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Improvement of Endosperm Hydration Counter the Negative Relationship Between Dormancy and Malt Quality in Barley (*Hordeum vulgare*)

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Keywords: barley | dormancy | hydration index | malt

ABSTRACT

Dormancy in barley has been thoroughly studied and shown to negatively impact malt quality, resulting in selection against dormancy. However, reduced dormancy coincides with increased preharvest sprout (PHS) risk, thus sparking a new interest in integrating dormancy back into American barley lines if the negative effects of dormancy on malt quality can be overcome. We evaluated the dormancy and hydration index (HYI) in a biparental mapping population to determine the genotypes that would protect against PHS but have good malt quality. We found 4 HYI QTLs and 4 dormancy QTLs, one of which was near the well-described SD2 QTL. The HYI QTLs were pleiotropically related to seed size (1H), dormancy (5H) and malt quality (2H). Lines with dormancy (5H) and increased HYI (2H and 3H) had malt quality similar to nondormant lines while maintaining PHS resistance, suggesting improvements in HYI could be the key to overcoming the negative effects of dormancy in malting.

1 | Introduction

Crops undergo breeding for properties that improve not only yield but also end-use characteristics. Unlike most cereals, barley (*Hordeum vulgare* L.) requires malting to extract the grain's nutritional content. The modern malting process precisely controls germination to modify the grain so that the endosperm cell walls and protein matrix are sufficiently degraded, freeing starch granules. The final malted grain product can then be used as a source of sugar, enzymes and flavour for food or beverages.

The quality of malt is determined by the degree of degradation, referred to as modification, achieved during the malting process. The enzymes that are responsible for endosperm modification are reliant on a hydrated endosperm to move through and degrade cell walls and storage structures. As germination begins, hormones and enzymes are activated in the embryo and move through the hydrated parts of the endosperm, signalling β-glucanase production to hydrolyze endosperm cell walls (Bamforth 2006). Water-activated transaminases and peptidases enter degraded endosperm cell walls and break down the protein matrix (Bourne and Wheeler 1984). The now-soluble protein can then be digested further into free amino nitrogen (FAN). As the protein structures are degraded, starch granules are exposed to digesting enzymes, but the malting process is stopped mid-modification through kilning to preserve starch, which is instead acted on by enzymes during brewing. Malt quality is a measurement of

the degree of modification and includes examining the levels of β-glucan (BG) (parts per million), soluble protein (percentage) and FAN (parts per million), as well as the amount of potential fermentable sugar (percent extract) and available enzymes (α-amylase [AA] and diastatic power [DP]). Malting is a balance where, if undermodified, the full potential of sugars and enzymes in the malt is not reached, while if overmodified, fermentable sugars are decreased after being used by the developing embryo.

Several studies have interrogated the hydration kinetics or water movement through the endosperm during germination, with changes in percent moisture serving as a common proxy (Miano and Augusto 2018). Identifying QTLs on 4H, 5H and 7H (Cu et al. 2016; Holopainen et al. 2014; Montanuci, Jorge, and Jorge 2013). However, in a recent GWAS paper identifying six endosperm hydration QTLs, there was no overlap with percent moisture QTLs (Jensen et al. 2023). These studies have identified several morphological traits related to endosperm hydration, including seed hardness and seed size. Softer kernels increase imbibition rates compared to harder kernels due to the greater porosity of the endosperm (Gamlath, Aldred, and Panozzo 2008; Psota et al. 2007). The malting industry has long recognized the impact of seed size on endosperm hydration, deploying the standard practice since the 1930s of sorting malt samples by seed size (Pollock 1962).

Dormancy, a seed's ability to resist germination, has been thoroughly studied in malt barley. Maltsters prefer a lack of dormancy in barley, eliminating the required delay between harvest and malting and reducing the potential negative impact of dormancy on malt quality even after it is broken (Rooney et al. 2023; Sweeney et al. 2022; Reuss and Cassells 2003). Unfortunately, lines without dormancy can prematurely germinate, known as preharvest sprout (PHS), when exposed to moisture postmaturation, which negatively impacts malt quality. Due to the importance of dormancy on seeds in general and malt barley quality in particular, the genetic control over dormancy has been thoroughly investigated (Wang et al. 2019). Although dormancy QTLs have been found on all seven chromosomes, there are two that control most of the genetic variation in barley (SD1 and 2) (Hori, Sato, and Takeda 2007; Ullrich et al. 1992). Both QTLs are located on the long arm of 5H, with SD1 (Qsd1) near the centromeric region and SD2 (Qsd2) more distal. The SD1 recessive dormant allele is most commonly found in wild barley but can also be found in some historic malting lines (e.g., Golden Promise) (Sato et al. 2016). The SD2 recessive dormant allele can be found in cultivars domesticated in East Asia, where dormancy is needed to prevent PHS (Nakamura et al. 2016). Of the minor dormancy QTLs, *Qsdw-4H* and *Qsdw-5H* appear to have the greatest impact on dormancy (Nakamura et al. 2017). However, due to selection against dormancy, these two QTLs may have been lost from modern malting germplasm (Wang et al. 2019).

Many studies have genetically dissected malt quality to understand the relationship between the traits and to develop markers for breeding. One such study was conducted on a half-sib population to identify important QTLs in the Montana State barley breeding programs for elite malting material (Pauli, Brown-Guedira, and Blake 2015). A reanalysis of this study highlighted the importance of three genes on malt quality, HvNam 1 and 2 and HvRGP, coincident with QTLs from the original study (Alptekin et al. 2022). We created a biparental mapping population, segregating for agronomic and malt quality traits, including dormancy and endosperm hydration, from two lines originally part of the previous two studies (Pauli, Brown-Guedira, and Blake 2015; Alptekin et al. 2022). The goal was to identify QTLs that improve malt quality in dormant lines while protecting against PHS, improving the security of the malt supply and decreasing grower and end-user risk.

2 | Methods

2.1 | Population

The biparental mapping population was made by crossing two Montana State University experimental lines, MT124128 (Hocket/*2 MT0101740) and MT124148 (Craft/MT010174). Hockett has been a successful malt line in Montana because of its plump seed and stable yields in rainfed environments. In 2022, Hockett was grown on 118,024 acres in Montana (NASS). However, Hockett has some disadvantages as a malt variety, including higher grain protein and extended malting times. Craft was created with the hope of acceptance by the craft brewing industry, but due to quality issues, it was not accepted by maltsters. MT010174 (Hockett//*3Lewis/Karl) resulted from backcrossing the low protein allele of *HvNam 1* from Karl into Hockett (Alptekin et al. 2022) to reduce grain protein. The two experimental lines were created as part of the breeding program and varied for dormancy, length of grain fill, yield, seed size, kernel hardness, grain protein, plumps, BG, extract and FAN. The progeny from this cross were selfed to F6 through single seed descent. Recombinant inbred lines (RILs) were derived from the population, totaling 139 lines.

2.2 | Genotyping

The population and parents were genotyped at the USDA-ARS lab in Fargo, North Dakota, using the Illumina 50 k barley SNP chip (Bayer et al. 2017). The SNPs were coded with 'A' for the MT124128 allele and 'B' for the MT124148 allele. Using the R qtl package (Broman et al. 2003), SNPs were dropped if monomorphic, redundant or missing in more than 10% of the genotypes, reducing the total number of SNPs from 44,040 to 779. A linkage map was then built using MapDisto (Heffelfinger, Fragoso, and Lorieux 2017) with anchor information provided by the Illumina 50 K SNP chip.

2.3 | Agronomics

This material was grown at the Bozeman Post Farm in 2018, 2019 and 2020 under dryland conditions. It was also grown under irrigated conditions in Bozeman in 2019 and at the Southern Agricultural Research Center (SARC) in Huntley, Montana, in 2019 and 2020. Plots at Bozeman were 5 m^2, with seeding rates of 30 g per 5 m^2 in dryland and 40 g per 5 m^2 in irrigated. Plots at SARC were 5.2 m^2, with seeding rates of 40 g per 5.2 m^2. The

2019 irrigated trial in Bozeman was hit by a significant rain event that resulted in PHS in some lines. The trial was grown in an augmented block design during all location years, with seven blocks containing four checks and 20 experimental lines each. The checks for this experiment were MT124128, MT124148, Craft and Hockett.

The field heading date, maturity date, length of grain fill and height were all evaluated using the following methods. The heading date was recorded in Julien days when 50% of a plot reached Zadoks Stage 59, where heads emerged completely from the boot (Zadoks, Chang, and Konzak 1974). A plot was considered to reach maturity when half the plants reached Zadoks Stage 89, or physiological maturity, and was recorded in Julian days. The length of grain fill was the number of Julian days between heading and maturity. Height was the average in centimetres of two measurements for each plot and was measured from the ground to the top of the head, excluding awns. Traits measured postharvest were yield, test weight, percent plump grain, percent grain protein, kernel hardness and kernel diameter. Yield was determined by dividing total plot weights by the plot area to get grain yield in bushels per acre. A Dickey-John Corporation's 2500-UGMA grain analysis computer was used to calculate the test weight. Percent plumps were evaluated by passing seed over a 6/64th sieve. Percent protein of the grain was determined on a Foss Infratec Nova NIR, while kernel diameter and hardness were found from the average of 50 seeds run through an SKCS 4100 (Perten Instruments, Springfield, IL, United States). Postharvest traits were not measured on the Bozeman 2019 irrigated trial, and no agronomic traits were collected on the SARC 2019 trial.

2.4 | PHS: AA SD Test

To determine sprout damage in a high-throughput manner, the AA level was measured on sprouted grain using the AA SD method of the Megazyme kit, as described in Mangan et al. (2016). Both the Megazyme kit and Mangan et al. (2016) provided curves showing the relationship between falling numbers and the AA SD test to determine acceptable levels for this test. To decrease confusion with malt quality AA, we will refer to this as PHS.

2.5 | Dormancy

Dormancy was measured in 2019 and 2020 on the MSU dryland trials, following the methods described in Jensen et al. (2023) for percent germination 3 days after harvest (%Germ@3) and days to reach 95% germination (DT95).

2.6 | Hydration Index (HYI)

The HYI and steep-out moisture were measured using the methods described by Jensen et al. (2023), where a 5.6-g subsample of grain was steeped, boiled and then cut into cross sections to score using the Chapon test. Percent at steep out moisture (SOM) was also measured using methods described in Turner et al. (2019).

2.7 | Malting and Malt Quality

After dormancy was broken, the samples were malted, as described by Turner et al. (2019), using the following changes. A 120-g subsample of unplumped grain was malted to ensure we captured the full genetic potential of each line. Also, samples were germinated for three rather than 4 days to emphasize the variation in modification. After malting, quality analysis was performed following ASBC methods and Turner et al. (2019) modifications to collect BG (parts per million), percent soluble protein, FAN (parts per million), percent extract, AA (DU), DP (ASBC) and the Kolbach index (soluble/total protein, S/T) (%).

2.8 | Statistical Analyses

Corrections due to field variation were performed using best linear unbiased predictors (BLUPs). The lme4 package (Bates et al. 2014) was used to run the model below for these corrections. Broad-sense heritability was determined with this BLUP model and the R code described by Bates et al. (2014).

$$Y_{\text{ijkl}} = \mu + \text{Check}_i + \text{Block}_j + \text{Location Year}_k + \text{Entry}_l + \varepsilon_{\text{ijkl}}$$

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

Principal component analysis (PCA) was also used to evaluate relationships between traits and QTLs identified in the population. This was done with the prcomp function with scaling and centering. PCs were then plotted and coloured based on QTLs for visual assessment.

The R qtl package (Broman et al. 2003) was used to perform QTL analysis on this population. Single interval mapping (SIM) was performed with the scanone function. Peaks identified from SIM were then assessed as potential covariates for composite interval mapping (CIM). The CIM function was used to perform CIM with reading windows of 10, 20 and 40 cM, along with the covariates from SIM. QTLs from CIM were then placed into a model for multiple interval mapping (MIM). Model assessment was then performed to see if terms needed to be added or removed, as well as searched for interactions between the terms using fitqtl, refineqtl, addint and addqtl functions. To confirm the results of manual model selection, an automated model selection method was also performed using the stepwiseqtl function in the qtl package. Significance thresholds for all QTL analysis methods were determined using 1000 permutations. Haley–Knott regression was used for all QTL analysis methods.

ANOVA was performed in R to estimate the variance between allelic combinations for the following QTLs: qD5H, qSS1H, qHYI2H, qHYI3H and Nam1. Eighteen combinations (haplotypes) were observed, and a Type 3 ANOVA was performed to model the impact on each trait. A post hoc analysis was then performed using Tukey's test and reported.

3 | Results

3.1 | Population

MT124128 and MT124148 are half-siblings each sharing a paternal parent, MT010174, with the female parent being Hockett or Craft, respectively. As a result of backcrossing, MT010174 is about 88% homologous to Hockett, while MT124128 is about 97% homologous to Hockett. MT124148 consists of about 50% Craft and 50% MT010174. Although MT124128 and MT124148 are closely related, they have complementary phenotypic variation for a number of traits, suggesting a cross could result in an improved line (Table 1). MT124148 was the most dormant of the two parents, with the lowest percent germination at 3 days postharvest, the longest time to 95% germination and the least PHS. However, it also had the highest HYI. MT124128 had earlier heading, resulting in longer grain fill and shorter stature. MT124128 also has lower grain protein and plumper seed, while MT124148 is higher yielding, with smaller and harder seeds. MT124128 outperforms MT124148 in every malt quality trait except BG and DP. The resulting F6 population varied with line means between the parental means for all traits except PHS, test weight, grain protein and DP, indicating transgressive segregation (Table 1).

When evaluating year-to-year variation within this population, each trait was correlated between location years and the BLUP-corrected average (Table S1). Therefore, the BLUP-corrected averages were used in QTL mapping. As expected, endosperm hydration, as measured by HYI, was negatively correlated with the three indicators of seed size (diameter, plumps and test weight). HYI was highly correlated with heading date (positively) and grain fill (negatively), likely due to the positive correlation between length of grain fill and seed size (Table 2). However, unlike other studies, kernel hardness was positively correlated with HYI, likely due to a relationship with seed size, since we observed that harder seed was correlated with smaller seed in this population. Of the malt quality traits, BG was most highly correlated with HYI, with BG decreasing with increasing HYI (Table 2). We also observed a negative correlation between percent extract and HYI, but one would expect more starch (extract) to be made available with higher hydration. However, since the malt recipe was consistent across genotypes and not adjusted for faster hydration, those samples with higher HYI could have been overmodified, resulting in reduced extract. There has been a belief in the industry that dormancy reduces malt quality even after dormancy is broken. Therefore, we delayed malting until dormancy was broken and 95% germination was achieved. Even so, we observe a negative correlation between dormancy and most malt quality traits. However, HYI is poorly correlated (<0.2) with dormancy and PHS, indicating endosperm hydration and thereby malt quality could be improved while maintaining resistance to PHS, encouraging this mapping study.

3.2 | QTL Mapping

The MT124128/MT124148 population map included 779 informative SNP markers, creating six linkage groups (Table 3). The population was fixed for all 4H markers, resulting in no 4H linkage group. Of the remaining six chromosomes, 2H was the

TABLE 1 | Summary statistics for all traits measured and mapped. The population means as well as the means for the two parents are reported in the first two rows. The standard deviation (SD), CV, minimum value, maximum value and broad sense heritability (H^2) are reported for the mapping population.

	Hydration index (points)	SOM (%)	%Germ@3	DT95	PHS (U/g)	Heading (Julian days)	Maturity (Julian days)	Length of grain fill (days)	Height (cm)	Yield (bushels/acre)	Kernel diameter (mm)	Kernel hardness (HI)	Test weight (lbs/bushel)	Plump (%)	Grain protein (%)	β-Glucan (ppm)	Soluble protein (%)	S/T (%)	FAN (ppm)	Extract (%)	AA (DU)	DP (ASBC)
MT124128	47.9	40.9	95	5	0.459	173	217	44	82.9	126.6	3.02	37.1	53.7	97.2	10.5	444	5.33	50.7	230	81.7	66	119
MT124148	57.9	42.6	68	20	0.304	181	216	35	87.6	141.8	2.83	45.4	53.5	90.1	11.3	212	4.37	39.3	188	78.0	56	122
Population Mean	54.3	41.9	90	9	0.471	178	217	38	85.2	132.7	2.92	41.1	53.9	94.8	11.3	363	5.03	45.2	221	79.5	57	134
SD	9.9	2.3	14	9.7	0.566	8.58	10.97	6.22	7.59	23.22	0.10	5.55	2.17	5.5	0.97	193	0.44	5.9	25	1.9	13	22
CV	0.18	0.06	0.16	0.86	1.20	0.04	0.05	0.16	0.089	0.17	0.037	0.13	0.04	0.05	0.08	0.533	0.08	0.13	0.11	0.02	0.23	0.17
Min	30	36	27	3	0.039	157	192	20	65.5	61.6	2.62	31.3	47.1	59.3	9.4	35	3.49	28.1	157	73.7	23	87
Max	85	50	100	47	4.086	192	236	57	102.0	193.3	3.25	58.9	58.1	99.8	14.3	987	5.96	59.3	301	83.9	100	210
H^2	0.507	0.120	0.259	0.480	NA	0.180	0.010	0.521	0.118	0.054	0.518	0.761	0.012	0.184	0.483	0.381	0.667	0.596	0.586	0.744	0.396	0.545

Abbreviations: %Germ@3: percent germination 3 days postharvest; AA: α-amylase; DP: diastatic power; DT95: days to 95% germination; FAN: free amino nitrogen; PHS: preharvest sprout; SOM: steep out moisture; S/T: soluble/total protein or Kolbach index.

TABLE 2 | Correlation matrix of the BLUP-corrected averages for each trait measured.

	Hydration index	SOM	%Germs@3	DT95	PHS	Heading	Maturity	Grain fill	Height	Yield	Diameter	Hardness	Test weight	Plump	Grain protein	β-Glucan	Soluble protein	S/T	FAN	Extract	AA	DP
Hydration index	1.00																					
SOM	0.39	1.00																				
%Germs@3	0.14	−0.12	1.00																			
DT95	−0.20	0.05	−0.81	1.00																		
PHS	0.16	−0.07	0.31	−0.32	1.00																	
Heading	0.64	0.33	−0.14	0.05	0.06	1.00																
Maturity	−0.36	−0.29	0.13	0.04	−0.10	−0.41	1.00															
Grain fill	−0.64	−0.37	0.15	−0.03	−0.08	−0.96	0.65	1.00														
Height	0.31	0.25	−0.11	−0.02	0.00	0.51	−0.42	−0.55	1.00													
Yield	0.33	0.22	−0.09	0.03	−0.05	0.65	−0.14	−0.59	0.31	1.00												
Diameter	−0.48	−0.39	0.09	−0.02	−0.06	−0.62	0.41	0.65	−0.20	−0.37	1.00											
Hardness	0.59	0.40	−0.31	0.22	−0.11	0.69	−0.33	−0.68	0.37	0.43	−0.65	1.00										
Test Weight	−0.36	−0.24	0.03	0.03	−0.06	−0.40	0.19	0.39	−0.08	−0.22	0.43	−0.37	1.00									
Plump	−0.43	−0.21	0.03	0.01	−0.13	−0.51	0.41	0.55	−0.07	−0.23	0.76	−0.61	0.47	1.00								
Grain protein	0.48	0.29	−0.21	0.06	0.01	0.74	−0.67	−0.82	0.59	0.39	−0.54	0.58	−0.23	−0.39	1.00							
β-Glucan	−0.68	−0.35	−0.12	0.27	−0.17	−0.53	0.41	0.57	−0.23	−0.30	0.57	−0.50	0.35	0.46	−0.43	1.00						
Soluble protein	0.04	−0.16	0.67	−0.64	0.30	−0.42	0.19	0.41	−0.20	−0.31	0.29	−0.48	0.12	0.22	−0.33	−0.14	1.00					
S/T	−0.27	−0.27	0.62	−0.50	0.20	−0.68	0.50	0.73	−0.46	−0.43	0.49	−0.65	0.22	0.34	−0.77	0.16	0.81	1.00				
FAN	0.28	−0.02	0.67	−0.70	0.28	−0.19	0.06	0.18	−0.08	−0.16	0.08	−0.21	0.00	0.00	−0.14	−0.36	0.89	0.64	1.00			
Extract	−0.38	−0.33	0.44	−0.33	0.11	−0.76	0.57	0.81	−0.49	−0.45	0.56	−0.70	0.33	0.45	−0.85	0.26	0.69	0.91	0.48	1.00		
AA	−0.10	−0.14	0.56	−0.50	0.24	−0.49	0.26	0.49	−0.36	−0.35	0.25	−0.41	0.12	0.15	−0.51	−0.20	0.76	0.78	0.71	0.71	1.00	
DP	0.33	0.22	−0.11	−0.03	0.04	0.50	−0.62	−0.61	0.37	0.31	−0.40	0.32	−0.29	−0.28	0.77	−0.45	−0.09	−0.47	0.01	−0.60	−0.23	1.00

Abbreviations: %Germ@3: percent germination 3 days postharvest; AA: α-amylase; DP: diastatic power; DT95: days to 95% germination; FAN: free amino nitrogen; PHS: preharvest sprout; SOM: steep out moisture; S/T: soluble/total protein or Kolbach index.

TABLE 3 | Summary statistics for each chromosome. The parent lines were monomorphic for 4H markers so no map of 4H.

Chromosome	Number of markers	Length (cM)	Average spacing between SNPs	Max spacing between SNPs
1H	55	86	1.6	24
2H	225	160	0.7	26
3H	160	106	0.7	19
5H	104	114	1.1	28
6H	106	64	0.6	22
7H	129	114	0.9	19
Overall	779	644	0.8	28

longest, 6H was the shortest chromosome and 1H contained the fewest SNPs. The average and maximum spacing between SNPs suggest regions of homology occurred across the genome, as well as on 4H (Table 3).

QTL analysis yielded 71 significant QTLs across all 22 measured traits (Table S2). Of these, 27 were related to agronomic characteristics, four to dormancy and 40 to malt quality traits. Many of these QTL were reported previously in similar locations, as summarized in Table S2. Four QTLs (Table 4) were associated with HYI, three being new and one (qHYI3H) in a similar position to a previously mapped QTL on 3H (Jensen et al. 2023). Three of the HYI QTLs cosegregated with other traits (Table 4). The HYI QTL on 1H not only had the largest effect on HYI but also on plant phenology and seed traits, explaining the most variation in heading date, maturity and grain fill as well as seed diameter and hardness. The allele from MT124148 contributed to shorter grain fill due to later heading and earlier maturity, resulting in smaller, harder seeds with faster hydration. The MT124148 allele at 1H was also negatively associated with malt quality traits, including lower extract and S/T but higher DP. The 2H MT124148 allele increased HYI, was associated with earlier heading and was positively associated with modification, thereby increasing AA, FAN, soluble protein and DP while lowering BG. The MT124148 allele at 5H decreased HYI and malt quality as measured by AA, extract, FAN, soluble protein and S/T, while increasing dormancy (DT95 and %germ), PHS. In this study, the positive allele for HYI 3H originated from MT124128 and was not associated with any other trait. Because 1H and 5H QTLs are likely impacting HYI pleiotropically, they were designated qSS1H and qD5H for seed size and dormancy, respectively. The HYI QTL on 2H and 3H appear to be having a more direct effect on HYI, and so are named qHYI2H and qHYI3H.

3.3 | Allele Combinations

The impact of seed size and dormancy on malt quality was further highlighted by PCA (Figure 1). We first examined all the genotypes used in the study, comparing those carrying both the large and small seed alleles for qSS1H (Figure 1A). PC1 explained seed size and most of the malt quality traits, while PC2 coincided with dormancy. To better reveal the variation explained by dormancy, PCA was next performed on the population after removing the large seeded qSS1H genotypes, since no large seeded dormant lines were in the population (Figure 1B). PC1 corresponded to dormancy and malt quality traits often related to modification, extract, BG, FAN, AA, soluble protein and S/T. PC2 explained variation in grain protein and several agronomic and developmental traits clustering based on the *HvNAM1* allele.

The PCAs suggest that HYI can increase with a reduction in seed size (Figure 1A) or a reduction in dormancy (Figure 1B). While poorly correlated (<0.2), the PCA results (Figure 1B) suggest that dormancy has some impact on HYI. To better understand this relationship, we made box and whisker plots comparing haplotypes varying for qD5H, qHYI2H and qHYI3H. Figure 2 reports the interaction of these haplotypes, excluding the large-seeded qSS1H genotypes. HYI increased with each positive allele in both nondormant (AA) and dormant (BB) genotypes, resulting in equivalent HYI between nondormant and dormant genotypes (Figure 2). When carrying both positive HYI alleles, an improvement in malt quality traits, particularly BG, FAN and AA, is observed; however, we do not see improvements in extract. Importantly, increased positive HYI alleles do not increase PHS (Figure 2). Unfortunately, only one allelic combination with the large-seeded allele was recovered, preventing us from interrogating the differential impact of the HYI QTL alleles on seed size and requiring further study.

4 | Discussion

Malting is guided by the Goldilocks Principle, which requires a number of factors to be balanced. Therefore, breeding for malt quality is also a balancing act. While it is tempting to maximize malt yield in the form of extract with larger seeds, large seeds are slower to hydrate, can result in higher BG and will cost the maltster time. Conversely, maximizing the speed of modification can also be costly for a maltster, as the temperature can build too quickly and kill the germinating seed. Maximizing malt readiness reduces dormancy and increases the risk of PHS. Grain protein can be too high slowing modification but can also be too low hurting malt quality through reduced DP and FAN. The current study verifies the importance of balancing traits to achieve the optimum malt quality.

Here, we confirm the industry knowledge that HYI correlates with overall better malt quality. This is not surprising since

TABLE 4 | Hydration index QTLs and all other QTLs that mapped to the same region. Summary statistics for LOD score, percent variance explained (%Var), *p* values based on *F* statistics, estimated effects of MT124148 and the standard error of the effect (SE) all being reported.

QTL name	Trait	Chromosome	Position (cM)	LOD	%Var	*p* (*F*)	Estimated effect	SE
qSS1H	Hydration index	1H	67	21.04	33.84	< 2e-16	15.59	1.33
	SOM	1H	67	5.00	15.58	1.99071e-06	1.35	0.27
	Kernel hardness	1H	67	29.02	65.91	< 2e-16	2.74	1.49
	Kernel diameter	1H	67	11.66	19.77	6.38e-13	−0.081	0.01
	Height	1H	67	5.49	10.23	0.000000843	5.83	0.58
	Heading	1H	67	63.60	35.55	< 2e-16	7.34	0.23
	Maturity	1H	67	3.40	4.63	0.000105	−1.82	0.20
	Length of grain fill	1H	67	74.07	60.88	< 2e-16	−8.82	0.23
	Grain protein	1H	67	33.32	43.50	< 2e-16	1.29	0.077
	S/T	1H	67	22.92	17.71	< 2e-16	−0.93	0.075
	Extract	1H	67	22.89	15.64	< 2e-16	−2.02	0.16
	DP	1H	67	7.22	7.77	2.67e-08	19.83	3.34
qHYI2H	AA	2H	155	5.53	7.06	0.00000077	5.74	1.11
	FAN	2H	155	10.08	6.87	4.97e-11	11.79	1.64
	Heading	2H	157	7.55	1.36	8.65e-09	−1.14	0.185
	S. protein	2H	159	8.05	8.78	2.46e-09	0.246	0.038
	Hydration index	2H	160	5.61	6.81	0.000000646	4.60	0.879
	BG	2H	160	17.59	22.98	< 2e-16	−104.82	13.74
	DP	2H	160	11.14	12.85	3.96e-11	9.25	2.099
qHYI3H	Hydration index	3H	53	7.56	9.50	7.54e-09	−7.32	1.18
qD5H	Hydration index	5H	95	5.73	6.98	0.000000482	−5.84	1.10
	PHS	5H	95	4.40	13.12	8.79e-06	−0.443	0.08
	%Germ@3	5H	95	33.87	68.21	< 2e-16	−14.22	0.85
	DT95	5H	95	29.55	63.20	< 2e-16	15.32	1.01
	Extract	5H	95	17.50	10.80	< 2e-16	−1.49	0.145
	S. protein	5H	95	27.63	43.38	< 2e-16	−0.59	0.04
	S/T	5H	95	27.71	23.48	< 2e-16	−0.97	0.068
	FAN	5H	95	42.34	53.95	< 2e-16	−36.59	1.83
	AA	5H	95	12.48	18.03	1.11e-13	−9.81	1.18

Abbreviations: %Germ@3: percent germination 3 days postharvest; AA: α-amylase; DP: diastatic power; DT95: days to 95% germination; FAN: free amino nitrogen; PHS: preharvest sprout; SOM: steep out moisture; S/T: soluble/total protein or Kolbach index.

water is required for germination. In fact, hydration of the endosperm is a requirement of malt quality since water transports modifying enzymes from the embryo and aleurone into the endosperm. The action of the modifying enzymes degrades cell walls and storage proteins, rendering starch accessible for fermentation. Our study also establishes endosperm hydration

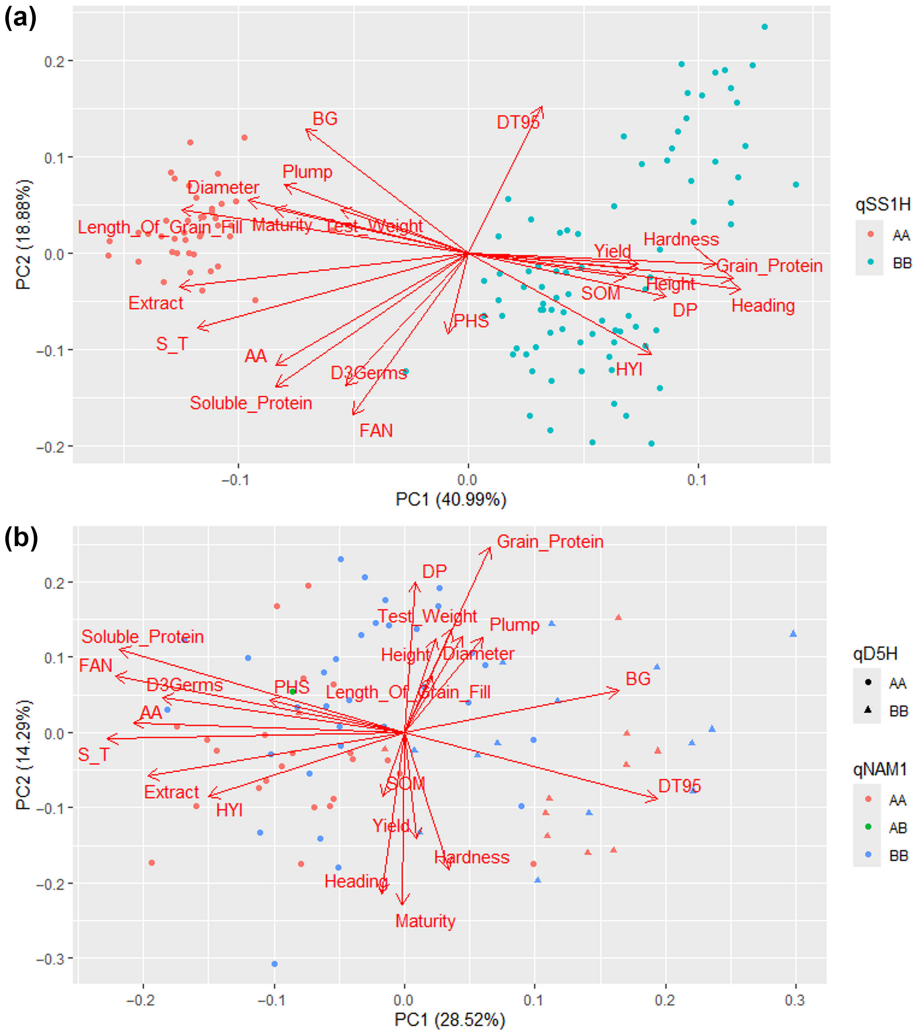

FIGURE 1 | (A) PC analysis of all genotypes and all measured traits. Red points indicate lines with the MT124128 (big seed) allele for qSS1H, while blue points indicate the MT124148 (small seed). Note: PC1 is primarily explained by variation in qSS1H. PC2 is primarily explained by dormancy. (B) PC analysis after removing lines with the 128 allele at qSS1H. Triangles indicate lines with dormant allele at qD5H, while circles indicate nondormant lines. Blue shapes indicate the 128 allele (low protein) at *HvNAM1*, while red indicates the 148 (high protein) allele. Note: PC1 is primarily explained by dormancy and accounts for much of the malt quality variation, while PC2 is partially explained by variation in HvNAM1 and accounting for length of grain fill and grain protein. The perpendicular relationship between PC1 and PC2 indicates a lack of interaction between *HvNAM1* and dormancy, such that *HvNAM1* cannot improve poor malt quality due to dormancy. SOM: steep out moisture; %Germ@3: percent germination 3 days postharvest; DT95: days to 95% germination; PHS: preharvest sprout; S/T: soluble/total protein or Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: diastatic power.

is at least partially under genetic control, explaining 54% of the additive variation across four QTLs for this population. The relationship between endosperm hydration and other traits is well established. For example, endosperm hydration correlates negatively with seed size (−0.48) but positively with several malt quality traits, as has been reported in other studies (Jensen et al. 2023). Conversely, HYI was negatively correlated with hardness (Gamlath et al. 2005; Psota et al. 2007), while here it was positively correlated, likely due to the correlation of hard seed with small seed.

Many of the QTLs reported here were reported previously (Table S2), supporting the validity of our results. Three of the four QTLs associated with HYI (qSS1H, qHYI2H and qD5H) also map with malt quality traits here and in other studies (Fang, Zhang, and Xue 2019; Panozzo et al. 2007; Pauli et al. 2014; Beattie et al. 2010; Mohammadi et al. 2015; Nice et al. 2019; Sweeney et al. 2022; Rooney et al. 2023). Similar findings identified a seed size QTL on 1H, and others have mapped malt quality traits to the same region (Table S2). The dormancy QTL (qD5H) was previously mapped, and the gene was identified as *MKK3* (Ullrich et al. 1992; Nakamura et al. 2016). Others have also negatively associated dormancy with malt quality (Sweeney et al. 2022; Rooney et al. 2023). Albeit qHYI2H was not previously mapped, the region on 2H is a hotspot for malt quality, as reported by a number of studies (Pauli, Brown-Guedira, and Blake 2015; Fang, Zhang, and Xue 2019; Beattie et al. 2010), suggesting endosperm hydration as a potential cause for improved quality. qHYI3H was not tightly linked with malt quality traits in this study; however, a HYI QTL was mapped to a similar location by Jensen et al. (2023). Also, the qHYI3H did impact malt quality traits in the allelic combination comparisons.

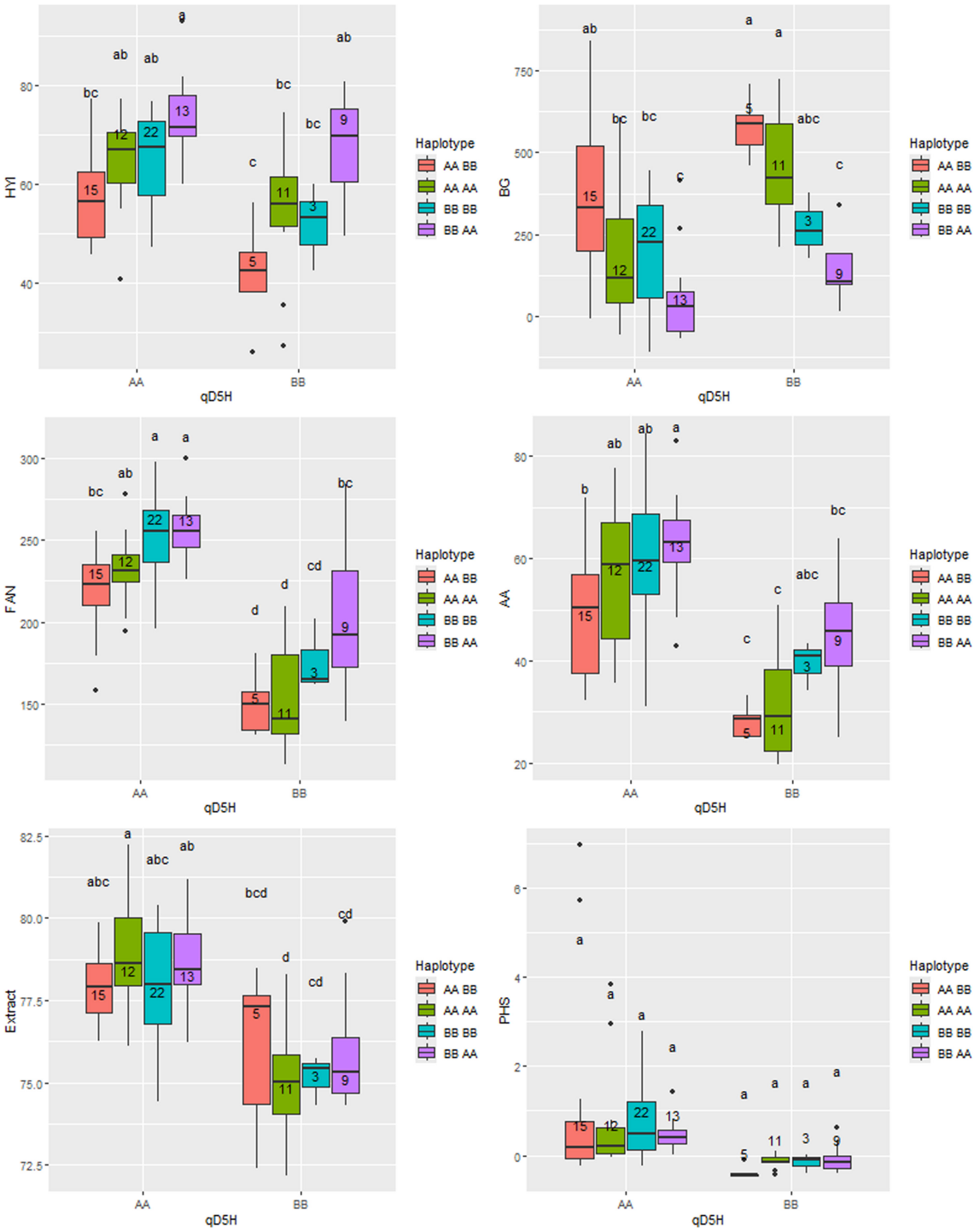

FIGURE 2 | Evaluation of haplotypes for the subset of the population with the small seed allele (BB) for qSS1H. BLUP-corrected averages of traits are reported on the *y*-axis, while the *x*-axis reports dormant (BB) or nondormant (AA) alleles with different combinations of the HYI alleles. The qHYI2H allele is indicated first in the legend and qHYI3H second. In the case of qHYI2H, AA reduces (–) HYI while BB increases (+) HYI, but for qHYI3H, AA increases (+) HYI while BB reduces (–) HYI. The box and whisker plots are distributed with an increasing number of positive HYI alleles from left to right. The value near the mean designates the total number of lines for each haplotype and does not change from plot to plot, while groups with the same letter are not detectably different based on Tukey's HSD test results. HYI: hydration index; BG: β-glucan; FAN: free amino nitrogen; AA: α-amylase; PHS: preharvest sprout damage from α-amylase SD test.

The lack of genetic diversity in this population limits the findings to only the HYI QTLs for which these two parents vary. Another study of a GWAS population with higher genetic diversity identified six HYI QTLs, with only one (qHYI3H) overlapping between this study and the association panel (Jensen et al. 2023). Here, as in Jensen et al. (2023), there is little overlap between SOM and HYI, with SOM only cosegregating with HYI at 1H in the current study. The heritability of SOM was lower than that of HYI in this study, like in Jensen et al. (2023). The lack of correlation between SOM and HYI could explain the lack

of overlap between this study and those previous studies mapping SOM (Cu et al. 2016; Holopainen et al. 2014; Montanuci, Jorge, and Jorge 2013). However, the lack of overlap could also be due to a lack of genetic diversity, especially considering one QTL for SOM previously mapped to 4H, which lacked polymorphisms in this study.

Importantly, this population varied for seed dormancy and qD5H mapped near the well described dormancy gene *MKK3* (Ullrich et al. 1992; Nakamura et al. 2016). Our findings match what others have reported where the dormant allele has a negative effect on quality (Sweeney et al. 2022; Rooney et al. 2023). In this population, HYI was poorly correlated with rate of germination or germination index which Sweeney et al. (2022) suggested could impact malt quality. Our results are the first indication that a dormancy allele (qD5H) can reduce hydration of the endosperm even after dormancy is broken, suggesting one reason dormant lines have poor malt quality. While it is currently unclear how dormancy negatively impacts HYI, reductions in endosperm hydration negatively impact enzyme movement, which results in reduced degradation or poor modification of the cell wall and protein structures (Fox 2009). This, in turn, can explain quality improvements for the dormant lines through the addition of positive HYI alleles (Figure 2 and Table S3). For some traits, the addition of two higher HYI alleles made dormant and non-dormant malt quality equal. The largest QTL for FAN in this population cosegregated with the dormancy QTL, explaining about 54% of the variation with dormant lines having lower FAN. Adding both positive HYI QTLs increases FAN, but on average, dormant lines even with both HYI QTLs have a lower FAN (Figure 2). The cross involving MT124128 × MT124148 was first initiated due to a request from all malt brewers for lower FAN malt. FAN unused by yeast as a nitrogen source and remaining in finished beer can cause off-flavours. Further study is needed to explore the impact on quality and the interactions of the HYI positive alleles identified in this and previous work.

Dormancy at harvest provides resistance to PHS but has been selected against due to the negative correlation with malt quality. Fortuitously, in 2019, rain during harvest exposed this population to PHS. Since this population is segregated for dormancy, this provided the opportunity to evaluate the effectiveness of dormancy and the impact of high HYI on resistance to PHS. Traditional methods of measuring PHS, for example, falling number, are highly labour-intensive. Therefore, we tested the utility of a proxy for PHS with the amount of AA in sprouted grain, where a score of approximately 0.129 was similar to a falling number of 300, the industry cutoff for sound grain and a score between 0.129 and 0.824 (falling number of 300–125) meets the industry standard for moderate pregermination with some risk of loss if not immediately malted and/or stored properly under cold, dry conditions (Mangan et al. 2016). Testing AA in the grain detected one QTL for PHS that mapped to the same SNP as qD5H, confirming the known relationship between dormancy and reduced PHS damage. The qHYI2H and qHYI3H alleles that increased HYI improved the malt quality of dormant lines but did not impact resistance to PHS (Figure 2), making it possible to deploy PHS resistance and meet malt quality standards.

Hydration of the endosperm is impacted by seed size, with larger seed having reduced hydration due to the greater area to be hydrated (Miano and Augusto 2018). The QTL with the largest impact on seed size in this population was qSS1H. The other two seed-size QTLs identified in this population have about half the estimated effect and were not associated with changes in HYI. Wang et al. (2019) also found the qSS1H region to impact seed size and contain the candidate genes *HvCO9*, regulating flowering time in barley, and *HvSMOS1*, producing small grains in rice. Although the big seed allele for qSS1H correlates with low HYI, it is associated with high malt quality and, most importantly, high extract. Therefore, it may be important not to select against seed size to improve HYI because of the decrease in extract. Instead, a breeder could select for larger seed combined with other QTLs that increase HYI. In fact, in this population, all the lines with the large-seeded allele also carried the two fast-hydration alleles and lacked dormancy. Selections were not intentionally made in the creation of this population. However, the haplotypes that would slow germination further (i.e., dormancy and lower HYI alleles) were likely selected against when associated with the largest seed by the inbreeding process. To complete three generations of inbreeding in a year in the greenhouse, we dry seed and then place it in a cold chamber for several days to break dormancy, but time is limited between harvest and planting. F2 and F3 seeds are placed in flats where the slower-to-germinate lines could be shaded out by faster lines, potentially eliminating slower lines from the population. Since this study lacked haplotypes of big-seeded lines varying in dormancy and HYI QTLs, we cannot establish the interactions we have with dormancy. In future work, we hope to create a big-seeded, dormant line with additional HYI QTLs identified in Jensen et al. (2023) to determine if we could have a dormant, large-seeded, fast-hydrating malt barley. We are currently developing markers to empower this selection.

Mutated *HvNAM1*, a nonfunctional NAC transcription factor, increases grain fill by delaying senescence, resulting in increased plumpness and reduced protein, thereby improving malt quality stability (Alptekin et al. 2022). Our population varied for *HvNAM1*, with the desirable Karl allele coming from MT124128. PC analysis indicates *HvNAM1* is associated with PC2 (Figure 1B), explaining plumps and protein consistency with Alptekin et al. (2022). However, dormancy and quality traits (PC1) see little interaction with *HvNAM1* in this figure. Likewise, haplotypes with the positive *HvNAM1* allele do not overcome the negative effects of dormancy unless paired with positive HYI alleles (Table 3). These interactions likely underline why dormancy has been selected against, since not all regions that improve quality balance the negative effects of dormancy, further emphasizing the importance of HYI QTLs for further breeding efforts where dormancy is desired.

Here, we identified two HYI QTLs that can be used to mitigate the negative impacts of large seed size and dormancy on malt quality. We previously identified five other HYI QTLs in a GWAS population (Jensen et al. 2023). An important next step is to determine the best combinations of seed size, dormancy and HYI for different growing regions. Having too many increased HYI QTLs could make malting too fast, increase temperatures and kill grain. However, multiple HYI QTLs could decrease malt time, saving water, time and labour.

Author Contributions

J.J. and J.S. were both responsible for the conceptualization of this study. J.J., H.U., G.L. and J.S. all worked on the methodology of this study with H.U. specifically working on malting and quality analysis while G.L. worked on field management and harvesting of material at Bozeman and K.K. at SARC. Formal analysis for this study was conducted by J.J. and J.L. while further investigation of the data and curation of the data were performed by J.J. X.Y. advised on industry standards for malting requirements. J.J. wrote the original draft of this manuscript, and all authors reviewed and edited it further. J.S. funded this project and was the project administrator.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in endosperm-hydration-vs.-dormancy at https://github.com/jensenjoseph/Endosperm-hydration-vs-dormancy.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.