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Genome-wide association study on seed dormancy in barley

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Abstract

Seed dormancy is an important trait associated with pre-sprouting and malting quality in barley. Genome-wide association studies (GWASs) have been used to detect quantitative trait loci (QTLs) underlying complex traits in major crops. In the present study, we collected 295 barley (*Hordeum vulgare* L.) accessions from Australia, Europe, Canada and China. A total of 25,179 single nucleotide polymorphism (SNP)/diversity arrays technology sequence markers were used for population structure, linkage disequilibrium and GWAS analysis. Candidate genes within QTL regions were investigated, and their expression levels were analysed using RNAseq data. Five QTLs for seed dormancy were identified. One QTL was mapped on chromosome 1H, and one QTL was mapped on chromosome 4H, while three QTLs were located on chromosome 5H. This is the first report of a QTL on the short arm of chromosome 5H in barley. Molecular markers linked to the QTL can be used for marker-assisted selection in barley breeding programmes.

Introduction

Freshly matured seeds have evolved to maintain dormancy until appropriate conditions are available for germination. Seed dormancy is a pivotal adaptive trait of many species that enables seeds to delay germination to avoid abiotic stress (Finkelstein et al., 2008). Many domesticated species have been selected for fast germination to establish rapid and uniform seedlings, which has resulted in a lack of or little dormancy. This could be a serious issue for crop species as it can cause pre-harvest sprouting and reduce seed longevity (Clerkx et al., 2003). There are two main types of dormancy: embryo dormancy and coat-enhanced dormancy (Bewley and Black, 1994). For embryo dormancy, the embryo is dormant and will not germinate, even if it is removed from the surrounding seed tissues (Kermode, 2005). This type of dormancy is very common in grasses, woody and Rosaceae species (Kermode, 2005). For coat-enhanced dormancy, isolated embryos can germinate. Inhibition factors include the surrounding seed tissues, such as endosperm, megagametophyte, testa, pericarp and perisperm (Kermode, 2005). In some species, water-impermeable seed coats prevent seed germination. 'Hard seededness' is present in the Leguminoses crop (Baskin and Baskin, 2000). Most cereal crops, conifers and some dicots exhibit this type of dormancy (Bewley and Black, 1994). To break dormancy, dormant seeds are often exposed to periods of warm, dry conditions, chilling, moisture or even smoke (Egerton-Warburton, 1998).

Seed dormancy varies in different species and even cultivars within the same species. The mechanisms underlying dormancy breaking remain comprehensive. Seed dormancy is related to changes in enzyme activity and hormone concentration, which is regulated by gene expressions in dry seed (Leubner-Metzger, 2005; Cadman et al., 2006; Finch-Savage et al., 2007). For example, a set of 442 genes had higher expression in all dormant seed states than the after-ripened states in Arabidopsis accession Cvi (Cadman et al., 2006).

The regulation of seed dormancy by hormones has been widely studied in plants. It is well known that abscisic acid (ABA) plays an important role in inducing dormancy during seed maturation (Finkelstein et al., 2002; Kermode, 2005; Finch-Savage and Leubner-Metzger, 2006). During early seed development, the accumulation of ABA in the embryo and surrounding tissues maintains embryos in a developmental stage (Kermode, 1990). Levels of ABA increase during the early stage of seed development, usually peak at the mid-maturation stage and then decrease during the late development stage (Taylor et al., 2005). The expression level of genes associated with the ABA biosynthesis enzyme zeaxanthin epoxidase correlates with the changes in endogenous ABA levels (Seo and Koshiba, 2002). ABA is considered to be synthesized in the embryo (Frey et al., 2004). However, the surrounding seed tissues play an important role in maintaining ABA biosynthesis in developing embryos (Frey et al.,

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2004). It is likely that some genes are involved in the regulation mechanism, thereby increasing the complexity of seed dormancy. It is believed that dormancy termination is attributed to changes in ABA biosynthesis, turnover and sensitivity (Frey et al., 2004).

Compared to ABA, gibberellins (GAs) act antagonistically to regulate dormancy breakage and germination (Yamauchi et al., 2004). The ABA:GA ratio controls seed dormancy and germination (Finch-Savage and Leubner-Metzger, 2006). In tobacco, the carotenoid and ABA biosynthesis inhibitor fluridone was used with GAs to effectively remove seed dormancy (Grappin et al., 2000). In yellow cedar, the application of GAs and fluridone also broke dormancy without any additional treatment (Schmitz et al., 2001). Using traditional methods, these seeds need 1 month of warm, moist conditions followed by 2 months of chilling to break dormancy (Ren and Kermode, 2000). However, a fluridone-only treatment is less effective in breaking dormancy, indicating that a decline in ABA content is insufficient and other changes may be necessary (Ren and Kermode, 2000).

Strong dormancy influences seed germination during sowing. However, the absence of seed dormancy can result in the rapid germination of mature seeds or pre-harvest sprouting in crops, which can cause substantial yield and quality losses (Simsek et al., 2014).

Dormancy mechanisms are complex in barley. Like in most cereals, dormancy in barley grain is imposed by the seed coat, but the coat exhibits different characteristics. For instance, the hull, palea and lemma represent a further constraint for germination, in addition to those already imposed by the pericarp and the endosperm (Benech-Arnold et al., 1999, 2000). Dormancy starts very early in barley (Benech-Arnold, 2001). Embryos can germinate if isolated from seed and incubated in water for 14–20 days following pollination (Benech-Arnold et al., 1999). Barley grains rarely release dormancy before seeds reach physiological maturity. Genotypic variability exists in the dormancy release pattern in barley germplasm. Some varieties release dormancy abruptly, some are gradual and last several weeks, while others remain dormant for several months (Benech-Arnold, 2001). For malting and brewing companies, the malting process requires a high germination rate. A low level of dormancy in malting barley is a desirable factor as the seeds can be malted immediately post-harvest, which will reduce storage costs (Benech-Arnold, 2001). However, short exposure to rainwater before harvest could lead to pre-harvest sprouting in grains with low dormancy levels (Benech-Arnold, 2001), preventing them from being used for malting. Therefore, barley breeders aim to breed barley varieties with appropriate dormancy levels for crop production and malting purposes.

Two major genes regulating grain dormancy have been reported in barley (Nakamura et al., 2016; Sato et al., 2016). A mitogen-activated protein kinase kinase 3 was mapped on barley chromosome 5HL, and the N260T substitution in the gene region was associated with seed dormancy (Nakamura et al., 2016). Another gene *Qsd1* was also mapped on chromosome 5H. The gene encoding alanine aminotransferase regulates dormancy and is expressed specifically in the embryo (Sato et al., 2016). Besides these two genes, from the wild barley 'H602', two more quantitative trait loci (QTLs) were identified at the end of chromosome 4H and in the middle of chromosome 5H (Nakamura et al., 2017). As the mechanisms regulating seed dormancy are very complex in barley, including coat dormancy and embryos, many genes could be involved in the dormancy regulatory pathway. In the present study, we used genome-wide association mapping analysis to map the QTL for seed dormancy in barley. These QTLs will be used for future studies on seed dormancy.

Materials and methods

Genotyping

Seedlings from 295 barley accessions were harvested and frozen below −80°C. Genomic DNA was extracted from each sample using the extraction method, according to Stewart and Via (1993). The diversity arrays technology sequence (DArTseq) method was used to genotype these barley accessions. DArTseq sequences the representations after complexity reduction using next-generation sequencing. The advantage of DArTseq over DArT is that it can generate tens of thousands of high-density markers (http://www.diversityarrays.com). Sequencing the genomic DNA will identify additional SNP markers among these barley accessions by aligning the reads to the barley Morex reference genome (http://webblast.ipk-gatersleben.de/barley_ibsc/). These marker-tagged sequences were conducted in a blast search against the barley cv 'Morex' genome and anchored to the physical positions. The marker names include the chromosome numbers and physical positions.

Phenotyping

The barley accessions were grown at three locations (Geraldton, Katanning and Esperance, see Supplementary Fig. S1) across Western Australia. They were grown for 2 years (2013 and 2014) in Geraldton and Katanning, and 1 year (2014) in Esperance. Barley's heads were harvested from the fields after maturation. The maturation time varied because the flowering time differed in the diversity accessions, so the seeds were dried for 2 months to maintain the same water content before the germination test. The seeds were visually tested without pre-harvest sprouting before they were used for germination. We placed 100 seeds of each accession in Petri dishes containing Whatman No.1 filter paper and 4 ml of water. The seeds were incubated at 20°C for 72 h. Seeds with exposed white roots were considered germinated. The number of germinated and non-germinated seeds was counted and germination rates were calculated.

Structure analysis

STRUCTURE (version 2.3) (Hubisz et al., 2009) was used to analyse the population structure. Genotypic data of the lines were imported into STRUCTURE software. The length of the burn-in period was set to 5,000, and the number of Markov Chain Monte Carlo (MCMC) reps after burn-in was 5,000. The admixture model was used to conduct a simulation running K (the number of populations) from 2 to 10. K was estimated according to the method described by Evanno et al. (2005).

TASSEL (v5.0) was used to measure the linkage disequilibrium (LD) between every two linear DArTseq markers. Squared allele frequency correlation (r^2 value) measures the correlation between a pair of markers.

$$r^2 = (p(\mathrm{AB}) \times p(\mathrm{ab}) - p(\mathrm{Ab}) \times p(\mathrm{aB}))/(p(\mathrm{A}) \times p(\mathrm{B}) \times p(\mathrm{a}) \times p(\mathrm{b})),$$

where p is the relevant allele and genotype frequencies (Fabbri et al., 2020). Genetic distances and r^2 values were selected to

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draw a LD decay figure. The 95th percentile of the distribution of r^2 was estimated as the critical threshold below which the LD could be considered as being caused by genetic linkage. The LD decay distance in the whole genome was calculated as the genetic distance when $r^2 = 0.2$ using the fitted equation. The intersection point between the r^2 threshold and the LD curve determined the LD decay value.

Association mapping

TASSEL (v5.0) was used for association mapping studies on seed dormancy in barley. Genotype, genetic map, population structure and traits were formatted and then imported into TASSEL5.0. Kinship was estimated using genetic markers. Association mapping for seed germination was analysed using the mixed linear model, which was calculated as follows: trait of interest = population structure + marker effect + individual + residual. To assess marker–trait associations, the significant threshold of *P*-value was calculated based on the false discovery rate (Benjamini and Hochberg, 1995) at a significance level of $P = 0.05$ after Bonferroni multiple test correction. The *P*-value was converted to $-\log10(p)$.

RNA extraction and sequencing

Embryos were collected from five barley cultivars (AC Metcalfe, Baudin, Stirling, Harrington and Bass) 24 and 48 h after germination and stored in liquid nitrogen for total RNA extraction. AC Metcalfe and Harrington are two-rowed Canadian malting barley, while the other three varieties are two-rowed Australian malting barley. Total RNA was isolated using the RNA purification kit with DNase I treatment in a column for 15 min (RNeasy Mini Kit, Qiagen). The RNA concentration was measured using NanoDrop 2000 (Thermo Scientific), and RNA integrity was checked on 1% agarose gel treated with DEPC (diethy pyrocarbonate). Transcript profiling using RNASeq was performed at BGI (Shenzhen, China), which also analysed the transcripts.

Results

Genotyping was carried out with SNP and DArTseq markers. A call rate of 95% was applied to filter the genotypes. These markers were conducted in a blast search against the 'Morex' genome. A total of 34,999 markers (SNP and DArTseq) were anchored to barley 1H to 7H chromosomes. After the markers with <2% allele frequency were removed, 25,179 markers were selected for structure, LD and genome-wide association analysis.

Population structure

Population structure was analysed in 295 barley accessions with STRUCTURE software to calculate *k* values. The cluster parameter *k* was set from 2 to 14 (Fig. 1). To determine the number of clusters suitable for association mapping analysis, the parameter Δk was used. When $k = 3$, Δk reached a maximum value of 21 (Fig. 1). According to the method of Evanno et al. (2005), the appropriate number of clusters was determined as three.

LD decay

At first, the LD decay (r^2) of the seven barley chromosomes was calculated separately. After which, all of the r^2 values from the barley genome were combined to calculate the average LD decay. The average LD decay in these 295 barley lines was 3.5 cM ($r^2 = 0.2$) (Fig. 2). A total of 25,179 markers were distributed across the barley genome and used for genome-wide association mapping analysis.

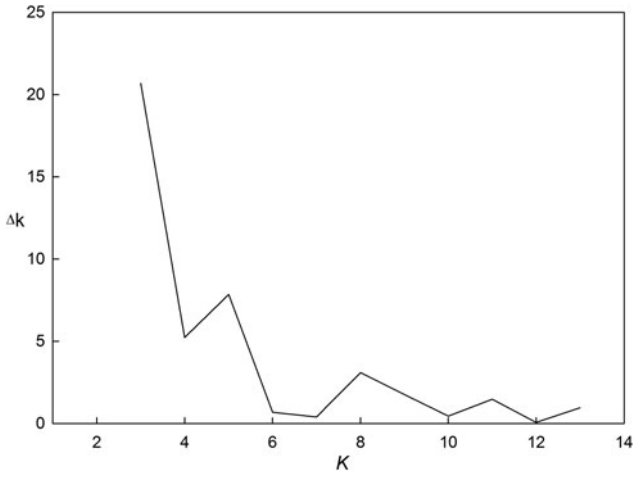


Figure 1. Estimation of the most probable number of clusters (*k*), ranging from 2 to 14.

Phenotyping variation

The average germination rate across the three sites ranged from 50.5 to 94.7% in 2013 and 55.5 to 83.7% in 2014. The distributions of seed dormancy in 2013 and 2014 (Fig. 3) show that the germination rates of most of the barley lines range from 50 to 100%. About 60% of the population varieties exhibited the germination rate of 70–100% (Fig. 3).

QTLs associated with seed dormancy

Five QTLs for seed germination were identified from the five trials. One QTL (*QTL-1HS*) was mapped on chromosomes 1H, one QTL and 4H (*QTL-4HL*), while three QTLs (*QTL-5HS*, *QTL-5HC* and *QTL-5HL*) were located on chromosome 5H. The QTLs were named by QTLs followed by their chromosome arms.

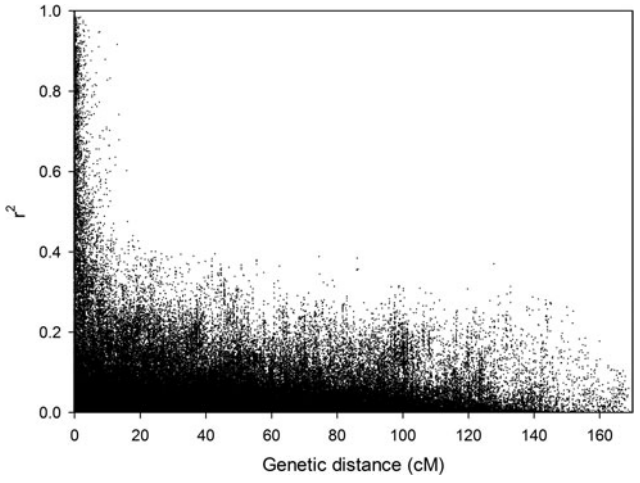


Figure 2. Decay of LD of the whole barley genome.

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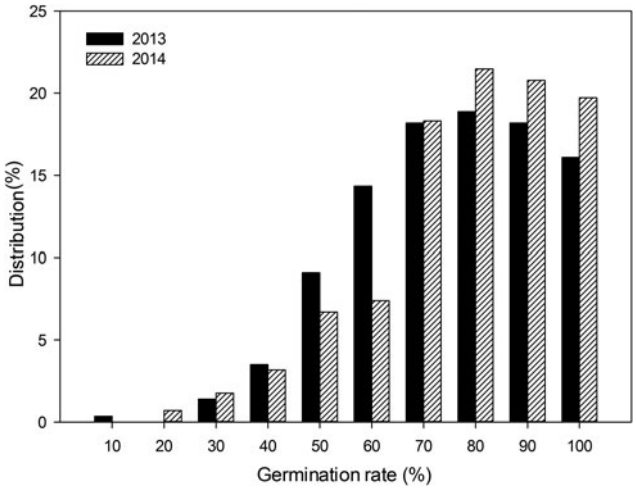


Figure 3. Distribution of germination rates from the 2013 and 2014 trials.

In the 2013 trials, the same QTL (*QTL-5HL*) was detected in the KAT (Katanning) and GER (Geraldton) trials, which was located on the long arm of chromosome 5H. The physical position ranged from 666.42 to 669.25 Mb (Table 1). The other two QTLs detected in the GER trial were located on the long arm of chromosome 4H (*QTL-4HL*) and the short arm of chromosome 5H (*QTL-5HS*) (Table 1). The physical positions for the two QTLs were 599.8 Mb (4H) and 205.5 Mb (5H), respectively. When the average value of the two trials was used for the analysis, four QTLs were identified: three (*QTL-4HL*, *QTL-5HS* and *QTL-5HL*) in the 2013 KAT and GER trials (Table 1) and one QTL (*QTL-5HC*) on the centromeric region of chromosome 5H from the average traits.

From the three trials in 2014 (GER, KAT and ESP), four QTLs were identified for germination. Of these, three (*QTL-4HL*, *QTL-5HC* and *QTL-5HL*) were reported in the 2013 trials (Table 1). One (*QTL-1HS*), which was located on chromosome 1HS, was only reported in the 2014 trial. In the 2014 ESP trial, one QTL was mapped on the centromeric region of chromosome 5H (Table 1). In the 2014 KAT trial, three QTLs were detected from chromosomes 1HS, 4HL and 5HL (Table 1). In the 2014 GER trial, one QTL was detected on chromosome 1HS (Table 1), which was also detected in the 2014 KAT trial. When the three trials were combined for the 2014 average, three of the four QTLs were detected, with 4H absent (Table 1). In 2013 and 2014, both *QTL-5HC* and *QTL-5HL* were identified. The other three QTLs, *QTL-1HS*, *QTL-4HL* and *QTL-5HS*, were detected either in 2013 or the 2014 average.

Investigation of the seed dormancy genes on chromosome 5H

Two genes for seed dormancy have been identified from previous studies on chromosome 5H (Nakamura et al., 2016; Sato et al., 2016), so we want to see whether these genes overlap with the QTL mapped in this study. The two major QTLs, *QTL-5HC* and *QTL-5HL*, were located in the 470.6–492.89 and 659.7–669.25 Mb regions on chromosome 5H, respectively. The other three minor QTLs were located at 31.63 Mb on 1H, 594.5–599.8 Mb on 4H and 205.5 Mb on 5H.

The *QTL-5HC* region has 185 annotated genes (Supplementary Table S1) spanning 22 Mb, while the *QTL-5HL* region has 239 annotated genes (Supplementary Table S2) flanking the 10 Mb region. The *QTL-5HC* region contains the gene HORVU5HrG062990.1 (*HvAA*) with the annotation of alanine aminotransferase 2 at the 489.0699 Mb position. The *QTL-5HL* region contains five protein kinase superfamily protein genes – HORVU5Hr1G121870.1 (*HvPK1*), HORVU5Hr1G124170.2 (*HvPK2*), HORVU5Hr1G125270.1 (*HvPK3*), HORVU5Hr1G125290.3 (*HvPK4*) and HORVU5Hr1G125710.2 (*HvPK5*) – and three PM19-like family protein genes – HORVU5Hr1G125450.1

Table 1. Association mapping of seed dormancy from three trial sites

Traits	Marker	Chr.	Position (bp)	–log10(*p*)	Marker-R2	QTL
2013 Average	D4H599801586	4H	599801586	4.07	0.05	*QTL-4HL*
2013 Average	D5H205503701	5H	205503701	4.67	0.06	*QTL-5HS*
2013 Average	D5H470603514–D5H483494300	5H	470603514–483494300	4.12–4.33	0.06	*QTL-5HC*
2013 Average	D5H666428368–D5H669246470	5H	666428368–669246470	4.95–6.04	0.07–0.09	*QTL-5HL*
2013 KAT	L5H668566901–D5H668940529	5H	668566901–668940529	3.95–4.51	0.09–0.11	*QTL-5HL*
2013 GER	D4H599801586	4H	599801586	4.01	0.05	*QTL-4HL*
2013 GER	D5H205503701	5H	205503701	4.61	0.06	*QTL-5HS*
2013 GER	D5H666428368–D5H669246470	5H	666428368–669246470	4.14–4.75	0.05–0.07	*QLT-5HL*
2014 Average	L1H031627329	1H	31627329	4.53	0.07	*QTL-1HS*
2014 Average	L5H668567022	5H	668567022	4.2	0.06	*QTL-5HL*
2014 Average	D5H482700403–D5H486008836	5H	482700403–486008836	4.28–5.54	0.07–0.09	*QTL-5HC*
2014 ESP	C5H469024140–D5H492894177	5H	469024140–492894177	4.30–4.37	0.06–0.07	*QTL-5HC*
2014 KAT	L1H031627329–L1H031627336	1H	31627329–31627336	4.34	0.06–0.06	*QTL-1HS*
2014 KAT	D4H594540778–D4H595349846	4H	594540778–595349846	4.25	0.06–0.06	*QTL-4HL*
2014KAT	D5H659743469–D5H669246470	5H	659743469–669246470	3.97–5.04	0.06–0.08	*QTL-5HL*
2014 GER	L1H031627336	1H	31627336	4.34	0.06	*QTL-1HS*

KAT: Katanning; GER: Geraldton; ESP: Esperance.

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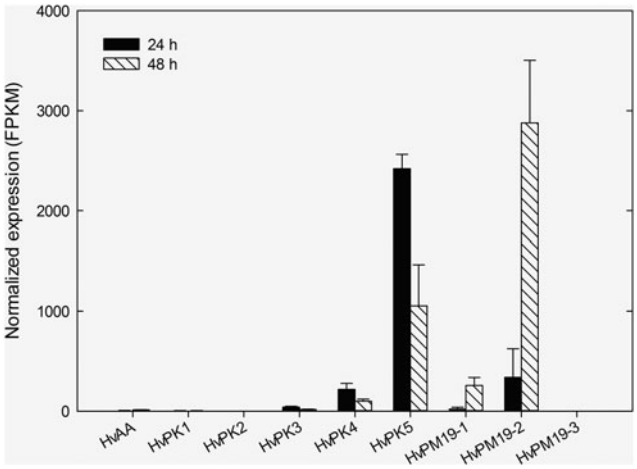


Figure 4. Normalized average gene expression levels from five cultivars at 24 and 48 h after germination. Five cultivars: AC Metcalfe, Baudin, Harrington, Stirling and Bass.

(*HvPM19-1*), HORVU5Hr1G125460.4 (*HvPM19-2*) and HORVU5Hr1G125500.1 (*HvPM19-3*).

RNAseq

The expression levels of the five protein kinase genes, three *PM19* genes and one alanine aminotransferase2 gene were investigated in germinated embryos. Fifty embryos were excised from each treatment 24 and 48 h after germination, and total RNA was extracted and sequenced.

Expression of the three genes (*HvPK1*, *HvPK2* and *HVPM19-3*) was not detected at 24 or 48 h after germination in any of the five varieties (Fig. 4). On average, the alanine aminotransferase2 gene *HvAA* exhibited slight expression at 24 and 48 h (Fig. 4). *HVPK5* had the highest expression level of the five *PK* genes with ~1,000–2,400, and *HvPM19-2* had the highest expression level of the three *PM19* genes with ~330–2,900 (Fig. 4). For the three protein kinase superfamily protein genes, *HvPK3*, *HvPK4* and *HvPK5*, their expression levels at 24 h were double that at 48 h, while for the two *PM19* genes, *HvPM19-1* and *HvPM19-2*, their expression patterns were approximately ten times greater at 48 h than those at 24 h (Fig. 4).

Barley lines with strong or weak dormancy

Barley accessions with weak or strong dormancy are listed in Table 2 based on their germination rates. Nine lines had weak dormancy, while six lines had strong dormancy. These lines can be used to improve barley cultivar dormancy or to clone genes responsible for different levels of dormancy.

The haplotypes of these lines based on the QTL for germination rate were investigated (Supplementary Table S3). The nine barley cultivars with weak dormancy had three haplotypes based on five QTLs on chromosomes 1H, 4H and 5H. The six cultivars or breeding lines with strong dormancy also had three haplotypes. Eight of the nine weak dormancy lines carry high germination rate alleles from four QTLs (*QTL-2HL*, *QTL-5HS*, *QTL-5HC* and *QTL-5HL*). Five of the six strong dormancy lines carry low germination rate alleles from three QTLs (*QTL-1HS*, *QTL-5HS* and *QTL-5HL*).

Table 2. List of barley accessions with weak or strong dormancy

Dormancy	Barley accessions
Weak	Manley, Granifen, Harrington, Merit, Rawson, Sublette, TR145, Tradition, Morex
Strong	Draught, Eunova, Chalice, O'Connor, Urambie, WABAR2547

Discussion

Populations used for association mapping

We used 295 barley accessions in this study, which is more than in other studies; Zhou et al. (2016) used 218 barley lines to map aluminium tolerance, Mamo et al. (2014) used 298 barley landraces from Ethiopia and Eritrea to detect QTLs for zinc and iron concentrations, and Wu et al. (2015) used 100 barley lines to map cadmium accumulation. The large number of lines with different genetic backgrounds helped us to identify QTLs with minor effects. In addition, the natural population is an important germplasm resource for breeders, and various levels of dormancy alleles can be used to improve current cultivars.

QTLs for the seed germination rate

The QTLs for germination were located on chromosomes 1H, 4H and 5H (Table 1). The identification of *QTL-1HS* on chromosome 1H was consistent with Hori et al. (2007), who detected a QTL on the short arm of barley chromosome 1H in a DH population and a RI3 population. For *QTL-4HL*, we anchored the QTL to the region of ~600 Mb on the long arm of chromosome 4H. Nakamura et al. (2017) also reported a QTL in the same region on chromosome 4H. Three QTLs were mapped on chromosome 5H, which plays an important role in seed dormancy. This is the first report of a QTL on the short arm of chromosome 5H. The other two QTLs on chromosome 5H (*QTL-5HC* and *QTL-5HL*) were consistent with those identified by Hori et al. (2007). Nakamura et al. (2017) also identified two major QTLs (*SD1* and *SD2*) near the centromeric region and at the end of the long arm of chromosome 5H. We anchored *QTL-5HC* and *QTL-5HL* to 470.6–492.89 and 659.7–669.25 Mb regions, respectively (Table 1).

In this study, we conducted the experiments for 2 years because we wanted to see whether environmental factors would influence the germination rate or not. Some barley lines displayed the difference in the germination rate between the 2 years at the same site, and the genome-wide association study (GWAS) results (Table 1) showed that different QTLs were detected from 2 years in GER and ESP sites. Environmental factors should be considered in the seed germination test.

Winter and spring barley were included in our research. Other studies indicated that the vernalization type (Auge et al., 2017) influences the germination rate in Arabidopsis. In barley, three vernalization genes (*VRN-H1*, *VRN-H2* and *VRN-H3*) were located on chromosomes 5H, 4H and 1H, respectively (von Zitzewitz et al., 2005). In the present study, five QTLs for germination were also mapped to the three chromosomes. The relationship between vernalization and seed germination needs further investigation. The seed germination rate may be influenced by the seed's low uniformity for the six-rowed barley, so we only selected the uniform seed in this study. The barley row-type

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gene *vrs1* was mapped to chromosome 2H (Komatsuda et al., 2007), but we did not detect the QTL for seed germination. It indicated that the six-rowed barley did not influence our GWAS analysis.

Two well-known major QTLs have been cloned (Nakamura et al., 2016; Sato et al., 2016). The first gene was cloned from the terminal region of chromosome 5H (Nakamura et al., 2016) and encodes a mitogen-activated protein kinase kinase 3; the N260T substitution in the gene region was responsible for seed dormancy (Nakamura et al., 2016). The physical position of the gene was anchored to 668.3 Mb on 5H. In the present study, this gene was mapped to *QTL-5HL* in the 659.7–669.25 Mb region, which covered the mitogen-activated protein kinase kinase 3 gene (*HvPK4*) (Table 1). There are 239 annotated genes in this 10 Mb region. A second gene-regulating seed dormancy has been identified on the centromeric region of chromosome 5H in the 489 Mb region (Sato et al., 2016) and encodes alanine aminotransferase (Sato et al., 2016). In the present study, *QTL-5HC* was mapped to the 470.6–492.89 Mb region, which spanned the alanine aminotransferase gene (*HvAA*) (Table 1). This indicates that the GWAS method can be used to identify QTLs associated with seed dormancy in barley.

The haplotypes of nine cultivars with weak dormancy and six with strong dormancy are listed in Supplementary Table S3. Obviously, the lines with weak dormancy alleles have a high germination rate, and vice versa, so germination rates in barley can be regulated by introducing different alleles from five QTLs (Supplementary Table S3).

RNAseq

Even though the seed dormancy gene encoding mitogen-activated protein kinase kinase 3 (*HvPK4*) has been cloned (Nakamura et al., 2016), there are four more protein kinase superfamily protein genes (*HvPK1*, *HvPK2*, *HvPK3* and *HvPK5*) in the *QTL-5HL* region. *HvPK1* and *HvPK2* were not expressed 24 or 48 h after germination (Fig. 4), and *HvPK3* showed slight expression at 24 and 48 h. *HvPK5* exhibited five-fold higher expression than the cloned gene *HvPK4* at 24 and 48 h after germination. The function of these four *PK* genes needs to be further investigated.

Barrero et al. (2015) identified *PM19-A1* and *A2* as candidates for a major dormancy QTL using a transcriptomic analysis method in wheat. Later, Shorinola et al. (2016) found that *Phs-A1* regulates seed dormancy in wheat, and they delimited this locus to a 0.3 cM interval distal to the PM19 genes. As a result, three *PM19* genes in the *QTL-5HL* region were investigated in this study. *HvPM19-3* did not show any expression at 24 and 48 h after germination, while *HvPM19-1* and *HvPM19-2* exhibited around 10-fold higher expression at 48 h than that at 24 h; furthermore, *HvPM19-2* had more than 10-fold higher expression than *HvPM19-1* at 24 and 48 h (Fig. 4). The function of these genes is unknown.

Conclusions

Five QTLs for the seed germination rate were detected across the barley genome, which revealed the genetic architecture of seed dormancy in barley. The markers for the QTL can be used for marker-assisted selection in barley breeding programmes. Two major genes have been cloned in barley, while other QTL underlying seed dormancy remains unknown. Barley lines with strong and weak seed dormancy were selected; these lines could be used to improve seed dormancy in the current barley varieties.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0960258524000114.

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Competing interests. The authors declare no conflict of interest.

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