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Genetic dissection of endosperm hydration in malting barley (*Hordeum vulgare*)

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Abstract

Hydration of the endosperm is a critical part of the malting process that ensures proper modification of the grain. However, little is known about the genetic controls of endosperm hydration and its relationship to agronomic and malt quality traits. The extent of endosperm hydration is estimated through hydration index (HYI). We measured HYI, agronomic, and malt quality traits on a 169-line subset of the NSGC Barley Core Panel, which includes global malt lines, some dating from the inception of European breeding programmes. Utilizing GWAS, 61 QTLs were identified for HYI, dormancy, agronomic, and malt quality traits. Of these, six were found to be related to HYI and were located on 1H, 2H, 3H, 6H, and 7H. We found HYI QTLs cosegregating with kernel size and hardness (1H and 3H), malting quality (2H and 6H), and dormancy (2H and 6H). These results indicate that endosperm hydration after steeping can be improved by selecting high HYI alleles on 2H, 6H, and 7H, positively impacting malting quality without negatively impacting kernel size or dormancy.

KEYWORDS
barley, dormancy, GWAS, hydration index, malt, quality

1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is a major cereal crop necessary to the malting and brewing industry. Unlike the results of wheat domestication, barley is not free-threshing. To utilize nutritional resources from barley despite the undigestible hull, early people developed a process of controlled germination and preservation called malting (Badr et al., 2000). The main goal of malting is to make nutritionally unavailable biopolymers such as cell-wall polysaccharides, proteins, and starch available for humans through a process called modification. In the 1870s in current Czechia, early malt barley selections were made from landraces (Psota et al., 2009). Early in the 1900s, European breeders also identified landraces with high malt quality. These landraces were incorporated into breeding programmes across Europe, resulting in a lack of genetic diversity in European malt lines (Fischbeck, 1992; Melchinger et al., 1994). Similarly, genetically narrow germplasm has been reported in North American Malt barley (Martin et al., 1991), and a recent focus on two-row malt has potentially narrowed the malt germplasm further. Although a lack of genetic diversity has been observed, there is still variation in malt quality. The burgeoning craft brewing industry requires diversity and expresses a preference for heritage malt (C. Swersey, Brewers Association, personal communication, March 2016), believing it has unique malt quality and flavour traits.

Malting begins with imbibition, where water enters the seed and eventually moves through the storage tissues and is a rate-limiting step in germination. However, little is known about the genetic

control of imbibition, although many industrialized food processes like cooking, extraction, fermentation, and germination all rely on adequately hydrated grain (Miano & Augusto, 2018). Most imbibition or hydration studies measure moisture uptake through the gross change in seed weight over time (Cu et al., 2016; Holopainen et al., 2014; Montanuci et al., 2013). While this measurement is fast and easy to collect, it does not distinguish the level of endosperm hydration, which is crucial to ensuring high-quality malt (Turner et al., 2019). The lag of endosperm hydration to moisture uptake has also been demonstrated through noninvasive MRI approaches (Yin, 2021) but is cost prohibitive for genetic mapping studies. The Chapon technique provides an inexpensive and detailed view of endosperm hydration by determining hydration index (HYI) (Chapon, 1959; Molina-Cano et al., 2002). Variation in HYI could be due to physical differences (seed size, seed hardness, cell wall barriers), hormonal differences (gibberellins and abscisic acid), metabolic activity, and/or enzymatic differences (Miano & Augusto, 2018). HYI at the end of steeping (steep out) also impacts malt quality traits (Turner et al., 2019).

The first step in modification is the degradation of the endosperm cell wall to access the grain protein and starch stored within (Meikle et al., 1994). During germination, water uptake signals the release of β-glucanase, which degrades the cell walls (Bamforth, 2006), exposing storage proteins. Enzymes, for example, transaminases and peptidases, when activated by water convert storage protein into soluble protein and free amino nitrogen (FAN) (Bourne & Wheeler, 1984). The breakdown of these protein structures exposes starch granules from which sugars are enzymatically released for fermentation. Malt quality parameters determine the success of endosperm modification by measuring β-glucan (ppm), solubilized protein (FAN [ppm], soluble protein [%], and soluble to total protein ratio [%]), and starch (% extract) available in an extract of malt called wort.

Seed morphology traits are directly related to barley imbibition. The malting industry has recommended sorting seed by size since 1933 (Pollock, 1962), to produce more uniform malt. Softer grains also imbibe and modify faster than harder grains (Gamlath et al., 2008; Mayolle et al., 2012; Psota et al., 2007). However, gross seed morphology does not completely explain all the variation in endosperm hydration.

Post-harvest dormancy inhibits germination until broken. In climates where winter temperatures can kill a developing seedling, seed dormancy protects from premature germination. Modern breeding for malt barley has selected for lines without significant dormancy, because breeders shorten generation times and select for lines with the immediate ability to malt. However, the cost of this selection is the increased likelihood of barley germinating in the field before harvest in a process called preharvest sprouting, resulting in loss of malt quality. The economic importance of seed dormancy in barley has resulted in many studies to identify the genetic controls of this trait (Bonnardeaux et al., 2008; Gong et al., 2014; Hickey et al., 2012; Nakamura et al., 2017; Ullrich et al., 2009). Dormancy quantitative trait loci (QTL) occur on all seven barley chromosomes (Bonnardeaux et al., 2008; Gong et al., 2014; Hickey et al., 2012; Nakamura et al., 2017; Ullrich et al., 2009), but the consensus is that seed dormancy QTLs SD1 (*Qsd1*) and SD2 (*Qsd2*) located near the centromeric region and distal end of the long arm of 5H control the majority of dormancy in barley (Hori et al., 2007).

To identify and utilize the genetic controls of malt traits, we performed a genome-wide association study (GWAS) on a subset of spring two-row lines with a malting history (Munoz-Amatriain et al., 2014). By focusing strictly on malting lines, we hoped to identify new malt quality QTLs by eliminating the impact of already mapped loci with large effects (e.g., two-row vs. six-row and lax vs. dense). Through this evaluation, it became clear that endosperm hydration impacted malt quality, but its genetic regulation was not understood (Miano & Augusto, 2018). Therefore, a second goal was to identify HYI QTLs and their potential impact on malt quality.

2 | METHODS

2.1 | Germplasm

A subset of the NSGC Barley Core Panel using 169 malting lines was utilized for this study (Munoz-Amatriain et al., 2014). Lines were selected based on row type (two-row) and evidence of a malting background to ensure identification of malt quality QTLs that may be undetected when larger effect loci are present (e.g., two-row vs. six-row). The included lines originated from 42 countries with 152 named lines, 13 landraces, and four derived from mutations (Table S1). Lines were ordered from the Germplasm Resources Information Network (GRIN) and increased in the field in short rows in 2017.

2.2 | Genotyping

Genotyping data for each line from the barley 9k SNP chip, with physical positions determined by the Morex 2012, IBSC physical map (Consortium, 2012), is available on T3 (Blake et al., 2012). Markers were selected using T3's marker selection software, removing those with a minor allele frequency (MAF) of less than 5% or missing more than 45% of the data, and resulting in 5716 markers used in association mapping.

2.3 | Agronomics

We grew material at the Bozeman Post Farm in 2018 under irrigated conditions and in 2019 under dryland conditions. Plots were 5 m^2 with seeding rates of 40 g per 5 m^2 in 2018 and 30 g per 5 m^2 in 2019. The trial was planted in an augmented block design both years. The 2018 trial consisted of four blocks with three checks replicated three times for a total of nine checks in each block. This trial had a total of 36 check plots and 210 experimental lines. The malting lines used for check plots were Craft, Hockett, and Genie. Some lines were lost to lodging in 2018, so the 210 experimental lines were reduced to 169. In 2019, we opted to use a more powerful and efficient design to fit the lower number of lines consisting of six blocks with four checks for a total of 24 check plots and 169 experimental lines. The checks consisted of the malt lines Craft, Hockett, Merit 57, and Metcalfe.

Test weight, per cent plump, per cent grain protein, kernel hardness, and kernel diameter were measured. A Dickey-John Corporation's 2500-UGMA grain analysis computer was used to calculate test weight. Per cent plumps were evaluated by passing seed over a 6/64th sieve. Per cent protein was determined using a Foss Infratec Nova NIR. Kernel hardness and diameter were determined with a SKCS 4100 (Perten Instruments, Springfield, IL, USA).

2.4 | HYI

HYI was measured by the Chapon test (Chapon, 1959; Molina-Cano et al., 2002). After completion of steeping, a subsample of each line was removed from the malt tank. The subsample was placed in boiling water for 1 min. Then 25 seeds from the subsample were cut longitudinally down the centre, and one half of the seed was scored based on the visual appearance of the endosperm. Chalky endosperm is unhydrated, while a shiny, translucent endosperm is hydrated. The seeds were scored by the degree of hydrated endosperm as follows: <50% (1 point), 50% to 75% (2 points), 75% to 100% (3 points), and complete (4 points). Total HYI points varied between 25 and 100. On the same subsample, per cent moisture uptake at steep out (SOM) was measured as described in Turner et al. (2019).

2.5 | Dormancy

In 2019, germination was determined using a modified ASBC method, of Barley-3 Germination. Twenty-four days post-harvest (% Germ@24), 100 seeds from each line were placed in a petri dish with two sheets of Whatman #1 filter paper and 4 mL of water and held constant at 20°C in a germination chamber. Germinated seeds were counted and removed at 24, 48, and 72 h. After 72 h, ungerminated seeds were suspended in a 0.75% hydrogen peroxide solution and left for 48 h. Any seeds that still had not germinated were noted as dead and not included in total germination potential. Because 95% germination is required for malting, we repeated the germination tests each week until all lines reached 95% germination to determine the days required to reach this threshold (DT95).

2.6 | Malt quality

Once dormancy was broken in all lines, the grain was malted as described in Turner et al. (2019) with the following changes (Table 1). The 120-g grain sample was not sorted by size, therefore unplumped, to capture the full phenotypic variation in the population. Malt quality was determined following ASBC methods with modifications as noted in Turner et al. (2019).

2.7 | Statistical analyses/GWAS

Data were corrected using best linear unbiased predictors (BLUPs) to adjust for field variation using the model below in R with the lme4 package (Bates et al., 2014). Broad sense heritability was also calculated using this model in the R code described by Matias et al. (2022).

$$Y_{ijkl} = \mu + Check_i + Block_j + Year_k + Entry_l + \varepsilon_{ijkl}$$

where Y_{ijkl} represents the traits for each line, block, year, and line type combination. $Check_i$ was modelled as a fixed factor representing the replicated check varieties. $Block_j$, $Year_k$, and $Entry_l$ were modelled as random factors, following $N(0, \sigma^2)$. Variation from malt tank to malt tank and day-to-day testing was monitored with control lines.

To identify relationships between agronomic and malt quality traits as well as country of origin and malt quality, principal components (PCs) were calculated using agronomic and quality data for all lines with the function prcomp with scaling and centring. PCs were plotted, and each line was coloured based on its origin. Finally, the plot was visually assessed for any clustering patterns based on origin.

GAPIT's FarmCPU method was used to analyse the data without the compression process (Wang & Zhang, 2021). To run GAPIT, the following packages were also loaded: multtest (Pollard et al., 2004),

TABLE 1 MSU pale base malt regime.

Stage of malting	Time	Temperature	Notes
Steeping			
	10 h	15°C	Water immersion
	18 h	15°C	Air rest
	6 h	15°C	Water immersion
	10 h	15°C	Air rest
	4 h	15°C	Water immersion
Germination			
	96 h	15°C	Moist air circulates for 1 min every 10 min. Grain turned for 5 min every 30 min.
Kilning			
	12 h	60°C	
	6 h	65°C	
	2 h	75°C	
	3 h	85°C	

gplots (Warnes, Bolker, et al., 2021), LDheatmap (Shin et al., 2006), genetics (Warnes, Gorjanc, et al., 2021), ape (Paradis & Schliep, 2019), EMMREML (Godfrey & Akdemir, 2015), compiler (Team RC, 2021), and scatterplot3d (Ligges & Mächler, 2003). The appropriate number of PCs to correct for the population structure of each trait was determined by running the model on 0, 1, 2, and 3 PCs to compare QQ plots. The QQ plots showed that the kinship matrix was sufficient for corrections; therefore, the PC correction was set to 0. The kinship matrix was also calculated in GAPIT and a dendrogram was produced from this output to help understand the genetic relationships between lines. To calculate significance thresholds for QTLs, we used GAPIT's Bonferroni correction where the negative log of alpha (.01) was divided by the number of markers (5716) to get a significance threshold of about 5.8 (Wang & Zhang, 2021). Any SNP above this threshold was recorded in Table S2.

We examined the six HYI QTLs to determine interactions by plotting the number of high HYI alleles versus the HYI of a line, using the yarrr package (Phillips, 2017). The trend of the mean value of each group of positive alleles was then visually evaluated to see if the HYI QTLs behaved additively.

To assess further relatedness of traits to the HYI QTLs, we took a generalist approach where we separated the lines based on their major and minor allele data from the most significant SNP for each HYI QTL. Then we ran two-sample unequal variances *t* tests on all measured traits. Any trait with a *p* value less than .05 was considered related to the HYI QTL being tested. Effects were evaluated by comparing the means of minor alleles between traits.

Epistatic interactions for the six HYI QTLs were also tested by looking at all possible combinations of two HYI QTLs. Interactions between two QTLs were tested with a two-way ANOVA (type III) using the car package (Fox & Weisberg, 2019) with the following linear model:

$$y_{ijk} = \mu + QTL1_i * QTL2_j + \varepsilon_k$$

where μ is the baseline mean, QTL1 is the i^{th} HYI QTL (i = *qHYI1H*, *qHYI2H*, *qHYI3H.a*, *qHYI3H.b*, *qHYI6H*, and *qHYI7H*), and QTL2 is the k^{th} HYI QTL that is not *i*. All interactions are reported in Table S3. Pirate plots made with yarrr (Phillips, 2017) show their effects (Figure S1).

3 | RESULTS

3.1 | Population

The 169 lines, with origins from around the world, were reported as being malting lines for their end-use quality. Of those, 152 were named varieties, for which 138 pedigrees were determined and 31 pedigrees unknown. Assessing the GAPIT PCA output, no clustering or grouping was observed even when geographic origin was included, so PCA structure corrections were not used for association mapping (data not shown). Evaluation of a line's country of origin and malting quality using PCA analysis suggests no association (Figure S2). Where possible, we investigated the pedigrees further. Six of the 14 highest quality lines came from Czechia, with Hanna, Gotland, and Valticky in the backgrounds of most. Diamant, an X-ray mutant of Valticky, is also prevalent in high-quality lines. Table 2 reports the high-quality lines along with their pedigrees and country of origin.

TABLE 2 The top 14 malting lines, pedigrees, and country of origin. Interbreeding between barley cultivars indicated by superscript with Valticky (1), Hanna (2), and Gull (3). Lines without superscript either did not have one of the three main parents or could not be traced back to one of them.

PI number	Name	Pedigree	Country
PI467811	Adorra	Eura II^{2}/Heine 1670–58	Austria
PI599628	Horal	Sladar^{1}/Minerva^{3}//Sladar^{1}/Amsel^{2,3}/3/Union^{2}/Diamant^{1,2}	Slovakia
PI599637	Malvaz	Z8-75/293-77//PI147-77	Czech Republic
PI330397	Diamant	Valticky_B^{1}/3/Hanna^{2}/Unknown//Unknown/4/Unknown	Czechoslovakia
PI564487	Alexis	Breun 1622/Triumph^{1}	Germany
PI592172	Donan	Trumpf^{1}/Ark Royal^{2,3}	United Kingdom
PI599621	Atlas	Mutant SS 55/Diamant^{1,2}	Czech Republic
PI599622	Safir	Valticky^{1}/Kneifel^{2}//Diamant^{1,2}/3/Arabische Zweilige	Czech Republic
PI599627	Rubin	Valticky^{1}/3/Algerian/Valticky^{1}/Union^{2}/4/Diamant^{1,2}/H.st.1373-64	Czech Republic
PI599633	Jarek|PI599633	KM 1192/Sladar^{1}/Opal^{2}	Czech Republic
PI467808	Perfekta|PI467808	Haisa II^{2}/North African variety//Carlsberg II^{2}	Austria
PI498435	Makomako	Unknown	New Zealand
PI365634	Lara	Research/Lenta^{2}	Australia
PI422233	PI422233	Unknown	Yemen

Heritability calculations for HYI showed that the trait was highly heritable (.688). These calculations also showed that β-glucan (.757) was the most heritable trait and soluble protein (.233) was the least heritable (Table 3). Heritability was not calculated for %Germ@24, DT95, Diameter, and Hardness because they were only measured for 1 year. Our PCA analysis of phenotypes indicated that traits related to modification were primarily explained by PC1 while variation in seed size was described by PC2. Some traits like enzymatic activity (AA and DP) along with protein traits (Soluble protein and FAN) were partially explained by both PCs, unsurprisingly suggesting seed size and modification impact their final concentrations (Figure S2).

3.2 | GWAS

Phenotypic averages from the 2018 and 2019 trials were used for GWAS (Table 3), because Pearson correlations between the trials indicated similar environments (Table S4). GWAS indicated 61 associations for all traits measured except SOM and AA (Table S2).

3.3 | HYI

Six HYI QTLs were found on five chromosomes—1H (*qHYI1H*), 2H (*qHYI2H*), 3H (*qHYI3H.a* and *qHYI3H.b*), 6H (*qHYI6H*), and 7H (*qHYI7H*) (Table 4). The effect of each QTL varied from about 1.7 to 2.9 points on a range of 25–100. The HYI QTLs appear to behave additively where on average there is a 10-point difference in HYI between lines with 4 or 1 HYI positive QTLs (Figure 1). Of the QTLs shown, evidence of nonadditive interactions were also observed. We detected an interaction between *qHYI1H* and *qHYI3H.a* (p value = .020578), where the three lines with both fast alleles have a decrease in HYI. There was also weak evidence (p value = .05504) of an interaction between qHYI6H and qHYI7H where lines with both fast alleles for *qHYI6H* and *qHYI7H* had a similar mean HYI to lines carrying a single fast allele (Table S3).

3.4 | Relationship of HYI to other traits

GWAS identified two malt quality traits, BG on 2H and extract on 6H, that co-segregated with HYI QTLs. In both cases higher HYI co-segregated with improved malt quality (lower BG and higher extract). To interrogate further the relationship between HYI and other traits, we compared the trait data of allelic groups for each HYI SNP (Table 5). The analysis indicates the expected relationship between steep out moisture and HYI at *qHYI1H*, *qHYI2H*, and *qHYI3H.b* (Table 5), where HYI increased with per cent moisture. Although several seed morphology QTLS were identified through GWAS, none co-segregated with HYI (Table S2). However, when observing allelic differences (Table 5), HYI increased with a decrease in test weight and per cent plumps at *qHYI1H*, HYI increased with increasing per cent plumps and decreasing kernel hardness at *qHYI3H.a*, while HYI increased with decreasing

TABLE 3 Summary table of average values of mapped traits for the population during the 2018 and 2019 growing seasons. All values are calculated from the average values of the 2 years except for %Germ@24 and DT95, which were only collected in 2019 and diameter and hardness which were only collected in 2018. H^2 shows the broad-sense heritability of each trait for the population and could not be calculated for traits with only a single location year of data.

	Hydration index	SOM	% Germ@24	DT95	Diameter	Hardness	Test weight	Plump	Protein	β-Glucan	Soluble protein	S/T	FAN	Extract	AA	DP
Mean	46.0266	0.4432	87.89	32.57	2.84	47.98	53.89	91.42	13.60	660.45	4.131	31.28	179.79	77.19	53.64	137.24
SD	10.2231	0.0145	18.20	13.33	0.08	6.66	1.67	6.46	1.12	359.7	0.6511	4.57	26.31	1.65	14.65	32.76
CV	0.2221	0.0327	0.2071	0.4093	0.0282	0.1388	0.031	0.0707	0.0822	0.5446	0.1576	0.1462	0.1464	0.0213	0.273	0.2387
Min	26	0.3622	9.32	24	2.64	29.79	46.3	64.20	11	54.68	2.6901	20.87	113.35	72.35	17.29	21.15
Max	78	0.4926	100	87	3.06	67.95	57.2	99.42	17.36	1683.49	6.758	49.18	284.68	82.31	134.79	270.06
H^2	.688	.239	NA	NA	NA	NA	.520	.458	.620	.757	.233	.521	.694	.656	.405	.469

Abbreviations: %Germ@24, per cent germination at 24 h; AA, α-amylase; DP, diastatic power; DT95, days to 95% germination; FAN, free amino nitrogen; S/T, soluble divided by total protein or the Kolbach index; SOM, per cent moisture of the grain at steep out.

TABLE 4 Hydration index (HYI) QTLs, with co-segregating traits and their effects. The most significant SNP for each QTL as indicated by low *p* values of FDR, with chromosome and base pair positions posted. The effect and frequency of the minor allele (MAF) are reported for each QTL. Effects of co-segregating traits are also reported.

QTL designation	SNP	Chromosome	Position	MAF	FDR adjusted *p* values	Effect	Co-segregating traits with effects
qHYI1H	11_11293	1H	76294035	0.16568	5.65E−04	2.578	
qHYI2H	SCRI_RS_219333	2H	2238515	0.23669	1.22E−05	2.9319	BG −73.22
qHYI3H.a	SCRI_RS_97417	3H	10631754	0.15385	1.67E−03	2.1132	
qHYI3H.b	SCRI_RS_1435	3H	550749370	0.49704	1.26E−03	−1.6981	
qHYI6H	SCRI_RS_167845	6H	498173284	0.39053	7.76E−05	−2.4261	Extract −0.215
qHYI7H	SCRI_RS_189107	7H	562630313	0.31361	5.35E−04	2.2613	

Abbreviation: BG, β-glucan.

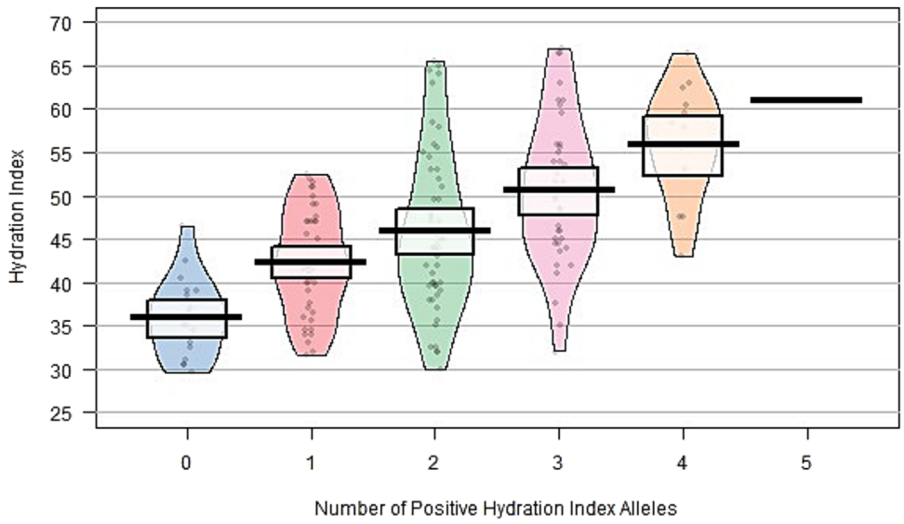

FIGURE 1 A pirate plot showing the hydration index versus the number of positive alleles. The grey dots represent each line tested. The bold line represents the mean hydration index for that number of positive alleles and the white band shows the confidence interval around the mean. Each bean functions as a smoothed density curve showing the distribution of the data points. There was only one line with five hydration index QTLs and 0 with all 6. [Color figure can be viewed at wileyonlinelibrary.com]

kernel diameter at *qHYI3H.b*. Endosperm hydration was also found to impact malt quality beyond the co-segregated traits. Allelic differences that increased HYI improved alpha-amylase while decreasing β-glucan at *qHYI2H*, while at *qHYI6H*, an increase in HYI was associated with an increase in extract and alpha-amylase levels while decreasing β-glucan levels and grain protein (Table 5).

Dormancy was mapped as %Germ@24 and DT95. For % Germ@24, we found QTLs on 2H, 4H, 5H, and 6H (Table S2). For DT95 we observed 5 QTLs on 1H, 2H, 5H, and 7H (Table S2). The 2H and 5H QTLs co-segregate for %Germ@24 and DT95. Of the six HYI QTLs, none coincided with DT95 or %Germ@24 QTLs. The closest dormancy QTL to *qHYI2H* is 6.5-Mb downstream while the closest dormancy QTL to *qHYI6H* is 33.7-Mb upstream. However, when comparing allelic patterns, at *qHYI2H* and *qHYI6H*, HYI increased with less dormancy (Table 5).

4 | DISCUSSION

4.1 | Population

When examining this population, we found minimal evidence for population structure. The population is a subset of a larger population also used for GWAS (Munoz-Amatriain et al., 2014). The lines selected from the larger population were all two-row lines with a malting background. Most of these lines (125) fell into subgroup 3, while the remainder (44) were outside of subgroup 3 of Munoz-Amatriain et al.'s (2014) findings. Interestingly, the pedigrees of the panel indicated shared parents across breeding programmes. For example, the named varieties Gull and Binder are parents of Dutch, British, Irish, French, Scandinavian, and German varieties (Aufhammer et al., 1968). Gull is a selection from the Gotland landrace and Binder is a selection from Hanna. In fact, the presence of a few parents is pervasive across this population demonstrating the exchange of malt lines between breeding programmes and explaining the lack of need for PCA corrections.

Evaluating the kinship matrix provided further perspective about the population structure. While we did see groupings, they were very deep in the relationships among lines (Figure S3), consistent with the pedigrees indicating shared parents throughout all the branches. We did notice the first branch lines were being separated based on occurrence in subgroup 3 from Munoz-Amatriain et al. (2014), but again shared parents throughout branches indicated relatedness. While the panel was genetically similar, we did see variation in malt quality. Although not exclusively, we noticed that lines of Czechia origin were present in many pedigrees and appeared to have better malt quality in

Montana. Psota et al. (2009) reviewed the early malt barley breeding efforts in the Czechia region. As early as 1870, Hanna was selected from a single grower's field from a region near the Hanna River due to its early heading and higher yield. In the 1920s, Hanna was crossed with a Turkish landrace to improve arid tolerance. Opavsky, also known as Kneifel, was selected as a landrace around 1880 due to its lack of dormancy, plump grain, and higher extract. Valticky selected from a landrace in 1930 was crossed with Kneifel to create an early arid tolerant line and an X-ray mutation of Valticky, Diamant, was widely used due to reduced height. Our results indicate these genetic backgrounds continue to provide positive malt quality traits.

4.2 | Endosperm hydration

Water uptake into the endosperm is a rate-limiting step for the germination of grain (Bewley & Black, 1978) and thereby can impact the efficiency of the malting process. Hydration of grain is not a simple process. Water must follow specified pathways to hydrate grain and can have different hydration patterns depending on the structure and composition of the grain (Miano & Augusto, 2018). While much work has been done to understand and model the mechanisms that impact imbibition, little is known about the genetic control of endosperm hydration (Miano & Augusto, 2018). Using a fixed malting recipe for all lines, we found six HYI QTLs.

Through further interrogation of traits based on HYI allelic differences, (Table 5), we were able to categorize each HYI QTL as related to grain morphology and malt quality to aid our understanding of these QTLs and identify the most beneficial. The HYI QTLs related to grain size are *qHYI1H* and *qHYI3H.b* where smaller seed has higher HYI. Because larger seeds are preferred by maltsters, these QTLs are unfavourable for breeding. However, at *qHYI3H.a* large seeds are associated with high HYI due to soft texture. The HYI QTLs *qHYI2H* and *qHYI6H* increase HYI and improve malt quality, but also may increase PHS risk. Interestingly, although *qHYI7H* has one of the largest impacts on HYI, it does not significantly relate to any other trait and therefore may be a candidate to improve endosperm hydration without negative impact, warranting further investigation. Where allelic variation at HYI QTLs correlates with variation in another trait, it is unclear whether that variation is due to pleiotropy or linkage. However, this examination helps determine that *qHYI2H*, *qHYI3H.a*, *qHYI6H* and *qHYI7H* are worth further investigation.

Although HYI QTLs appear to act additively, there is a large amount of variation in HYI for lines with two or three QTLs (Figure 1). Epistatic interactions indicate that *qHYI1H* and *qHYI3H.a* decreased HYI when both positive HYI alleles were present. Although *qHYI3H.a's* important effect is likely related to seed hardness, the two fast alleles at *qHYI1H* and *qHYI3H.a* had opposite effects on seed size perhaps resulting in this negative interaction. The interaction between *qHYI6H* and *qHYI7H* did not increase HYI when both positive alleles were present (Figure S2). Although further study is needed to understand these interactions, they do explain the low HYI of some lines with two high HYI alleles, but it does not explain why some lines with only

TABLE 5 The impact of the hydration index (HYI) QTLs on other traits. HYI QTLs are designated as *qHYI1H* (1H 11_11293), *qHYI2H* (2H SCRI_RS_219333), *qHYI3H.a* (3H SCRI_RS_97417), *qHYI3H.b* (3H SCRI_RS_1435), *qHYI6H* (6H SCRI_RS_167845), and *qHYI7H* (7H SCRI_RS_189107). HYI reports the effects of the minor allele on hydration index. For the remaining traits the difference between the mean of the lines with minor and major alleles is reported. Results from *t* tests indicate if the differences are meaningful.

QTLs	HYI	SOM	%Germ@24	DT95	Diameter	Hardness	Test weight	Plump	β-glucan	Protein	Soluble protein	S/T	FAN	Extract	AA	DP
qHYI1H	2.578	0.0094**	−0.81	−0.65	−0.0221	1.47	−0.89**	−2.87*	−75.99	0.14	0.1583	1.14	1.77	−0.1288	4.43	2.47
qHYI2H	2.9319	0.0076**	6.91***	−6.41**	−0.0161	0.81	−0.47	−1.54	−219.85***	−0.24	0.0954	1.47*	6.42	0.2754	5.19*	3.60
qHYI3H.a	2.1132	0.0022	4.98	−3.77	0.0314	−3.18**	0.54	2.75**	−113.15	−0.28	0.0539	0.99	−0.28	0.2415	−0.36	−4.29
qHYI3H.b	−1.6981	−0.0079***	−1.76	0.35	0.0321**	−0.43	0.39	1.26	66.84	0.26	0.0788	−0.02	4.29	0.1219	−0.03	5.37
qHYI6H	−2.4261	−0.0029	−7.48*	4.97*	0.0097	1.01	−0.42	−1.38	173.99**	0.53**	−0.0653	−1.66**	−1.98	−0.7976**	−3.69*	7.81
qHYI7H	2.2613	0.0011	−2.33	1.29	−0.0118	0.99	0.26	0.03	−41.43	−0.07	0.0422	0.40	2.90	0.4915	0.47	3.13

Note: Asterisk (*) indicates where major and minor alleles are not equal with *p* values equal to * <.05, ** <.01, and *** <.0001.

Abbreviations: %Germ@24, per cent germination at 24 h; AA, α-amylase; DP, diastatic power; DT95, days to 95% germination; FAN, free amino nitrogen; S/T, soluble divided by total protein or the Kolbach index; SOM, per cent moisture of the grain at steep out.

two QTLs have such high HYI values. Our ability to observe epistasis was likely limited by the low frequencies of some two gene combinations. Also, other alleles could occur with such minor frequency that a significant association was not observed. This is further supported by our heritability calculations which show that HYI is heritable ($H^2 = .688$), but the genetic variation explained by the HYI QTLs was only about 14%. To better understand variation in HYI, we are developing bi-parental mapping populations to identify any unmapped HYI QTLs

4.3 | Steep out moisture

When gauging water uptake other studies have used SOM (Cu et al., 2016; Holopainen et al., 2014; Montanuci et al., 2013), identifying QTLs on 4H, 5H, and 7H in a set of double haploid lines (Cu et al., 2016). Although we did not identify any SOM QTLs through GWAS, allelic comparisons associated SOM with HYI at *qHYI1H*, *qHYI2H*, and *qHYI3H.b* suggest some connection between the traits (Table 3). However, the lack of a relationship between all six HYI QTLs and SOM QTLs from other studies emphasizes variation in the genetic control of the two measurements. Turner et al. (2019) came to similar conclusions that these traits are related, but not perfectly correlated.

4.4 | Dormancy

Dormancy is another important trait for malting barley and has likely been selected against by the modern breeding process of advancing generations as quickly as possible. QTLs for dormancy have been identified on all seven barley chromosomes (Bonnardeaux et al., 2008; Gong et al., 2014; Nakamura et al., 2017; Ullrich et al., 2009), but *SD1* and *SD2* on 5H consistently have the greatest effects on dormancy (Hori et al., 2007). For our two traits that assessed dormancy (% Germ@24 and DT95), we found associations near SD2. We also found an association near the QTLs reported in Gong et al. (2014) on 1H, 2H, 4H, and 6H for both traits. Although no dormancy QTLs co-segregated with HYI QTLs through GWAS, there were some relationships between hydration and dormancy traits at *qHYI2H and qHYI6H*. Importantly, for the most part, QTLs for malt quality and dormancy do not overlap. Providing the opportunity to increase dormancy, while also improving malt performance with alleles for increased HYI.

4.5 | Morphology

Because grain size, structure, and composition impact hydration pathways (Miano & Augusto, 2018), the malting industry has historically sorted grain by size to ensure even hydration (Pollock, 1962). Also, softer grains are preferred because harder grains imbibe slower than softer grains (Gamlath et al., 2008; Psota et al., 2007). The kernel size QTL on 6H (Table S2) matches the findings of Wang et al. (2019) while grain hardness association on 4H and 5H matches the findings of Walker et al. (2013) and Fox et al. (2007), respectively. While none of these morphological QTLs co-segregate with the HYI QTLs, allelic comparisons indicate seed size and hardness have some impact on endosperm hydration. *qHYI1H*, *qHYI3H.a*, and *qHYI3H.b* were all related to seed size and hardness traits, confirming previous findings, although results of *qHYI3H.a* indicate that larger seeds can have a higher HYI with softer kernels. Importantly, HYI QTLs on 2H, 6H, and 7H were not associated with seed size or hardness traits suggesting a deeper level of complexity for this trait.

Protein content and protein structure are one piece of a very complicated interaction between grain size, hardness, porosity, starch, and fat content that all impact hydration (Miano et al., 2018). QTLs related to grain protein have been mapped to all seven chromosomes except 3H (Emebiri et al., 2005; Fan et al., 2017). NAC transcription factors on 6H (*HvNAM1*) and 2H (*HvNAM2*) have been related to delayed senescence, larger seed, lower grain protein and malt quality (Alptekin et al., 2022). We mapped QTLs for grain protein to the same six chromosomes, but none co-segregated with HYI QTLs. Examination of allelic variation indicates that *qHYI6H* has some relation with grain protein; however, this population is fixed for the functional allele of *HvNAM1* on 6H suggesting *qHYI6H* is controlled by another gene. Although this population does segregate for *HvNAM2* on 2H, *qHYI2H* localized 400 Mb from this gene.

4.6 | Malt quality

HYI related to malt quality on 2H and 6H, where increased HYI correlates with improved malting quality. Pauli et al. (2015) also report a group of malt quality QTLs on 2H in a population of Montana breeding lines, but the QTL is more proximal compared to *qHYI2H*. On 6H there is another malt quality hot spot identified by Mohammadi et al. (2015), which contained QTLs for DP, AA, grain protein, and extract. This region appears near *qHYI6H* and matches our findings. Both the 2H and 6H associations lacked associations with any of the seed morphology traits suggesting that we could select for increased HYI to improve malting quality without decreasing grain size.

To observe genetic differences, we malted all lines with the same recipe, targeting appropriate modification of high HYI lines, thereby under modifying lower HYI lines. Thus, ensuring the high HYI lines were not overmodified with subsequent negative impacts on malt quality and allowing the detection of the positive relationship between HYI and extract.

4.7 | Breeding impacts

The relatedness and lack of structure for these historic malting barley lines pose a unique problem for breeders and have been reported by others (Fischbeck, 1992; Martin et al., 1991; Melchinger et al., 1994). Limited genetic variation could limit the ability to improve malt quality. However, we observed variation in malt quality that could in part be explained by variation in endosperm hydration. We are currently

evaluating traits in this population not evaluated during malting quality analysis including metabolite differences that might be used to further improve malt.

HYI QTLs, *qHYI2H*, and *qHYI6H*, both co-segregate with improved malt quality. However, *qHYI1H* and *qHYI3H.b* are related to seed size, where the beneficial HYI allele co-segregates with decreased seed size suggesting these QTL are not beneficial for malting barley. While *qHYI3H.a* is also related to seed size, the minor allele that increases HYI also increases plumps while decreasing kernel hardness. This suggests that kernel hardness is more impactful on HYI than kernel size, allowing breeders to select for large seeds that do not hinder HYI. A concern for breeders is the potential negative interaction of some of the HYI QTLs. For example, the high HYI allele for *qHYI1H* and *qHYI3H.a* interacted to reduce HYI. Also, *qHYI6H* and *qHYI7H* may not be additive when both are present. Importantly, all the genetic variation was not explained for HYI. Therefore, we are developing biparental mapping populations to identify minor QTLs. We are also further exploring *qHYI2H*, *qHYI6H*, and *qHYI7H* with NIL populations to confirm function and look for candidate genes.

Endosperm hydration is a key part of the imbibition of grain and its effect on malting barley is extremely important. Our associations on 2H and 6H indicate that we can improve malt quality by increasing the HYI. Increasing the HYI of barley could also improve the efficiency of the malting process. The first step in the malting process, steeping, requires the flooding of seed with water interspersed with air rests. Modern malt recipes try to limit the number of steeps to save time and water, attempting to achieve modification with two steeps, but sometimes requiring three. Each steep consumes up to 1.39 metric tons of water per metric ton of barley which is roughly 8 gal of water per bushel (Yin, 2021). Lines with higher HYI could ensure fewer steeps saving a malt house time and money.

AUTHOR CONTRIBUTIONS

Joseph Jensen and Jamie Sherman were both responsible for the conceptualization of this study. Joseph Jensen, Hannah Turner, Greg Lutgen, and Jamie Sherman all worked on the methodology of this study with Hannah Turner specifically working on malting and quality analysis while Greg Lutgen worked on field management and harvesting of material. Formal analysis for this study was conducted by Joseph Jensen and Jennifer Lachowiec while further investigation of the data and curation of data was performed by Joseph Jensen. Joseph Jensen wrote the original draft of this manuscript and all authors reviewed and edited it further. Jamie Sherman collected the funding for this project and was the project administrator.

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CONFLICT OF INTEREST STATEMENT

All other authors declare they have no financial interest or competing interest that would be impacted by this paper.

DATA AVAILABILITY STATEMENT

The dataset generated during the current study is in the process of being placed on T3.

CONSENT TO PARTICIPATE

No human subjects were involved in this study.

CONSENT TO PUBLISH

All images present in this manuscript have consent to publish.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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