

A new major-effect QTL for waterlogging tolerance in wild barley (*H. spontaneum*)

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Abstract

Key message We report the first study on the unique allele from wild barley that can improve waterlogging tolerance in cultivated barley with a substantially higher contribution to aerenchyma formation.

Abstract Waterlogging is one of the major abiotic stresses that dramatically reduce barley crop yield. Direct selection on waterlogging tolerance in the field is less effective due to its viability to environment. The most effective way of selection is to choose traits that make significant contributions to the overall tolerance and are easy to score. Aerenchyma formation under waterlogging stress is one of the most effective mechanisms to provide adequate oxygen supply and overcome stress-induced hypoxia imposed on plants. In this study, a new allele for aerenchyma formation was identified from a wild barley accession TAM407227 on chromosome 4H. Compared to that identified in cultivated barley, this allele not only produced a greater proportion of aerenchyma but made a greater contribution to the overall

waterlogging tolerance. The QTL explained 76.8% of phenotypic variance in aerenchyma formation with a LOD value of 51.4. Markers co-segregating with the trait were identified and can be effectively used in marker assisted selection.

Introduction

Waterlogging dramatically reduces the yield of crops and the problem is exacerbated by the need to increase crop production to feed an increasing human population. The yield of barley is dramatically reduced under waterlogging stress (de San Celedonio et al. 2014) and the cost caused by waterlogging is more than 60 billion Euro annually in all the crops (www.dartmouth.edu/~floods/Archives/2005sum.htm). In the USA, 16% of soils are affected by waterlogging and waterlogging is also a major problem in India, Pakistan and China (Boyer 1982).

Waterlogging mainly results from heavy rainfall and poor soil drainage (Voesenek et al. 2014; Zhang et al. 2015a). Gas diffusion under waterlogging stress is ten thousand-fold slower than that in air (Armstrong 1979), resulting in a lack of oxygen in waterlogged plants (Voesenek et al. 2016). Aerenchyma formation is one of the mechanisms to overcome waterlogging stress. Aerenchyma is the gas space in cortical tissues that improves oxygen transportation from shoots to waterlogged roots (Colmer 2003). In many wetland species, aerenchyma is well developed even in drained conditions and can be further enhanced under waterlogging stress (Evans 2004). In barley, waterlogging tolerant genotypes are able to form inducible lysigenous aerenchyma under waterlogged conditions (Zhang et al. 2015b). Aerenchyma formation is also the most effective mechanism for waterlogging tolerance in barley.

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The development of waterlogging tolerant varieties is an effective and economical approach to improve crop production under waterlogging conditions. However, the progress of developing waterlogging tolerant barley varieties is slow due to the complexity of waterlogging conditions resulting from different water depth, soil type, duration of waterlogging, nutrient ions and temperature (Setter and Waters 2003; Setter et al. 2009; Zhang et al. 2015b). Waterlogging tolerance is also a complex trait, controlled by many genes including some with small effects (Zhou 2010). Molecular markers have provided plant breeders with a method to improve selection accuracy and accelerate breeding programs (Ribaut and Hoisington 1998).

Many QTL for waterlogging tolerance in barley have been detected (Li et al. 2008; Zhang et al. 2016b; Zhou et al. 2012); however, accurate phenotyping remains the main challenge for improving waterlogging tolerance in breeding. Different traits were used in different studies, such as leaf scoring system, aerenchyma formation and other agronomic traits (Zhang et al. 2016a). The leaf scoring system and aerenchyma formation under waterlogging conditions have been shown to be the most reliable method for screening waterlogging tolerance in barley (Zhang et al. 2016b; Zhou 2011).

Wild relatives of cultivated crop species are often used as the donor parents in breeding because of their tolerance to biotic and abiotic stresses. Wild relatives of maize are able to form constitutive aerenchyma under aerated conditions (Mano and Omori 2013). This favourable trait for waterlogging tolerance has been successfully used to improve waterlogging tolerance of maize (Mano and Omori 2013, 2015). Higher root porosity and lower radial oxygen loss from a wild relative *Hordeum marinum* were successfully transferred into cultivated wheat to improve

waterlogging tolerance (Malik et al. 2011). The wild barley TAM407227 showed significantly higher potential for enhancing waterlogging tolerance in barley (Zhang et al. 2015b). Compared with waterlogging tolerant cultivated barley, TAM407227 performed much better with regard to the tolerance to waterlogging with a greater proportion of aerenchyma formation under waterlogging conditions.

In this study, a new linkage map between cultivated barley Franklin and wild barley TAM407227 was constructed. A number of different QTL for different traits under waterlogging and control conditions were detected from this population. Importantly, a new major allele showed much greater effect on aerenchyma formation and waterlogging tolerance and is an ideal candidate gene for use in barley breeding programs.

Materials and methods

Evaluation of waterlogging tolerant traits

A total of 163 double haploid (DH) lines from the cross between Franklin and wild barley accession TAM407227 (*H. spontaneum*) were used in this study. TAM407227 was introduced from Australian Grains Genebank and showed better waterlogging tolerance and aerenchyma formation than the cultivated tolerant barley Yerong (Fig. 1) (Zhang et al. 2015b). Franklin is a malting barley but susceptible to waterlogging stress. DH lines and parent varieties were grown in a field screening facility (Figure S1). Twenty seeds of each line/variety were sown in a 60-cm row with 15 cm between rows. The controls were sown in well-drained beds. Three replicates were applied for both waterlogging treatment and controls. The trial was sown in late

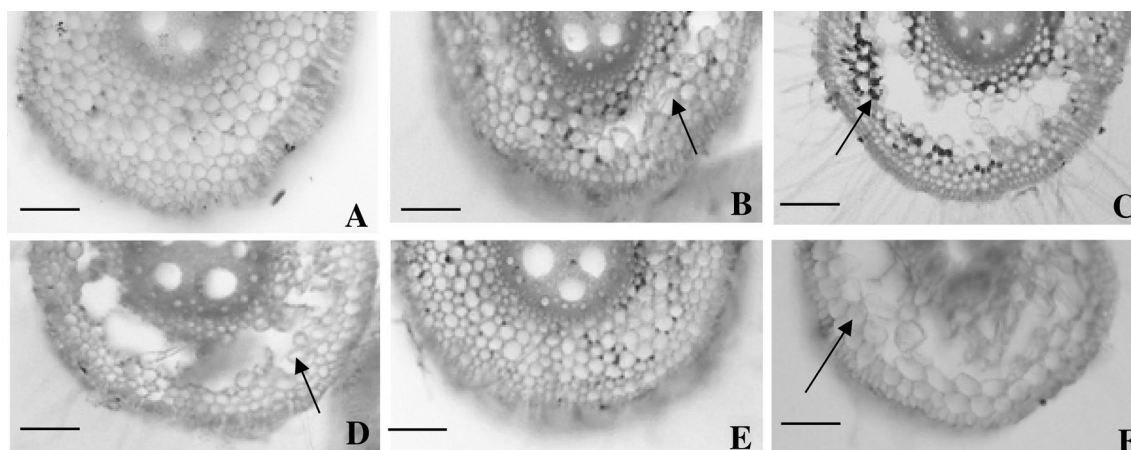


Fig. 1 Light micrographs of cross section of adventitious roots demonstrating aerenchyma formation (arrows) after 7 days of waterlogging treatment in different DH lines: TAMF42 (a), TAMF56 (b), and

TAMF135 (c). Wild barley TAM407227 (d) had larger proportion of aerenchyma than Franklin (e) and Yerong (f). Bar 100 μ m. The scores for aerenchyma formation are a = 0, b = 1, c = 4, d = 4, e = 0, f = 3

April and waterlogging treatment began at the three-leaf stage. Waterlogging tolerance was scored based on plant survival and leaf senescence (0 = plants died from waterlogging, 10 = not affected by waterlogging) (Zhou 2011). At maturity, different traits were measured for each DH line and parent variety under both control and waterlogging conditions. The traits include plant height under control (CPH) and waterlogging (WPH), the number of tillers under control (CT) and waterlogging (WT), and grain yield under control (CY) and waterlogging (WY) conditions. Relative changes (waterlogging/control) in differences are also used as waterlogging tolerance indicators.

Aerenchyma formation

Aerenchyma formation of DH lines and parents were detected based on the method by Zhang et al. (2016b). Plants were grown in 50-L bins, filled with pine bark/loam-based potting mixture with premixed slow release fertiliser. At the three-leaf stage, adventitious roots were sampled in each DH line after 7 days of waterlogging. Approximately 2 cm long root segments were taken from the mature zone and about 6 cm from the root apex. Cross sections were cut by free-hand using razor blades and examined under a bright field light microscope (Olympus BX41). The proportion of aerenchyma was visually scored based on digital images from Olympus DP20: 0 = no aerenchyma, 4 = well-formed aerenchyma (Fig. 1) (Zhang et al. 2016b). The experiment was repeated three times in the glasshouse in January–March, 2016.

Genetic map construction

Diversity Arrays Technology (DArT) and SNP markers were developed and conducted by Triticarte Pty. Ltd. A total of around 15,000 DArT markers and 14,500 SNP markers were shown to be polymorphic between Franklin and TAM407227. JoinMap 4.0 was used in this study to construct the linkage map (Van Ooijen and Kyazma 2009). Before map construction, markers with more than 10%

missing data and duplicate markers (markers located at same/similar positions) were deleted. The relatively lower density map was used to conduct the preliminary QTL analysis. Further high-density mapping was conducted in the region on 4H where the major QTL was located.

QTL analysis

The software package MapQTL 6.0 was used to identify different QTL (Van Ooijen and Kyazma 2009). After interval mapping (IM), the closest marker at each QTL was selected as a cofactor in the multiple QTL model (MQM). A logarithm of the odds (LOD) threshold value of 3.0 was used to detect the presence of a QTL. To determine the effects of waterlogging tolerance on other traits (WPH, WCPH, WT, WCT, WY and WCY), different QTL were re-analysed by using various traits as covariates. The percentage of variance explained by each QTL (R^2) was obtained with restricted MQM mapping. The linkage maps showing the QTL positions were made with MAPCHART (Voorrips 2002). The sequence of flanking SNP markers were used to check the position of QTL on the barley physical map (http://barleygenomeapplications.com/default_2.aspx) and the barley Morex reference genome.

Results

Waterlogging tolerance related traits of DH lines

Franklin and TAM407227 showed significant difference ($P < 0.01$) in plant height, tiller number and grain yield under both waterlogging and control conditions (Table 1) with TAM407227 showing significantly higher aerenchyma formation as well as waterlogging tolerance based on plant survival ($P < 0.01$) (Fig. 1). Compared with Franklin, TAM407227 had significantly higher plant height, more tillers, but lower yield under control conditions ($P < 0.01$). However, in terms of relative change of traits, TAM407227 was less affected by

Table 1 Phenotypic values of traits measured in the DH population, Franklin and TAM407227

	Aerenchyma	Waterlogging tolerance	CPH	WPH	WCPH	CY	WY	WCY	CT	WT	WCT
Average	1.7	4.3	101.0	69.4	0.7	74.7	34.3	0.5	6.9	3.1	0.5
Min	0.0	0.0	70.0	44.0	0.1	20.9	10.5	0.1	5.0	1.0	0.2
Max	4.0	7.6	131.3	102.5	0.6	126.8	82.2	0.9	9.0	4.8	0.8
Franklin	0.3	0.0	95.0	37.5	0.4	80.4	21.2	0.3	5.0	2.0	0.4
TAM407227	3.5	6.0	121.7	115.0	0.9	58.2	38.0	0.7	7.0	3.3	0.5
Heritability	0.7	0.7	0.8	0.8	0.5	0.5	0.6	0.3	0.6	0.3	0.2

CPH control plant height, WPH waterlogging plant height, WCPH waterlogging/control plant height), CY control yield, WY waterlogging yield, WCY waterlogging/control yield, CT control number of tillers, WT waterlogging number of tillers, WCT waterlogging/control number of tillers

Fig. 2 Frequency distribution for waterlogging tolerance (a) and aerenchyma formation (b) in DH lines derived from the cross of Franklin and TAM407227

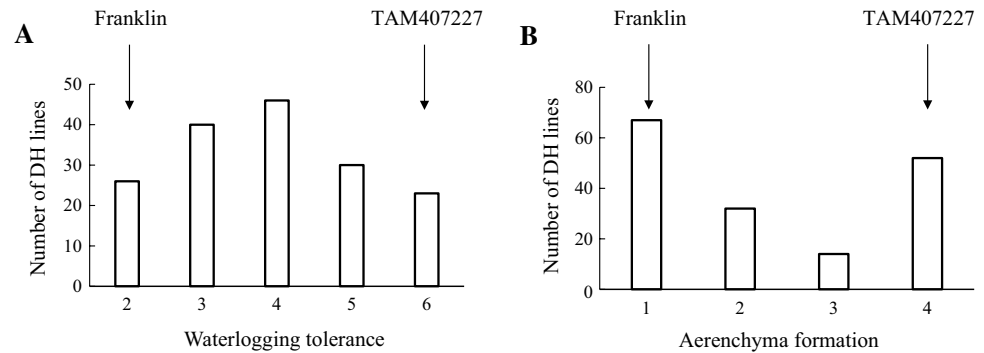


Table 2 Correlation between waterlogging tolerance and all other traits measured in DH population

	Waterlogging tolerance
Aerenchyma formation	0.63*
WPH	0.26*
WCPH	0.52*
WY	0.70*
WCY	0.54*
WT	0.47*
WCT	0.39*

CPH control plant height, WPH waterlogging plant height, WCPH waterlogging/control plant height), CY control yield, WY waterlogging yield, WCY waterlogging/control yield, CT control number of tillers, WT waterlogging number of tillers, WCT waterlogging/control number of tillers

* Significant at the 5% level

waterlogging stress. In DH populations, waterlogging stress reduced plant height, tiller number and yield significantly ($P < 0.01$). DH lines showed a wide segregation in waterlogging tolerance (Table S1). Figure 2 shows frequency distributions of waterlogging tolerance based on plant survival and aerenchyma formation in all DH lines. As shown in Table 2, waterlogging tolerance showed very high positive correlations with both grain yield ($r = 0.70$, $P < 0.01$) and aerenchyma formation ($r = 0.63$, $P < 0.01$) under waterlogging stress. Waterlogging tolerance was also significantly correlated with both plant height and tiller number under waterlogging stress as well as with relative plant height, relative tiller numbers and relative grain yield (Table 2).

Figure 3 shows the correlation between waterlogging tolerance and aerenchyma formation under waterlogging stress. Aerenchyma formation was clearly grouped into two clusters, one with the scores of more than 2 and the other with the scores of less than 2. In general, nearly all the lines with high scores of aerenchyma formation showed good waterlogging tolerance. However, a few lines with low scores of aerenchyma also showed good waterlogging

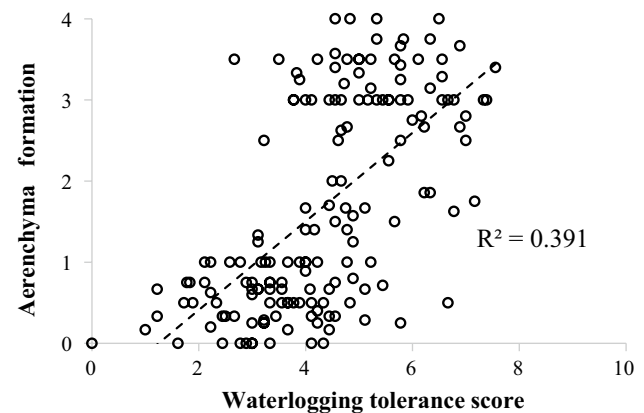


Fig. 3 Correlation between waterlogging tolerance score and aerenchyma formation after 7 days of waterlogging treatment among 165 DH lines

tolerance, indicating the possible existence of some other tolerance mechanisms.

QTL for waterlogging tolerance

A total of 19 QTL were identified for different traits under control and waterlogging conditions (Table 3; Fig. 4). Three QTL for waterlogging tolerance were based on plant survival. One major QTL was on chromosome 4H at 98.8 cM, with a LOD value of 19.2, explaining 34.6% of the phenotypic variance. Other two minor QTL for waterlogging tolerance were found on chromosomes 6H and 7H, determining 6.3 and 5.3% of the phenotypic variance, respectively. Only one major QTL for aerenchyma formation under waterlogging conditions was identified on chromosome 4H, explaining 76.8% of the phenotypic variation with a LOD value of 51.4. This QTL was located at the same position of a QTL for waterlogging tolerance. A high-density map on chromosome 4H was further constructed (Fig. 5) and the QTL was mapped to the region between 97.5 and 99.10 cM on the published consensus map (<http://barleygenomeapplications.com>) with around 20 markers

Table 3 QTL for waterlogging tolerant related traits under different conditions

Traits	QTL	Chromosome	LOD score	Percent phenotypic variation explained (%)	Allele effects	Physical map position	2-LOD interval	Nearest marker ^a
Aerenchyma formation	QTL-AER	4H	51.4	76.8	1.12	98.8	99.2–99.3	3255355S4
Waterlogging tolerance	QTL-WL-4H	4H	19.2	34.6	0.92	98.8	96.1–104.2	3254813S4
	QTL-WL-6H	6H	4.4	6.3	0.47	24.5	16.6–32.8	3260661S6
	QTL-WL-7H	7H	3.7	5.3	0.50	70.7	55.5–74.5	3256877S7
Control plant height	QTL-CPH-3H	3H	32.9	60.8	11.16	108.4	103.0–105.7	3255136S3
Waterlogging plant height	QTL-WPH-3H	3H	13.0	31.0	6.00	117.6	102.1–113.2	4011783S3
	QTL-WPH-4H	4H	3.9	7.2	2.94	103.7	102.2–119.5	6437034D
Waterlogging/control plant height	QTL-WCPH-3H	3H	3.6	7.6	0.02	83.6	63.9–109.3	3274569D
	QTL-WCPH-4H	4H	9.4	21.8	−0.04	98.8	94.4–96.1	7934461D4
Control yield	QTL-CY-3H	3H	9.8	20.3	−11.57	109.8	98.4–110.6	3254867S3
	QTL-CY-5H	5H	3.0	5.1	−6.40	114.9	78.4–110.1	3255097S5
	QTL-CY-7H	7H	5.7	11.1	−8.15	48.7	31.1–65.2	6277000S7
Waterlogging yield	QTL-WY-3H	3H	3.1	7.0	−4.00	105.9	86.9–110.6	3264662S3
	QTL-WY-4H	4H	9.2	25.0	7.05	98.8	96.1–102.4	3255355S4
Waterlogging/control yield	QTL-WCY-4H	4H	5.2	12.7	−0.07	98.8	86.9–100.3	3254813S4
	QTL-WCY-7H	7H	4.0	9.7	−0.07	126.3	116.1–124.1	3258238S7
Waterlogging tillers	QTL-WT-4H	4H	4.2	11.3	0.20	100.0	102.0–104.2	7244354D4
Waterlogging/control tillers	QTL-WT-4H	4H	5.0	12.7	−0.05	98.8	84.3–104.3	3254813S4
	QTL-WCT-5H	5H	4.1	10.3	0.05	98.1	73.6–92.0	3257423S5

^a Detailed information of the nearest markers are shown in Table S2

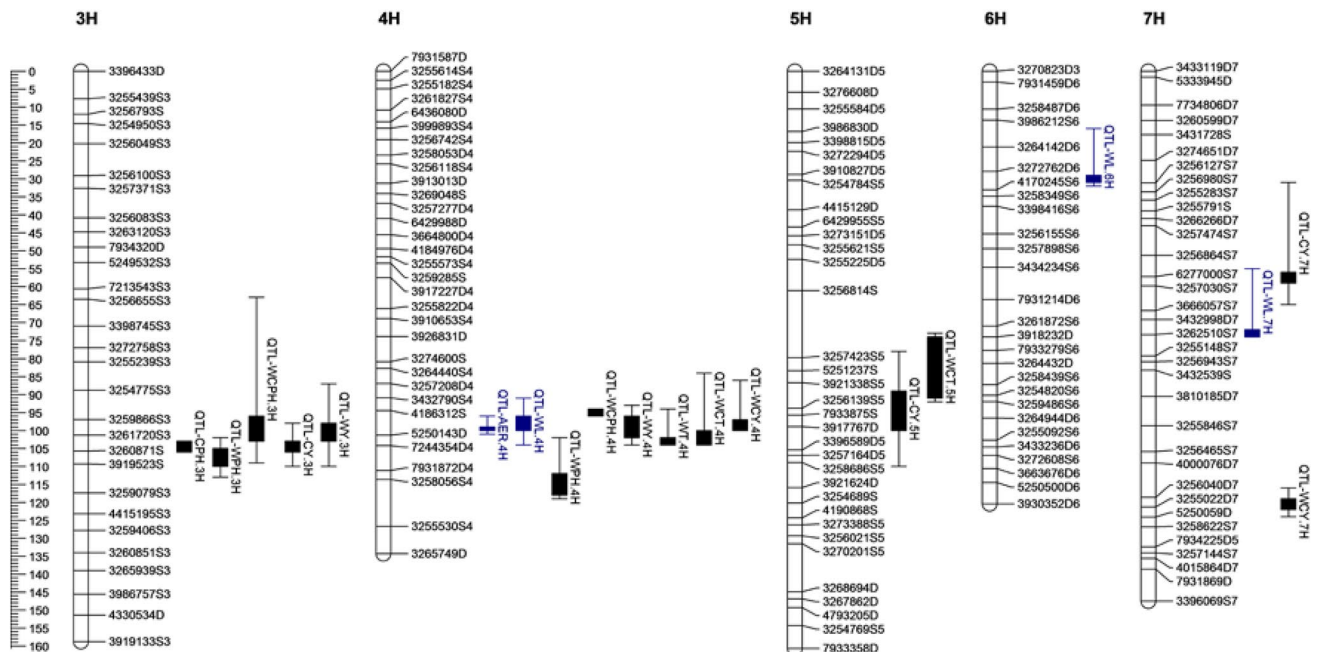


Fig. 4 Genetic linkage map of Franklin/TAM407227 and QTL identified for different traits in the population. Only selected markers are shown

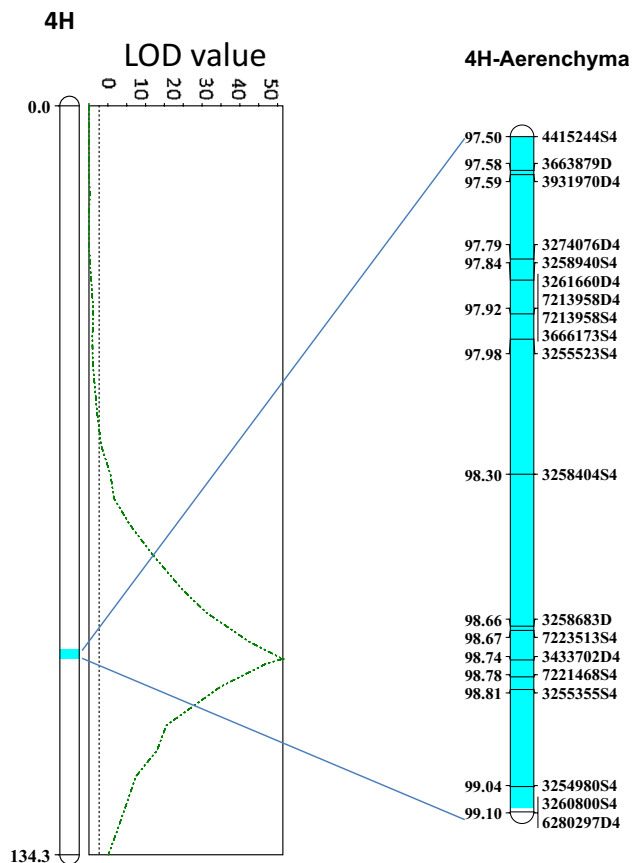


Fig. 5 QTL for aerenchyma formation (QTL-AER) after 7 days of waterlogging treatment and QTL for waterlogging tolerance (QTL-WL-4H) on a high density map of chromosome 4H. All the markers are projected on the barley physical map position

co-segregating with the traits, which can be used in breeding programs and help to identify candidate genes.

Six other QTL for WPH, WCPH, WY, WCY, WT and WCT were also identified on chromosome 4H at the same position as those for waterlogging tolerance and aerenchyma formation. Another cluster of QTL was found on chromosome 3H at 110 cM based on the barley physical map, controlling CPH, WPH, WCPH, CY and WY (Fig. 4).

The contribution of aerenchyma formation to overall waterlogging tolerance

Figure 4 shows that the QTL for aerenchyma formation under waterlogging stress is located on a similar position to the major QTL for waterlogging tolerance, WPH, WCPH, WT, WCT, WY and WCY. To confirm their relationships between aerenchyma formation and other waterlogging tolerance-related traits, the scores for aerenchyma formation was used as a covariate while analysing QTL for other traits. Aerenchyma formation under waterlogging made a significant contribution to waterlogging tolerance, as the

major QTL on 4H for traits that are used as the indices for waterlogging tolerance became insignificant (Table 4; Fig. 6). In contrast, the QTL for these traits on other chromosomes were not affected by using aerenchyma formation as a covariate. Waterlogging scores based on plant healthiness (combined leaf chlorosis and plant survival) also showed to be a good indicator of grain yield under waterlogging condition. When using the tolerance scores as covariates, QTL for grain yield, plant height and tiller numbers under waterlogging on 4H all became insignificant (Table 4). Similarly, waterlogging scores contributed less to QTL on other chromosomes for these traits.

Discussion

A new allele for aerenchyma formation from wild barley can be more effective in breeding for waterlogging tolerance in barley

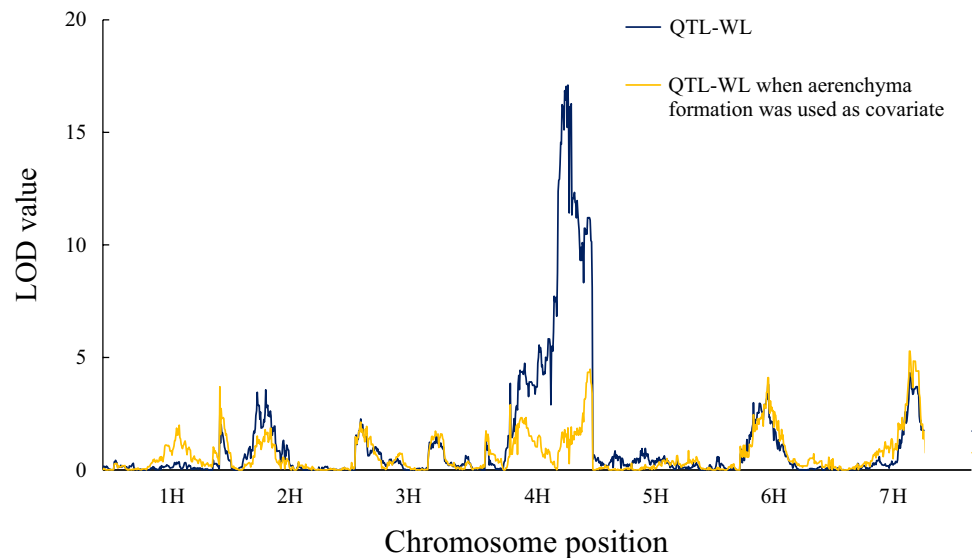
A total of three QTL for waterlogging tolerance were identified from this population with all three tolerance alleles being from wild barley. The major allele controlling waterlogging tolerance from wild barley was located on chromosome 4H at 98.8 cM on the barley physical map. This QTL was located on the same position as the QTL identified from several other populations (Zhou 2011; Zhou et al. 2012). This QTL identified from the current population explained much better phenotypic variation (34.6%) than those from other populations, including Yerong/Franklin 23.9% (Zhou 2011) and YYXT/Franklin 7.0% (Zhou et al. 2012). The minor QTL on 6H was not identified in previous reports. The minor QTL on 7H is at a similar position to that identified for leave chlorosis in the Yerong/Franklin and TX9425/Franklin populations (Li et al. 2008). However, this QTL from the Yerong/Franklin population became nonsignificant after further long-term waterlogging treatment (Zhou 2011). A QTL for waterlogging tolerance was reported at 125 cM on 7H from a Chinese landrace (Xu et al. 2012), which is far away from the QTL identified in this study (71 cM on chromosome 7H).

A high-density map of chromosome 4H provided enough markers for further marker assisted selection to improve waterlogging tolerance in barley. The location of QTL on chromosome 4H is the main region controlling waterlogging tolerance in barley and 58 candidate genes have been identified (http://barleygenomeapplications.com/default_2.aspx). Among all the identified 58 candidate genes, the NAC domain transcription factor and glutathione-S-transferase genes were also candidate genes identified for lysigenous aerenchyma formation in maize (Rajhi et al. 2011; Zhang et al. 2016a). The sequence of nearest marker (3255355S4) for aerenchyma formation

Table 4 Changes in the significance and percentage variation determined by the QTL after aerenchyma or waterlogging tolerance scores were used as covariates

QTL	Covariate	Linkage group	Nearest marker	Position	LOD	R^2
QTL-WL-4H	Aerenchyma formation				ns	ns
QTL-WL-7H	Aerenchyma formation	7H	3434116S7	63.9	5.3	8.5
QTL-WL-6H	Aerenchyma formation	6H	3260661S6	31.1	4.1	6.7
QTL-WPH-3H	Aerenchyma formation	3H	3917582D	111.8	14.4	31.7
QTL-WPH-4H	Aerenchyma formation				ns	ns
QTL-WCPH-3H	Aerenchyma formation				ns	ns
QTL-WCPH-4H	Aerenchyma formation				ns	ns
QTL-WY-3H	Aerenchyma formation					ns
QTL-WY-4H	Aerenchyma formation				ns	ns
QTL-WCY-4H	Aerenchyma formation				ns	ns
QTL-WCY-7H	Aerenchyma formation	7H	3258238S7	121.9	3.9	9.7
QTL-WT-4H	Aerenchyma formation				ns	ns
QTL-WCT-4H	Aerenchyma formation				ns	ns
QTL-WCT-5H	Aerenchyma formation	5H	3257423S5	79.6	3.8	9.2
QTL-WPH-3H	Waterlogging tolerance	3H	3254867S3	104.2	17.7	36.9
QTL-WPH-4H	Waterlogging tolerance				ns	ns
QTL-WCPH-3H	Waterlogging tolerance				ns	ns
QTL-WCPH-4H	Waterlogging tolerance				ns	ns
QTL-WY-3H	Waterlogging tolerance				ns	ns
QTL-WY-4H	Waterlogging tolerance				ns	ns
QTL-WCY-4H	Waterlogging tolerance				ns	ns
QTL-WCY-7H	Waterlogging tolerance	7H	3258238S7	121.9	3.9	8.2
QTL-WT-4H	Waterlogging tolerance				ns	ns
QTL-WCT-4H	Waterlogging tolerance				ns	ns
QTL-WCT-5H	Waterlogging tolerance				ns	ns

ns not significant

Fig. 6 QTL for waterlogging tolerance identified from the DH population of Franklin and TAM407227 before (black line) and after (yellow line) aerenchyma formation is used as a covariate (color figure online)

on chromosome 4H was used to search for barley genome sequences using the Morex reference genome. The marker 3255355S4 was blasted to Morex_contig_1572026. The Morex_contig_1572026 sequence was then searched on

NCBI blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Results suggested the gene MIR171_1 (Genbank ID: JX195502.1) is a possible candidate gene for aerenchyma formation and waterlogging tolerance in barley. MIR171

was involved in the regulation of metabolic adaptations to the waterlogging conditions in maize (Zhang et al. 2008). Further experiments will be required to confirm the genes for aerenchyma formation and waterlogging tolerance in barley.

The region on chromosome 4H controlling aerenchyma formation identified from the population of Franklin/TAM407227 is at the same position as that from both Yerong/Franklin and YYXT/Franklin populations (Zhou 2011; Zhou et al. 2012). The allele originating from wild barley TAM407227 not only exhibited a higher percentage of phenotypic variation [76.8% in TAM407227 vs 44% in Yerong (Zhang et al. 2016b) and 39% in YYXT (Broughton et al. 2015)], but also made a much greater contribution to waterlogging tolerance than the allele from cultivated barley varieties. Of the total percentage of phenotypic variation determined by three significant QTL (46.2%, Table 3), the allele on 4H contributed 34.6% (75% of total contribution). In contrast, the allele from Yerong contributed 23.9% to the overall waterlogging tolerance and 42% of all the contributions by four QTL (Zhou 2011). The allele from YYXT contributed only 5.2% to the overall waterlogging tolerance, which is only 11% of all the contributions by four QTL (Zhou et al. 2012). Together this further confirms that aerenchyma formation is one of the most effective mechanisms for waterlogging tolerance (Armstrong 1979). However, some of the waterlogging tolerant DH lines did not form large amounts of aerenchyma, indicating the possible existence of other mechanisms involved in waterlogging tolerance, such as development of adventitious roots (Mano et al. 2005), formation of the barrier to radial oxygen loss (Colmer and Voeselek 2009), or increased tolerance to elemental or metabolite toxicity (Shabala et al. 2014). These mechanisms play more important roles in waterlogging tolerance in cultivated barley, while in wild barley TAM407227 the allele controlling aerenchyma formation was shown to be most effective in improving waterlogging tolerance and thus can be effectively used in future breeding programs. Similar results have been reported in other crops. Wild relatives of maize are able to form aerenchyma without waterlogging stress (Mano et al. 2006). Wild relatives of wheat showed higher root porosity and lower radial oxygen loss under waterlogging conditions (Malik et al. 2009). These favourable traits of waterlogging tolerance in wild relatives of maize and wheat have been successfully transferred to cultivated maize and wheat (Malik et al. 2011; Mano and Omori 2013).

Selecting for waterlogging tolerance

Higher yield under waterlogging stress is always an important target in plant breeding in high rainfall areas. However, the heritability of yield under waterlogging

conditions is relatively low (Collaku and Harrison 2005) and, therefore, difficult to be directly used in breeding programs. The genes contributing to high yield under abiotic stresses might be the same as those controlling higher yield under well-drained conditions with nothing to do with stress tolerance (Jones 2007). Thus, relative changes in different parameters (stressed/control) are always used as indicators for stress tolerance. QTL for yield and other useful agronomic traits (plant height and tiller number) under control and waterlogging conditions were also identified. Two QTL for WPH and WCPH were identified at the same positions on chromosomes 3H and 4H. A QTL for CPH was identified at the same position of a QTL for WPH and WCPH on chromosome 3H. When aerenchyma formation and waterlogging tolerance were used as covariates, QTL for WCPH became insignificant. However, the QTL for WPH on chromosome 3H was not affected by aerenchyma formation and waterlogging tolerance. This suggested the importance of using relative changes in different parameters (stressed/control) as indicators for waterlogging tolerance.

In this work the correlation coefficient between waterlogging tolerance and WY was the highest among all the traits ($r = 0.7$, $P < 0.01$) and the QTL for WY and WCY on chromosome 4H is the same position of QTL for waterlogging tolerance based on plant survival. The QTL for WY and WCY on chromosome 4H could not be detected when aerenchyma formation and waterlogging tolerance were used as covariates. This further confirmed the effectiveness of using aerenchyma formation and waterlogging tolerance as the selection criteria to improve the yield under waterlogging conditions. The QTL for waterlogging tolerance on chromosome 7H is in the same position as the QTL for yield under hypoxia in barley (Bertholdsson et al. 2015; Zhang et al. 2016a).

Plant architecture traits, such as plant height and tillers, are reported to be possible target traits to improve yield (Khush 2001). A QTL on chromosome 3H at around 110 cM was identified for CPH, WPH, WCPH, CY and WY. This region also controls drought tolerant QTL, such as plant height and peduncle length under drought stress (Korff et al. 2008; Zhang et al. 2016a). QTL analysis for CY using CPH as a covariate suggested that plant height did not have a significant effect on yield. This position on chromosome 3H provided a useful resource for breeders to improve yield of barley.

In conclusion, a new allele for aerenchyma formation under waterlogging stress was identified from a wild barley accession. This allele showed a much better ability in forming aerenchyma and was the major contributor to waterlogging tolerance. A high-density linkage map helped identify several co-segregating markers that can be directly used in breeding programs.

Author contribution statement XZ conducted the experiment and wrote the paper; YF, helped genotyping; SS, AK, LS contributed to writing; PJ, HH helped phenotyping; MZ designed the experiments 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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