected onto the reference map

because they did not have common markers with the reference map or genetic linkage map. The remaining 90 initial QTL that were projected onto all 10 chromosomes ranged from 5 to 12 QTL on each Chr. Chromosome 9 had the least (5), whereas Chr 8 had the largest (16) number of QTL. In this part, a total of 13 MQTL were distributed on eight chromosomes, with the exception of Chr 3 and 7 (Figure S1). The MQTL of Chr 3 and 7 were discarded due to less than three initial QTL projected on them. There were 2 MQTL on each of Chr 1, 2 and 5; whereas Chr 4, 6, 9 and 10 contained 1 MQTL each. Meanwhile, on Chr 8, sixteen initial QTL were aggregated to 3 MQTL and positioned at 68.68 cm, 119.05 cm and 152.36 cm, with the confidence interval narrowed to 6.63 cm, 3.90 cm and 8.47 cm, respectively. The initial QTL of each MQTL ranged from 3 to 6, with the R<sup>2</sup> ranging from 6.0% to 29.0% (Table 3).

Table 3. Meta-QTLs for yield, flowering date and plant type related traits under drought stress conditions identified by meta-analysis

Trait	Meta-QTL	Chr	AIC Value	Position of MQTL	CI	No of initial QTL	Mean R <sup>2</sup> for initial QTL	Flanking markers of the position	Interval of physical map	Related gene loci
Yield-related	MQTL1	1	281.62	207.88	7.04	10	13.4	IDP2420-TIDP4556	4,758,454-4,767,124	
	MQTL2			<b>222.37</b>	<b>4.11</b>	<b>12</b>	<b>11.7</b>	<b>gpm774d-TIDP8795</b>	<b>21,419,789-22,381,090</b>	<b>tip rpe1</b>
	MQTL3			<b>235.7</b>	<b>4.88</b>	<b>7</b>	<b>11.29</b>	<b>IDP8569-IDP8612</b>	<b>60,047,657-60,678,493</b>	<b>dvr1 sod4</b>
	MQTL4			253.04	5.91	5	18.4	w18-umc2047	266,933,205-263,011,985	
	MQTL5		<b>99.34</b>	<b>174.92</b>	<b>4.15</b>	<b>7</b>	<b>7.57</b>	<b>umc11-umc53b</b>	<b>21,419,789-22,003,995</b>	<b>k1s2</b>
	MQTL6	2	106.61	72.4	11.2	5	11.2	umc259b-bzip121	31,889,777-34,239,593	
	MQTL7			<b>83.74</b>	<b>2.86</b>	<b>5</b>	<b>9.2</b>	<b>IDP2468-umc1454</b>	<b>63,944,183-69,704,680</b>	<b>mde1</b>
	MQTL8		66.4	122.74	8.49	3	13.33	IDP7614-csu64a(grf)	224,335,836-224,605,071	
	MQTL9			140.32	9.23	5	12.6	IDP2512-npi294a	232,489,953-232,824,897	
	MQTL10	3	233.9	<b>35.62</b>	<b>4.7</b>	<b>7</b>	<b>12.86</b>	<b>TIDP3332-npi71</b>	<b>1,713,115-2,270,923</b>	<b>npi71</b>
	MQTL11			57.7	3.96	7	11.71	<b>pza00749-IDP9134</b>	<b>9,843,447-10,080,572</b>	<b>pld1 tip4 myb40</b>
	MQTL12			<b>80.59</b>	<b>3.75</b>	<b>8</b>	<b>9.75</b>	<b>umc165a-bnl6.16a</b>	<b>176,248,449-191,054,985</b>	<b>gst8 got1 taf1</b>
	MQTL13	4	<b>150.63</b>	<b>61.39</b>	<b>2.64</b>	<b>7</b>	<b>10</b>	<b>bnlg1217-pza03385</b>	<b>41,977,452-42,016,246</b>	<b>su1 fie1</b>
	MQTL14			100.21	6.51	5	11.4	umc2365-umc1051	189,494,506-193,438,448	
	MQTL15			125.43	10.07	4	12	gpm350b-gpm87a	237,822,149-239,027,984	
	MQTL16	5	39.68	80.12	5.24	3	21	pza00818-IDP3834	1,080,009-1,538,562	
	MQTL17			98.96	10.46	4	12.25	umc40-umc1679	87,290,927-3,429,663	
	MQTL18		165.25	136.43	4.41	5	8.8	IDP4864-bcd207b	20,922,705-23,141,360	
	MQTL19			<b>151.53</b>	<b>3.48</b>	<b>7</b>	<b>8.57</b>	<b>bnl10.12-umc1264</b>	<b>175,145,318-175,677,720</b>	<b>rps27b</b>
	MQTL20			196.97	19.97	4	6.25	umc1792-bnl61695	218,451,914-221,681,657	
	MQTL21	6	125.01	225.4	5.69	3	26.33	TIDP3537-mmp160	87,122,202-66,438,133	
	MQTL22			253.53	7.22	5	8	gpm250b-IDP4314	134,804,842-142,486,587	
	MQTL23			268.86	10.5	4	14.25	gpm631-roa1	153,555,627-154,285,844	
	MQTL24			281.52	5.77	3	35	umc237-umc1490	161,024,525-162,276,992	
	MQTL25	7	96.07	50.05	7.01	3	12.67	TIDP4658-csu251a	4,549,325-4,816,431	
	MQTL26			<b>66.28</b>	<b>4.09</b>	<b>5</b>	<b>10.8</b>	<b>gpm804-rps1</b>	<b>14,539,980-17,234,112</b>	<b>rps15 rps1 taf1</b>
	MQTL27			<b>78</b>	<b>4.78</b>	<b>4</b>	<b>12</b>	<b>umc1660-umc2630</b>	<b>141,594,488-151,703,776</b>	<b>nthr3 ocl3 nac1</b>
	MQTL28	8	168.68	85.16	15.81	5	8.2	gpm934-IDP9127	20,305,710-20,699,697	
	MQTL29			122.68	7.59	6	10.17	umc2357-mmp64	170,061,917-170,127,656	
	MQTL30			<b>132.22</b>	<b>3.83</b>	<b>6</b>	<b>11.5</b>	<b>obf4-npi315a</b>	<b>171,334,858-175,442,935</b>	<b>psei2</b>
	MQTL31	9	122.22	102.76	6.79	3	10	znod1-gpm79a	16,979,346-18,607,113	
	MQTL32			<b>129.93</b>	<b>3.9</b>	<b>9</b>	<b>9.33</b>	<b>csu355(ext)-polm2</b>	<b>131,908,055-154,478,416</b>	<b>vacsl phyB2 apx1 asn4 pck2</b>
	MQTL33		<b>40.02</b>	<b>145.79</b>	<b>4.72</b>	<b>5</b>	<b>15</b>	<b>pco063085-telomere9L</b>	<b>149,480,368-156,132,972</b>	
MQTL34	10	<b>176.32</b>	<b>117.4</b>	<b>2.44</b>	<b>13</b>	<b>9.69</b>	<b>mpk6-gpm840a</b>	<b>84,128,565-88,611,477</b>	<b>gst2 rps3 nac1</b>	
MQTL35			128.05	8.05	8	9.75	gpm503-IDPCP59559	133,899,843-125,630,737		
Flowering date	MQTL36	1	196.91	206.44	22.3	3	7.67	mHbrBT90-Mol7-dpr1	2,978,024-3,792,043	
	MQTL37			242.54	8.88	8	9.67	npi234-umc1568	34,652,377-15,808,040	
	MQTL38			272.21	9.63	8	9	umc2396-gpm260a	198,104,270-200,547,561	
	MQTL39		<b>38.52</b>	<b>303.15</b>	<b>0.24</b>	<b>5</b>	<b>9</b>	<b>pza00978-mab18</b>	<b>283,187,143-287,192,923</b>	<b>ao1 ccs1</b>
	MQTL40	2	63.08	129.77	11.54	4	10.25	IDP1657-mmc0271	202,196,007-198,625,109	
	MQTL41		84.82	47.04	5.54	3	18	TIDP5226-pco102445	5,818,068-6,534,813	
	MQTL42			157.67	14.9	4	9.5	IDP4819-TIDP6098	222,275,941-222,468,376	
	MQTL43	3	<b>130.27</b>	<b>67.31</b>	<b>1.82</b>	<b>6</b>	<b>7.67</b>	<b>IDP2399-gpm713</b>	<b>3,840,177-4,379,610</b>	<b>kch4</b>

			<b>MQTL44</b>	<b>110.27</b>	<b>3.16</b>	<b>3</b>	<b>10</b>	<b>nrp1-umc92a</b>	<b>29,838,476-31,245,032</b>	<b>nrp1 e4</b>	
			MQTL45	123.21	14.44	3	8.67	agrp97-tda64	136,086,344-137,548,200		
			MQTL46	4 74.97	132.83	11.04	6	9.33	gst26-bnl17.09	36,299,524-36,460,064	
			MQTL47	5 96.45	197.49	11.5	3	7.33	ucsd64a-IDP3964	7,052,510-7,265,191	
			MQTL48		292.29	25.51	3	6	umc68-bnl5.24	205,440,973-217,012,402	
			MQTL49	6 79.13	266.21	13.63	5	9.8	csu116a-csu382a(cld)	107,401,374-132,064,555	
			MQTL50		284.09	5.65	3	34.67	bnl8.08c-umc1859	154,096,569-154,494,718	
Flowering date			<b>MQTL51</b>	<b>7 122.15</b>	<b>81.85</b>	<b>0.69</b>	<b>4</b>	<b>8.75</b>	<b>IDP4192-TIDP5555</b>	<b>124,265,889-129,111,170</b>	<b>asc1</b>
			MQTL52		104.81	11.14	3	15.67	gpm392-IDP4527	153,455,716-153,584,620	
			MQTL53		137.63	9.49	4	8	gpm683b-agrr44	165,268,333-166,901,107	
			<b>MQTL54</b>	<b>8 123.15</b>	<b>120.82</b>	<b>1.68</b>	<b>6</b>	<b>6.5</b>	<b>bnl17.20-rack2</b>	<b>39,115,777-74,024,212</b>	<b>prp1</b>
			MQTL55		151.76	11.48	4	13	IDP6608-umc1121	141,912,596-147,824,187	
			MQTL56		190.04	8.11	3	24.67	npi328a-cdo187	165,636,122-166,244,750	
			MQTL57	9 143.2	73.74	13.14	3	12	gpm117a-TIDP6487	4,382,875-5,538,490	
			<b>MQTL58</b>		<b>130.71</b>	<b>3.29</b>	<b>4</b>	<b>18.75</b>	<b>asg37-IDP8628</b>	<b>95,980,842-96,199,121</b>	<b>fdh2 mrpa pep1</b>
			MQTL59		162.13	12.51	5	9.2	IDP1691-rps22a	134,286,389-143,421,988	
			MQTL60		195.06	28.19	3	7	jpsb596-pco088782b	150,643,209-153,998,887	
		MQTL61	10 52.88	129.12	31.33	3	8	pco072368-TIDP5776	85,246,955-92,538,469		
		MQTL62	1 53.45	334.99	9.41	3	13	bif2-bnlgl1908	173,096,051-218,832,513		
		MQTL63		405.02	7.57	3	29	umc2589-umc1500	283,187,143-287,192,923		
		MQTL64	2 92.29	55.93	28.87	3	11.33	pza02208-nrpd2/e2	5,764,847-5,967,363		
		<b>MQTL65</b>		<b>101.8</b>	<b>4.66</b>	<b>6</b>	<b>10.83</b>	<b>wt1-AY110266</b>	<b>29,500,958-32,443,663</b>	<b>myb49 gpa1 sam2</b>	
		MQTL66	4 75.09	123.78	6.8	3	8.33	isu144b-mmp111	15,257,930-17,982,099		
		MQTL67	5 88.99	166.45	8.28	3	6	umc2298-dupsr7	87,032,692-112,385,161		
		MQTL68		210.61	8.65	4	10	IDP6595-IDP7362	173,381,107-155,810,019		
		MQTL69	6 65.71	325.66	9.42	6	11	umc238a-uaz81	164,260,857-165,096,553		
		MQTL70	8 146.96	68.68	6.63	6	8.5	IDP6944-TIDP5457	4,805,115-4,918,906		
		<b>MQTL71</b>		<b>119.05</b>	<b>3.9</b>	<b>3</b>	<b>11.67</b>	<b>mmp120-bnlgl1067</b>	<b>24,558,727-25,351,500</b>	<b>rgp2</b>	
		MQTL72		152.36	8.47	5	25.2	rap2-1g4	132,473,577-132,963,880		
		<b>MQTL73</b>	<b>9 31.3</b>	<b>149.79</b>	<b>4.01</b>	<b>3</b>	<b>9.33</b>	<b>umc1357-umc1494</b>	<b>135,613,607-138,953,873</b>	<b>gols3</b>	
		MQTL74	10 37.06	123.69	5.85	4	8.5	umc1938-TIDP5692	83,332,319-83,950,011		

Note. QTL = quantitative trait loci; MQTL = meta-QTL; Chr = chromosome; AIC = Akaike Information Criterion; CI = confidence interval; cm = centi Morgan;  $R^2$  = phenotypic variance.

### 3.4 Gene Mining With MQTL Intervals Associated With Maize Drought Tolerance

With the help of flanking marking between MQTL, we observed 9 859 genes. MQTL32 of Chr 9 had the most (2 415) number of genes whilst there were no genes in the MQTL13 interval. After sorting out the loci information from the IBM2 2008 Neighbors map, 15 991 marker loci were found to confer 4 170 genes, among which 1 392 genes got annotated functions (Li et al., 2010). In the current study, 118 genes were located within or close to the hot regions detected by meta-analysis. According to functional annotation from the NCBI, 45 gene loci were inferred to 24 hot MQTL regions linked to maize drought tolerance. We downloaded the protein and gene sequences of the hot MQTL regions from the online website PlantGDB. Then, using “Batch search tool” on Pfam website, we analysed the conserved domains of all the sequences. Subsequently, the conserved domain information of these genes was compared with the gene loci information from NCBI. Resultantly, we found out that the gene loci *sod4* and gene sequence GRMZM2G169890\_T01 of interval MQTL3 had the same conserved domain and the sequence homology ratio of up to 100%.

The conserved domain of *taf1* and *rps1* in the MQTL12 and MQTL26 were homologous to the gene sequences GRMZM2G002276\_T01 and GRMZM5G895282\_T01, with sequence homology ratios of 84% and 87%, respectively. On MQTL27, three gene loci's (*nthr3*, *oc13* and *bas1*) conserved domains were 73%, 100% and 97% identical to the gene sequences GRMZM2G073377\_T01, GRMZM2G116658\_T01 and GRMZM2G077673\_T01, respectively. On the MQTL32 interval, there were also three gene loci (*apx1*, *asn4* and *pck2*) possessing the same conserved domains with the gene sequences GRMZM2G054300\_T02, GRMZM2G078472\_T01 and GRMZM5G870932\_T01, respectively, and sequence homology ratios of 100%, 89% and 99%, respectively. Besides, the conserved domain of *ao1* and *kch4* on the MQTL39 and MQTL43 intervals were homologous to the gene sequences GRMZM2G141535\_T02 (78%) and GRMZM2G093313\_T02 (99%), respectively (Table 4).



Table 4. Gene loci in MQTL intervals possessing identical conserved domains with predicted genes from maize sequence database

Trait	MQTL	Chr	Gene loci	Gene ID	Conserved proportion (%)
Yield	MQTL3	1	<i>sod4</i>	GRMZM2G169890_T01	100%
	MQTL12	3	<i>taf1</i>	GRMZM2G002276_T01	84%
	MQTL26	7	<i>rps1</i>	GRMZM5G895282_T01	87%
	MQTL27	7	<i>nthr3</i>	GRMZM2G073377_T01	73%
	MQTL27	7	<i>oc13</i>	GRMZM2G116658_T01	100%
	MQTL27	7	<i>bas1</i>	GRMZM2G077673_T01	97%
	MQTL32	9	<i>apx1</i>	GRMZM2G054300_T02	100%
	MQTL32	9	<i>asn4</i>	GRMZM2G078472_T01	89%
	MQTL32	9	<i>pck2</i>	GRMZM5G870932_T01	99%
	MQTL34	10	<i>nac1</i>	GRMZM2G015605_T01	93%
	MQTL34	10	<i>gst2</i>	GRMZM2G132093_P01	99%
Flowering date	MQTL39	1	<i>aol</i>	GRMZM2G141535_T02	78%
	MQTL43	3	<i>kch4</i>	GRMZM2G093313_T02	99%

Note. Chr = chromosome.

### 3.5 Extraction of Maize Drought Tolerance Candidate Genes by Gene Comparative Mapping

By scavenging the *Oryza sativa* Japonica website GRAMENE, 47 genes were found located on all the 11 *Oryza sativa* Japonica chromosomes, except on Chr 12. Then, the gene ATG6, located in region 28.070-28.076 cm on Chr 1 of *Oryza sativa* genetic map IGCN 1998, was transposed onto Chr 8 between the marker rpgc131b and rgpc112 of maize genetic map IBM2008 by 'Cmap' function of the website GRAMENE (Figure 1). Subsequently, the physical map was digged and the *Oryza sativa* gene was positioned on the region of 13.150-162.498 cm on the maize physical map B73 RefGen\_v2. The online website PlantGDB was used to download predicted gene protein sequences within the range, and the conserved domains were compared with *Oryza sativa* drought related gene ATG6. Finally, homologous genes GRMZM2G027857\_T01 and GRMZM2G027857\_T02 on Chr 8 (160,471-160,478 cm) were found (Figure 2). Through NCBI local blast, genes GRMZM2G027857\_T01 and GRMZM2G027857\_T02 had the same sequence as gene ATG6. It became clear then that gene GRMZM2G027857\_T01 and GRMZM2G027857\_T02 were candidate genes for response to drought stress in maize.

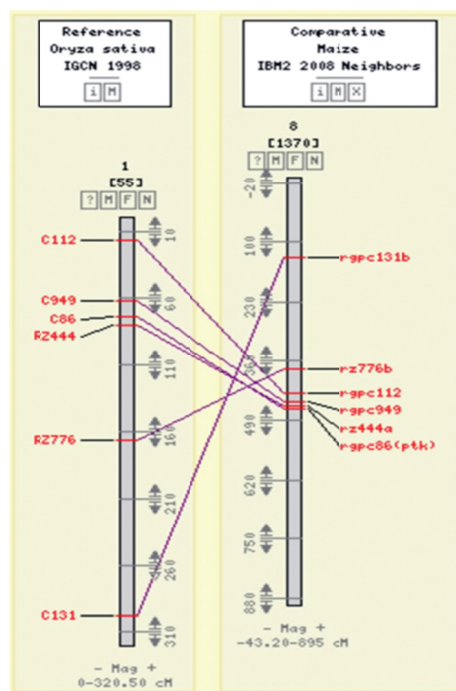


Figure 1. Colinearity comparison of the drought related rice gene *ATG6* to maize genes onto an IBM2 Neighbors 2008 map

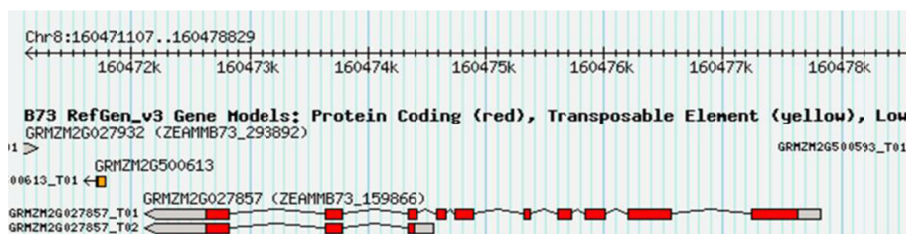


Figure 2. Mapping of maize genes (onto maize B73 RefGen\_v3 map) homologous to rice gene *ATG6*

#### 4. Discussion

Drought stress is the major abiotic factor causing a series of complex physiological and biochemical alterations in maize, resulting in yield reduction (Hao et al., 2010). The genetic improvement of maize drought tolerance is therefore critical for sustained food security in the face of the changing global climate. Molecular breeding, encompassing MAS, presents a quicker and better way of improving crop drought tolerance, consequently leading to sustained higher yields (Xu et al., 2014). To date, marker assisted selection has been successfully used in crop genetics and breeding programs in various crops (Aslam et al., 2015).

Efficient molecular breeding scheme entails the dissection of genetic bases underpinning agronomic traits. This involves the correct identification of these traits. The majority of agriculturally important traits are quantitative, meaning that they are controlled by QTL (Wang et al., 2016). Moreover, most important traits are controlled by numerous genes with minor effects. Therefore, an in-depth integration and re-analysis of QTL information is critical for precise location of QTL and quantitative trait genes (QTGs) and understanding the molecular basis underlying these traits and for proper designing of molecular breeding schemes (Blum, 2011; Wang et al., 2016).

Since drought tolerance is best explained as the crop's ability to maintain stable productivity under drought stress, grain yield definitely becomes the final target for selection and breeding for drought tolerance (Hao et al., 2010). QTL mapping of grain yield related traits under drought stress conditions in maize have been widely studied (Lu et al., 2006; Hao et al., 2010; Li et al., 2010). In addition, an array of researches has shown that there is a significant correlation between plant height and drought tolerance under water-limited conditions (Edmeades, 1999; Ziyomo & Bernardo, 2013; Rex & Yu, 2017). Further, it is well known that the most vital stage for yield

loss due to moisture deficit is just before and during flowering; thus flowering date can be an important indicator of drought tolerance (Tuberosa, 2012).

Previously, an array of QTL has been found in maize under drought stress conditions (Veldboom et al., 1994; Frova et al., 1999; Messmer et al., 2009). However, the QTL identified in individual studies usually had too large confidence intervals, which made it difficult to identify the candidate genes related to drought and conduct positional cloning in maize (Arcade et al., 2014). Therefore, integrating QTL data from various researches and narrowing the confidence intervals (CIs) can be essential in eventual QTL cloning and MAS breeding. The meta-analysis methodology proposed by Goffinet and Gerber (2000) has been widely applied in QTL studies. This method is a powerful tool for integrating QTLs detected in different genetic backgrounds and environments and refining QTL position and accuracy, which could be further pursued for MAS or the identification of candidate genes. Peleman and van der Voort (2003) also put forward the technical system of molecular breeding design which suggested that QTL of related agronomic traits should be located first, then allelic variation of these loci evaluated, and lastly, molecular breeding design carried out. This method has been used in integrated QTL of wheat (Zhao et al., 2006), tomato (Sarfatti et al., 1991; Van der Beek et al., 1992), *Lactuca sativa* (Witsenboer et al., 1995) and maize (Wang et al., 2016).

In the present study, we compiled 457 QTLs related to maize drought tolerance conferring for 18 traits and from 26 previously published researches, and detected 24 MQTLs through meta-analysis strategy. As observed by Goffinet and Gerber (2000), the number meta-QTLs and the size of their CIs depend on the initial QTL number, position and CI estimates. Herein our study, the distribution of 443 QTLs under drought stress condition was such that QTL clusters were greatly universal in ten chromosomes. Even our observed MQTLs covered all the chromosomes of the maize genome, this suggesting that maize drought tolerance is a complex quantitative trait controlled by numerous genes with complicated mechanisms (Li et al., 2010). In agreement, Yan et al. (2004) reported that QTLs affecting five vital agronomic traits of maize clustered in chromosomes. Wang et al. (2006) collected 1201 published QTLs affecting 68 traits and imported them into a local CMap software to construct an integrated QTL map, which found that QTL clusters were greatly universal in every chromosomes. In our current study, we observed that QTL clusters existed in chromosomes. For instance, the intervals 200-210 cm and 220 cm – 230 cm on Chr 1 had more than 10 QTLs and these QTLs have been confirmed to control different yield and yield related traits. Tuberosa et al. (2002) made several possible explanations regarding the observed QTL distribution pattern, the most persuasive of which may be one-factor multi-effect or a close linkage between genes that control different traits. Probably, the QTL controlling same traits might not be at random distribution, but there might be QTL concentration on the chromosome that controls a particular trait which might be related to the presence of gene clusters. By comparing the research results from different experiments, here, our finding verifies the universality of QTL cluster distribution. Further, our findings provide a reference for the selection of multi-effect regions in molecular MAS.

Identification and cloning of QTLs can be hampered by imperfect QTL mapping. Those QTLs identified from individual mapping studies usually contain broad confidence interval, where too many genes are mapped and true quantitative trait genes (QTGs) are obscured (Wang et al., 2006). Herein, our integrated maize QTL map had high density of molecular markers from meta-analysis, which clearly reduced the confidence intervals in the MQTL compared with the original maps. The average genetic distance of consistent QTL was 155.05 cm and there were 32% MQTL with confidence interval of < 5 cm which were selected to be hot MQTL. These ‘real’ QTLs with precise locations were then compared with genes related to drought tolerance in maize and rice, and some candidate genes for drought tolerance were obtained. A total 118 genes were located within or close to the detected hot MQTL regions; and 45 genes from these regions were inferred to 24 maize drought tolerance hot MQTL regions (Table 3).

Predicted genes similar to drought tolerance related genes were identified in many MQTL regions. After comparing the gene loci information (from NCBI) and the gene sequences (from the online website PlantGDB) of these hot MQTL regions, we observed that thirteen gene loci (*sod4*, *tafl*, *rps1*, *nthr3*, *oc13*, *bas*, *apx1*, *asn4*, *pck2*, *nac1*, *gst2*, *ao1* and *kch4*) had the same conserved domains with their corresponding maize gene sequences, with sequence homology ratios ranging from 73% to 100% (Table 4). This result confirmed the reliability of MQTLs detected. In agreement, the gene loci *fdh2*, *pep1*, *phyB2*, and *nac1*, along with the gene of *gst* and *rps* family were related to maize drought-tolerance QTLs (Li et al., 2010). This confirms that our meta-analysis can be relied upon and the hot MQTL regions identified herein can be useful in QTG cloning.

However, not all maize genes were overlapped with ‘real’ QTLs. This could be explained by the fact that some maize genes affecting morphology were not mapped onto IBM2 2008 Neighbors map. Additionally, QTLs with small effect are concealed by mapping populations with lower detection power and ‘overview’ analysis that tend

to exclude QTLs with small effect (Wang et al., 2006). To address this, the combination of meta-analysis within a species of interest and synteny-based projections from a related model plant could be an effective way of identifying new candidate genes for trait variation (Chardon et al., 2004).

Our gene comparative mapping analysis identified 47 maize drought tolerance related genes that were located on 11 *Oryza sativa* Japonica chromosomes, except on Chr 12. By transposing the rice gene *ATG6* (located between 28.070-28.076 cm on Chr 1 of *Oryza sativa* IGCN 1998 genetic map), onto Chr 8 (between rpgc131b and rgpc112 markers) of maize IBM2008 genetic map, we observed that the rice gene was located between 13.150-162.498 cm on the B73 RefGen\_v3 maize physical map (Figure 2). Subsequently, analysis using bioinformatics tools identified that *ATG6* is homologous to maize genes *GRMZM2G027857\_T01* and *GRMZM2G027857\_T02* on Chr 8 (Figure 2), and that these two were candidate genes for maize drought tolerance. This validates that the employment of a comparative genomics approach in QTL meta-analysis improves the power to detect promising MQTLs and mining out candidate genes underlying drought stress tolerance as has already been reported by previous researchers (Hao et al., 2010; Swamy et al., 2011; Wang et al., 2016).

## 5. Conclusion

In summary, our meta-analysis using 457 original QTLs from diverse studies, conferring 18 traits for yield, plant morphology and flowering time under drought stress in maize, detected 24 MQTLs through the integration of these original QTLs. A total of 47 drought related gene loci were identified and several candidate genes of the hot MQTL were reorganized by bioinformatics techniques. Thirteen gene loci of hot MQTL regions were homologous to their corresponding gene sequences from the PlantGDB database. Further, we used a comparative genomics approach to identify the homologous regions of MQTL in rice database and observed that drought-related rice gene *ATG6* was homologous to maize candidate genes *GRMZM2G027857\_T01* and *GRMZM2G027857\_T02*. The identified hot MQTL regions with narrowed CIs and the candidate genes identified in the current study can serve as valuable reference for MAS and discovering novel genomic segments conferring drought tolerance, and cloned, respectively, to further unravel the molecular mechanisms underpinning maize drought tolerance.

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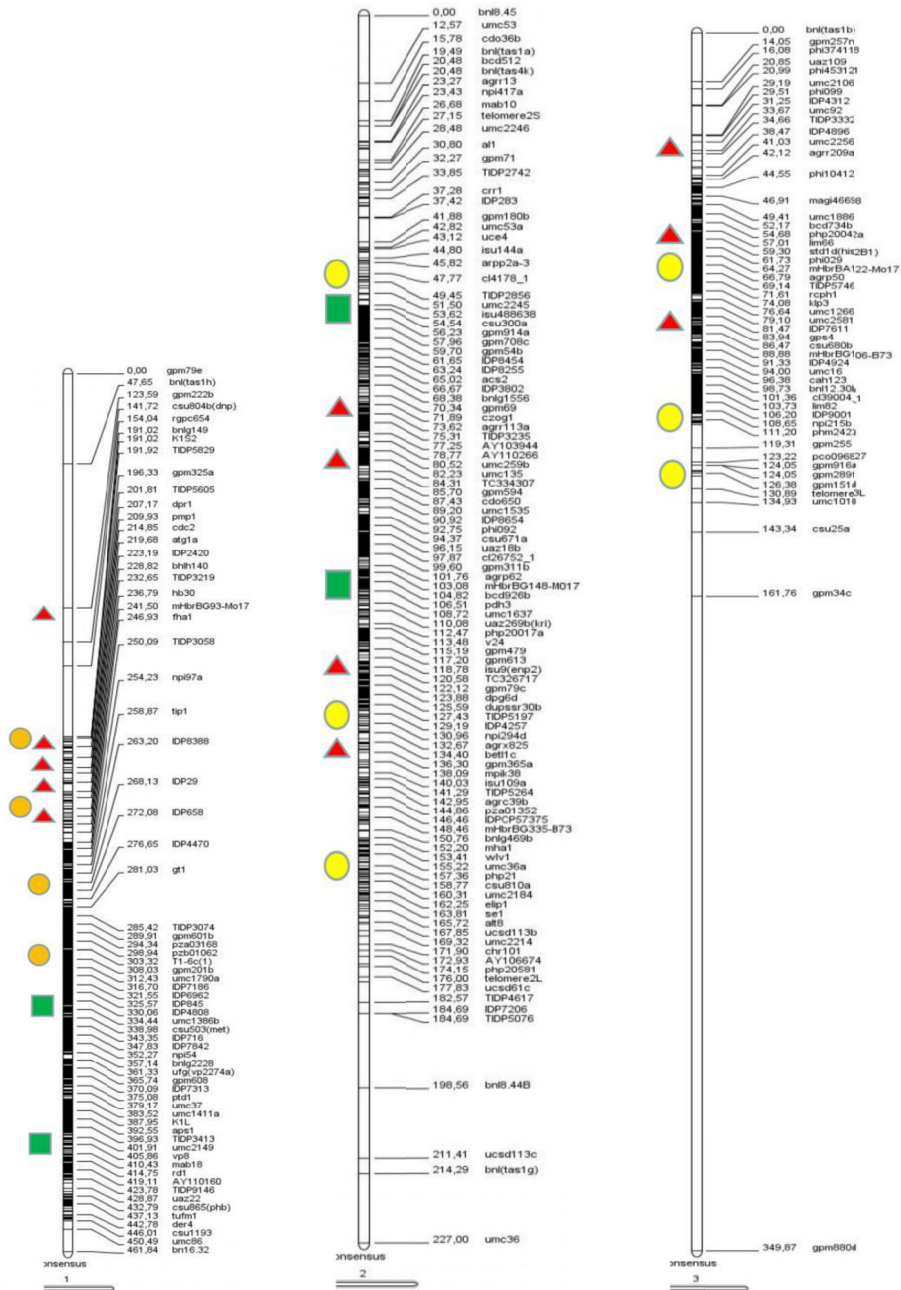
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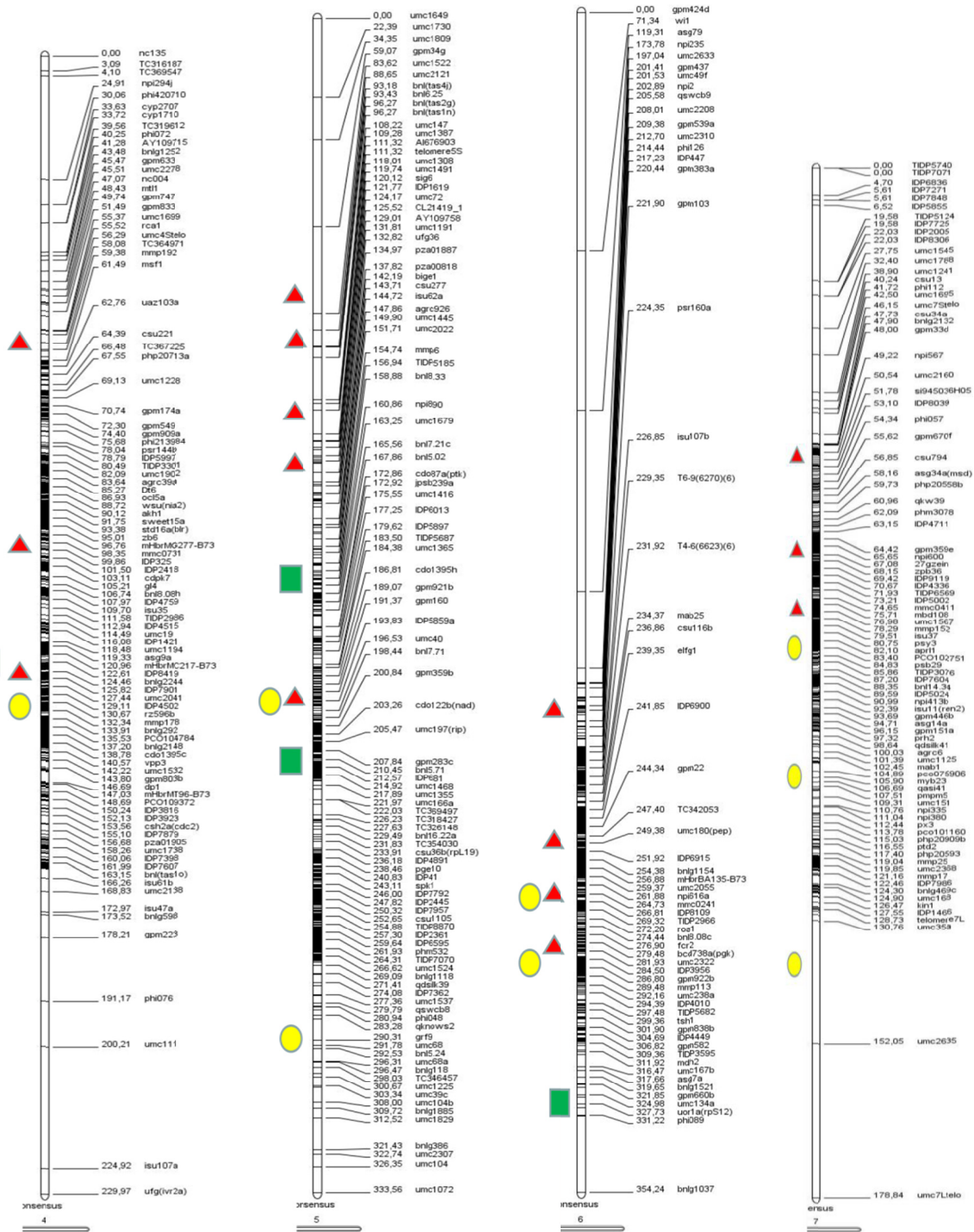
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Appendix A





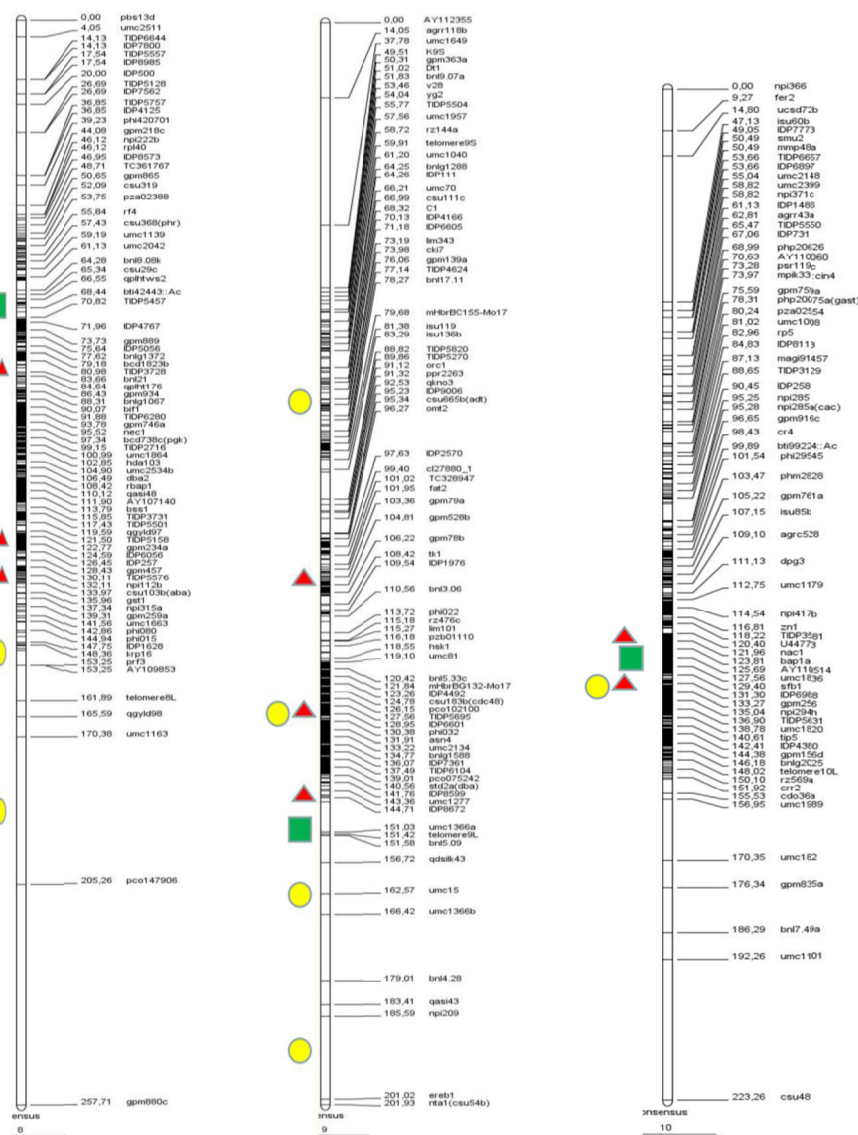


Figure A1. MQTLs revealed by meta-analysis of reported grain yield-, flowering date- and plant type related traits under drought stress conditions, on maize chromosomes, from 1 to 10. Red triangles represent MQTLs related to grain yield, yellow circles represent MQTLs related to flowering date, whereas green squares represent MQTLs related to plant height

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