THE SD1 LOCUS AFFECTS PRIMARY SEED DORMANCY IN WINTER MALTING BARLEY

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# Abstract

Preharvest sprouting(PHS) and seed dormancy are key targets for malting barley(*Hordeum vulgare L.*) in environments with high chances of rain events at harvest. The seed dormancy locus, *SD1* on 5H has been extensively studied in seed dormancy mapping populations, however characterization has been limited in winter malting barley populations. Using a modified half sib winter malting barley breeding population, germination traits and PHS were mapped over eight timepoints to identify genetic association of dormancy loss during the after ripening period. We found that *HvAlaAT* was significantly associated with the rate of primary seed dormancy loss in the winter malting barley population but not associated with PHS susceptibility. Additional QTL from mapping were also identified. The identification ALaAT and other QTL for seed dormancy provide finer precision in selecting for lines that have high PHS resistance but short dormancy periods to produce high quality malt.

# 1 Introduction

Barley(*Hordeum vulgare L*) is grown worldwide for food consumption, animal feed and malt for brewing and distilling. A significant portion of barley acreage in United States and Canada is grown for malting and distilling purposes due to the high premiums compared to animal feed and food consumption. Changes in climate, shifting cropping systems and demand for locally farmed products are pushing the adoption of winter malting barley to be grown in nontraditional environments. Growing barley in novel environments is challenging, as much of the current barley germplasm has been tailored to environments where rain does not occur at harvest and conditions. Selection of malting barley for environments where rain is a frequent threat at harvest requires a delicate balance to select for grain that does not sprout at harvest but can also provide high quality malt within minimal storage time.

Seed dormancy and pre harvest sprouting(PHS) are critical traits for the production of malting barley. Seed dormancy is defined as the inability of a seed to germinate under favorable conditions ([Bewley et al. 2013](#ref-bewley2013)) and developed as an evolutionary mechanism to avoid stress environments. Dormancy in barley is induced during seed development and is influenced by genetic and environmental factors such as temperature, water context, oxygen availability and light([Gubler, Millar, and Jacobsen 2005](#ref-gubler2005)). Seed dormancy is regulated by the ratio of Abscisic acid (ABA) to Giberillic acid(GA) in the barley grain. (Finch-Savage and Leubner-Metzger [2006](#ref-finch-savageSeedDormancyControl2006)) Peak ABA content occurs in the middle of seed development and decreases as physiological maturity approaches Benech-Arnold([2006](#X217593c0e77bf7dc326969337dcb6159ba0b853)). High ABA/GA ratio maintains dormancy and a decreasing amount of ABA to GA over time results in a loss of dormancy ([Gómez-Cadenas et al. 1999](#Xc886f86a13171cb4eeb304f3ac45b93c587c62b); [Piskurewicz et al. 2008](#X7b94c8d9d24d92511a00d56afd2bc4242f4d1d0)) and degrades over a period of time after harvest. The rate of dormancy loss varies widely from days to months based on genotype and environmental conditions during grain fill Rodríguez et al. ([2015](#ref-rodriguezDormancyCerealsNot2015)) Gong et al. ([2014](#ref-gongSeedDormancyBarley2014)) Sweeney et al. ([2021](#ref-sweeney2021_QTL)b). Prolonged seed dormancy is undesirable for malting as germination is uneven, has low vigor and is expensive to store for long periods of time. Barley that has little to no dormancy at physiological maturity is prone to PHS, where wet and humid conditions induce starch degradation and germination in the field before harvest ([Bradford 1995](#Xe49deafe0f2bb39033b5ebd9db7f8a7c2a1371e)) . PHS can be visible in the form of visible radicle and collectible emergence or in pre germination where starch in the grain begins to degrade Li et al. ([2003](#ref-liMajorQTLControlling2003)).

Achieving the balance between PHS resistance and high-quality malt is a key breed target for barley breeding in environments where rain occurs at harvest. Genetic sources of PHS resistance and seed dormancy have been well characterized in barley. The large effect QTL loci of SD1 on chromosome 5H has been mapped extensively in a wide range of barley germplasm. Lin et al. ([2009](#ref-linQTLMappingDormancy2009)) ([Gao et al. 2003](#ref-gaoMolecularDissectionDormancy2003)) have been cloned at each locus: alanine aminotransferase at SD1 HvAlaT1 ([Sato et al. 2016](#Xa619781977bcc33d8759a173a2a841e3d9c5a82)) and a mitogen-actived protein kinase kinase 3 at SD2 HvMKK3 ([Nakamura et al. 2016](#ref-nakamura2016)). Alleles for both AlaAT and MKK3 have been identified by ([Vetch et al. 2020](#ref-vetchMutationsHvMKK3HvAlaAT12020)) for spring and winter barley germplasm. The mutation L214F in AlaAT results in a loss of dormancy and an increased reduction of ABA/GI. ALaAT has been one of the first targets of Cas9-induced mutagenesis in barley prolonging dormancy in mutant types ([Hisano et al. 2022](#ref-hisano2022)) Despite the known effect of AlaAT on the role of long-term primary seed dormancy, little is known about the exact mechanism. ([Wei et al. 2019](#X0ebe6e6e2c674cc3ee45acc5e93d8a19c2c8a1c))

Genome wide associations studies(GWAS) have become the standard practice to identify traits of interest in various types of breeding populations. Advances in barley marker platforms such as the Illumina barley 50K SNP chip ([Bayer et al. 2017](#ref-bayerDevelopmentEvaluationBarley2017)) and the development of improved barley reference genomes ([Mascher et al. 2021](#ref-mascherLongreadSequenceAssembly2021)) have made for higher resolution mapping of relevant malting barley traits. Numerous GWAS methods are available, and improvement to models that account for population structure ([Yu and Buckler 2006](#ref-yuGeneticAssociationMapping2006)) using principal component analysis Price et al. ([2006](#ref-pricePrincipalComponentsAnalysis2006)) and the inclusion of kinship and Q matrix allow for mapping association studies to be conducted across structured populations. Increased computational efficiency and flexibility of models in package “ASRgwas” ([Galli et al. 2022](#ref-ASRgwas2022)) allow for single step analysis GWAS, which includes increased flexibility of terms to be included in the model and also reduces the loss of information that can be associated with two step fixed models ([Möhring and Piepho 2009](#Xd8872a6267b401567dada093beca241c933a74f)) lack of degression using two step random effect models ([Garrick, Taylor, and Fernando 2009](#X92243c07a006347d94051ae9c641cdbb9c2dd5c).)

The objectives of this study aimed to genetically characterize pre-harvest sprouting and seed dormancy loss over time in a connected half sib winter malting barley breeding population. Our goals for this research were to a) Identify QTL for PHS, seed dormancy and seed dormancy loss over time standardizing for physiological maturity b) conduct a single step GWAS of seed dormancy traits over all year and timepoint combinations and c) model seed dormancy loss over time to assess optimal selection for seed dormancy. Understanding the genetic basis for PHS, seed dormancy, and seed dormancy loss over time will be invaluable information for breeders to malting barley that has high PHS resistance while also maintain a short seed dormancy period to ensure high malting quality.

# 2 Methods

## 2.1 Plant populations

The populations used in study were part of the winter malting barley breeding population at Cornell University. Four bi-parental half sibling populations were developed by crossing a common parent ‘Lightning’ ([Hayes et al. 2021](#ref-hayesRegistrationLightningBarley2021)) , a facultative type barley, to four winter malting barley cultivars; ‘KWS Scala’, ‘Flavia’, ‘SY Tepee’ and ‘Wintmalt’. Double haploids were developed from the crosses at Oregon state University using anther culture of seed from each cross ([Cistué et al. 2003](#ref-cistueBarleyAntherCulture2003)) . Double haploid lines were planted in fall 2019 and fall 2020 in two fields each season in Ithaca, NY. Trials for the 2019-2020 growing season were planted in a modified augmented design of single m rows with all parent lines(5), and check ‘Charles’ replicated in blocks across the field. 544 lines were evaluated at each locations. The ratio of checks to experimental lines was 10%. Trials for the 2020-2021 were planted as a preliminary yield trial according to a randomized 480 plot augmented block design of 3 x 1 meter trimmed plots. Parent lines “Lightning” and “KWS Scala” and the line “Endeavor” were used as checks. The ratio of checks to experimental lines was 11%.

## 2.2 Field Phenotyping and sampling

Physiological maturity(PM) was recorded as the date when 50% of the of plot lost green color from the peduncle and spike.([Copeland and Crookston 1985](#Xae1ec155daf9e45d97994ff3abfc0314146d93f)) Two days after PM, bundles of 15-20 selected mature spikes were harvested, dried for 2 days at 38 C, hand threshed, and stored at -20C to pause after-ripening. ([Nagel et al. 2019](#ref-nagelNovelLociRole2019)) The standardization of harvesting and freezing spikes 2 days after the PM of the plot enabled observation of traits at the same physiological state for all lines.

For seed dormancy assays in 2020, a total of 450 lines were sampled from each location. Lines with poor winter survival and poor agronomic quality were excluded. For seed dormancy assays in 2021, the complete trial at Ketola of 435 lines(480 plots) was sampled, and 120 lines at the McGowan location were sampled. PHS was measured by harvesting 5 spikes per headrow at physiological maturity (PM), after-ripening for 3 days, and then misting in a greenhouse for 3 days, after which the spikes were assessed for PHS on a 0 to 9 scale. ([Anderson et al. 1993](#ref-andersonRFLPAnalysisGenomic1993)) Due to labor constraints brought on by the pandemic in the 2020 field season, phenotyping capacity was limited and seed dormancy sampling was prioritized for that year. PHS was phenotyped on a sub-sample of 100 lines from one location. The sub-sample consisted of all facultative types across all four families, parental checks, and Charles. For 2021, all plots from both locations were sampled.

## 2.3 Post harvest germination assay

To measure dormancy loss, all samples were removed from the freezer at the same time, beginning the after-ripening process at the same physiological state for all lines. Samples were stored at ambient room temperature for the duration of the experiment. Germination assays were measured with petri plate assay tests that followed the American Society of Brewing Chemists (ASBC) ([Kuester et al. 1997](#Xab117d8a952fca661189e15a55de9a8d64a2886)) with modifications and subsequent steps followed in Sweeney et al. ([2021](#ref-sweeneyInteractionsBarleySD12021)). The first modification was the use of 30 kernels instead of 100 kernels. The second modification was an extended germination count from 3 days to 5 days in lieu of counting for 3 days and using H2O2 to break dormancy. Germination energy(GE) as a measure of seed dormancy was determined as

(1)

Where corresponds to the the number of germinated kernels at 24, 48, and 72 hours after the start of the assay and is the total number of germinated and ungerminated kernels. Complete seed dormancy loss is defined when GE values per line reach 95% and mean seed dormancy loss across lines reached 90%.

Germination Index was calculated as a germination rate(Frančáková et al. [2012](#ref-francakova2012));

(2)

(3)

where , , were the number of germinated kernels at 24, 48, and 72 hours after the start of the assay. GI was scaled by GE as to account for low germination at earlier timepoints. At later timepoints, was used instead of for the inverse reason of using scaling at earlier timepoints. GE and GI were measured at five time points for 2020: PM5 (TP1), PM19 (TP2), PM47 (TP3), PM96 (TP4), and PM152 (TP5) days post PM. GE and GI measured at eight time points for 2021: PM5(TP1), PM12(TP1.5), PM19 (TP2), PM33(TP2.5), PM47(TP3), PM68 (TP3.5), PM96 (TP4) and PM152(TP5) days post PM.

**Table 1:** Summary statistics for each timepoint, year, combined years, and trait combination used for single timepoint genome wide association studies. Variance components and heritability were estimated by the ASRgwas package.

| **ID** | **trait** | **Year** | **PM datea** | **mean** | **range** | **o2g** | **h2** | **h2\_PEV** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| GE-2020-PM\_5 | GE | 2020 | 5 | 0.347 | 0.00-1.00 | 0.057 | 0.843 | 0.633 |
| GE-2020-PM\_19 | GE | 2020 | 19 | 0.605 | 0.00-1.00 | 0.166 | 0.891 | 0.716 |
| GE-2020-PM\_47 | GE | 2020 | 47 | 0.929 | 0.13-1.00 | 0.010 | 0.561 | 0.607 |
| GE-2020-PM\_96 | GE | 2020 | 96 | 0.988 | 0.77-1.00 | 0.000 | 0.105 | 0.234 |
| GE-2020-PM\_152 | GE | 2020 | 152 | 0.993 | 0.87-1.00 | 0.000 | 0.412 | 0.544 |
| GI-2020-PM\_5 | GI | 2020 | 5 | 1.483 | 0.00-8.82 | 1.137 | 0.852 | 0.637 |
| GI-2020-PM\_19 | GI | 2020 | 19 | 2.865 | 0.00-8.49 | 5.462 | 0.923 | 0.727 |
| GI-2020-PM\_47 | GI | 2020 | 47 | 4.757 | 0.48-10.00 | 2.093 | 0.869 | 0.707 |
| GI-2020-PM\_96 | GI | 2020 | 96 | 5.591 | 3.55-10.00 | 0.324 | 0.649 | 0.635 |
| GI-2020-PM\_152 | GI | 2020 | 152 | 5.685 | 3.56-8.25 | 0.155 | 0.444 | 0.559 |
| GE-2020/2021-PM\_5 | GE | 2020/2021 | 5 | 0.203 | 0.00-1.00 | 0.043 | 0.720 | 0.668 |
| GE-2020/2021-PM\_19 | GE | 2020/2021 | 19 | 0.508 | 0.00-1.00 | 0.113 | 0.649 | 0.433 |
| GE-2020/2021-PM\_47 | GE | 2020/2021 | 47 | 0.753 | 0.00-1.00 | 0.032 | 0.451 | 0.340 |
| GE-2020/2021-PM\_96 | GE | 2020/2021 | 96 | 0.972 | 0.21-1.00 | 0.000 | 0.025 | 0.347 |
| GE-2020/2021-PM\_152 | GE | 2020/2021 | 152 | 0.987 | 0.52-1.00 | 0.000 | 0.141 | 0.015 |
| GI-2020/2021-PM\_5 | GI | 2020/2021 | 5 | 0.854 | 0.00-8.82 | 0.980 | 0.737 | 0.445 |
| GI-2020/2021-PM\_19 | GI | 2020/2021 | 19 | 2.376 | 0.00-8.49 | 3.763 | 0.851 | 0.474 |
| GI-2020/2021-PM\_47 | GI | 2020/2021 | 47 | 3.818 | 0.00-10.00 | 2.382 | 0.741 | 0.433 |
| GI-2020/2021-PM\_96 | GI | 2020/2021 | 96 | 5.356 | 0.89-10.00 | 0.539 | 0.665 | 0.410 |
| GI-2020/2021-PM\_152 | GI | 2020/2021 | 152 | 5.611 | 2.32-9.09 | 0.294 | 0.500 | 0.359 |
| PHS-2020/2021-PM\_3 | PHS | 2020/2021 | 3 | 0.25 | 0-6.4 | 0.175 | 0.372 | 0.593 |
| GE-2021-PM\_5 | GE | 2021 | 5 | 0.094 | 0.00-1.00 | 0.045 | 0.831 | 0.667 |
| GE-2021-PM\_12 | GE | 2021 | 12 | 0.262 | 0.00-1.00 | 0.095 | 0.803 | 0.657 |
| GE-2021-PM\_19 | GE | 2021 | 19 | 0.374 | 0.00-1.00 | 0.162 | 0.868 | 0.680 |
| GE-2021-PM\_33 | GE | 2021 | 33 | 0.447 | 0.00-1.00 | 0.389 | 0.960 | 0.936 |
| GE-2021-PM\_47 | GE | 2021 | 47 | 0.50 | 0.00-1.00 | 0.135 | 0.795 | 0.885 |
| GE-2021-PM\_68 | GE | 2021 | 68 | 0.789 | 0.00-1.00 | 0.351 | 0.958 | 0.790 |
| GE-2021-PM\_96 | GE | 2021 | 96 | 0.949 | 0.21-1.00 | 0.033 | 0.890 | 0.732 |
| GE-2021-PM\_152 | GE | 2021 | 152 | 0.978 | 0.52-1.00 | 0.000 | 0.046 | 0.000 |
| GI-2021-PM\_5 | GI | 2021 | 5 | 0.377 | 0.00-7.25 | 1.294 | 0.864 | 0.749 |
| GI-2021-PM\_12 | GI | 2021 | 12 | 1.127 | 0.00-7.89 | 4.801 | 0.877 | 0.750 |
| GI-2021-PM\_19 | GI | 2021 | 19 | 1.711 | 0.00-7.89 | 4.137 | 0.900 | 0.707 |
| GI-2021-PM\_33 | GI | 2021 | 33 | 2.106 | 0.00-7.56 | 4.669 | 0.892 | 0.704 |
| GI-2021-PM\_47 | GI | 2021 | 47 | 2.476 | 0.00-9.09 | 4.023 | 0.838 | 0.900 |
| GI-2021-PM\_68 | GI | 2021 | 68 | 3.906 | 0.00-8.11 | 0.559 | 0.706 | 0.351 |
| GI-2021-PM\_96 | GI | 2021 | 96 | 5.023 | 0.89-10.00 | 0.516 | 0.812 | 0.667 |
| GI0-2021-PM\_152 | GI0 | 2021 | 152 | 5.624 | 3.55-9.09 | 0.391 | 0.597 | 0.583 |
| *Note:*ID- Unique timepoint trait year combination; PM date: days after physiological maturity; :h2 narrow sense heritability using percent error variances by Cullis et. al 2006 | | | | | | | | |
| atraits of GE-Germination Energy, GI-Germination index adjusted by germination energy, GI0-Germination index unadjusted by GE, and PHS-preharvest sprouting score | | | | | | | | |

Summary statistics for each timepoint, year, combined years, and trait combination used for single timepoint genome wide association studies. Variance components, narrow sense heritability and percent error variance heritability were estimated by the ASRgwas package([Galli et al. 2022](#ref-ASRgwas2022))

## 2.4 Genotyping

The winter malting barley double haploid population was genotyped with the 50K Barley Illumina SNP array ([Bayer et al. 2017](#ref-bayerDevelopmentEvaluationBarley2017)) at the USDA small grains research laboratory in Fargo, ND. Marker positions were based on the Morex version 3 and gene annotations referenced the Morex version 3 assembly ([Mascher et al. 2021](#ref-mascherLongreadSequenceAssembly2021)) and version 2 ([Monat et al. 2019](#X14ff13f1d88fe3af8a9e316ac0563d686c41cae)) Markers were filtered a maximum heterozygosity level of 0.01 and a minimum minor allele frequency of 0.05 using rTASSEL ([Monier et al. 2022](#ref-monierRTASSELInterfaceTASSEL2022)), resulting in a total of 13,452 markers. The R package “SNPRelate” (Zheng et al. 2012) was used to Linkage Disequlibrium (LD) prune markers using a sliding base pair window of 2000 markers and a maximum LD threshold of 0.9. PCA analysis was also conducted with the SNPRelate package. After LD pruning, 9,628 markers remained for analysis. KASP markers were used to genotype *HVAlaAT* and *HvMKK3* following casual mutations discovered in Sato et al. ([2016](#Xa619781977bcc33d8759a173a2a841e3d9c5a82)) and Nakamura et al. ([2016](#ref-nakamura2016)) respectively. Details of KASP marker development can be found in Sweeney et al. ([2021](#ref-sweeneyInteractionsBarleySD12021)a).

## 2.5 Statistical Analysis

Given the advancement in computational efficiency, per time point GWAS was analyzed using a single step approach. The package ASRgwas Galli et al. ([2022](#ref-ASRgwas2022)) was used to develop the K and Q matrices, filter out missing information, and conduct single step GWAS using the raw data as input for each timepoint and trait ASRgwas model flexibility allowed for the integration of fixed, random and residual factor integration. The base model for GWAS per timepoint was as follows:

Where is the response variable for GI, or GE, is the overall mean, is a vector of fixed effects(i.e. Location, replication), is the vector of addivitive genotype effects associated with the genomic additive relationship matrix with , is a matrix of vectors describing population structure and corresponds to the number of vectors(PCAs) of the Q matrix to be included to account for population structure and where is either a incidence matrix corresponding independently and identically distributed residuals or a heteroscadistic error structure. A summary of model terms included for each timepoint is presented in supplementary table 2

KASP markers for AlaAT and MKK3 were ran on the experimental winter double haploid lines, parent lines and checks. Details about the interactions of MKK3, AlaAT in PHS data of spring and winter malting barley trials are examined in more detail in Sweeney et al. ([2021](#ref-sweeney2021_QTL)b). The common parent Lightning and parent Flavia contained the dormant(D) AlaAT allele while parents KWS Scala, SY Tepee and Wintmalt contained the N allele for AlaAT. This resulted in variation for the AlaAT in all families crossed to Lightining except for the Lightning x Flavia family, where lines were monomorphic for AlaAT. All experimental lines and parent genotypes were monomorphic for dormant(D) HvMKK3. The check lines Charles and Endeavor used as a PHS susceptibility and germination check in 2020 and 2021 respectively conatined the highly non-dormant () allele for MKK3 but the D allele for AlaAT Given the low frequency of the N\* MKK3 haplotype represented in this population , we are limited in what inferences can be made regarding the MKK3 loci.

All lines were genotyped with the 50k Illumina Infinium iSelect SNP array at the USDA Small Grains Genotyping Lab in Fargo, ND. After filtering poor quality markers, minor allele frequency (MAF) below 0.05, and monomorphic sites, 15,467 polymorphic markers remained and were used for genome-wide association (GWA). After conducting linkage disequilibrium (LD) pruning to reduce high LD blocks that exist in double haploid populations, 9258 markers were retained for analysis. Models were run for all trait/time point combinations.

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# 3 Results

## 3.1 Phenotypic Distribution

Preharvest sprouting scores presented were averaged across years as there was a lack of sufficient phs data in the 2020 year alone. PHS score distribution was skewed towards resistance(Figure 1). 95% of genotypes were classified as resistant(0 to 2 score), 4.4% were moderately resistant and 1.6% classified as PHS susceptible. Average GE had significant variation based on year as mean GE in 2021 was lower than mean GE at equivalent time points. Heritability for GE was high at early timepoints(0.8) but decreased as primary dormancy loss occurred over time. The slight increase of heritability of GE at later timepoints is most likely attributed to diseased kernels as seeds were not surface sterilized due to population size. GI was initially low(1-1.5) at early timepoints and increased as primary dormancy loss occurred. Unlike GE, GI variation remained after dormancy resulting in higher heritability(0.75) at later timepoints. Similar to GE, there was significantly more dormancy in 2021 compared to 2020, as initial genetic variation and heritability was low for PM5 in 2021. After observed dormancy loss, GI plateaued at a mean GI of 5.6(Figure 2). Phenotypic correlations between GE and GI were significant between all timepoints except for PM152. (Supplementary Table S1). PHS phenotypic correlations were not significant to GE and GI. Genotypic correlations were high for all traits, suggesting shared genetic control.

Chart

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Figure 1— Distribution of mean pre-harvest sprouting scores per genotype for 2020/2021 by AlaAT allele. Shaded regions indicate the classification for pre-harvest sprouting resistance/susceptibility with green indicating high preharvest sprouting resistance(0 to 2), yellow indicating moderate preharvest sprouting resistance(2 to 4) and red indicating preharvest sprouting susceptibility(4 to 9)

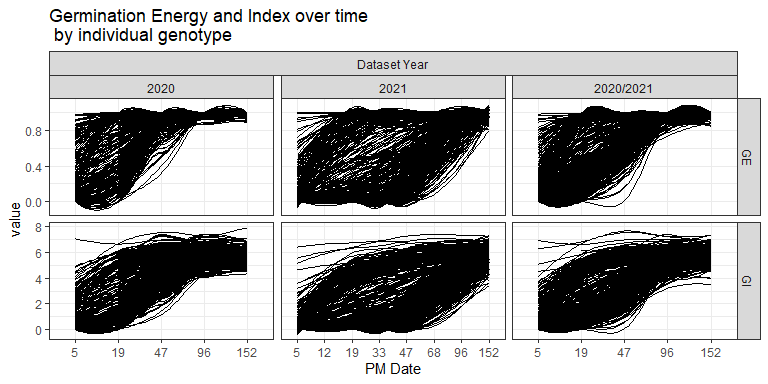


Figure 2- Germination trait values grouped by genotypes across years. GE, Germination Energy; GI, Germination Index: PM Date, Days post physiological maturity; 2020/2021 combined analysis for both years

## 3.2 Genetics

### 3.2.1 KASP marker results

The winter barley population for this experiment was developed by crossing parent Lightning to four malting barley parents of SY Tepee, Scala, Flavia and Wintmalt. KASP marker analysis revealed that the common parent Lightning and parent Flavia contained the dormant “C” allele for AlaAT and parents KWS Scala, SY Tepee and Wintmalt containing the non-dormant “G” allele, making Lightning the donor for the dormant AlaAT. Experimental lines and the parents were monomorphic for the dormant MKK3 allele, a loci identified in spring malting barley to be indicative of high malting quality but also high PHS susceptibility. Check varieties Charles and Endeavor had the highly non dormant allele(N\*) for MKK3. An analysis of different barley haplotypes for AlaAT and MKK3 by Sweeney et al. ([2021](#ref-sweeney2021_QTL)b) found that winter barley haplotypes with the highly N\* MKK3 allele had significantly higher phs scores than any other group, including spring barley N\* MKK3 haplotypes. However, given the low frequency of N\* allele to the D allele for MKK3 in this population, we are limited in what inferences can be made regarding MKK3. Nonetheless, lines Charles and Endeavor served as useful positive controls for PHS and germination throughout the experiment.

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Figure 3: Distribution of germinations over timepoints by AlaAT haplotype across the 2020 and 2021 year. GE: Germination Energy: GI Germination Index; Timepoint- Physiological date after maturity

### 3.2.1 Population structure

Principal component analysis Principal component analysis revealed variation attributed to the first, second and third PCs at 14.55% and 5.69 and 4.9 % respectively. The PCA plot displays the first two PCs on the x and y axis.(Figure 4) The first component separated SY Tepee populations from the Wintmalt, KWS Scala and Flavia populations, with Lightning being a common intermediate between the two parents. Position of genotypes remained largely unchanged when separate recombinant inbred line population of crosses not including Lightning were included(data not shown). Wintmalt, Scala and Flavia families have closer degree of relatedness.

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| Figure 4: Principal component plot of the WMB DH population. Points represent individual accessions. PC1 and PC2 were scaled to zero for interpretation |

### Model fitting using asrGWAS

Single step genome wide association analysis was conducted using the ASRgwas Galli et al. ([2022](#ref-ASRgwas2022)) package across all trait, year, PM date and year combinations(36 analysis). Not all combinations resulted in identified marker trait associations. Fixed effects were identified by wald tests and if models did not converge with reduced inclusion of effects. Within years, significant fixed effects included Location, Replication(Rep) and the spatial dimension of Row.(Supplementary Table S2). In analysis across years, Year was identified as a significant term as either a fixed effect or residual term for all GI trait models and all GE trait models except for GE 2020/2021 at PM date 152. Not all models converged, particularly models for the GE trait as variation was low at early and later timepoints. After accounting for model terms, significant marker traits were identified based on false discovery rate(FDR) adjusted p-values using the R package p-adjust within each GWAS analysis. Traits were considered significant if the FDR adjusted p-value was below 0.05(alpha value. The highest unadjusted p-value that was below the FDR threshold identified in this study was

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| --- | --- | --- |
| |  | | --- | | (a)  Chart  Description automatically generated with low confidence  (b) | | Chart  Description automatically generated  Figure 5 : Manhattan plot summary of marker trait association for germination traits across all chromosomes(a) and chromosome 5(b) across years and timepoints represented in days after physiological maturity. GE, germination energy, GI, germination index; PHS, preharvest sprouting. | |

### Genome wide association analysis results

Using a false discovery rate of alpha=0.05, 48 unique marker trait associations were identified across the genome. Centimorgan(cM) values were used from the genetic consensus mapped developed by Mascher et al. ([2017](#X8a817d4cc6f62daa2f9d671cd9b8311899f41a3)). Given the highly structured breeding populations, the LD in this population was high and markers in LD were reported as single group if r2>0.9 between markers on specific chromosome. A range of the minimum and maximum positions of significant markers within each LD group are presented as we do not have the resolution to determine where in LD blocks the casual variant is located. Six individual marker trait associations and one LD group located on 5H were detected for PHS. For germination traits a total of 48 unique locations were detected across 36 timepoint-trait-year combinations. For GE, three markers were detected on 1H, ten markers identified on 2H, four markers identified on 3H, one LD group identified on 4H, ten markers plus two LD groups on 5H, three markers on 6H and three markers and one LD group on 7H. For GI and GI0, one marker was detected on 1H, two markers were detected on 2H, two LD groups on 4H, nine markers and one LD group detected on 5H, and one LD group detected on 7H.

The region between 435mb to 448 Mb on 5H included the KASP marker AlaAT\_L214F with detected markers explaining 2.63-38.22% PVE for all timepoint and GI, GE, and GI0 combinations except for PM5. The KASP AlaAT was not the highest PVE marker itself, but all 50K SNP in high LD(r=0.95) of AlaAT explained the highest percentage of variance in all GWAS models. An abscisic aldehyde oxidase 3(HORVU.MOREX.r2.5HG0399320) was also identified in this region. Other significant regions include the marker region JHI\_Hv50k\_2016\_310278 to JHI\_Hv50k\_2016\_314246 located in the 462 Mb to 486 Mb region on 5H. The region was associated with PHS and GE but was not associated with AlaAT LD region as the LD was r=0.2.(Supplementary Figure S3) Potential gene candidates in this region include a GRAM containing/ABA-responsive protein a 1-4-alpha glucan branching, and a root meristem growth factor, all associated with seed germination and starch degradation associated with barley germination. Other regions of interest include Dimeric alpha-amylase inhibitor, mitogen activated protein kinase, beta amylase and 1,4- alpha glucan branching enzyme located on 2H, a IAA amino acid hydrolase identified on 3H, and QTL associated with diastatic power(QDp.DiMo-4H) and beta glucan(QBgnm.MT2-4H) identified in Mohammadi et al. ([2015](#X080e289fd9581d602069bb2b273f4b78c073569)) . After accounting for LD blocks, gene annotation from barley genome reference versions 2 and 3 were gathered into a list of all markers 2 Mb upstream and downstream of either single markers or the minimum and maximum of an LD block using modifications of the GALLO package. Lists associated with each marker are documented in supplemental table 4.

# 4 Discussion

Efforts to identify sources of PHS resistance that do not compromise malting quality has been a key target in malting barley breeding, Numerous barley mapping studies have identified dormancy loci such as SD1 and SD2 on 5H and SD3 on 7H, but few have evaluated dormancy loss with extensive germination assays in a winter malting barley breeding population. We found that the *HvAlaAT* at the SD1 locus contributed to significant difference in rate dormancy loss over time in winter malting barley from 5 days(PM5) to 152 days(PM152) after physiological maturity. In addition to the SD1, we found numerous other regions of low to moderate effect associated with dormancy loss, including markers associated with alpha-amylase inhibitors and beta amylase glucan branching enzymes on 2H, a marker two regions for Beta glucan and Diastatic power on 4H, and SD3 on 7H Romagosa et al. ([1999](#ref-romagosaIndividualLocusEffects1999)). Identifying multiple regions associated with dormancy loss provides breeders with a greater ability to select for a desired balance of seed dormancy in target environments.

Observed preharvest sprouting was low in the WMB DH population(Figure 1). Preharvest scoring overlaps at the same post PM range as first timepoint observed in dormancy assays as phs samples are harvested at physiological maturity, after ripened for 4 days and scored 3 days after misting, equivalent to PM 4 when given misting conditions and PM 7 when scored. Despite similar physiological states, the phenotypic correlation of PHS and GE and GI at early timepoints was low(Supplementary Table S1)This is most likely due to the high dormancy observed in 2021 both with PHS and seed dormancy as well as sampling error.

Mean seed dormancy loss occurred between timepoints and in 2020 and between timepoints and in 2021(Table 1)(Figure 2) Genetic variation for GE was initially high in 2020 and signficantly reduced once seed dormancy loss was near complete. Genetic varaition for GE in 2021 increased slightly and decreased once dormancy loss occurred, albeit at later timepoints compared to 2020.GI genetic variation for 2020 and 2021 followed similar rates but mean GI values were higher at earlier timepoints in 2020 to 2021(Table 1). Compared to GI of spring malting barley populations, winter malting barleys were observed to have lower 1 day germination values, even after dormancy loss, which is reflected as a penalty in the calculated GI score and high genetic variation at later timepoints. The low phs incidence, prolonged dormancy and moderate GI values suggests that selection for barley with shorter dormancy times and higher GI should be a priority in winter malting barley.

Genetic correlations between GE, GI and PHS were surprisingly high at early and later timepoints(Table S1). GWAS analysis results of this region suggest some common MTAs associations between GE, GI and PHS but only at a few specific combinations. AlaAT was found to not be associated with this identified region based on statistical analysis of including AlaAT as an effect in the PHS GWAS model and due to the separate observed LD group identified for AlaAT on 5H (Figure S3). PHS and GI are similar measurements as both observe rate of germination over time, whereas GE is a binary observation of seed germination. Marker trait association associated with GE at was in low LD compared to surrounding upstream and downstream markers and most likely was associated with a different factor contributing to GE, such as disease.

## Statistical modeling for GWAS

Fitting single step models can reduce the amount of information compared to using linear mixed models in a two-stage analysis. Often errors associated with the first step analysis of fixed effect models are different, and unless if accounted for with weights in the second stage, results in a loss of information ([Möhring and Piepho 2009](#Xd8872a6267b401567dada093beca241c933a74f)). Using random effects in two stage analysis requires that BLUPS be degressed after the first stage, particularly if genotypes in the population are unbalanced ([Garrick et al. 2009](#X92243c07a006347d94051ae9c641cdbb9c2dd5c)).Fitting single step models for each timepoint produced challenges however, as some models did not converge for some timepoints. (Supplementary Table 2). The lack of convergence issues associated with GE and early and late time points was most likely due low variation of the trait. Other model terms such as location were significant across many combinations, indicating the potential of either field effects or sampling error within locations. Typically, each replication of the seed dormancy timepoint was scored by one individual, and error of Replication may have been associated with scorer error. Fixed effect terms such Location:Row most likely correspond to some form of sampling error in the field. Qsd1 as tested as a fixed effect in model building to determine potential epistatic effects associated with the Qsd1 locus. No novel QTL were identified and QTL that had reduced h2\_PEV in timepoints where the AlaAT locus explained the most variance were detected in different timepoint, trait and year combinations.

## MTA associations

### Role of HvAlaAT (SD1) in long term seed dormancy

The locus SD1 was initially discovered as a seed dormancy QTL in barley by Oberthur et al. ([1995](#ref-oberthurGeneticAnalysisSeed1995)) and has since validated by many researchers (Romagosa et al. [1999](#ref-romagosaIndividualLocusEffects1999)) (Han et al. [1996](#ref-han1996)). HvAlaAT was cloned by [Sato et al. (2016](#Xa619781977bcc33d8759a173a2a841e3d9c5a82)) and sanger sequence by [Vetch et al. (2020](#ref-vetchMutationsHvMKK3HvAlaAT12020)) and Sweeney et al. ([2021](#ref-sweeneyInteractionsBarleySD12021)a). *HvAlaAT* and the *SD1* locus have often been studied in the context of epistatic interactions with *MKK3* of the SD2 locus. This study provided a greater understanding of the gene effect on seed dormancy of winter malting barley conditioned on the dormant allele state of MKK3. AlaAT was detected at the most timepoint, trait and year combinations but was not detected at PM5 or in PHS GWAS. In a target CRISPR-Cas9 mutagenesis experiment [Hisano et al. (2022](#ref-hisano2022)) performed a targeted modification of the L214F casual loci of AlaAT. They found that the knockout of AlaAT was delayed, but did not inhibit germination, which was validated by our results.

### ABI5 and /ABA-responsive protein on 5H

Three potential gene candidates were found in the 5H 462 Mb to 486 Mb LD region based on gene annotations and previous literature. The first includes gene HORVU.MOREX.r3.5HG0486380 identified as *ABI5(Abscisic Acid INSENSITIVE 5)* Seiler et al. ([2014](#ref-seilerAbscisicAcidFlux2014)) Collin et al. ([2020](#ref-collinBarleyABI5Abscisic2020)) in response to drought tolerance and stress. The second includes a includes a GEM GRAM-containing/ABA-responsive protein(HORVU.MOREX.r3.5HG0487770.1) Mauri et al. ([2016](#ref-mauriGEMMemberGRAM2016)) located downstream of asABI5. They found that mutant types of the GEM would delay germination . Mauri et al. ([2016](#ref-mauriGEMMemberGRAM2016)) also discovered that 24 hour cold treatment eliminated the effects of GEM, which could explain why the loci was detected in PHS assays at a higher prevalence than GE and GI assays, as PHS samples did not undergo any cold treatment. Increases in potential ABA content in the 2021 year could also prolonged the effect of this gene in for GI in 2021. Conditions for grainfill in 2021 were dry up until harvest which could have induced higher ABA associated with this gene. A third potential gene related to seed germination identified by gene annotation includes a 1,4-alpha-glucan branching enzyme(GlgB(HORVU.MOREX.r3.5HG0488090.1) identified in morex version 3, a key regulator in amylopectin degradation Sun et al. ([1998](#ref-sunTwoGenesEncoding1998)), Regina et al. ([2010](#ref-reginaControlStarchBranching2010)). Isoamylase HvISA3 (HORVU.MOREX.r2.5HG0404420) of similar function was identified in the same region for morex version 2 Shu and Rasmussen ([2014](#X0c81dccd21fc9f4b865921f2e03d6f9f74b1470)). 1,4-alpha-glucan branching,a key regulator in amylopectin degradation Sun et al. ([1998](#ref-sunTwoGenesEncoding1998)), Regina et al. ([2010](#ref-reginaControlStarchBranching2010)). Isoamylases are importanct for starch degradation as they hydrolyze α-(1,6) glycosidic linkages and debranch amylopectin during grain filling Gous and Fox ([2017](#ref-gousReviewAmylopectinSynthesis2017)). Starch with higher amylose content is hydrolyzed more slowly by amylolytic enzymes and higher amylose content has been hypothesized to be a contributing factor either grain dormancy or delay in germination rate observed by moderate GI values.

### 2H

Three potential candidate genes were identified 160 Mb to 469 Mb region on 2H potentially related to seed dormancy. An alpha-amylase inhibitor ([Mena et al. 1992](#ref-menaMajorBarleyAllergen1992)) (*Bdai-1*)(HORVU.MOREX.r2.2HG0176960) was located in this region and is associated with bakers asthma and with foam retention in beer. The inhibition of alpha amylase could delay the breakdown of starch and potentially delay germination. An activated protein kinase and a 1,4-alpha-glucan-branching enzyme (Regina et al. [2010](#ref-reginaControlStarchBranching2010)) were also identified. Alpha beta-glucan branching enzymes are involved in the breakdown of complex starches.

### 4H

On 4H two marker regions were of potential interest for seed dormancy were found. The first region included another alpha amylase inhibitor as well as a QTL identified for protein content QGpc.StMo-4H ([Mohammadi et al. 2015](#X080e289fd9581d602069bb2b273f4b78c073569)). The second LD region on 4H located on the far distal end of 4H was identified in numerous trait timepoint combinations. The malting quality QTLs QDp.DiMo-4H(Diastatic Power) and QBgnm.MT2-4H(Beta Glucan) Higher diastatic power could result in faster degradation of starches in the barley grain, resulting in barley grains. Variation in beta glucan content could be an indirect indicator of seed dormancy, as higher beta glucan can indicate that some seed dormancy exists. On 7H marker located near the centromeric regions 14595309-387792817 were identified to have a  
beta-amylase(HORVU.MOREX.r2.7HG0614220), ABA responsive binding factor(HORVU.MOREX.r2.7HG0533970), Glucan endo-1,3-beta-glucosidase(HORVU.MOREX.r2.7HG0535140) and protein(HORVU.MOREX.r2.7HG0535020). The region was also identified as SD3 in Romagosa et al. ([1999](#ref-romagosaIndividualLocusEffects1999))

**Table 2** Marker trait associations for preharvest sprouting(PHS), Germination Energy(GE), Germination Index(GI) and unadjusted germination index(GI0) in the WMB DH population

| **Ch** | **SNP** | **Positiona** | **cMab** | **PM date(s)** | **trait(s)c** | **year** | **FDR adjusted p-valuea** | **MAFa** | **PVE** | **Gene candidate(s)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | JHI\_Hv50k\_2016\_14932 | 17847065-17847065 | 30.27 | 5 | GE, GI | 2021 | 0.0003 | 0.469 | 11.739-16.013 |  |
| 1 | SCRI\_RS\_154140, SCRI\_RS\_135092, JHI\_Hv50k\_2016\_38954 | 469434018-470037309 | 67.58 | 96 | GE | 2021 | 0.0003 | 0.464-0.468 | 18.224-21.567 |  |
| 1 | JHI\_Hv50k\_2016\_55371 | 515345160-515345160 |  | 5 | GE | 2021 | 0.0001 | 0.49 | 14.62 |  |
| 2 | JHI\_Hv50k\_2016\_64721 | 9741175-9741175 |  | 33, 19 | GE, GI | 2021 | 0.0002 | 0.411-0.414 | 9.588-18.699 | Protein kinase family protein(HORVU.MOREX.r3.2HG0098680.1) |
| 2 | JHI\_Hv50k\_2016\_75388 | 29443758 | 34.76 | 68 | GE | 2021 | 0.0002 | 0.363 | 16.058 |  |
| 2 | JHI\_Hv50k\_2016\_75392 | 29444702-29444702 |  | 68 | GE | 2021 | 0.0001 | 0.349 | 17.407 |  |
| 2 | SCRI\_RS\_140819 | 29447608-29447608 | 34.765625-34.765625 | 68 | GE | 2021 | 0.0003 | 0.361 | 15.667 |  |
| 2 | JHI\_Hv50k\_2016\_75638 | 30726173-30726173 | 37.109375-37.109375 | 96 | GE | 2021 | 0.0000 | 0.068 | 12.905 |  |
| 2 | JHI\_Hv50k\_2016\_75682 | 30850871-30850871 | 37.109375-37.109375 | 96 | GE | 2021 | 0.0000 | 0.068 | 12.905 |  |
| 2 | JHI\_Hv50k\_2016\_78167, JHI\_Hv50k\_2016\_78425, JHI\_Hv50k\_2016\_78947 | 42266412-45212736 |  | 152 | GE | 2021 | 0.0000 | 0.062-0.076 | 3.103-3.674 | Anthocyanin 3-O-betaglucosyltransferase  (HORVU.MOREX.r3.2HG0113240.1) |
| 2 | SCRI\_RS\_135633, JHI\_Hv50k\_2016\_90999, SCRI\_RS\_132839 | 160022796-469196911 |  | 96 | GE, GI | 2020, 2020/2021, 2021 | 0.0003 | 0.179-0.339 | 1.439-26.835 | Dimeric alpha-amylase inhibitor(HORVU.MOREX.r2.2HG0176960),  mitogen-activated protein kinase 1(HORVU.MOREX.r3.2HG0135700.1),  Beta-amylase(HORVU.MOREX.r3.2HG0138420.1),  1,4-alpha-glucan-branching enzyme(HORVU.MOREX.r3.2HG0165780.1) |
| 2 | JHI\_Hv50k\_2016\_141424 | 664208343-664208343 |  | 33 | GE | 2021 | 0.0003 | 0.341 | 14.698 |  |
| 3 | SCRI\_RS\_154973, JHI\_Hv50k\_2016\_164292, JHI\_Hv50k\_2016\_164365 | 37347178-38198969 |  | 5 | GE | 2020 | 0.0003 | 0.314-0.321 | 13.073-13.646 |  |
| 3 | SCRI\_RS\_16934 | 460169870-460169870 | 49.0234375-49.0234375 | 33, 47 | GE | 2021 | 0.0002 | 0.083 | 6.165-12.478 | IAA-amino acid hydrolase ILR1,putative(HORVU.MOREX.r2.3HG0235350) |
| 3 | JHI\_Hv50k\_2016\_197534 | 538591368-538591368 | 66.40625-66.40625 | 68 | GE | 2021 | 0.0000 | 0.161 | 17.139 |  |
| 3 | SCRI\_RS\_198609 | 538592488-538592488 | 66.40625-66.40625 | 68 | GE | 2021 | 0.0000 | 0.161 | 13.118 |  |
| 4 | JHI\_Hv50k\_2016\_262688, JHI\_Hv50k\_2016\_262688, JHI\_Hv50k\_2016\_262937 | 588576545-589392408 |  | 96 | GI | 2021 | 0.0000 | 0.438-0.462 | 9.935-12.202 | Alpha amylase inhibitor protein(HORVU.MOREX.r3.4HG0409620.1)  .Alpha-1,4-glucan-protein synthase [UDP-forming] 1(  HORVU.MOREX.r3.4HG0409970.1), QGpc.StMo-4H(Protein Content) |
| 4 | JHI\_Hv50k\_2016\_275586, JHI\_Hv50k\_2016\_275686, JHI\_Hv50k\_2016\_276209 | 621542632-623890428 |  | 68, 152, 47, 12, 19, 33 | GI, GI0, GE | 2021, 2020/2021, 2020 | 0.0003 | 0.466-0.487 | 4.873-11.893 | QDp.DiMo-4H(Diastatic Power)/QBgnm.MT2-4H(Beta Glucan) |
| 5 | JHI\_Hv50k\_2016\_282096 | 10610153-10610153 |  | 33, 47 | GE, GI | 2021 | 0.0004 | 0.116-0.117 | 6.723-16.454 |  |
| 5 | JHI\_Hv50k\_2016\_282745 | 11905816-11905816 | 35.546875-35.546875 | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 |  |
| 5 | JHI\_Hv50k\_2016\_282781 | 11975993-11975993 |  | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 | Aminotransferase-like,  plant mobile domain-containing protein(HORVU.MOREX.r3.5HG0425960.1) |
| 5 | JHI\_Hv50k\_2016\_282768 | 11978422-11978422 |  | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 | Aminotransferase-like,  plant mobile domain-containing protein(HORVU.MOREX.r3.5HG0425960.1) |
| 5 | JHI\_Hv50k\_2016\_283159 | 14264799 |  | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 | Aminotransferase-like,  plant mobile domain-containing protein(HORVU.MOREX.r3.5HG0425960.1) |
| 5 | SCRI\_RS\_136706 | 14267092 |  | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 | Aminotransferase-like,  plant mobile domain-containing protein(HORVU.MOREX.r3.5HG0425960.1) |
| 5 | JHI\_Hv50k\_2016\_283231, SCRI\_RS\_144042, JHI\_Hv50k\_2016\_283898 | 14268330-16611284 |  | 96 | GI | 2020/2021 | 0.0003 | 0.173 | 7.593 |  |
| 5 | JHI\_Hv50k\_2016\_283903 | 16611510- |  | 47, 152 | GE, GI | 2021 | 0.0004 | 0.096-0.097 | 2.45-8.109 |  |
| 5 | JHI\_Hv50k\_2016\_283960 | 16616554-16616554 |  | 96 | GI | 2020/2021 | 0.0003 | 0.173 | 7.593 |  |
| 5 | JHI\_Hv50k\_2016\_285061 | 20461364-20461364 | 42.1875-42.1875 | 33 | GE | 2021 | 0.0002 | 0.324 | 18.232 |  |
| 5 | AlaAT\_L214F, JHI\_Hv50k\_2016\_308790, JHI\_Hv50k\_2016\_308899 | 435636564-448089400 |  | 19, 47, 152, 33, 96, 12, 68 | GE, GI, GI0 | 2020, 2020/2021, 2021 | 0.0003 | 0.26-0.355 | 2.637-38.211 | ***HvAlaAT*-**Aminotransferase(HORVU.MOREX.r3.5HG0481320.1),abscisic aldehyde oxidase 3(HORVU.MOREX.r2.5HG0399320) |
| 5 | JHI\_Hv50k\_2016\_310278, JHI\_Hv50k\_2016\_312229, JHI\_Hv50k\_2016\_314246 | 462656156-486764914 |  | 3, 152 | PHS, GE | 2020/2021, 2020 | 0.0002 | 0.08-0.101 | 4.27-10.666 | GRAM-containing/ABA-responsive protein  (HORVU.MOREX.r3.5HG0487770.1),  1,4-alpha-glucan branching enzymeGlgB  (HORVU.MOREX.r3.5HG0488090.1)  root meristem growth factor  (HORVU.MOREX.r3.5HG0486840.1),  ***ABI5***  ( AbscisicAcid INSENSITIVE 5)  HORVU.MOREX.r3.5HG048638 |
| 5 | JHI\_Hv50k\_2016\_311161 | 470081584-470081584 |  | 5 | GI | 2020/2021 | 0.0002 | 0.303 | 6.506 | root meristem growth factor(HORVU.MOREX.r2.5HG0403260 |
| 5 | JHI\_Hv50k\_2016\_311178 | 470514378-470514378 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.088 | 7.384 |  |
| 5 | BOPA1\_9745\_628 | 470515911-470515911 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.088 | 7.384 |  |
| 5 | JHI\_Hv50k\_2016\_314183, JHI\_Hv50k\_2016\_314454, JHI\_Hv50k\_2016\_316395 | 487301428-493098639 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.081-0.088 | 8.571-8.727 |  |
| 5 | JHI\_Hv50k\_2016\_314406 | 487385179-487385179 |  | 152 | GE | 2021 | 0.0002 | 0.145 | 3.13 |  |
| 5 | JHI\_Hv50k\_2016\_316548 | 493263818-493263818 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.088 | 8.571 |  |
| 5 | JHI\_Hv50k\_2016\_316787 | 493829674-493829674 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.088 | 8.571 |  |
| 5 | JHI\_Hv50k\_2016\_316813 | 494501277-494501277 |  | 3 | PHS | 2020/2021 | 0.0000 | 0.088 | 8.571 |  |
| 5 | JHI\_Hv50k\_2016\_323633 | 515253612-515253612 |  | 68 | GE | 2021 | 0.0003 | 0.189 | 12.802 |  |
| 6 | JHI\_Hv50k\_2016\_372982 | 9705929-9705929 |  | 96 | GE | 2021 | 0.0000 | 0.414 | 20.523 |  |
| 6 | BOPA1\_5993\_2383 | 9712203-9712203 |  | 96 | GE | 2021 | 0.0000 | 0.414 | 20.523 |  |
| 6 | JHI\_Hv50k\_2016\_373096 | 10463350-10463350 |  | 96 | GE | 2021 | 0.0000 | 0.414 | 20.523 |  |
| 7 | JHI\_Hv50k\_2016\_450348 | 14595309-14595309 |  | 19 | GI | 2020 | 0.0003 | 0.395 | 14.163 | beta-amylase  (HORVU.MOREX.r2.7HG0614220)  ABA responsive binding factor  (HORVU.MOREX.r2.7HG0533970)  Glucan\_endo-1,3-beta-glucosidase,putative  (HORVU.MOREX.r2.7HG0535140)  Seed maturation protein  (HORVU.MOREX.r2.7HG0535020) |
| 7 | JHI\_Hv50k\_2016\_483509 | 387792817-387792817 | 57.421875-57.421875 | 33 | GE | 2021 | 0.0002 | 0.1 | 16.564 | beta-amylase(HORVU.MOREX.r2.7HG0614220)/ABA responsive binding factor  (HORVU.MOREX.r2.7HG0533970)  Glucan\_endo-1,3-beta-glucosidase ,putative  (HORVU.MOREX.r2.7HG0535140)  Seed\_maturation protein  (HORVU.MOREX.r2.7HG0535020) |
| 7 | JHI\_Hv50k\_2016\_486280 | 429860543-429860543 | 57.421875-57.421875 | 19 | GE | 2020/2021 | 0.0002 | 0.077 | 1.886 | Endoglucanase  (HORVU.MOREX.r2.7HG0584740) |
| 7 | JHI\_Hv50k\_2016\_498356 | 589591547-589591547 |  | 96 | GE | 2021 | 0.0002 | 0.317 | 14.711 |  |
| 7 | JHI\_Hv50k\_2016\_507218, JHI\_Hv50k\_2016\_507370, JHI\_Hv50k\_2016\_508103 | 610941346-613874053 |  | 5, 19 | GE, GI | 2020 | 0.0004 | 0.19-0.247 | 5.446-13.965 | beta glucosidase 17  (HORVU.MOREX.r2.7HG0609800) |
| *Note:* Chr: Chromosome(H); SNP-Single nucleotide polymorphism; cM-centiMorgans; PM date(s)-Days since Physiological Maturity timepoints; FDR-False discovery rate; MAF- minor allele frequency; PVE- Percent variance explained | | | | | | | | | | |
| aA range of values indicates that multiple markers(more than 3) were included in this region. The first, median and last marker of the region are included in the SNP column | | | | | | | | | | |
| bcM based on nearest marker identified in Morex x Steptoe consesus map in Manscher et al. 2017 | | | | | | | | | | |
| cGE: Germination Energy;GI: Germination Index; GI0: unadjusted germination index; PHS; preharvest sprouting | | | | | | | | | | |

### Selection of seed dormancy

For a barley variety to malt well, seed dormancy loss needs to be complete before it reaches the malt house. In our seed dormancy analysis, we found that the mean dormancy loss occurred at PM45 to PM67 in 2020 and PM67 to PM96 in 2021. If we assume that winter malting barley harvested does not reach the malthouse until 60 to 90 days after harvest, most lines in this experiment would have complete dormancy loss by the malting stage. Germination percentage(GE) however, is not equivalent to germination index and the rate at which barley kernels germinate is another important consideration for malting. Many experimental lines observed in this population reached peak GI of 5.5 compared to Charles, Endeavor and KWS Scala GI values in the ranges of 7 to 9. Initial germination delay could be caused by a number of previously described genes. Future efforts in selection for malting barley should focus on higher germination index once dormancy loss is complete.

**PHS and seed dormancy considerations**

The observed dormancy for most non dormant haplotypes at earlier post physiological maturity (PM) days and low PHS correlations with the AlaAT haplotype is encouraging in determining the balance of high PHS resistance but minimal seed dormancy. In an analysis of the effect of weather covariates on PHS susceptibility, Sweeney et al. ([2021](#ref-sweeney2021_QTL)b) found that winter malting barley PHS were highly sensitive to increases in temperature during grain fill, but this was conditioned on varying states of the MKK3\* allele. In the context of dormant MKK3 haplotypes, PHS scores were low(0 to 2) to moderate(2 to 4). This was a similar trend observed in our population(Figure 1). While more observations of PHS of winter malting barley lines are needed, particularly in years where conditions during grainfill would results in less seed dormancy, we are cautiously optimistic that we can select for less dormancy in this winter malting barley population while still maintaining strong PHS resistance.

**Improvement of seed dormancy assays**

Dormancy assays are currently limited throughput, as many considerations were made to balance the statistical power effectiveness and allocation of labor to maximize observation of dormancy loss over time. Imaging technologies have the potential to recognize barley kernels, map their location in a petri plate, and potentially measure the kernel rate of growth over time. A fine tuning measurement of the germination index or rate could provide more insights to the critical three day period of barley germination

# Conclusion

Results from single timepoint GWAS analysis combined with KASP markers validate the significant effect of *HvAlaAT* in primary seed dormancy of a winter malting barley population. *HvAlaAT* was observed to significantly affect germination rate, germination energy and seed dormancy length from 12 days post physiological maturity to complete dormancy loss and that the growing year environment had a significant effect on seed dormancy. Genetic regions in high LD with *HvAlaAT* KASP marker explained the most variance for seed dormancy traits and were observed at most timepoint trait combinations. In addition to *HvAlaAT,* we also found a distinct LD region on 5H associated with GE and PHS. *HvAlaAT*  was not found to affect PHS susceptibility in the winter malting breeding population. Understanding the role of seed dormancy loss in a winter malting barley breeding population allows more informed selection decisions to reduce seed dormancy negatively associated with malting quality while also maintaining strong PHS resistance in the field.

### Supplementary info

**Table S1:** Correlation table for 2020-2021 analysis

| **Variable** | **PHS** | **GE, PM5** | **GE, PM12 a** | | **GE, PM19** | **GE, PM33a** | **GE, PM47** | **GE, PM68a** | **GE, PM96** | | **GE, PM152** | | **GI, PM 5** | **GI,PM 12a** | **GI, PM 19** | | **GI, PM 33a** | **GI, PM 47** | **GI, PM 68a** | **GI, PM96** | **GI, PM152** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PHS** |  | 0.62\* | | 0.67\* | 0.6\* | 0.69\* | 0.65\* | 0.43\* | | 0.14 | | -0.57\* | 0.64\* | 0.54\* | 0.59\* | 0.59\* | | 0.63\* | 0.44\* | 0.12 | -0.61\* |
| **GE, PM5** | 0.13 |  | | 0.77\* | 0.78\* | 0.76\* | 0.77\* | 0.57\* | | 0.33\* | | -0.39\* | 0.99\* | 0.74\* | 0.79\* | 0.76\* | | 0.77\* | 0.53\* | 0.25\* | -0.17\* |
| **GE, PM12** | -0.09 | 0.86\* | |  | 0.76\* | 0.98\* | 0.79\* | 0.56\* | | 0.32\* | | -0.36\* | 0.78\* | 0.72\* | 0.78\* | 0.81\* | | 0.79\* | 0.59\* | 0.25\* | -0.24\* |
| **GE, PM19** | -0.22 | 0.79\* | | 0.97\* |  | 0.81\* | 0.93\* | 0.77\* | | 0.26\* | | -0.39\* | 0.77\* | 0.77\* | 0.99\* | 0.91\* | | 0.94\* | 0.61\* | 0.33\* | -0.22\* |
| **GE, PM33** | -0.23 | 0.74\* | | 0.96\* | 0.98\* |  | 0.84\* | 0.62\* | | 0.34\* | | -0.37\* | 0.77\* | 0.74\* | 0.83\* | 0.86\* | | 0.84\* | 0.62\* | 0.31\* | -0.26\* |
| **GE, PM47** | -0.28 | 0.76\* | | 0.95\* | 0.97\* | 0.98\* |  | 0.81\* | | 0.3\* | | -0.41\* | 0.77\* | 0.74\* | 0.93\* | 0.91\* | | 0.99\* | 0.65\* | 0.42\* | -0.26\* |
| **GE, PM68** | -0.51\* | 0.34 | | 0.65\* | 0.76\* | 0.82\* | 0.82\* |  | | 0.36\* | | -0.13 | 0.57\* | 0.6\* | 0.76\* | 0.76\* | | 0.81\* | 0.68\* | 0.64\* | -0.09 |
| **GE, PM96** | -0.59\* | -0.28 | | -0.07 | 0.04 | 0.14 | 0.14 | 0.62\* | |  | | 0.36\* | 0.31\* | 0.24\* | 0.27\* | 0.3\* | | 0.27\* | 0.07 | 0.64\* | 0.34\* |
| **GE, PM152** | -0.55\* | -0.79\* | | -0.71\* | -0.61\* | -0.6\* | -0.55\* | -0.23 | | 0.25 | |  | -0.38\* | -0.26\* | -0.37\* | -0.28\* | | -0.41\* | -0.37\* | 0.34\* | 0.79\* |
| **GI,**  **PM5** | 0.13 | 1\* | | 0.86\* | 0.79\* | 0.74\* | 0.76\* | 0.34 | | -0.28 | | -0.79\* |  | 0.78\* | 0.78\* | 0.77\* | | 0.77\* | 0.53\* | 0.24\* | -0.17\* |
| **GI, PM12** | -0.09 | 0.85\* | | 1\* | 0.97\* | 0.96\* | 0.95\* | 0.66\* | | -0.06 | | -0.71\* | 0.85\* |  | 0.79\* | 0.88\* | | 0.75\* | 0.5\* | 0.24\* | -0.16 |
| **GI, PM19** | -0.19 | 0.81\* | | 0.97\* | 1\* | 0.98\* | 0.97\* | 0.75\* | | 0.01 | | -0.63\* | 0.81\* | 0.97\* |  | 0.93\* | | 0.94\* | 0.61\* | 0.33\* | -0.2\* |
| **GI, PM33** | -0.22 | 0.76\* | | 0.97\* | 0.99\* | 1\* | 0.98\* | 0.8\* | | 0.12 | | -0.62\* | 0.76\* | 0.97\* | 0.98\* |  | | 0.92\* | 0.6\* | 0.36\* | -0.16 |
| **GI, PM47** | -0.25 | 0.77\* | | 0.95\* | 0.99\* | 0.98\* | 0.99\* | 0.81\* | | 0.1 | | -0.57\* | 0.77\* | 0.95\* | 0.99\* | 0.99\* | |  | 0.67\* | 0.41\* | -0.25\* |
| **GI, PM68** | -0.46 | 0.44 | | 0.74\* | 0.84\* | 0.88\* | 0.88\* | 0.99\* | | 0.51\* | | -0.32 | 0.44 | 0.75\* | 0.83\* | 0.87\* | | 0.88\* |  | 0.38\* | -0.27\* |
| **GI, PM96** | -0.56\* | 0.31 | | 0.55\* | 0.71\* | 0.72\* | 0.74\* | 0.93\* | | 0.55\* | | -0.12 | 0.31 | 0.56\* | 0.7\* | 0.71\* | | 0.76\* | 0.93\* |  | 0.37\* |
| **GI, PM152** | -0.61\* | 0.04 | | 0.3 | 0.48\* | 0.47 | 0.49\* | 0.77\* | | 0.5\* | | 0.12 | 0.04 | 0.31 | 0.47 | 0.46 | | 0.53\* | 0.76\* | 0.9\* |  |
| *Note:*phs, preharvest sprouting; GE Germination Energy; GI Germination Index; PM Physiological maturity date. Lower diagonal corresponds to phenotypic correlation and the upper diagonal corresponds to genotypic correlations.   \* Significant at *p < 0.05* using a pearson correlation | | | | | | | | | | | | | | | | | | | | | |
| aTimepoints were only measured in 2021 | | | | | | | | | | | | | | | | | | | | | |

**Supplementary table S2.** Summary of the fixed and residual model terms fitted at each timepoint across germination energy, germination index, and preharvest sprouting for genome wide association modeling**.**

| **traita** | **year** | **PM date** | **Q matrix?** | **PCs** | **Fixed effect term(s)b** | **Residual termsb** | **Converged?** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| GE | 2020 | 5 | yes | 2 | Location |  | yes |
| GE | 2020 | 19 | yes | 2 | Location | Location | yes |
| GE | 2020 | 47 | yes | 2 | Location:Rep + Location + Rep |  | yes |
| GE | 2020 | 96 | yes | 2 | Location |  | no |
| GE | 2020 | 152 | yes | 2 | Location |  | yes |
| GI | 2020 | 5 | yes | 2 | Location + Rep |  | yes |
| GI | 2020 | 19 | yes | 2 | Location | Location | yes |
| GI | 2020 | 47 | yes | 2 |  |  | yes |
| GI | 2020 | 96 | yes | 2 |  |  | yes |
| GI | 2020 | 152 | yes | 2 |  |  | yes |
| GE | 2020/2021 | 5 | yes | 2 | Year+ Year:Location |  | no |
| GE | 2020/2021 | 19 | yes | 2 |  | Year | yes |
| GE | 2020/2021 | 47 | yes | 2 | Year:Location + Location |  | yes |
| GE | 2020/2021 | 96 | yes | 2 | Year:Location |  | yes |
| GE | 2020/2021 | 152 | yes | 2 |  |  | yes |
| GI | 2020/2021 | 5 | yes | 2 | Year:Location | Year | yes |
| GI | 2020/2021 | 19 | yes | 2 | Location:Year |  | yes |
| GI | 2020/2021 | 47 | yes | 2 | Year:Rep | Year | yes |
| GI | 2020/2021 | 96 | yes | 2 | Year + Year:Rep |  | yes |
| GI | 2020/2021 | 152 | yes | 2 | Year + Year:replication |  | yes |
| PHS | 2020/2021 | 7 | yes | 2 | Year+ Year:Location |  | yes |
| GE | 2021 | 5 | yes | 2 |  | Location | no |
| GE | 2021 | 19 | yes | 2 |  | Rep | yes |
| GE | 2021 | 22 | yes | 2 |  |  | yes |
| GE | 2021 | 33 | yes | 2 | replication:Row |  | yes |
| GE | 2021 | 47 | yes | 2 |  |  | yes |
| GE | 2021 | 68 | yes | 2 | Location:Row + Row |  | yes |
| GE | 2021 | 96 | yes | 2 | Location:Row | Location | no |
| GE | 2021 | 152 | yes | 2 | Location:Row |  | no |
| GI | 2021 | 5 | yes | 2 |  | Location | yes |
| GI | 2021 | 19 | yes | 2 |  | Rep | yes |
| GI | 2021 | 22 | yes | 2 |  |  | yes |
| GI | 2021 | 33 | yes | 2 | Rep |  | yes |
| GI | 2021 | 47 | yes | 2 |  |  | yes |
| GI | 2021 | 68 | yes | 2 | Location |  | yes |
| GI 0 | 2021 | 96 | yes | 2 | Location:Row + replication | Row | yes |
| GI 0 | 2021 | 152 | yes | 2 | Location |  | yes |
| *Note:*PM date: days after physiological maturity; PCs: Number of Principal components included in the model | | | | | | | |
| aGE: Germination Energy, GI: Germination index, GI 0:unadjusted germination index, PHS: Preharvest sprouting | | | | | | | |
| bRep:Replication of dormancy assay measured within each timepoint; Row: spatial effect of Row in the field | | | | | | | |

Chart

Description automatically generated

**Supplementary Figure S3:** Linkage disequilibrium(LD) of significant marker trait associations on barley chromosome 5. At least three distinct LD blocks were identified and are highlighted in red

Anderson, James A., Mark E. Sorrells, and Steven D. Tanksley. 1993. “RFLP Analysis of Genomic Regions Associated with Resistance to Preharvest Sprouting in Wheat.” *Crop Science* 33 (3): cropsci1993.0011183X003300030008x. <https://doi.org/10.2135/cropsci1993.0011183X003300030008x>.

Bayer, Micha M., Paulo Rapazote-Flores, Martin Ganal, Pete E. Hedley, Malcolm Macaulay, Jörg Plieske, Luke Ramsay, et al. 2017. “Development and Evaluation of a Barley 50k iSelect SNP Array.” *Frontiers in Plant Science* 8. <https://www.frontiersin.org/articles/10.3389/fpls.2017.01792>.

Benech-Arnold, Roberto L., Nicolas Gualano, Juliette Leymarie, Daniel Côme, and Françoise Corbineau. 2006. “Hypoxia Interferes with ABA Metabolism and Increases ABA Sensitivity in Embryos of Dormant Barley Grains.” *Journal of Experimental Botany* 57 (6): 1423–30. <https://doi.org/10.1093/jxb/erj122>.

Bewley, J. Derek, Kent J. Bradford, Henk W. M. Hilhorst, and Hiro Nonogaki. 2013. “Dormancy and the Control of Germination.” In, edited by J. Derek Bewley, Kent J. Bradford, Henk W. M. Hilhorst, and Hiro Nonogaki, 247–97. New York, NY: Springer. <https://doi.org/10.1007/978-1-4614-4693-4_6>.

Bradford, Kent J. 1995. “Seeds: Physiology of Development and Germination J. D. Bewley and M. Black, Xv + 445 Pp. Second Edition. Plenum Press, New York, London, 1994. ISBN 0-306-44747-9 (Hardbound) 39.50.” *Seed Science Research* 5 (2): 127–28. <https://doi.org/10.1017/S0960258500002713>.

Cistué, L., M. P. Vallés, B. Echávarri, J. M. Sanz, and A. Castillo. 2003. “Barley Anther Culture.” In, edited by M. Maluszynski, K. J. Kasha, B. P. Forster, and I. Szarejko, 29–34. Dordrecht: Springer Netherlands. <https://doi.org/10.1007/978-94-017-1293-4_5>.

Collin, Anna, Agata Daszkowska-Golec, Marzena Kurowska, and Iwona Szarejko. 2020. “Barley ABI5 (Abscisic Acid INSENSITIVE 5) Is Involved in Abscisic Acid-Dependent Drought Response.” *Frontiers in Plant Science* 11. <https://www.frontiersin.org/articles/10.3389/fpls.2020.01138>.

Copeland, Philip J., and R. Kent Crookston. 1985. “Visible Indicators of Phsiological Maturity Barley1.” *Crop Science* 25 (5): cropsci1985.0011183X002500050028x. <https://doi.org/10.2135/cropsci1985.0011183X002500050028x>.

Finch-Savage, William E., and Gerhard Leubner-Metzger. 2006. “Seed Dormancy and the Control of Germination.” *New Phytologist* 171 (3): 501–23. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>.

Frančáková, Helena, Miriam Líšková, Tatiana Bojňanská, and Ján Mareček. 2012. “Germination Index as an Indicator of Malting Potential.” *Czech Journal of Food Sciences* 30 (4): 377–84. <https://doi.org/10.17221/314/2010-CJFS>.

Galli, Giovanni, Salvador A. Gezan, Didier A. Murillo, and Darren Murray. 2022. *ASRgwas: An r Package to Perform Complex Genome-Wide Association Studied (GWAS)*. Hemel Hempstead, HP1 1ES, UK: VSN International Ltd.

Gao, W., J. A. Clancy, F. Han, D. Prada, A. Kleinhofs, and S. E. Ullrich. 2003. “Molecular dissection of a dormancy QTL region near the chromosome 7 (5H) L telomere in barley.” *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 107 (3): 552–59. <https://doi.org/10.1007/s00122-003-1281-5>.

Garrick, Dorian J., Jeremy F. Taylor, and Rohan L. Fernando. 2009. “Deregressing Estimated Breeding Values and Weighting Information for Genomic Regression Analyses.” *Genetics Selection Evolution* 41 (1): 55. <https://doi.org/10.1186/1297-9686-41-55>.

Gómez-Cadenas, Aurelio, Steven D. Verhey, Lynn D. Holappa, Qingxi Shen, Tuan-Hua David Ho, and M. K. Walker-Simmons. 1999. “An Abscisic Acid-Induced Protein Kinase, PKABA1, Mediates Abscisic Acid-Suppressed Gene Expression in Barley Aleurone Layers.” *Proceedings of the National Academy of Sciences* 96 (4): 1767–72. <https://doi.org/10.1073/pnas.96.4.1767>.

Gong, Xue, Chengdao Li, Meixue Zhou, Yumiko Bonnardeaux, and Guijun Yan. 2014. “Seed Dormancy in Barley Is Dictated by Genetics, Environments and Their Interactions.” *Euphytica* 197 (3): 355–68. <https://doi.org/10.1007/s10681-014-1072-x>.

Gous, Peter W., and Glen P. Fox. 2017. “Review: Amylopectin Synthesis and Hydrolysis Understanding Isoamylase and Limit Dextrinase and Their Impact on Starch Structure on Barley (Hordeum Vulgare) Quality.” *Trends in Food Science & Technology* 62 (April): 23–32. <https://doi.org/10.1016/j.tifs.2016.11.013>.

Gubler, Frank, Anthony A Millar, and John V Jacobsen. 2005. “Dormancy Release, ABA and Pre-Harvest Sprouting.” *Current Opinion in Plant Biology*, Genome studies and molecular genetics / Plant biotechnology, 8 (2): 183–87. <https://doi.org/10.1016/j.pbi.2005.01.011>.

Han, F., S. E. Ullrich, J. A. Clancy, V. Jitkov, A. Kilian, and I. Romagosa. 1996. “Verification of Barley Seed Dormancy Loci via Linked Molecular Markers.” *Theoretical and Applied Genetics* 92 (1): 87–91. <https://doi.org/10.1007/BF00222956>.

Hayes, P., D. R. Carrijo, T. Filichkin, S. Fisk, L. Helgerson, J. Hernandez, B. Meints, and M. E. Sorrells. 2021. “Registration of ‘Lightning’ Barley.” *Journal of Plant Registrations* 15 (3): 407–14. <https://doi.org/10.1002/plr2.20129>.

Hisano, Hiroshi, Robert E. Hoffie, Fumitaka Abe, Hiromi Munemori, Takakazu Matsuura, Masaki Endo, Masafumi Mikami, Shingo Nakamura, Jochen Kumlehn, and Kazuhiro Sato. 2022. “Regulation of Germination by Targeted Mutagenesis of Grain Dormancy Genes in Barley.” *Plant Biotechnology Journal* 20 (1): 37–46. <https://doi.org/10.1111/pbi.13692>.

Kuester, H., E. Austin, S. Chan, R. Fasset, B. Sanders, R. Hills, G. Smith, G. Laycock, J. Lowe, and M. Munar. 1997. “Simultaneous Determination of Germination Energy, Water Sensitivity, and Germination Capacity in Barley.” *Journal of the American Society of Brewing Chemists* 55 (4): 179182. https://doi.org/<https://doi.org/10.1094/ASBCJ-55-0179>.

Li, C. D., A. Tarr, R. C. M. Lance, S. Harasymow, J. Uhlmann, S. Westcot, K. J. Young, et al. 2003. “A Major QTL Controlling Seed Dormancy and Pre-Harvest Sprouting/Grain α-Amylase in Two-Rowed Barley (Hordeum Vulgare L.).” *Australian Journal of Agricultural Research* 54 (12): 1303–13. <https://doi.org/10.1071/ar02210>.

Lin, R., R. D. Horsley, N. L. V. Lapitan, Z. Ma, and P. B. Schwarz. 2009. “QTL Mapping of Dormancy in Barley Using the Harrington/Morex and Chevron/Stander Mapping Populations.” *Crop Science* 49 (3): 841–49. <https://doi.org/10.2135/cropsci2008.05.0269>.

Mascher, Martin, Heidrun Gundlach, Axel Himmelbach, Sebastian Beier, Sven O. Twardziok, Thomas Wicker, Volodymyr Radchuk, et al. 2017. “A Chromosome Conformation Capture Ordered Sequence of the Barley Genome.” *Nature* 544 (7651): 427–33. <https://doi.org/10.1038/nature22043>.

Mascher, Martin, Thomas Wicker, Jerry Jenkins, Christopher Plott, Thomas Lux, Chu Shin Koh, Jennifer Ens, et al. 2021. “Long-Read Sequence Assembly: A Technical Evaluation in Barley.” *The Plant Cell* 33 (6): 1888–1906. <https://doi.org/10.1093/plcell/koab077>.

Mauri, Nuria, María Fernández-Marcos, Celina Costas, Bénédicte Desvoyes, Antonio Pichel, Elena Caro, and Crisanto Gutierrez. 2016. “GEM, a Member of the GRAM Domain Family of Proteins, Is Part of the ABA Signaling Pathway.” *Scientific Reports* 6 (March): 22660. <https://doi.org/10.1038/srep22660>.

Mena, M., R. Sanchez-Monge, L. Gomez, G. Salcedo, and P. Carbonero. 1992. “A major barley allergen associated with baker’s asthma disease is a glycosylated monomeric inhibitor of insect alpha-amylase: cDNA cloning and chromosomal location of the gene.” *Plant Molecular Biology* 20 (3): 451–58. <https://doi.org/10.1007/BF00040604>.

Mohammadi, Mohsen, Thomas K. Blake, Allen D. Budde, Shiaoman Chao, Patrick M. Hayes, Richard D. Horsley, Donald E. Obert, Steven E. Ullrich, and Kevin P. Smith. 2015. “A Genome-Wide Association Study of Malting Quality Across Eight U.S. Barley Breeding Programs.” *Theoretical and Applied Genetics* 128 (4): 705–21. <https://doi.org/10.1007/s00122-015-2465-5>.

Möhring, J., and H.-P. Piepho. 2009. “Comparison of Weighting in Two-Stage Analysis of Plant Breeding Trials.” *Crop Science* 49 (6): 1977–88. <https://doi.org/10.2135/cropsci2009.02.0083>.

Monat, Cécile, Sudharsan Padmarasu, Thomas Lux, Thomas Wicker, Heidrun Gundlach, Axel Himmelbach, Jennifer Ens, et al. 2019. “TRITEX: Chromosome-Scale Sequence Assembly of Triticeae Genomes with Open-Source Tools.” *Genome Biology* 20 (1): 284. <https://doi.org/10.1186/s13059-019-1899-5>.

Monier, Brandon, Terry M. Casstevens, Peter J. Bradbury, and Edward S. Buckler. 2022. “rTASSEL: An R Interface to TASSEL for Analyzing Genomic Diversity.” *Journal of Open Source Software* 7 (76): 4530. <https://doi.org/10.21105/joss.04530>.

Nagel, Manuela, Ahmad M. Alqudah, Marlène Bailly, Loïc Rajjou, Sibylle Pistrick, Gabriele Matzig, Andreas Börner, and Ilse Kranner. 2019. “Novel Loci and a Role for Nitric Oxide for Seed Dormancy and Preharvest Sprouting in Barley.” *Plant, Cell & Environment* 42 (4): 1318–27. <https://doi.org/10.1111/pce.13483>.

Nakamura, Shingo, Mohammad Pourkheirandish, Hiromi Morishige, Yuta Kubo, Masako Nakamura, Kazuya Ichimura, Shigemi Seo, et al. 2016. “Mitogen-Activated Protein Kinase Kinase 3 Regulates Seed Dormancy in Barley.” *Current Biology* 26 (6): 775–81. <https://doi.org/10.1016/j.cub.2016.01.024>.

Oberthur, L., Thomas K. Blake, W. R. Dyer, and S. E. Ullrich. 1995. “Genetic Analysis of Seed Dormancy in Barley (Hordeum Vulgare l.).” <https://wheat.pw.usda.gov/jag/papers95/paper495/dormancy.html>.

Piskurewicz, Urszula, Yusuke Jikumaru, Natsuko Kinoshita, Eiji Nambara, Yuji Kamiya, and Luis Lopez-Molina. 2008. “The Gibberellic Acid Signaling Repressor RGL2 Inhibits Arabidopsis Seed Germination by Stimulating Abscisic Acid Synthesis and ABI5 Activity.” *The Plant Cell* 20 (10): 2729–45. <https://doi.org/10.1105/tpc.108.061515>.

Price, Alkes L., Nick J. Patterson, Robert M. Plenge, Michael E. Weinblatt, Nancy A. Shadick, and David Reich. 2006. “Principal Components Analysis Corrects for Stratification in Genome-Wide Association Studies.” *Nature Genetics* 38 (8): 904–9. <https://doi.org/10.1038/ng1847>.

Regina, Ahmed, Behjat Kosar-Hashemi, Samuel Ling, Zhongyi Li, Sadequr Rahman, and Matthew Morell. 2010. “Control of Starch Branching in Barley Defined Through Differential RNAi Suppression of Starch Branching Enzyme IIa and IIb.” *Journal of Experimental Botany* 61 (5): 1469–82. <https://doi.org/10.1093/jxb/erq011>.

Rodríguez, María V., José M. Barrero, Francoise Corbineau, Frank Gubler, and Roberto L. Benech-Arnold. 2015. “Dormancy in Cereals (Not Too Much, Not so Little): About the Mechanisms Behind This Trait.” *Seed Science Research* 25 (2): 99–119. <https://doi.org/10.1017/S0960258515000021>.

Romagosa, Ignacio, Feng Han, Janet A. Clancy, and Steven E. Ullrich. 1999. “Individual Locus Effects on Dormancy During Seed Development and After Ripening in Barley.” *Crop Science* 39 (1): cropsci1999.0011183X003900010012x. <https://doi.org/10.2135/cropsci1999.0011183X003900010012x>.

Sato, Kazuhiro, Miki Yamane, Nami Yamaji, Hiroyuki Kanamori, Akemi Tagiri, Julian G. Schwerdt, Geoffrey B. Fincher, Takashi Matsumoto, Kazuyoshi Takeda, and Takao Komatsuda. 2016. “Alanine Aminotransferase Controls Seed Dormancy in Barley.” *Nature Communications* 7 (1): 11625. <https://doi.org/10.1038/ncomms11625>.

Seiler, Christiane, Vokkaliga T. Harshavardhan, Palakolanu S. Reddy, Götz Hensel, Jochen Kumlehn, Lennart Eschen-Lippold, Kalladan Rajesh, et al. 2014. “Abscisic Acid Flux Alterations Result in Differential Abscisic Acid Signaling Responses and Impact Assimilation Efficiency in Barley Under Terminal Drought Stress1[c][w][OPEN].” *Plant Physiology* 164 (4): 1677–96. <https://doi.org/10.1104/pp.113.229062>.

Shu, Xiaoli, and Søren K. Rasmussen. 2014. “Quantification of amylose, amylopectin, and β-glucan in search for genes controlling the three major quality traits in barley by genome-wide association studies.” *Frontiers in Plant Science* 5: 197. <https://doi.org/10.3389/fpls.2014.00197>.

Sun, C., P. Sathish, S. Ahlandsberg, and C. Jansson. 1998. “The two genes encoding starch-branching enzymes IIa and IIb are differentially expressed in barley.” *Plant Physiology* 118 (1): 37–49. <https://doi.org/10.1104/pp.118.1.37>.

Sweeney, Daniel W., Travis E. Rooney, Jason G. Walling, and Mark E. Sorrells. 2021a. “Interactions of the Barley SD1 and SD2 Seed Dormancy Loci Influence Preharvest Sprouting, Seed Dormancy, and Malting Quality.” *Crop Science* 62 (1): 120–38. <https://doi.org/10.1002/csc2.20641>

Sweeney, Daniel W., Karl H. Kunze, and Mark E. Sorrells. 2021b. “QTL x Environment Modeling of Malting Barley Preharvest Sprouting.” *Theoretical and Applied Genetics* 135 (1): 217–32. <https://doi.org/10.1007/s00122-021-03961-5>.

.

Ullrich, S. E., H. Lee, J. A. Clancy, I. A. del Blanco, V. A. Jitkov, A. Kleinhofs, F. Han, D. Prada, I. Romagosa, and J. L. Molina-Cano. 2009. “Genetic Relationships Between Preharvest Sprouting and Dormancy in Barley.” *Euphytica* 168 (3): 331–45. <https://doi.org/10.1007/s10681-009-9936-1>.

Vetch, Justin M., Jason G. Walling, Jamie Sherman, John M. Martin, and Michael J. Giroux. 2020. “Mutations in the HvMKK3 and HvAlaAT1 Genes Affect Barley Preharvest Sprouting and After-Ripened Seed Dormancy.” *Crop Science* 60 (4): 1897–1906. <https://doi.org/10.1002/csc2.20178>.

Wang, Jiabo, and Zhiwu Zhang. 2021. “GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction.” *Genomics, Proteomics & Bioinformatics*, Bioinformatics Commons, 19 (4): 629–40. <https://doi.org/10.1016/j.gpb.2021.08.005>.

Wei, Wenxin, Xiaoyu Min, Siyao Shan, Hao Jiang, Jiajia Cao, Li Li, Jianfeng Wang, et al. 2019. “Isolation and Characterization of TaQsd1 Genes for Period of Dormancy in Common Wheat (Triticum Aestivum L.).” *Molecular Breeding* 39 (10): 150. <https://doi.org/10.1007/s11032-019-1060-x>.

Yu, Jianming, and Edward S. Buckler. 2006. “Genetic association mapping and genome organization of maize.” *Current Opinion in Biotechnology* 17 (2): 155–60. <https://doi.org/10.1016/j.copbio.2006.02.003>.

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. <https://doi.org/10.1093/bioinformatics/bts606>