


Meta-analysis of major QTL for abiotic stress tolerance in barley and implications for barley breeding

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Abstract

Main conclusion We projected meta-QTL (MQTL) for drought, salinity, and waterlogging tolerance to the physical map of barley through meta-analysis. The positions of these MQTL were refined and candidate genes were identified.

Drought, salinity and waterlogging are three major abiotic stresses limiting barley yield worldwide. Breeding for abiotic stress-tolerant crops has drawn increased attention, and a large number of quantitative trait loci (QTL) for drought, salinity, and waterlogging tolerance in barley have been detected. However, very few QTL have been successfully used in marker-assisted selection (MAS) in breeding. In this study, we summarized 632 QTL for drought, salinity and waterlogging tolerance in barley. Among all these QTL, only 195 major QTL were used to conduct meta-analysis to refine QTL positions for MAS. Meta-analysis was used to map the summarized major QTL for drought, salinity, and waterlogging tolerance from different mapping populations on the barley physical map. The positions of identified meta-QTL (MQTL) were used to search for candidate genes for drought, salinity, and waterlogging tolerance in barley. Both MQTL3H.4 and

MQTL6H.2 control drought tolerance in barley. Fine-mapped QTL for salinity tolerance, *HvNax4* and *HvNax3*, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL2H.1 and MQTL5H.3 were also the target regions for improving salinity tolerance in barley. MQTL4H.4 is the main region controlling waterlogging tolerance in barley with fine-mapped QTL for aerenchyma formation under waterlogging conditions. Detected and refined MQTL and candidate genes are crucial for future successful MAS in barley breeding.

Keywords Barley · Meta-analysis · Drought · Salinity · Waterlogging

Introduction

Continued crop improvement is of paramount importance for feeding an increasing human population. Global breeding efforts over the past century have made significant contributions to increased yield potential and stability, as well as cultivars with more durable levels of tolerance to a diverse array of abiotic (drought, freezing, salinity and waterlogging) stresses (Khush 2001). Breeding crops that are tolerant to abiotic stresses is still the best approach to increase crop production (Gill and Tuteja 2010; Tester and Langridge 2010).

Quantitative trait loci (QTL) analysis is a powerful tool in agriculture and other fields. It provides knowledge of the chromosomal location of the target loci and can be applied in breeding programs using marker-assisted selection (MAS). Molecular markers linked to specific QTL have provided plant breeders with a method to improve selecting desirable recombinants from superior varieties and accelerating breeding programs (Khush 2001). MAS, combined

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with conventional breeding, has been utilized in many parts of the world and on many crops (Singh et al. 2009).

The number of publications reporting the identification of new QTL has been increasing tremendously during the past two decades, involving many crop plants and all types of agronomic traits (Xu and Crouch 2008). However, reports of QTL mapping, to date, are mostly based on a relatively low amount of markers, providing limited marker–trait association; and few of the QTL reported have been efficiently used for MAS in plant breeding (William et al. 2007). Many QTL could be identified for one trait, but most of them explain a small proportion of phenotypic variances of the traits (Tuberosa 2012). Therefore, plant breeding programs have not been able to take full advantage of these QTL (Eagles et al. 2001; Xu and Crouch 2008). Positional cloning (DNA sequence identification) of the QTL that explains more than 15 % phenotypic variance can greatly increase the effectiveness of using MAS in breeding programs (Salvi and Tuberosa 2005).

Drought, salinity and waterlogging are three major abiotic stresses limiting the yield of crops, causing extensive losses worldwide (Mittler 2006; Qin et al. 2011). Numerous QTL for drought, salinity and waterlogging tolerance in barley have been described. A meta-analysis can be used to combine different experimental results in one single study. At the QTL level, meta-analysis is able to map the QTL on the same linkage group from different mapping populations of different traits and lower the confidence of interval of QTL to identify more effective candidate genes (Goffinet and Gerber 2000). So far, meta-analysis has been successfully used in studying QTL for flowering time in maize (Chardon et al. 2004; Wang et al. 2016b), drought tolerance in rice (Khowaja et al. 2009), agronomic traits in cotton (Said et al. 2015), leaf senescence in *Arabidopsis* (Chardon et al. 2014) and yield-related traits in wheat (Zhang et al. 2010).

In this study, we summarized 632 QTL for drought, salinity and waterlogging tolerance in barley. Among all these QTL, only 195 major QTL were used to perform meta-analysis to refine QTL positions for MAS. We also identified candidate genes for each of the meta-QTL. Identified meta-QTL from meta-analysis provide resources for further MAS and various omics studies.

Materials and methods

Development of databases

Overall, 632 QTL identified from 1994 to 2015 for drought, salinity and waterlogging tolerance from 32 peer-reviewed publications were summarized in barley (Supplementary Table S1). Each QTL represents QTL for

different traits from different studies with some of them being located in similar positions. Major QTL with the LOD value above 3 and the value of phenotypic variance exceeding 10 % were selected for the meta-analysis, as only QTL with these qualities can potentially be used in MAS and positional cloning (Collard et al. 2005). Parameters under control conditions are able to provide the tolerance coefficients caused by stresses with the relative changes of parameters (stressed/control). Therefore, many QTL for the tolerance coefficients and QTL for traits under control conditions are reported. We included QTL for the tolerance coefficients, but excluded QTL for the traits under control conditions. Although plant architecture traits, such as reduced height, increased number of tillers and erect leaves were also reported to be effective in breeding under control conditions (Khush 2001), these QTL are not relevant to the present investigation. Therefore, we reduced the number of QTL to 195 (Supplementary Table S2). All of these 195 major QTL were used for meta-analysis.

Consensus map and QTL projection

The physical map of barley was used as the consensus map in this study (http://barleygenomeapplications.com/default_2.aspx). BioMercator V4.2 (Arcade et al. 2004) (<https://urgi.versailles.inra.fr/Tools/BioMercator-V4>) was used to project QTL and refine QTL positions from different populations and studies onto one consensus map. The projection of QTL on barley physical map was based on LOD scores, phenotypic variation explained by each QTL, confidence intervals and QTL positions. The positions of the 195 major QTL were based on the positions of flanking markers on the consensus map. In terms of markers without physical positions, the closest markers of the QTL flanking markers from the reference were used to project QTL on the physical map. For those QTL lacking flanking markers and confidence intervals, positions of the closest markers to these QTL were selected as the positions of QTL on the reference map. A 95 % confidence interval was calculated based on the approach: confidence interval = $530/N \times R^2$ (Darvasi and Soller 1997), where N was the population size and R^2 was the proportion of phenotypic variance of QTL.

Meta-analysis of QTL

A meta-QTL is an integrated QTL resulting from several experiments. It is the “actual” QTL location underlying the distribution of the observed QTL on the genome (Goffinet and Gerber 2000). Meta-analysis was conducted with BioMercator V4.2, including algorithms from the MetaQTL software (Arcade et al. 2004; Veyrieras et al. 2007; Goffinet and Gerber 2000; Sosnowski et al. 2012)

(<https://urgi.versailles.inra.fr/Tools/BioMercator-V4>). Meta-analysis first determined the number of meta-QTL (MQTL) in the physical map on each chromosome from different experiments based on AIC (Akaike information content), AICc (AIC correction), AIC3 (AIC 3 candidate models), BIC (Bayesian information criterion) and AWE (average weight of evidence). The number was considered the best fit to carry out meta-analysis when the values of the model selection criteria were the lowest in at least three of the five models (Chardon et al. 2014). Calculated QTL from the optimum model are regarded as the meta-QTL (MQTL) (Goffinet and Gerber 2000). The positions and 95 % confidence intervals of each MQTL were calculated.

Searching for candidate genes

The confidence intervals (cM) of identified MQTL on the barley physical map were used to search for the candidate genes in barley on the website (http://barleygenomeapplications.com/default_2.aspx) with ‘annotated gene’ tool.

Results

The 195 major QTL for abiotic stress tolerance were projected on different chromosomes (Fig. 1; Table 1). Chromosome 2H had the largest number of major QTL (55) and chromosome 6H had the least number of major QTL (15) for abiotic stress tolerance. There were 72 major QTL for drought tolerance, 70 major QTL for salinity tolerance, 48 major QTL for waterlogging tolerance, and 5 major QTL for combined salinity and waterlogging tolerance in barley. Each chromosome had at least seven major QTL for drought tolerance. Most major QTL for salinity tolerance were on chromosomes 2H (23) and 5H (21). In terms of waterlogging tolerance, chromosome 2H had the most number of major QTL (15).

A total of 37 MQTL (~19 %) of the initial 195 major QTL for abiotic stress tolerance were detected based on meta-analysis (Fig. 1; Table 2). Apart from chromosome 5H, all the other chromosomes showed the peaks of density curve (Fig. 1), suggesting the target regions to improve abiotic stress tolerance in barley. There were six MQTL on chromosome 2H, with 53 initial major QTL. Each MQTL on chromosome 2H was formed with at least three initial QTL. Only three MQTL were detected on chromosome 6H. Among all the 37 identified MQTL, two MQTL were formed with QTL from six different populations and four MQTL were formed with QTL from five different populations. The QTL from different populations appeared to be unique. Meta-analysis also reduces the confidence intervals of MQTL from original 18.7 cM on average to 5.5 cM on average of each MQTL. Each MQTL had an average of

112 candidate genes (Supplementary Table S3) based on the physical positions of MQTL (Table 2). MQTL6H.1 had the lowest confidence interval of 0.1 cM (38.1–38.2 cM on chromosome 6H), resulting in no candidate genes on MQTL6H.1. No candidate genes were found on MQTL2H.6 and MQTL7H.5 due to confidence intervals of less than 1.5 cM. There were more than 600 candidate genes on MQTL3H.2 and MQTL4H.2.

Discussion

Drought tolerance in barley

Among all the abiotic stresses limiting crop yield, drought is one of the most important in agriculture, and breeders have made great efforts trying to improve drought tolerance in crops (Cattivelli et al. 2008; Tuberosa and Salvi 2006). Drought is a complex quantitative trait, controlled by many genes and numerous physiological mechanisms, such as early flowering time, plant height, higher K⁺ contents and osmotic adjustment (Cattivelli et al. 2008; Shabala and Pottosin 2014). Accurate phenotyping of drought tolerance remains the challenge for plant breeders to select drought-tolerant genotypes (Hu and Xiong 2014; Tuberosa 2012). Different traits have been used to identify drought-tolerance QTL (Supplementary Table S2). These traits include late leaf senescence (Guo et al. 2008; Sayed et al. 2012), root system (Chen et al. 2010), osmotic adjustment (Diab et al. 2004), relative water content (Teulat et al. 2003) and yield-related traits (Korff et al. 2008).

MQTL1H.4 were formed with five initial QTL for drought tolerance as shown in two studies (Korff et al. 2008; Sayed et al. 2012). The traits used as tolerance criteria include wilting score (Sayed et al. 2012), heading date, early vigour, days of maturity and days of grain-filling period. All of these traits were positively correlated with yield (Korff et al. 2008). Early flowering has been regarded as an effective trait to improve drought tolerance (Blum 2005; Salvi and Tuberosa 2005), escaping drought stress during flowering stage (Tuberosa 2012). Meta-analysis of flowering traits also refined positions of QTL in maize (Chardon et al. 2004).

On MQTL1H.4, totally 30 candidate genes were identified. Putative ATP-dependent Clp protease ATP-binding subunit ClpX1 (CLPX) and cytochrome P450 family protein were both expressed in drought-susceptible rice (Rabello et al. 2008). Overexpression of *lipid transfer protein 3* enhanced drought tolerance in *Arabidopsis* (Guo et al. 2013).

MQTL3H.3 included four QTL for drought tolerance from two different studies (Fan et al. 2015; Korff et al.

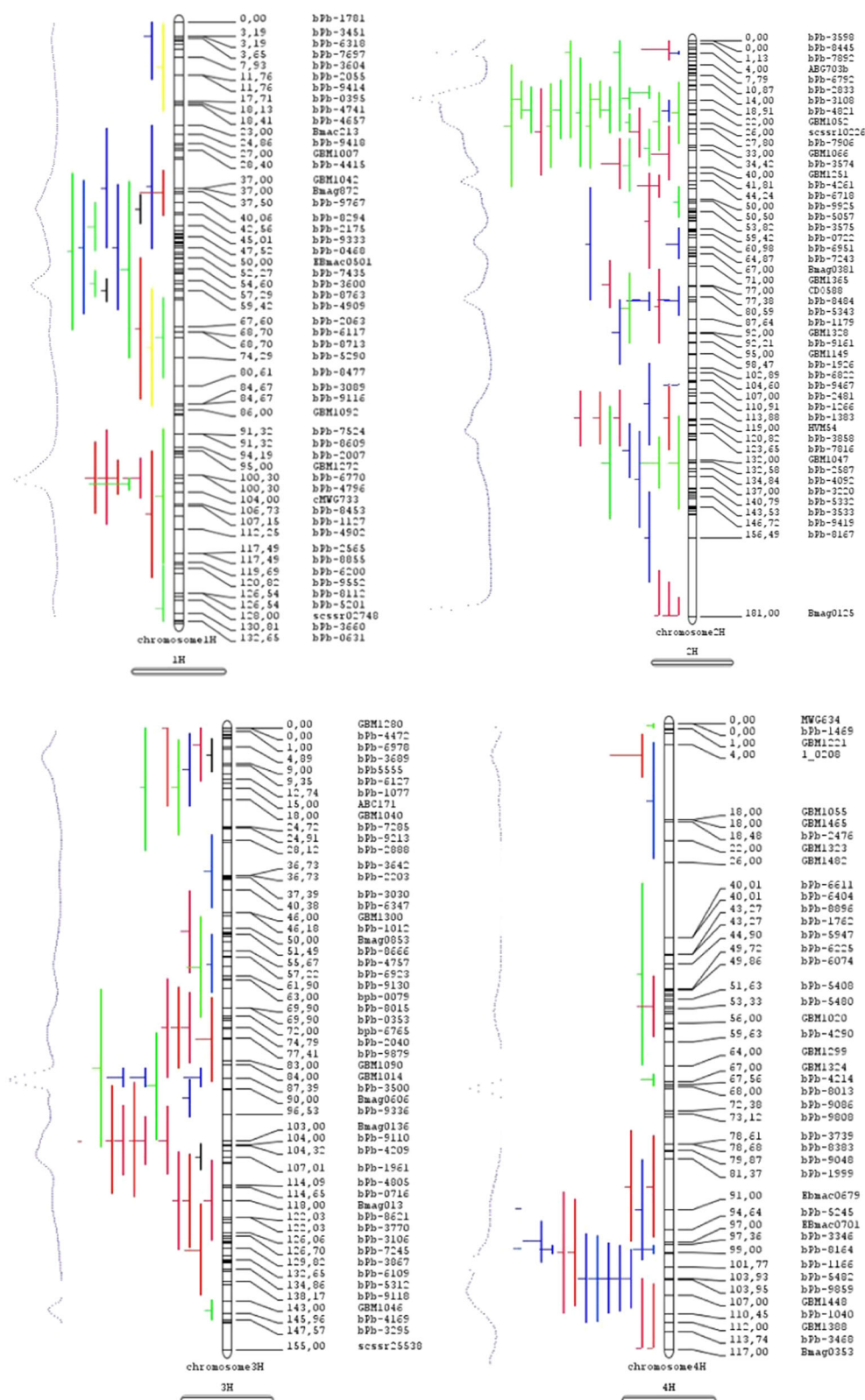


Fig. 1 Summarized major QTL for abiotic stress tolerance [drought (red), salinity (green), waterlogging (blue), combined salinity and waterlogging (yellow), and calculated MQTL (black)] on the barley physical map. Common used markers and genetic distance (cM) are

shown on the right of chromosomes. The dotted line on the left of chromosome is the density curve of QTL on each chromosome (Chardon et al. 2004)

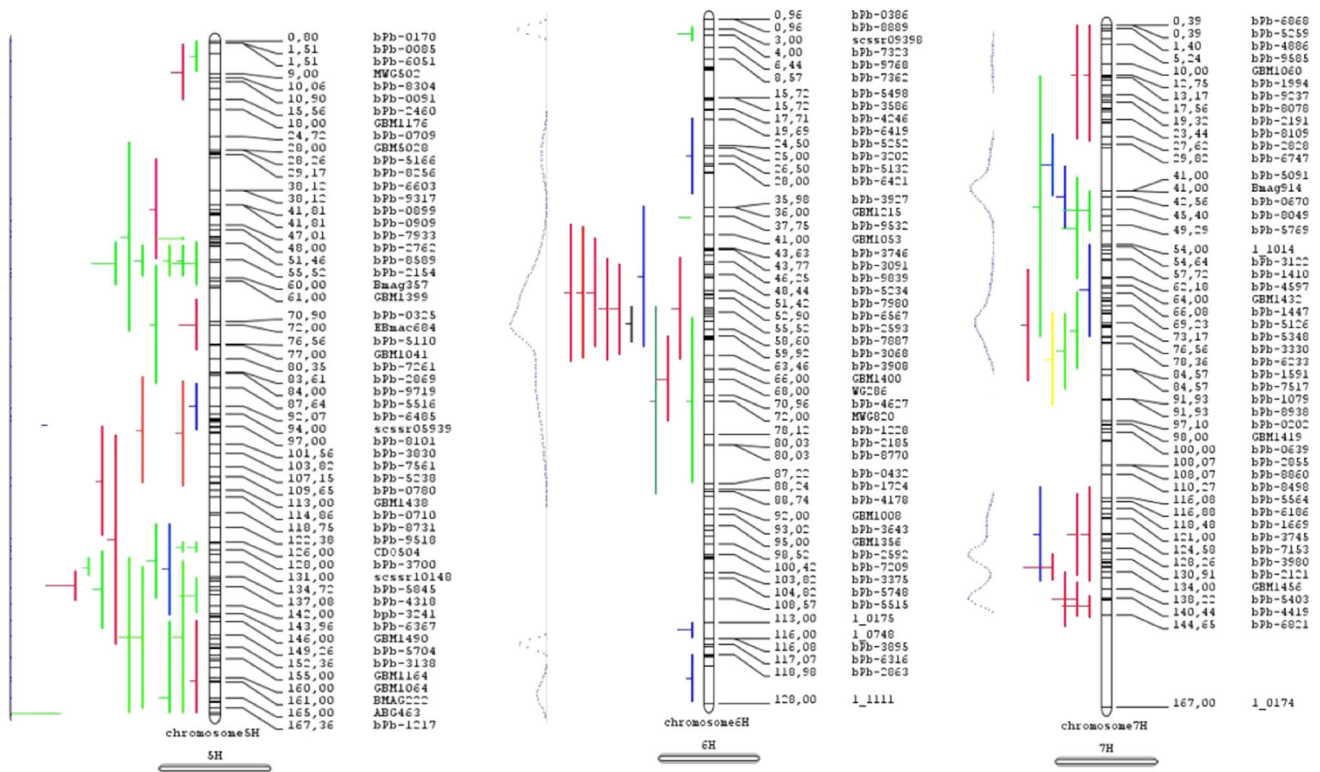


Fig. 1 continued

Table 1 The number of major QTL for abiotic stress tolerance on different chromosomes

	Drought	Salinity	Waterlogging	Combined salinity and waterlogging	Total
1H	7	8	5	2	22
2H	16	23	15	1	55
3H	16	6	7		29
4H	8	3	10		21
5H	9	21	3	1	34
6H	7	4	4		15
7H	9	5	4	1	19
Total	72	70	48	5	195

2008) with three being based on agronomic traits, i.e., plant height, peduncle length and peduncle extrusion (Korff et al. 2008). MQTL3H.3 included 51 candidate genes. Different zinc-finger protein genes were found to improve drought tolerance in different plant species. Expression of CCCH-type zinc-finger gene *OsTZF1* is induced by drought stress in rice (Jan et al. 2013). A C2H2-type zinc-finger protein gene *GmZFP3* in soybean showed negative impact on drought tolerance in transgenic *Arabidopsis* (Zhang et al. 2016a). *IBZFP1* is encoding a C2/H2 zinc-finger protein gene from sweet potato, improving drought tolerance in transgenic *Arabidopsis* (Wang et al. 2016a). Overexpression of another C2H2-type zinc-finger protein gene

GsZFP1 in transgenic *Arabidopsis* also enhanced drought tolerance (Luo et al. 2012).

MQTL3H.4 was formed with eight droughttolerant QTL from two populations based on different physiological traits, wilting score, peduncle length, water-soluble carbohydrate contents, and plant height (Sayed et al. 2012; Diab et al. 2004; Korff et al. 2008). MQTL6H.2 was formed with seven QTL for drought tolerance based on grain yield, kernel weight, and plant height (Korff et al. 2008) and chlorophyll content (This et al. 2000). MQTL3H.4 and MQTL6H.2 had relatively large confidence intervals (7.0 and 6.8 cM), resulting in the large amount of candidate genes (more than 100).

Table 2 Summary of the detected MQTL for abiotic stress tolerance

MQTL	Chromosome	Flanking markers	MQTL position	MQTL confidence interval (cM)	Number of initial QTLs	Number of studies	Number of populations	Candidate genes
MQTL1H.1	1H	bPb-1781-bPb-9718	5.4	10.8	2	2	2	114
MQTL1H.2	1H	bPb-8481-GBM1451	41.7	6.7	6	3	3	70
MQTL1H.3	1H	Glb1-ABC160	59.5	5.3	6	5	3	195
MQTL1H.4	1H	ABC257	102.2	2.6	7	4	4	30
MQTL1H.5	1H	scssr02748-bPb-3201	129.8	10.4	1	1	1	112
MQTL2H.1	2H	bPb-6792	18.8	2.3	21	8	6	25
MQTL2H.2	2H	GBM1251-bPb-4875	44.8	4.7	6	4	4	54
MQTL2H.3	2H	Bmac684-Bmag0381	63.9	9.8	3	3	3	199
MQTL2H.4	2H	bPb-4377	81.9	0.3	8	4	4	11
MQTL2H.5	2H	HVM54-bPb-6688	124.2	6.5	10	4	3	62
MQTL2H.6	2H	Bmag0125	180.1	1.5	4	3	3	0
MQTL3H.1	3H	bPb-6978-bPb-5555	7.1	8.5	5	4	4	63
MQTL3H.2	3H	GBM1300-GBM1110	51.5	9.5	4	4	4	613
MQTL3H.3	3H	GBM1014-Bmag0606	87.1	3.0	10	5	5	51
MQTL3H.4	3H	Bmag0136-bPb-3630	107.1	7.0	8	2	1	125
MQTL3H.5	3H	GBM1046	144.7	1.7	2	2	2	15
MQTL4H.1	4H	MWG634-GBM1221	0.6	1.0	3	3	3	20
MQTL4H.2	4H	scssr20569-GBM1509	52.4	11.0	2	2	2	695
MQTL4H.3	4H	GBM1299-GBM1324	66.7	2.4	1	1	1	45
MQTL4H.4	4H	EBmac0701-bPb-9859	98.6	1.2	13	6	4	58
MQTL4H.5	4H	Bmag0353	117.0	5.2	2	1	1	3
MQTL5H.1	5H	scssr02306-MWG502	6.0	7.0	2	2	2	36
MQTL5H.2	5H	bPb-2762-ABC324	50.5	1.0	2	2	2	47
MQTL5H.3	5H	Bmag337-Bmag357	56.0	4.0	6	4	3	21
MQTL5H.4	5H	ABC302-GBM1041	72.0	12.2	2	2	2	137
MQTL5H.5	5H	scssr05939-bPb-8101	95.4	9.2	5	5	3	165
MQTL5H.6	5H	CDO504-bPb-3700	127.4	2.1	3	2	2	14
MQTL5H.7	5H	scssr10148-GBM1054	135.1	3.6	10	7	5	55
MQTL5H.8	5H	scssr03907	168.8	1.0	3	2	2	43
MQTL6H.1	6H	GBM1215	38.1	0.1	2	2	2	0
MQTL6H.2	6H	cdo497-bPb-3746	57.7	6.8	10	5	3	380
MQTL6H.3	6H	1_0748	115.4	0.5	2	1	1	3
MQTL7H.1	7H	bPb-6868-bPb-8660	13.2	20.4	2	1	1	206
MQTL7H.2	7H	bPb-5091-bPb-9601	43.3	7.0	5	5	4	67
MQTL7H.3	7H	bPb-2379-GBM1472	76.0	9.7	5	4	4	299
MQTL7H.4	7H	Ebmac755-GBM1456	131.3	6.1	4	2	2	122
MQTL7H.5	7H	BMAG135	142.0	1.3	3	2	2	0

Salinity tolerance in barley

Salinity tolerance is also a complex trait, controlled by many minor QTL (Flowers and Flowers 2005). Slow progress was made to improve salinity tolerance with MAS in crops during the past few years although many QTL for salinity tolerance were identified (Ashraf and Foolad 2013). There are two phases of growth reactions in crops to salinity stress (Munns and Tester 2008). The first phase is the osmotic effect to crops, reducing water uptake

by crops, that is similar to drought effects. The second phase is the ion toxicity caused by Na^+ and/or Cl^- that inhibit crop growth.

Many physiological traits are regarded as salinity-tolerant mechanisms (Colmer et al. 2005; Munns 2005). This includes: osmotic adjustment; Na^+ exclusion from uptake; control of xylem ion loading; efficient vacuolar Na^+ sequestration; reactive oxygen species (ROS) detoxification; and cytosolic K^+ homeostasis (Flowers and Colmer 2008; Munns and Tester 2008).

The fine-mapped QTL for salinity tolerance were on chromosome 1H and 7H. *HvNax4* is the locus lowering the shoot Na^+ contents in barley on MQTL1H.4 (Rivandi et al. 2011). This locus was fine-mapped and 34 candidate genes were identified (Rivandi et al. 2011). Possibly, the detected QTL for salinity tolerance at seedling stage on MQTL1H.4 had the same genes with *HvNax4* (Mano and Takeda 1997). MQTL1H.4 was also the hot spot to improve drought tolerance, including five drought-tolerant QTL (discussed above), showing the possibility of improving drought tolerance and salinity tolerance simultaneously.

Among the identified 30 candidate genes on MQTL1H.4, overexpression of heavy metal transport/detoxification superfamily protein was detected in transgenic *Arabidopsis* under salinity conditions (Yokotani et al. 2013). Based on meta-analysis in rice, the pentatricopeptide repeat (PPR)-containing protein-like gene was identified as the candidate gene for improving rice yield on different chromosomes (Swamy et al. 2011).

MQTL7H.2 formed a fine-mapped major locus for salinity tolerance *HvNax3*, explaining 51 % phenotypic variance with an LOD value of 9.9 (Shavrukov et al. 2010). Neither *HvNax3* nor *HvNax4* was able to influence K^+ contents in barley (Rivandi et al. 2011; Shavrukov et al. 2010), while *HvNax3* was shown to lower the sodium accumulation in leaves. The physiological mechanisms of this reduction remain a matter of conjecture. Several candidate genes were identified in the *HvNax3* locus with colinearity in rice and *Brachypodium*. From meta-analysis, 67 candidate genes were also identified in the locus *HvNax3*. Which of these candidate genes play a role in controlling Na^+ content in the shoot remain to be investigated in future experiments. It was suggested earlier that other *Nax* loci, *Nax 1* and *Nax 2*, enhance the retrieval of Na^+ back into the root stele via HKT1;4 or HKT1;5 (Munns et al. 2012). However, more recent studies have shown that *Nax* loci also reduce the rate of Na^+ loading into the xylem via SOS1 Na^+/H^+ exchanger in wheat (Zhu et al. 2016). It remains to be determined which of these mechanisms is conferred by *Nax3* loci. QTL for germination speed under salinity stress (Mano and Takeda 1997) and salinity tolerance score (Fan et al. 2015) were also located in MQTL7H.2. It is probable that these two QTL are also controlled by the locus *HvNax3*.

The *TaMyb1* gene was suggested to be involved in the signalling pathways of waterlogging and salinity stresses (Lee et al. 2007). Overexpression of another Myb transcription factor gene, *JAmyb*, contributed to salinity tolerance by stimulating abiotic stress-tolerant genes, such as osmotic adjustment and ROS scavenging, in rice and *Arabidopsis* (Yokotani et al. 2013). Another Myb transcription factor, SRM1, is able to regulate the ABA biosynthesis and signalling-related genes in *Arabidopsis*

under salinity stress (Wang et al. 2015). A calmodulin-like protein OsMSR2 identified in rice was found to improve drought and salinity tolerance by regulating stress-related genes in ABA-mediated pathways. Expression of OsMSR2 showed improved drought and salinity tolerance in *Arabidopsis* (Xu et al. 2011).

Generally, root K^+ retention ability is strongly associated with salinity tolerance in barley (Chen et al. 2005, 2007). One QTL for root K^+ under salinity stress was identified on chromosome 1H, located on MQTL1H.3 (Nguyen et al. 2013). MQTL1H.3 was formed with four initial QTL for salinity tolerance from three different experiments from the same mapping population (Steptoe/Morex). Different traits were used among these three initial QTL: leaf injury, root K^+ , and chlorophyll content. All of these three salinity-tolerant QTL on MQTL1H.3 were from the seedling growth stages.

MQTL2H.1 was formed with 21 major QTL, including two for waterlogging tolerance, one for combined salinity and waterlogging tolerance, three for drought tolerance, and 15 for salinity tolerance. This region is the main area contributing to salinity tolerance in barley, at both seedling and vegetative growth stages. MQTL2H.1 included QTL for salinity tolerance based on leaf yellowing, number of yellow leaves, leaf dry matter, and proline, Na^+ , K^+ , and Cl^- contents in leaves.

There were 25 candidate genes on MQTL2H.1, including two candidate genes of particular interest. In soybean seedlings, the protein flavonol 4'-sulfotransferase was downregulated when placed under combined salinity and waterlogging conditions (Alam et al. 2011). The cytochrome P450-like gene was upregulated in waterlogged rape seedlings (Lee et al. 2014). These two candidate genes on MQTL2H.1 can be further explored to improve abiotic stress tolerance in barley.

MQTL5H.3 was formed with six QTL for salinity tolerance from three different studies. Glutamate receptor was the candidate gene on MQTL5H.3. Glutamate receptor is one of the factors inducing K^+ efflux under abiotic stresses (Demidchik et al. 2014). Maintaining high cytosolic K^+ level with lower K^+ efflux is crucial for abiotic stress tolerance in barley (Shabala and Pottosin 2014), and a causal link exists between cytosolic K^+ concentration and the ability of a cell to undergo programmed cell death (e.g., senescence) (Demidchik et al. 2014; Shabala et al. 2010).

Waterlogging tolerance in barley

The factor that has impeded the progress of improving waterlogging tolerance in barley is the low heritability of plant yield under waterlogging conditions (Collaku and Harrison 2005; Zhou 2010). Despite the advanced

genotyping technology, accurate phenotyping remains to be a challenge in plant breeding for waterlogging tolerance (Zhou 2011). Agronomic traits were widely used to screen waterlogging tolerance in barley, rice and maize (Qiu et al. 2007; Xu and Mackill 1996; Zhou 2010). Visual symptom of leaf yellowing is the main indicator of waterlogging tolerance in barley breeding programs (Supplementary Table S1). Utilizing physiological traits associated with waterlogging tolerance, such as higher K^+ contents, is required in waterlogging breeding programs (Shabala 2011; Shabala et al. 2014). Even in breeding, only a few physiological traits have been utilized and none of the genes encoding these traits have been cloned (Collins et al. 2008). More convenient and reliable physiological traits should be further explored to screen waterlogging tolerance.

Aerenchyma formation in roots is a reliable and faster method to detect waterlogging tolerance, compared with leaf chlorosis (Zhang et al. 2015, 2016b). Root porosity is the percentage of gas volume per root volume, widely used as an indicator of aerenchyma formation (Colmer 2003). Aerenchyma provides an internal system of gas-filled spaces to improve oxygen supply in waterlogged roots (Evans 2004). MQTL4H.4 was formed with nine QTL for waterlogging tolerance, including one fine-mapped QTL for aerenchyma formation and two QTL for root porosity under waterlogging conditions (Zhang et al. 2016b). The seven QTL were from the population of Yerong/Franklin and two QTL from YYXT/Franklin (Broughton et al. 2015; Li et al. 2008; Zhou 2011; Zhou et al. 2012). MQTL4H.4 was positioned at 98.6 cM with confidence interval of 1.2. MQTL4H.4 and can be used in MAS in breeding to improve waterlogging tolerance in barley.

There were 58 candidate genes on MQTL4H.4 contributing to waterlogging tolerance in barley. Members of the family of NAC domain proteins were increased during leaf senescence in *Arabidopsis* (Buchanan-Wollaston et al. 2005). Also, the NAC domain-containing gene ANAC102 was induced as an important regulator of seed germination under waterlogging conditions (Christianson et al. 2009). In waterlogging-tolerant maize, calcium-dependent lipid-binding (CaLB domain) protein showed increasing abundance (Yu et al. 2015). Catalase was one of the antioxidant enzymes reducing the oxidative stress under waterlogging conditions (Zhang et al. 2015). Cytochrome P450-like gene and glutathione-S-transferase on MQTL4H.4 were upregulated in waterlogged rape seedlings (Lee et al. 2014). However, glutathione-S-transferase gene was downregulated in waterlogged cucumber (Qi et al. 2012). Glycosyltransferase genes, which are involved in cytokinin inactivation, showed decreased expression under waterlogging conditions (Christianson et al. 2010; Qi et al. 2012). *LOB DOMAIN-CONTAINING PROTEIN 41*

(*LBD41*) is likely a repressing factor in submerged *Arabidopsis* (Voesenek et al. 2016). The mitochondrial serine acetyltransferase gene was upregulated in waterlogged rape seedlings (Christianson et al. 2010).

Lysigenous aerenchyma formation candidate genes have been identified in maize (Rajhi et al. 2011). The identified candidate genes, NAC domain transcription factor gene and glutathione-S-transferase gene, were both located to MQTL4H.4 (Rajhi et al. 2011). MQTL4H.4 also included one fine mapped QTL for aerenchyma formation under waterlogging conditions in barley (Zhang et al. 2016b). Further studies are needed to identify genes controlling aerenchyma formation under waterlogging conditions in barley.

Based on chlorophyll fluorescence, there were two major QTL under hypoxia conditions identified on chromosome 6H, explaining 39.8 % of the phenotypic variance (Bertholdsson et al. 2015). This suggests that QTL can also be fine mapped and used for MAS. Meta-analysis projected these two QTL on MQTL6H.4 on the physical map.

MQTL7H.2 also included two QTL for waterlogging tolerance. Ethylene response factors gene is also located in the region of MQTL7H.2 and can be the candidate gene for waterlogging-tolerant QTL on MQTL7H.2 (Xu et al. 2006). Increased transcripts of a Myb transcription factor *TaMyb1* gene were identified in wheat under waterlogging conditions and combined salinity and waterlogging stress (Lee et al. 2007).

Combined drought and salinity stresses

Plants are usually subjected to combined drought and salinity in both natural and agricultural systems (Roy et al. 2011). The direct effect from drought and salinity stresses is the reduction of photosynthesis and cell growth (Chaves et al. 2009). Osmotic adjustment is one of the crucial mechanisms of drought tolerance in crops, enhancing photosynthetic rates through water uptake and cell turgor (Cattivelli et al. 2008). Osmotic adjustment is also the key trait for salinity tolerance in the first phase of salinity stress (Munns and Tester 2008). MQTL2H.4, MQTL5H.1 and MQTL6H.2 were all from one QTL for leaf osmotic potential under drought stress (Teulat et al. 2001, 1998) and QTL for salinity tolerance. These findings suggested the possibility of using osmotic adjustment to improve drought and salinity tolerance simultaneously, as well as improving combined drought and salinity tolerance.

MQTL1H.4 was formed with five QTL for drought tolerance and two QTL for salinity tolerance. One QTL for salinity tolerance, *HvNax4*, which lowers the shoot Na^+ content in barley, is mapped to a 200-kb interval within this region (Rivandi et al. 2011). MQTL1H.4 can be a possible region controlling combined drought and salinity tolerance

in barley. Until now, experiments regarding plant response to combined drought and salinity stresses are limited (Ahmed et al. 2013). QTL for combined drought and salinity tolerance have not been identified yet.

Drought would aggravate the ion toxicity caused by Na^+ and Cl^- , thereby impeding plant growth (Ahmed et al. 2013). Under combined drought and salinity stresses, relatively more tolerant wild barley genotypes were shown to have higher K^+ contents and K^+/Na^+ ratio than the relatively intolerant genotypes (Ahmed et al. 2013). MQTL2H.1 was formed with 16 QTL for salinity tolerance and three QTL for drought tolerance. One QTL for salinity tolerance was based on shoot K^+ contents. MQTL3H.1 was formed with two drought-tolerant QTL from two different populations (Diab et al. 2004; Zhang et al. 2005), and two salinity-tolerant QTL based on the plant Na^+/K^+ ratio and shoot Na^+/K^+ ratio (Nguyen et al. 2013). MQTL2H.1 and MQTL3H.1 both illustrated that maintaining higher K^+ contents helped plants to adapt better to the drought, salinity, and combined drought and salinity stresses.

Combined salinity and waterlogging stresses

Waterlogged soils can be also affected by salinity. Under combined salinity and waterlogging stresses, severe damage occurs in barley (Colmer et al. 2005). Oxygen deprivation in waterlogged soils inhibited the ATP production in plants (Bailey-Serres and Voesenek 2008). Reduced ATP in plants leads to increased Na^+ and decreased K^+ levels in leaves under combined stress (Barrett-Lennard and Shabala 2013; Zeng et al. 2013). Aerenchyma provides an internal system of gas-filled spaces to improve oxygen supply to waterlogged roots, leading to increased energy in plants (Evans 2004). Therefore, it is proposed that aerenchyma formation can be an effective mechanism in plants under combined salinity and waterlogging stresses (Colmer and Flowers 2008). Maintaining lower Na^+ and higher K^+ content in leaves is another key mechanism for improving combined salinity and waterlogging tolerance (Zeng et al. 2013). So far, there is only one experimental study detecting QTL for combined salinity and waterlogging tolerance in barley (Ma et al. 2015).

In our study, MQTL4H.4 was formed with nine QTL for waterlogging tolerance, including one fine-mapped QTL for aerenchyma formation and two QTL for root porosity under waterlogging conditions (Zhang et al. 2016b). However, no salinity-tolerant QTL were located on MQTL4H.4. Another QTL for root porosity under waterlogging conditions was on MQTL6H.2, and without any QTL for salinity tolerance. Also, MQTL1H.4 with fine-mapped salinity-tolerant QTL *HvNax4*, was not formed with QTL for waterlogging tolerance. MQTL7H.2 was

formed with fine-mapped salinity-tolerant QTL *HvNax3* and two QTL for waterlogging tolerance.

QTL for combined salinity and waterlogging tolerance were projected on MQTL1H.1, MQTL1H.3, MQTL2H.1, MQTL5H.6, and MQTL7H.3 (Ma et al. 2015). MQTL2H.1 and MQTL7H.3 both included QTL for salinity tolerance and waterlogging tolerance. MQTL2H.1 and MQTL7H.3 indicated the possibility to improve combined salinity and waterlogging tolerance.

Importance of marker validation and limitations of meta-analysis

A major objective of QTL studies is to find QTL that can be implemented into breeding programs via MAS. The major objective of barley breeding is high yield, combined with greater malting quality and insensitivity to biotic and abiotic stresses. QTL has been successful for introgressing and pyramiding major-effect genes. However, there are still many traits of interest facing great challenges since traits are controlled by many QTL with small effects.

A meta-analysis of QTL associated with abiotic stresses has been performed in barley (Li et al. 2013). Overall, 35 experiments under both control and stress conditions, with 337 major or minor QTL on drought, salinity, waterlogging, low temperature, mineral toxicity or deficiency were included in their study (Li et al. 2013). In our study, a larger number and the latest QTL (632 overall) for drought, salinity, and waterlogging tolerance were investigated. Before meta-analysis, we excluded the parameters under control conditions and the QTL with minor effects. The QTL controlling the yield-related traits under stresses might be the QTL for yield-related traits, rather than the stress-tolerant QTL (Jones 2007). The change of traits under stress conditions should be compared with the traits under control conditions. We only used major QTL for stress tolerance in barley to perform meta-analysis since MAS was successful in crop breeding with one or two major genes controlling stress tolerance. To our knowledge, this is the first meta-analysis that projected all the QTL on the barley physical map, with previous meta-analysis generating the consensus map from the markers common to the different population maps (Khowaja et al. 2009; Li et al. 2013; Zhang et al. 2010). A limited number of common markers from different populations resulted in the inaccurate QTL positions on the consensus map. In our study, we used the position of the QTL flanking markers on the barley physical map to refine the positions of abiotic stress-tolerant QTL from different studies. The positions of MQTL on the barley physical map were also used to search the candidate genes. Identified candidate genes on the physical map provide meaningful information for further MAS and positional cloning.

Meta-analysis is able to integrate the different QTL from different populations into one consensus map. Meta-analysis has also successfully validated the major QTL for abiotic stress tolerance in barley reducing the confidence interval of MQTL. After primary QTL mapping, the mapped QTL was located within a chromosome region so that the confidence interval was up to 50 cM (Supplementary Table S2). Chromosome regions within 10 cM include several hundreds of genes (de Dorlodot et al. 2007; Salvi and Tuberosa 2005). Fine mapping is widely used to refine the QTL less than 1 cM between flanking markers to search for candidate genes and positional cloning of QTL for abiotic stress tolerance (de Dorlodot et al. 2007; Rivandi et al. 2011; Semagn et al. 2013; Shavrukov et al. 2010). QTL cloning has enhanced the exploitation of functions of tolerant genes and the allelic variation in germplasm (Ashraf and Foolad 2013). Meta-analysis provided another method to refine the locations of QTL by lowering the confidence interval (de Dorlodot et al. 2007). The calculated meta-QTL provides breeders with target regions on consensus map for further MAS. However, how effective and accurate is the reduction of confidence interval for searching candidate genes by meta-analysis remains unknown unless that the number of observed QTL is more than five (Veyrieras et al. 2007). Also, recombination might break the linkage between markers and target QTL. Further experiments are, therefore, required to explore the MQTL from meta-analysis before it can become an effective tool in crop breeding.

Conclusions

Both MQTL3H.4 and MQTL6H.2 were target regions controlling drought tolerance in barley. Further experiments are required to fine-map these regions for the effective use of MAS in drought tolerance breeding in barley. Fine-mapped QTL for salinity tolerance, *HvNax4* and *HvNax3*, were validated on MQTL1H.4 and MQTL7H.2, respectively. MQTL1H.4 was formed with fine-mapped salinity-tolerant *HvNax4* and five initial major QTL for drought tolerance. MQTL1H.4 provides breeders with the possibility of improving drought tolerance and salinity tolerance simultaneously and thereby improving barley performance under combined drought and salinity stresses. MQTL7H.2 was formed with a fine-mapped major locus for salinity tolerance, *HvNax3*, two other QTL for salinity tolerance and two QTL for waterlogging tolerance. Genes for ethylene response factors and Myb transcription factor are possible candidate genes for salinity-tolerant locus *HvNax3*. Improved salinity tolerance, waterlogging tolerance, and combined salinity and waterlogging tolerance can be achieved by selecting MQTL7H.2. MQTL2H.1

and MQTL5H.3 were also target regions improving salinity tolerance. MQTL4H.4 is the main region controlling waterlogging tolerance in barley, including fine-mapped QTL for aerenchyma formation under waterlogging conditions. The genes for NAC domain transcription factor and glutathione-S-transferase are candidate genes for aerenchyma formation under waterlogging conditions in barley. Identified MQTL and candidate genes provide breeders with target regions to improve drought, salinity, and waterlogging tolerance in barley.

Author contribution statement XZ conducted the data analysis and wrote the paper; SS, AK, and LS contributed to writing; MZ designed and wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

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