QTL x environment modeling of preharvest sprouting in spring and winter two-row malting barley

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**Abstract**

Preharvest sprouting (PHS) can severely damage barley (*Hordeum vulgare* L.) malting quality but selection for PHS resistance often results in poor malting quality. Seed dormancy is closely related to PHS and increased temperature during grain fill causes seed dormancy to decrease in barley. Several large effect seed dormancy quantitative trait loci (QTL) have been identified in barley, but genetic components of seed dormancy temperature sensitivity are poorly understood. Six years of PHS data from Cornell University field experiments were used to fit QTL x environment mixed models incorporating molecular marker haplotypes from seed dormancy genes *HvAlaAT1*, *HvGA20ox1,* and *HvMKK3* and weather covariates in spring and winter two-row malting barley. Variation in winter barley PHS was best modeled by average temperature range during grain fill and spring barley PHS by total precipitation during grain fill. Average high temperature during grain fill also accurately modeled PHS for both datasets. *HvGA20ox1* determined baseline PHS susceptibility and *HvAlaAT1* and *HvMKK3* were responsible for environmental sensitivity. Substantial polygenic variation for PHS within haplotype was detected. Haplotype response to environmental covariates was similar across winter and spring datasets but winter entries showed more extreme high and low average PHS. Residual genotype and QTL by environment interaction variance indicated additional environmental and genetic factors involved in PHS control. Entries with dormant *HvGA20ox1* and *HvMKK3* were most stable across environments. These models provide insight into genotype and environmental regulation of barley seed dormancy, a method for PHS forecasting, and a tool for breeders to improve PHS resistance.

**Introduction**

Seed dormancy, the inability of a seed to germinate under conducive environmental conditions (DePauw and McCaig, 1991; Bewley et al., 2013), has a number of physiological determinants but the ratio of and sensitivity to abscisic acid (ABA) and gibberellin (GA) hormones are particularly important (Finch-Savage & Leubner-Metzger, 2006). ABA induces and maintains seed dormancy and GA promotes germination. Seed dormancy in barley is primarily imposed by seed covering structures, the glumellae, pericarp, and endosperm (Lenoir et al., 1986; Benech-Arnold et al., 1999). Excised embryos readily germinate as soon as 22 days after pollination but retain sensitivity to exogenous ABA through maturity (Benech-Arnold et al., 1999). Dormancy is not determined by absolute ABA content or GA content (Jacobsen et al., 2002); rather, embryo sensitivity to ABA is correlated with dormancy imposition and maintenance (Van Beckum et al., 1993; Benech-Arnold et al., 1999; 2006; Bradford et al., 2007). Physiological regulation of seed dormancy is also sensitive to environmental factors, with temperature playing an especially important role. Increased temperature during grain fill has been linked to reduced seed dormancy in wheat (*Triticum aestivum* L.) (Biddulph et al., 2005; 2007; Nakamura et al., 2011) and barley (Rodriguez et al., 2001; Li et al., 2003; Gualano & Benech-Arnold, 2009; Gong et al., 2014). In barley, maximum ABA content is reached in the middle of seed development and decreases as physiological maturity approaches (Goldbach & Michael, 1976; Benech-Arnold et al., 1999) with similar results observed in wheat (King, 1976). Timing of peak ABA accumulation and total ABA content at maturity are known to be temperature sensitive in barley (Goldbach & Michael, 1976; Walker-Simmons & Sesing, 1990; Chono et al., 2006). In rice (*Oryza sativa* L.), GA accumulation peaks before ABA and decreases as physiological maturity approaches (Liu et al., 2014) but little is known about GA accumulation or temperature sensitivity during grain fill in barley.

Preharvest sprouting occurs when excess moisture prematurely initiates seed germination, either visibly or non-visibly, in the field before harvest and can severely reduce barley malting quality. This phenomenon is related to seed dormancy as varieties that quickly lose primary dormancy are at high risk for PHS damage. A number of QTL have been mapped for PHS in barley but the *SD1* and *SD2* seed dormancy loci on chromosome 5H are the most consistent and largest effect QTL (Oberthur et al., 1995; Han et al., 1996; Hori et al., 2007; Lin et al., 2009). An alanine aminotransferase, *HvAlaAT1*, has been cloned at *SD1* (Sato et al., 2016) and a mitogen associated protein kinase kinase (*HvMKK3*) has been cloned in the *SD2* region (Nakamura et al., 2016). AlaAT is likely involved in ABA signaling but the exact mechanism is unknown (Wei et al., 2019) and in rice, MKK3 is part of a ABA signaling pathway regulating expression of *MOTHER OF FLOWERING TIME* (*OsMFT*) (Mao et al., 2019). In modern North American spring malting barley, *HvMKK3* variants were associated with PHS (Vetch et al., 2020). Gibberellin 20-oxidases (GA20ox) are important enzymes in bioactive gibberellin production (Spielmeyer et al., 2004). GA20ox1 is a key part of active gibberellin synthesis during seed germination and is highly expressed in late stages of grain development and during germination (Sreenivasulu et al., 2008; Liu et al., 2014; Betts et al., 2019). *HvGA20ox1* is located in the *SD2* region 1.7 Mb proximal to *HvMKK3* and its role in PHS has been suggested in barley (Li et al., 2004; Nagel et al., 2018) and sorghum (Perez-Flores et al., 2003; Rodriguez et al., 2012) and in germination rate in rice (Abe et al., 2012).*HvGA20ox1* is the main determinant of primary dormancy in two-row spring malting barley and interactions between *HvGA20ox1* and *HvMKK3* explain a large proportion of variation for PHS (**hopefully Sweeney et al., 2021**). Genetic evidence supporting the correlation between increased temperature and PHS susceptibility has been reported. *SD2*-associated dormancy in Steptoe x Morex increased in environments with lower average temperatures during grain fill (Gao et al., 2003). Germination percentage QTL in the *SD1* and *SD2* regions were detected in a Stirling x Harrington doubled haploid population with variable effects across environments differentiated by temperature (Gong et al., 2014). Increased temperature during embryo development in the twenty days after flowering was correlated with reduced seed dormancy.

Phenotypic stability is one of the main objectives in crop genotype by environment interaction studies. Often, breeders are interested in identifying lines that perform similarly across diverse environments rather than optimizing performance in a subset of environments. QTL by environment interaction (QEI) models are a special case of genotype by environment interaction models that incorporate known QTL information to estimate change in QTL effects across environment. Genetic effects are partitioned into main QTL and residual polygenic effects and genotype by environment interaction (GEI) effects into QEI and residual polygenic GEI effects (Malosetti et al., 2004). Variation in QTL effects can be further modeled by introducing environmental covariates such as temperature or precipitation into a QEI mixed model framework (van Eeuwijk et al., 2005). Malosetti et al. (2004) estimated a QTL main effect, environment specific effects, and QTL sensitivity to temperature during heading for a grain yield QTL on chromosome 2H in barley. PHS susceptibility in South American barley varieties has been modeled as a function of temperature within grain fill intervals defined by thermal time, revealing strong linear relationships between increased temperature in late grain fill stages and PHS (Rodriguez et al., 2001; Gaulano and Benech-Arnold, 2009), but a QEI framework has not been implemented to model barley PHS. Temperature sensitivity of *HvAlaAT1*, *HvMKK3* and *HvGA20ox1* has not been formally described and temperature sensitivity models are not available for European and North American malting barley germplasm. Spring malting barley tends to be more susceptible to PHS than winter malting barley but is it unknown if this difference is genetic, environmental, or a combination of both.Preharvest sprouting is often negatively correlated with malting quality so additional genetic sources of PHS resistance could be useful for combining high malting quality potential with PHS resistance. Modeling the response of *HvAlaAT1*, *HvMKK3* and *HvGA20ox1* to environmental covariates could identify climate stable PHS resistance and reveal residual polygenic variation for PHS resistance or environmental sensitivity. Improved understanding of genetic and environmental causes of PHS variability for commonly grown varieties would also help growers adjust management strategies to minimize risk from PHS-inducing weather.

The objective of this study was to use seed dormancy QTL haplotypes and weather covariates to model genotypic and environmental influences on annual variation in PHS using historical data from 59 two-row spring malting barley entries in twenty-five environments over six years and 21 two-row winter malting barley entries in nine environments over five years. Three facultative entries planted in both winter and spring were also analyzed. The seed dormancy haplotypes of these entries were classified using Kompetitive Allele Specific Primers (KASP) assays for *HvAlaAT1*, *HvGA20ox1,* and *HvMKK3.* Five temperature and precipitation covariates were tested in QTL x E models to estimate seed dormancy QTL by environmental covariate interaction effects, residual polygenic variance, and residual QEI and GEI variance.

**Materials and methods**

Two datasets, 1) seven spring barley experiments planted across six years and 2) three winter barley experiments planted across five years, were used for analysis. Spring and winter datasets were always analyzed separately. These experiments were highly unbalanced across years and included both commercial variety yield trials, cooperative regional nurseries, and preliminary breeding trials. A description of each experiment can be found in Table 1. A total of 59 spring entries and 21 winter entries that represented the range of PHS scores in each dataset and were present in multiple experiments across years either as checks or experimental entries were used for analysis (Table 2). Complete datasets will be uploaded to T3. The entries were all derived from modern European or North American two-row malting barleys, with the exception of the NakedReg experiment which was composed of spring hulless, or naked, barley entries and a hulled check. Winter 2015 data was removed due to collection and sample preparation discrepancies. Environments were defined as year-location combinations. Within year, different experiments planted at the same location on the same day with shared checks were classified in the same environment. For each experiment, heading date was recorded as the Julian date that 50% of the spikes in a plot had fully emerged from the flag leaf. Heat, drought, and semi-dwarfism can cause heading date to be delayed in spring barley, making accurate measurement of the grain fill period difficult. To improve estimates of the grain fill period, in 2019 and 2020 spring barley experiments, tipping date was recorded as the date that 50% of the plot showed awn protrusion of 2 mm. Anthesis in spring barley occurs at this stage (Zadoks scale Z49), not at heading date as in winter barley (Alqudah & Schnurbusch, 2017). The period between heading date and spike sampling for winter barley and the period between tipping date and spike sampling for spring barley were defined as the grain fill period. The average difference between tipping date and heading date in 2019 and 2020 spring experiments, 9 days, was added to the heading date of spring samples collected from 2015-2018 to estimate tipping date. In all experiments, five spikes per plot were sampled at physiological maturity (defined as the loss of green color from the peduncle), after-ripened at ambient temperature for three days, misted in an artificial greenhouse mist chamber, and scored on a 0-9 scale as described by Anderson et al. (1993) with 0 indicating no visible PHS and 9 indicating advanced radicle and coleoptile development on all caryopses on the spike. Winter barley experiments in 2019 and 2020 were after-ripened for four days to increase variation for selection. Preharvest sprouting scores of 0-2 can be classified as resistant, 3-5 as susceptible, and 6-9 as highly susceptible.

Daily high, low, and average temperature and daily precipitation were downloaded from the Northeast Regional Climate Center station (http://www.nrcc.cornell.edu/wxstation/ithaca/ithaca.html) at the Game Farm Road station, which is centrally located between the testing locations for all trials (average distance of 1100 m). Weather and planting date summaries for each environment can be found in Table 3. For each plot in each environment, the environmental covariates of average high temperature during grain fill (Tmax), average low temperature during grain fill (Tmin), average temperature during grain fill (Tavg), average difference between high and low temperature during grain fill (Tr), and total precipitation during grain fill (Psum) were calculated.

Within each experiment/environment combination, single spike scores within entry were aggregated and outlier spike scores with a z-score greater than two were removed. Spike scores were then averaged per plot within experiment/environment. All experiments were randomized complete block designs except S2MET and CU1, which were planted in an augmented design with replicated checks. Replication was not significant for either dataset and was not included in the models. QTL by environment models of the following form were fit in a single step with *ASReml-R* (Butler et al., 2009) for each environmental covariate within each dataset:

yijk ~μ + 𝐸j + 𝑄i + 𝑄i: Zj + *Gk* + *Gk:Zj* *+ Gk:Ej + Qi:Ej + Ej(samplel)* + 𝑒ijk

where yijk is the phenotypic observation for PHS, μ is the overall mean, *Ej* is the fixed effect for environment, *Qi* is the fixed effect for seed dormancy haplotype with six levels for spring entries and four levels for winter entries (Table 2), *Zj* is the zero-centered environmental covariate, and *Gk* is the random effect of entry with *Gk* ~ N(0, σg2 ). Random effects are underlined. All phenotypic observations were included in the models to accurately estimate environmental effects. A dummy variable was added to differentiate observations with and without haplotype data. For each *Gk* main or interaction effect, the ASReml-R function at() was used to estimate separate variance components for entries with and without haplotype data (i.e. at(dummy, c(1,2)):Entry where 1 indicates lines without a haplotype and 2 indicates lines with a haplotype). Variance components for entries without haplotype data were not included in the results. QEI was partitioned into QTL by environmental covariate interaction (*QiZj*) and residual QTL by environment interaction (*QiEj*) effects with *QiEj* ~ N(0, σQE2). GEI was similarly partitioned as entry by environmental covariate interaction, *GkZj* ~ N(0, σGZ2), and entry by environment interaction, *GkEj* ~ N(0, σGE2) (Malosetti et al., 2004). Spike sampling date was nested within environment and specified as *Ej(samplel)* ~ N(0, σE(s)2). Heterogeneous residuals were fitted to estimate a unique residual variance in each environment with *e*ijk ~ N(0, **I**σe2 ). Model summaries are presented in Table 5 and QZ effects in Table 6. Fixed effect significance for *E*, *Q*, and *QZ* terms was tested with Wald tests. Best model fit was determined by Akaike information criterion (AIC) estimated using icREML to compare models with different fixed environmental covariates (Verbyla, 2019). Briefly, the icREML method decomposes full likelihood into residual and conditional likelihood using restricted maximum likelihood (REML) and derives AIC from the full log-likelihood.

Kompetitive Allele Specific Primers (KASP) markers were developed for each of the three seed dormancy genes of interest (Table 7). Primers were developed to amplify the causal L214F polymorphism in *HvAlaAT1*described by Sato et al. (2016), which was confirmed to be present in North American germplasm by Vetch et al. (2020). This marker was named HvQsd1.Primer development for *HvGA20ox1* and *HvMKK3* was based on markers on the Illumina 50k SNP chip. Sweeney et al. (2021) identified SCRI\_RS\_121526 and SCRI\_RS\_121501 as candidate markers in high linkage disequilibrium with *HvGA20ox1* and JHI-Hv50k-2016-367342 as a candidate marker in high linkage disequilibrium with *HvMKK3*.SCRI\_RS\_121526 and SCRI\_RS\_121501 were highly similar (r=0.99) in the Sweeney et al. (2021) dataset but primer design for SCRI\_RS\_121501 was unsuccessful and only SCRI\_RS\_121526 was used in this study. SCRI\_RS\_121526 is 0.2 Mb distal to *HvGA20ox1.*JHI-Hv50k-2016-367342 is 152 bp proximal to *HvMKK3.* KASP polymerase chain reaction (PCR) was performed in 384 well plates. Forward primer candidates were developed using OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html) and reverse primer candidates were developed using NCBI Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers were diluted to 100 uM and primer master mix was composed of 12 uL allele 1 specific primer, 12 uL allele 2 specific primer, 30 uL common reverse primer, and 46 uL dd H2O. Master mix was prepared with 315 uL PCR Allele Competitive Extension (PACE) master mix (3CR Bioscience, Essex, UK) and 8.7 uL primer mix for each 384 well plate. 2.5 uL master mix and 2.5 uL DNA were added to each well for PCR. The following amplification protocol was used: initial denaturation at 94 C for 15 minutes, 10 cycles each of 94 C for 20 seconds followed by a stepdown to 65 C by 0.8 C per minute, and 36 cycles each of 94 C for 20 seconds followed by 57 C for 1 minute. Fluorescence was measured on a ViiA 7 real time PCR system (Applied Biosystems, ThermoFisher Scientific). For AC Metcalfe and CDC Copeland, *HvAlaAT1* and *HvMKK3* alleles were downloaded from Vetch et al. (2020) and SCRI\_RS\_121256 markers were downloaded from the T3 database (Blake et al., 2015). SCRI\_RS\_121526 and JHI-Hv50k-2016-367342 data for DH130939 and DH130004 was taken from 50k Illumina sequencing results. **Still waiting for sample of DH130718 from OSU to confirm genotype**.Haplotypes were defined as the combination of alleles at HvQsd1, SCRI\_RS\_121526, and JHI-Hv50k-2016-367342, respectively, coded as NNN for non-dormant alleles at all three markers, DNN for dormant HvQsd1 and non-dormant SCRI\_RS\_121526 and JHI-Hv50k-2016-367342, DDD for dormant alleles at all three markers, NDD for non-dormant HvQsd1 and dormant SCRI\_RS\_121526 and JHI-Hv50k-2016-367342, NDN for non-dormant HvQsd1 and JHI-Hv50k-2016-367342 and dormant SCRI\_RS\_121526, and DDN for dormant HvQsd1 and SCRI\_RS\_121526 and non-dormant JHI-Hv50k-2016-367342. A single NND haplotype was detected in the spring dataset that was omitted and no DND haplotypes were present.

**Results**

The spring dataset contained six seed dormancy haplotypes but the number of observations for each haplotype was unbalanced (NNN=133, DNN=366, DDD=115, NDD=524, NDN=347, DDN=90). Triple non-dormant (NNN) lines are typically selected against in Cornell spring regional testing due to high PHS susceptibility but the PHS susceptible DNN lines AAC Synergy and Newdale were used as malting quality checks in the majority of the experiments, which accounts for the high number of DNN observations. The DDN and DDD haplotypes were not observed in any spring commercial lines and were only present in Cornell experimental lines or the facultative variety Lightning (DDD). Experimental and commercial lines containing NDD and NDN haplotypes were common in spring regional and experimental testing. The winter dataset contained four seed dormancy haplotypes which were more balanced (NNN=103, DNN=99, DDD=129, NDD=241) but only two winter lines, Charles and Endeavor, contained the DNN haplotype. These lines were used as malting quality checks in all winter experiments. Not all Cornell winter barley breeding lines were available for analysis, resulting in a smaller number of entries for the winter dataset. The average observed PHS scores for the spring dataset were μNNN = 5.81, μDNN = 4.99, μDDD = 1.88, μNDD = 1.99, μNDN = 2.5, μDDN = 2.29 and for the winter dataset were μNNN = 6.36, μDNN = 5.76, μDDD = 0.46, μNDD = 0.67. Observed average PHS scores for winter non-dormant *HvGA20ox1* haplotypes, NNN and DNN, were significantly higher (p < 0.05) than equivalent spring haplotype means but winter dormant *HvGA20ox1* haplotype means, DDD and NDD, were significantly lower (p < 0.01) than equivalent spring haplotype means.

Models were fitted for five environmental covariables: Tmax, Tmin, Tavg, Tr, and Psum. Average temperature and precipitation during the months of May to August from 2015 to 2020 were mostly representative of weather variation in those months in the past 30 years ~~(Figure 1).~~ For both winter and spring datasets, Tmax and Psum were negatively correlated (Table 4), partially confounding the effects of increased temperature and decreased precipitation. Tmax and Tmin had low correlations in both datasets.Environment and QTL effects were highly significant (Pr(Chisq) < .001) for all spring and winter models. QZI was significant (Pr(Chisq) < .05) for spring Tmax and Tavg and winter Tavg, Tmin, and Psum (Table 5). Spring Psum and winter Tr had the best model fit as determined by AIC estimated with icREML. For the spring Psum model, polygenic background variation due to entry (G) accounted for 32% of the within-environment variance. Entry by environment variance (GE), QTL by environment (QE), and entry by precipitation (GZ) explained 17%, 21%, and 0.1% of the within-environment variance, respectively. The winter Tr model had a lower proportion of within-environment variance explained by G (16%), higher proportion by QE (51%), and similar proportions by GZ (<.001%), and GE (21%). Variance component estimates for other models within dataset were similar (Table 5). The percentage of variance explained by QE was consistently higher in winter than spring models. QZ interactions and deviations due to G and GZ are plotted for spring Psum (Figure 2), spring Tmax (Figure 3), winter Tr (Figure 4), and winter Tmax (Figure 5).

Haplotypes with a non-dormant *HvGA20ox1* allele (NNN, DNN) exhibited higher baseline PHS susceptibility than dormant *HvGA20ox1* haplotypes (Figures 2-5). Both spring and winter non-dormant *HvGA20ox1* haplotypes were sensitive to Tmax , Tavg, and Tmin, but winter haplotypes were more sensitive than springs (Table 6, Figure 3 and 5). Non-dormant *HvGA20ox1* spring and winterhaplotypes showed contrasting sensitivities to Tr and Psum. Spring NNN haplotypes had no sensitivity to Tr and Psum, spring DNN haplotypes were sensitive to Tr but not Psum, winter NNN haplotypes were sensitive to Tr and Psum (negative slope), and winter DNN haplotypes were sensitive to Psum but not Tr. Haplotypes with dormant *HvGA20ox1* and *HvMKK3* alleles (DDD, NDD) in spring and winter datasets were generally less temperature sensitive than non-dormant *HvGA20ox1* haplotypes and displayed baseline PHS resistance. Winter NDD haplotypes showed sensitivity to Tmax, Tavg, Tmin, and Psum but winter DDD haplotypes were less sensitive to the same covariates. Spring DDD haplotypes had lower PHS as Tmax, Tavg, and Tmin increased but NDD haplotypes were only moderately sensitive to Tmin and Tr. Neither spring DDD nor NDD were precipitation sensitive. Spring dormant *HvGA20ox1* and non-dormant *HvMKK3* haplotypes (NDN, DDN) were highly temperature sensitive but had baseline PHS resistance similar to dormant *HvGA20ox1* and dormant *HvMKK3* haplotypes. Spring NDN and DDN haplotypes were not precipitation sensitive. Overall, winter haplotypes were noticeably more sensitive to Tmin and P than spring haplotypes. Within haplotype for both winter and spring datasets, there was considerable variation for baseline PHS resistance due to entry. Although the variance due to GZ was small for spring datasets, the change in haplotype temperature sensitivity due to entry was large enough to change PHS resistance ranks for entries within spring NNN, NDD, NDN haplotypes as Tmax increased (Figure 3). Winter GZ variance was negligible for all models and did not result in rank changes across environmental covariates. ( *was GZ neglible because there was no sensitivity of winter germplasm or because there was not enough variation in precipitation during PM compared to what we have had for spring at PM?)*

**Discussion**

Annual variation in two-row winter and spring malting barley PHS across six years was modeled with environmental covariates related to temperature and precipitation. The years used in this analysis, 2015-2020, were representative of the past 30 years in Ithaca, NY and captured a wide range of temperature and precipitation during grain fill. Annual PHS variation was partitioned into genetic, environmental, and GEI effects. Genetic effects were partitioned into seed dormancy QTL and residual polygenic entry effects and environmental effects were partitioned into year-location and environmental covariate effects. Although the testing sites sampled in this dataset are physically close, there is substantial environmental variation between and within sites for soil type, management history, topography, and disease pressure. These factors were not quantified and may have contributed to GEI, QEI, and error. Uniform sampling of physiologically mature spikes for PHS assays is affected when disease pressure is high or temperature increases sharply just before sampling, leading to discoloration of the peduncle or premature senescence. Heading date accuracy was affected by incomplete spike emergence in spring barley.Severe winter injury can substantially delay heading date and cause irregular maturities within plot in winter barley, biasing sampling date and grain fill intervals. The grain fill period for both winter and spring datasets varied substantially across years and within entry, making accurate comparisons of developmental stages across years and experiments difficult. Despite these challenges, PHS response to temperature was similar to previously reported results (Rodriguez et al., 2001; Gaulano & Benech-Arnold, 2009). Temperature has been shown to have a highly positive correlation with PHS during the late grain fill stages defined by thermal time (Gualano and Benech-Arnold, 2009). Increased temperature in the first several weeks of grain fill, during embryo development, has also been correlated with a reduction in seed dormancy (Gong et al., 2014). Environmental sensitivity of ABA and GA biosynthesis and catabolism genes at critical developmental timepoints may play a role in determining PHS susceptibility. Expression of the ABA biosynthesis gene *HvNCED1* and the ABA catabolism gene *HvCYP707A1* varied during grain fill across three years and changed ABA content and germination percentage, indicating sensitivity to environmental factors (Chono et al., 2006). Change in GA content during grain fill and the impact of growth environment on GA content, ABA/GA ratio, and sensitivity to ABA and GA is poorly understood in barley. Change in expression of large effect seed dormancy genes during grain fill across varieties and environmental conditions, particularly *HvAlaAT1* and *HvMKK3*, is also not understood. Identification of seed dormancy haplotype sensitivity to environmental conditions in specific phenological intervals during winter and spring barley seed development was beyond the scope of this study.

Average observed PHS for winter non-dormant *HvGA20ox1* haplotypes was higher than spring non-dormant *HvGA20ox1* haplotypes but winter dormant *HvGA20ox1* haplotypes had consistently lower observed PHS than comparable spring dormant *HvGA20ox1* haplotypes. These differences between spring and winter datasets may have a genetic component, but Endeavor and a number of the winter DH entries have spring germplasm in their pedigree, reducing the probability that winter germplasm specific loci alone are conferring additional sources of PHS variation. Environmental components, particularly temperature, are a more likely cause of the more extreme observed PHS haplotype means in the winter dataset. Average Tmax, Tavg, and Tmin were each about 2.5 C higher in the spring dataset than the winter dataset. Tmax never exceeded 27 C for the winter dataset but 50% of the spring Tmax observations exceeded 27 C. Limited data from three facultative lines included in spring and winter datasets also supports the role of temperature in observed differences between winter and spring PHS. Average observed PHS in winter and spring, respectively, was 0 and 1.44 for Lightning (DDD), 0 and 3.34 for DH131055 (NDD), and 1.07 and 2.14 for DH130935 (NDD) (Table 2). Cooler temperatures during grain fill may have increased ABA content, decreased ABA decay rate, or increased ABA sensitivity during grain fill in the winter dataset, all of which could have induced stronger primary dormancy in dormant *HvGA20ox1* haplotypes. As previously mentioned, temperature induced changes in GA content, decay, and sensitivity are unknown in malting barley. The physiological mechanism behind primary seed dormancy mediated by *HvGA20ox1* is also unknown but cooler temperatures would also likely increase ABA in non-dormant *HvGA20ox1* entries. Unless variants at ABA biosynthesis, catabolism, or sensitivity loci are present, increased observed PHS in winter non-dormant *HvGA20ox1* haplotypes may be due to temperature mediated increases in GA content or GA sensitivity that counterbalance any increase in ABA.

**Environmental and genetic sources of PHS variation**

The environmental covariates that produced the best model fit as determined by AIC were not Tmax as expected but Psum for spring and Tr for winter datasets. The winter Tmax, Tavg, and Tmin models indicated substantial QZ interaction for NNN, DNN, and NDD haplotypes but the Tr model only indicated QZ interaction for NNN (Table 6). This result was not observed for the spring Tr model. The two winter experiments grown in 2016 had significantly (p < 2.2 e-16) higher average Tr (μ=14.7 C) than experiments in other years (μ=12.7 C) and Tr was less correlated with Tmax in 2016 (r=0.584) than in other years (r=0.933). The smaller size of the winter dataset may have been more sensitive to years with large differences in temperature during grain fill than the spring dataset, as this relationship was not observed for spring. The ability of Tr to capture variation in Tmin may have been more informative under the cooler growth conditions of winter barley. The spring Psum model indicated very low sensitivity to precipitation for all haplotypes but an increase in genetic variance was observed compared to other spring models, indicating greater variation in PHS resistance in drought conditions. These results were in stark contrast to the winter Psum model which had highly significant QZ interactions (Pr(Chisq<.01)) but inconsistent precipitation sensitivity across haplotypes. The two non-dormant winter haplotypes showed contrasting QZ effects (NNN= -0.095, DNN=0.153) as did the dormant winter haplotypes (DDD = -0.082, NDD = 0.106). These results might also be partially explained by the smaller winter dataset and the presence of only two entries for DNN. Differences between baseline haplotype effect may be exacerbated with smaller sample size, especially if the entries within haplotype are highly variable in their baseline PHS in drought conditions.

Variance component estimates for QE were consistently large compared to G and GE variance components in the winter dataset. Winter GE variance components were also consistently larger than G variance components, unlike the spring dataset. Several environmental factors may have increased QE and GE variance in the winter dataset. The limited number of DNN entries may have contributed to larger QE variance, as both DNN lines, Charles and Endeavor, are susceptible to the foliar disease scald, caused by *Rhyncosporium secalis* (Oudem.), which can infect seeds and may affect seed viability. Charles was a check for all winter experiments, due to its success in western growing regions, but it is poorly adapted to New York and had the largest phenotypic variance (σp2=7.1) across both datasets. In 2019 and 2020, an additional after-ripening day was added for winter barley in an attempt to increase the variation for PHS for selection purposes. Adding this information as a covariate in the winter model had no effect but still may have biased environmental effects upwards for those years, particularly for non-dormant *HvGA20ox1* haplotypes. Bias in heading and sampling date due to winter injury may have also contributed.

Spring two-row malting barley experiments typically have less annual PHS stability than winter malting barley. These results show spring two-row barley had more seed dormancy haplotype combinations, more polygenic variation, and more variation in temperature sensitivity. However, almost three times more spring than winter entries were evaluated in this study and more genetic variation may be present in winter germplasm. Winter malting barley is a relatively new breeding target in New York and fewer entries have been evaluated for PHS. Additional spring polygenic sensitivity to temperature may be due to ABA or GA synthesis, catabolism, and signaling loci. The increased G and GE variation within haplotype in the spring dataset is of particular interest. ND Genesis and Pinnacle are NDN entries from the same breeding program (NDSU) but had observed PHS means of 1.55 and 3.43, respectively. Despite overall haplotype Tmax instability for NDN, some NDN entries showed low baseline PHS and little change as temperature increased. Further mapping is needed in two-row spring malting barley germplasm within *HvGA20ox1* allelic state to better understand the basis of quantitative variation for PHS and PHS temperature sensitivity. Within the DDD, DNN, and NDD spring haplotype groups, several entries had noticeably higher baseline PHS BLUPs than average. This may be due to genotyping errors, modifiers of *HvAlaAT1, HvGA20ox1,* or *HvMKK3*, or other unknown large effect loci. One entry with NND haplotypes was identified in this work but was not present in enough experiments for analysis. This haplotype is a target of future research.

**Physiological basis of preharvest sprouting temperature sensitivity**

Dormant *HvGA20ox1* and non-dormant *HvMKK3* spring haplotypes displayed temperature sensitivity similar to non-dormant *HvGA20ox1* haplotypes. Non-dormant *HvMKK3* haplotypes in pairs of spring haplotypes differing by *HvMKK3* allelic state (DDD/DDN and NDD/NDN) were more sensitive to Tmax and Tavg than the dormant *HvMKK3* counterpart (Table 6). Both observations indicate temperature sensitivity of the non-dormant *HvMKK3* allele. Although the physiological function of *HvMKK3* is still unknown, results from wheat and rice provide a potential model for temperature sensitivity of *HvMKK3*. *TaMKK3-A* has been identified as the causal gene at the Phs-A1 locus for PHS resistance on chromosome 4A in wheat (Torada et al., 2016; Shorinola et al., 2017). The ABA hypersensitive *ENHANCED RESPONSE TO ABA8* (*ERA8*) mutant in wheat is likely a novel allele of *TaMKK3-A* (Martinez et al., 2020) that results in increased ABA sensitivity at physiological maturity through the after-ripening period but does not increase ABA content (Martinez et al., 2016). In rice (*Oryza sativa* L.), *OsMKK3* is part of a MAPK cascade system composed of MKKK62-MKK3-MAPK7/14 (Mao et al., 2019). Overexpression of MKKK62 reduced seed dormancy, reduced ABA sensitivity, and reduced expression of *OsMFT* while knockouts of MKK3 and MAPK7/14 increased seed dormancy and expression of *OsMFT*. *MOTHER OF FT AND TFL1* (*MFT*) is a highly conserved regulator of seed germination in the phosphatidylethanolamine-binding protein family, which also includes *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER1* (*TFL1*) (Xi et al., 2010). A wheat *MFT* homolog is the underlying causal gene for the *TaPHS1* locus on chromosome 3AS (Nakamura et al., 2011; Liu et al., 2013) and positively regulates seed dormancy. *TaMFT* displays differential temperature sensitivity before and after seed physiological maturity. Low grain fill temperatures (13 C) increased *TaMFT* expression and embryo dormancy and high grain fill temperatures (25 C) reduced *TaMFT* expression and embryo dormancy (Nakamura et al., 2011) but *TaMFT* expression is reduced by low germination temperatures (4 C overnight treatment) in after-ripened seed, leading to increased germination (Lei et al., 2013). *OsMFT2* was recently identified as a positive regulator of ABA signaling, and thus seed dormancy, in rice through interactions with three basic leucine zipper transcription factors (Song et al., 2020). Vetch et al. (2020) sequenced *HvMFT* in North American spring malting germplasm but did not detect sequence variants. In wheat, *TaMKK3* is likely involved in ABA signaling but has not been directly associated with *TaMFT,* a temperature sensitive positive regulator of seed dormancy. It is unknown if *TaMFT* is directly temperature sensitive or regulated by upstream temperature sensitive factors but several ABA-regulated MAPK cascades are known to be induced by abiotic stress (Danquah et al., 2015; Colcombet & Hirt, 2008). *HvMKK3* may be involved in ABA signaling and regulation of *HvMFT.* The MAPK cascade including *HvMKK3* may not be directly temperature sensitive, but it is likely ABA sensitive and barleyseed ABA content is temperature sensitive. Reduced ABA sensitivity in an ABA-mediated MAPK cascade conferred by a non-dormant *HvMKK3* allele might lead to a baseline reduction in *HvMFT* expression, and therefore seed dormancy, that would further be reduced by decreased ABA content resulting from high temperature during grain fill. Spring haplotypes with a non-dormant *HvMKK3* allele have higher baseline PHS and higher temperature sensitivity than dormant *HvMKK3* haplotypes which supports this hypothesis. Temperature sensitivity of *HvGA20ox1* cannot be estimated in these datasets as non-dormant *HvGA20ox1* and dormant *HvMKK3* haplotypes were not observed in this study, confounding temperature sensitivity of non-dormant *HvGA20ox1* with temperature sensitivity of non-dormant *HvMKK3.*

*HvAlaAT1* also showed evidence of temperature sensitivity, with differences in slope for haplotype pairs differing by allelic state at *HvAlaAT1* (NNN/DNN, DDD/NDD, NDN/DDN) in winter and spring datasets (Table 6). The physiological mechanism of *HvAlaAT1* imposed seed dormancy is also unknown but has also been speculated to be connected to ABA signaling (Sato et al., 2016). This hypothesis is consistent with the temperature sensitivity results of this study. *HvAlaAT1* has been observed to have a larger effect on dormancy duration than primary dormancy level (Sato et al., 2016; Vetch et al., 2020). *TaQsd1* was identified as a dormancy period QTL in wheat and was found to encode AlaAT, with variants in *TaQsd1-5B* (Wei et al., 2019). *TaQsd1-5B* variants associated with prolonged dormancy had higher expression of the ABA biosynthesis genes, *TaNCED1* and *TaNCED2*, decreased expression of the ABA catabolism gene *TaCYP707A1*, and increased ABA content compared with short dormancy *TaQsd1-5B* variants. Temperature sensitivity of *HvAlaAT1* may be similarly related to the change in *HvNCED1* and *HvCYP707A1* expression across environments noted by Chono et al. (2006). The *SD1* locus has rarely been detected in PHS mapping studies with two-row by two-row parentage (Li et al., 2003; Ullrich et al., 2009; Hickey et al., 2012; Sweeney et al., 2020). The exceptions are Bonnardeaux et al. (2008) and Gong et al. (2014) who both detected *SD1* as a minor effect QTL in a Stirling x Harrington doubled haploid population grown in three and ten environments, respectively. However, *SD1* was not detected in all environments and had variable effects across environment. Gong et al. (2014) speculated that growth conditions did not favor *SD1* expression or that germination test conditions favored rapid germination, masking the dormancy release rate effects of *SD1*. This supports the findings of this study that *HvAlaAT1* has variable effects across temperature regimes and in combination with *HvGA20ox1* and *HvMKK3*.

**Conclusion**

Our results demonstrated differential seed dormancy haplotype response to temperature and precipitation covariates in winter and spring malting barley. These results provide valuable information for barley breeders looking to select stable PHS resistance and may be useful for wheat breeding as well since MKK3 and AlaAT are known to affect PHS in both species. Marker assisted selection is a promising tool to use in breeding for PHS resistance due to the presence of several large effect seed dormancy QTL but background polygenic effects and additional polygenic temperature sensitivity suggest the potential for a genomic selection approach. This quantitative variation indicates that further breeding progress may be possible for combining PHS resistance with good malting quality. The impact of temperature on malting quality and its relationship with PHS susceptibility in the spring and winter datasets is unknown. In regions with high annual PHS risk and large variation in spring and summer temperatures, DDD and NDD haplotypes are the most stable source of PHS resistance for winter and spring barley. Triple dormant lines may exhibit excessive primary dormancy in spring germplasm (Sweeney et al., 2021), which is undesirable for malting and may explain why no commercial spring DDD lines were observed in this study. Several of the winter DDD entries are known to have good malting quality, suggesting possible differences in dormancy release rate or ABA sensitivity between winter and spring DDD entries. For areas with cooler average summer temperatures, the NDN haplotype may provide adequate PHS resistance for spring germplasm but the increased polygenic variation in this haplotype group requires further local testing. Winter and spring entries with the NNN and DNN haplotypes have consistent high risk for PHS in all environments and must be carefully managed in high moisture environments. These models provide a practical model for forecasting PHS risk by variety with simple weather data to prioritize harvest in high risk years.

Tables and figures

Table 1: Summary of preharvest sprouting experiments. Experiments consisted of replicated yield trial plots and early generation meter long headrow nurseries. (**Tables 1-3 likely supplementary material**).

|  |  |  |  |
| --- | --- | --- | --- |
| **Dataset** | **Experiment** | **Years** | **Type** |
| Spring | SMBReg | 2015-2020 | yield |
| Spring | ESBN | 2015-2020 | yield |
| Spring | S2MET | 2015-2016 | yield |
| Spring | CU\_TP | 2017 | headrow |
| Spring | CUReg | 2018-2019 | yield |
| Spring | CU1 | 2019-2020 | headrow |
| Spring | NakedReg | 2018-2020 | yield |
| Winter | WMBReg | 2016-2020 | yield |
| Winter | WMBCoop | 2016-2020 | yield |
| Winter | OSUmalt | 2016-2018 | yield |

Table 2: Summary of genotyped entries in spring and winter datasets. Haplotypes were defined by allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele. CU entries signify Cornell University experimental entries, OSU, Oregon State University experimental entries, MSU, Montana State University experimental lines, and NDSU, North Dakota State University experimental lines. All entries are two-row malting barleys except for CDC\_CLEAR, DH133529, and DH133535, which are two-row spring naked barleys intended for food and malting use. Three entries, LIGHTNING, DH130935, and DH131055, exhibit a facultative growth habit (labeled as F) and were evaluated in spring and winter experiments. The number of preharvest sprouting (PHS) observations on a per plot basis for each entry is indicated by n and PHS phenotypic mean and variance are indicated by μ and σp2, respectively. **Entries with an asterisk indicate commercial lines that may need to be given a pseudonym.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dataset | Entry | Source | Haplotype | n | PHS μ | PHS σp2 |
| Spring | AC\_METCALFE | commercial | NNN | 15 | 5.43 | 2.08 |
| Spring | BENTLEY | commercial | NNN | 48 | 5.19 | 2.39 |
| Spring | CDC\_CLEAR | commercial | NNN | 14 | 6.84 | 1.35 |
| Spring | CDC\_COPELAND | commercial | NNN | 12 | 5.93 | 2.73 |
| Spring | FULL\_PINT | commercial | NNN | 20 | 5.71 | 2.34 |
| Spring | SG293-3 | CU | NNN | 8 | 6.96 | 0.75 |
| Spring | SG542-1 | CU | NNN | 8 | 6.58 | 0.83 |
| Spring | SP362R-2 | CU | NNN | 8 | 6.63 | 0.58 |
| Spring | 08MT-03 | MSU | NDD | 14 | 2.03 | 2.04 |
| Spring | 09N2-65 | NDSU | NDD | 14 | 3.14 | 0.90 |
| Spring | 09N2-96 | NDSU | NDD | 14 | 5.22 | 3.46 |
| Spring | 2ND32529 | NDSU | NDD | 15 | 5.44 | 1.89 |
| Spring | ACCORDINE\* | commercial | NDD | 15 | 1.99 | 0.85 |
| Spring | CONLON | commercial | NDD | 83 | 2.44 | 2.19 |
| Spring | CRAFT | commercial | NDD | 70 | 1.10 | 1.07 |
| Spring (F) | DH130935 | OSU | NDD | 12 | 2.14 | 1.28 |
| Spring (F) | DH131055 | OSU | NDD | 6 | 3.34 | 0.52 |
| Spring | DH133529 | OSU | NDD | 14 | 1.46 | 2.53 |
| Spring | DH133535 | OSU | NDD | 14 | 1.39 | 3.10 |
| Spring | ESMA\* | commercial | NDD | 29 | 0.91 | 0.46 |
| Spring | EXPLORER\* | commercial | NDD | 32 | 0.32 | 0.44 |
| Spring | EXPO\* | commercial | NDD | 15 | 0.84 | 0.76 |
| Spring | KWS\_CHRISSIE\* | commercial | NDD | 15 | 2.04 | 1.52 |
| Spring | KWS\_JESSIE\* | commercial | NDD | 18 | 1.55 | 1.31 |
| Spring | KWS\_TINKA\* | commercial | NDD | 102 | 2.41 | 1.22 |
| Spring | SANGRIA\* | commercial | NDD | 26 | 1.09 | 1.37 |
| Spring | ST1453-4 | CU | NDD | 16 | 2.63 | 1.95 |
| Spring | 09N2-16 | NDSU | NDN | 9 | 4.68 | 3.04 |
| Spring | 09N2-84 | NDSU | NDN | 8 | 4.51 | 4.34 |
| Spring | EIFEL\* | commercial | NDN | 18 | 1.76 | 1.00 |
| Spring | LCS\_GENIE\* | commercial | NDN | 29 | 1.43 | 1.67 |
| Spring | ND\_GENESIS | commercial | NDN | 116 | 3.43 | 2.07 |
| Spring | PINNACLE | commercial | NDN | 113 | 1.55 | 2.93 |
| Spring | SG5123-1 | CU | NDN | 17 | 2.59 | 3.43 |
| Spring | SP333R-1 | CU | NDN | 14 | 3.18 | 2.86 |
| Spring | SP572-3 | CU | NDN | 13 | 2.01 | 1.28 |
| Spring | SP575-1 | CU | NDN | 10 | 2.80 | 1.13 |
| Spring | AAC\_CONNECT | commercial | DNN | 9 | 6.44 | 1.69 |
| Spring | AAC\_SYNERGY | commercial | DNN | 217 | 5.10 | 1.41 |
| Spring | NEWDALE | commercial | DNN | 94 | 4.61 | 2.45 |
| Spring | SB182R-3 | CU | DNN | 8 | 5.89 | 2.29 |
| Spring | SN391R-1 | CU | DNN | 14 | 4.91 | 2.76 |
| Spring | SR556-3 | CU | DNN | 10 | 4.62 | 2.96 |
| Spring | SR591R-3 | CU | DNN | 14 | 4.70 | 2.45 |
| Spring (F) | LIGHTNING | commercial | DDD | 26 | 1.44 | 0.97 |
| Spring | SC662R-1 | CU | DDD | 12 | 3.22 | 3.63 |
| Spring | SR575-2 | CU | DDD | 10 | 1.86 | 2.98 |
| Spring | SR6122R-1 | CU | DDD | 14 | 1.93 | 0.92 |
| Spring | SR632R-3 | CU | DDD | 12 | 0.82 | 1.01 |
| Spring | ST1442R-2 | CU | DDD | 12 | 1.54 | 1.33 |
| Spring | ST1453-2 | CU | DDD | 14 | 1.65 | 0.60 |
| Spring | ST1481R-1 | CU | DDD | 15 | 2.91 | 1.35 |
| Spring | SC742R-2 | CU | DDN | 15 | 1.60 | 1.70 |
| Spring | SC795-4 | CU | DDN | 16 | 2.03 | 2.34 |
| Spring | SG223R-1 | CU | DDN | 12 | 3.40 | 3.27 |
| Spring | SG231R-1 | CU | DDN | 12 | 2.81 | 1.52 |
| Spring | SP323R-2 | CU | DDN | 12 | 2.79 | 3.00 |
| Spring | SP382R-3 | CU | DDN | 10 | 2.24 | 3.49 |
| Spring | SP585-2 | CU | DDN | 13 | 1.45 | 1.41 |
| Winter | DH130004 | OSU | NNN | 12 | 6.47 | 3.62 |
| Winter | **DH130718** | OSU | NNN | 21 | 6.46 | 2.47 |
| Winter | DH130939 | OSU | NNN | 24 | 6.61 | 2.90 |
| Winter | DH140088 | OSU | NNN | 30 | 5.84 | 3.64 |
| Winter | THUNDER | commercial | NNN | 16 | 6.78 | 3.74 |
| Winter (F) | DH131055 | OSU | NDD | 18 | 0.00 | 0.00 |
| Winter | DH131738 | OSU | NDD | 20 | 0.28 | 0.15 |
| Winter (F) | DH130935 | OSU | NDD | 21 | 1.07 | 0.81 |
| Winter | KWS\_DONAU\* | commercial | NDD | 30 | 1.40 | 1.60 |
| Winter | KWS\_SCALA\* | commercial | NDD | 30 | 0.4 | 0.34 |
| Winter | KWS\_SOMERSET\* | commercial | NDD | 30 | 0.28 | 0.18 |
| Winter | NECTARIA\* | commercial | NDD | 21 | 1.29 | 0.87 |
| Winter | SY\_TEPEE\* | commercial | NDD | 27 | 0.19 | 0.08 |
| Winter | VIOLETTA\* | commercial | NDD | 18 | 0.08 | 0.02 |
| Winter | WINTMALT\* | commercial | NDD | 26 | 1.46 | 1.29 |
| Winter | CHARLES | commercial | DNN | 51 | 4.66 | 7.1 |
| Winter | ENDEAVOR | commercial | DNN | 48 | 6.94 | 2.39 |
| Winter | CALYPSO\* | commercial | DDD | 24 | 0.28 | 0.25 |
| Winter (F) | LIGHTNING | commercial | DDD | 42 | 0.00 | 0.00 |
| Winter | FLAVIA\* | commercial | DDD | 39 | 0.88 | 0.88 |
| Winter | SU\_MATEO\* | commercial | DDD | 24 | 0.77 | 0.92 |

Table 3: Environmental summaries with planting date, average heading date, average daily high temperature (Tmax), average daily low temperature (Tmin), average daily temperature (Tavg), and total precipitation (Psum) during the grain fill period. Temperatures in Celsius and precipitation in centimeters.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Dataset | Environment | Planting date | Average heading date | Tmax | Tavg | Tmin | Psum |
| Spring | Ketola2015 | 5/4/2015 | 6/30/2015 | 25.3 | 19.1 | 13.4 | 12.4 |
| Spring | Snyder2015 | 4/27/2015 | 6/19/2015 | 24.8 | 19.0 | 13.1 | 16.5 |
| Spring | S2MET\_KT2015 | 5/5/2015 | 6/29/2015 | 25.0 | 18.9 | 13.2 | 12.2 |
| Spring | Helfer2016 | 4/27/2016 | 7/2/2016 | 29.1 | 21.2 | 14.4 | 3.8 |
| Spring | Ketola2016 | 4/22/2016 | 6/25/2016 | 29.5 | 20.4 | 14.2 | 2.8 |
| Spring | S2MET\_Hel2016 | 4/20/2016 | 6/29/2016 | 28.4 | 20.9 | 14.1 | 3.6 |
| Spring | S2MET\_Ket2016 | 4/28/2016 | 6/25/2016 | 28.3 | 20.6 | 14.0 | 2.8 |
| Spring | Helfer2017 | 4/27/2017 | 6/23/2017 | 27.2 | 20.2 | 14.2 | 14.0 |
| Spring | Ketola2017 | 4/23/2017 | 6/24/2017 | 27.5 | 20.3 | 14.4 | 14.5 |
| Spring | CU\_TP\_Hel2017 | 5/12/2017 | 7/9/2017 | 25.9 | 20.3 | 15.0 | 15.0 |
| Spring | CU\_TP\_Sny2017 | 5/12/2017 | 7/12/2017 | 26.1 | 20.4 | 14.7 | 13.2 |
| Spring | Caldwell2018 | 4/30/2018 | 6/22/2018 | 27.2 | 19.6 | 13.1 | 4.6 |
| Spring | Helfer2018 | 5/7/2018 | 6/29/2018 | 27.3 | 20.3 | 13.4 | 9.1 |
| Spring | Ketola2018 | 5/6/2018 | 6/28/2018 | 27.3 | 20.3 | 13.4 | 7.4 |
| Spring | Naked\_Freeville2018 | 5/8/2018 | 7/1/2018 | 27.3 | 20.6 | 13.8 | 10.9 |
| Spring | Ketola2019 | 4/17/2019 | 6/22/2019 | 27.3 | 20.2 | 14.7 | 8.1 |
| Spring | Snyder2019 | 4/9/2019 | 6/17/2019 | 27.4 | 19.9 | 14.3 | 13.0 |
| Spring | CU1\_Caldwell2019 | 4/12/2019 | 6/24/2019 | 27.2 | 20.1 | 14.4 | 5.6 |
| Spring | CU1\_Ketola2019 | 4/12/2019 | 6/28/2019 | 27.6 | 20.6 | 14.8 | 3.8 |
| Spring | Naked\_Caldwell2019 | 4/23/2019 | 6/25/2019 | 26.9 | 20.8 | 14.7 | 12.4 |
| Spring | Naked\_Freeville2019 | 4/22/2019 | 6/22/2019 | 26.8 | 20.7 | 14.6 | 13.2 |
| Spring | Helfer2020 | 4/6/2020 | 6/20/2020 | 27.4 | 20.7 | 14.0 | 11.2 |
| Spring | CU1\_Caldwell2020 | 4/22/2020 | 7/1/2020 | 28.4 | 22.1 | 15.7 | 12.2 |
| Spring | CU1\_Helfer2020 | 4/15/2020 | 6/28/2020 | 28.2 | 21.7 | 15.2 | 12.2 |
| Spring | Naked\_Freeville2020 | 5/4/2020 | 6/24/2020 | 27.9 | 21.4 | 14.8 | 11.4 |
| Winter | Ketola2016 | 10/5/2015 | 5/23/2016 | 25.2 | 17.8 | 10.5 | 3.6 |
| Winter | Snyder2016 | 9/23/2015 | 5/20/2016 | 24.7 | 17.2 | 10.0 | 3.6 |
| Winter | Ketola2017 | 10/6/2016 | 5/24/2017 | 23.3 | 17.4 | 11.4 | 12.4 |
| Winter | McGowan2017 | 9/28/2016 | 5/20/2017 | 23.2 | 17.1 | 11.2 | 10.9 |
| Winter | Ketola2018 | 10/3/2017 | 5/25/2018 | 24.4 | 17.8 | 11.4 | 4.8 |
| Winter | Snyder2018 | 10/2/2017 | 5/27/2018 | 24.5 | 18.1 | 11.7 | 6.1 |
| Winter | Snyder2019 | 10/10/2019 | 5/31/2019 | 23.7 | 17.6 | 11.4 | 15.7 |
| Winter | Ketola2020 | 9/27/2019 | 5/25/2019 | 25.2 | 18.4 | 11.7 | 3.6 |
| Winter | Snyder2020 | 9/26/2019 | 5/26/2019 | 25.4 | 18.7 | 11.9 | 6.4 |

Table 4: Phenotypic correlations between environmental covariates average daily high temperature (Tmax), average daily low temperature (Tmin), average daily temperature (Tavg), average daily temperature range (TR), and the sum of precipitation (Psum) during the grain fill period for winter and spring (shaded gray) datasets.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Tmax | Tavg | Tmin | TR | Psum |
| Tmax | 1 | 0.81 | 0.21 | 0.70 | -0.67 |
| Tavg | 0.83 | 1 | 0.74 | 0.16 | -0.29 |
| Tmin | 0.23 | 0.73 | 1 | -0.79 | 0.29 |
| TR | 0.54 | 0.02 | -0.61 | 1 | -0.79 |
| Psum | -0.68 | -0.20 | 0.46 | -0.82 | 1 |

Table 5: QTL x environment interaction model summaries for spring and winter models fitting environmental covariates (Z) as average daily high temperature (Tmax), average daily low temperature (Tmin), average daily temperature (Tavg), average daily temperature range (TR), and the sum of precipitation (Psum) during the grain fill period. Wald tests were conducted for environment (E), QTL (Q), and QTL by environmental covariate (QZ) fixed effects. Wald test significance is designated as Pr(Chisq) < .001 (\*\*\*), Pr(Chisq) < .01 (\*\*), Pr(Chisq) < .05 (\*), or not significant (ns). Variance component estimates for entry (G), entry by environmental covariate interaction (GZ), entry by environment interaction (GE), and QTL by environment interaction (QE) are presented. Error variance (e) is presented as the average of heterogeneous error variance estimates at each environment. All variance component estimates presented here are for entries with haplotype data. Separate genetic variance component estimates for lines without haplotype data were omitted from the table. AIC was estimated with icREML to enable comparison of models with different fixed effects (Verbyla, 2019).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Spring** | Fixed | Random | Tmax | Tavg | Tmin | TR | P |
| Wald | E |  | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* |
|  | Q |  | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* |
|  | QZ |  | \* | \* | ns | ns | ns |
| Variance |  | G | 0.577 | 0.668 | 0.713 | 0.498 | 0.712 |
|  |  | GZ | 3.54E-02 | 5.12E-02 | 1.70E-02 | 4.24E-02 | 4.81E-04 |
|  |  | GE | 0.392 | 0.402 | 0.408 | 0.363 | 0.378 |
|  |  | QE | 0.421 | 0.412 | 0.425 | 0.491 | 0.463 |
|  |  | e | 0.708 | 0.709 | 0.728 | 0.723 | 0.69 |
| AIC |  |  | 8844.27 | 8838.86 | 8825.71 | 8858.71 | 8814.87 |
| **Winter** | Fixed | Random | Tmax | Tavg | Tmin | TR | P |
| Wald | E |  | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* |
|  | Q |  | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* |
|  | QZ |  | ns | \* | \* | ns | \*\*\* |
| Variance |  | G | 0.348 | 0.34 | 0.306 | 0.362 | 0.368 |
|  |  | GZ | 2.68E-08 | 2.68E-08 | 3.51E-03 | 2.68E-08 | 3.75E-07 |
|  |  | GE | 0.454 | 0.447 | 0.442 | 0.458 | 0.461 |
|  |  | QE | 1.03 | 1.102 | 1.27 | 1.15 | 1.53 |
|  |  | e | 0.276 | 0.276 | 0.275 | 0.275 | 0.276 |
| AIC |  |  | 819.2 | 820.71 | 821.55 | 799.7 | 809.11 |

\*\*\*: Pr(chisq) < .001

\*\*: Pr(chisq) < .01

\*: Pr(chisq) < .05

ns: Pr(chisq) > .05

Table 6: QTL by environmental covariate interaction effects and standard errors for spring and winter datasets. Haplotypes are defined as allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dataset | Haplotype | Tmax | Tavg | Tmin | TR | Psum |
| Spring | NNN | .230 (.176) | .358 (.209) | 0.352 (.186) | -0.034 (.194) | .005 (.034) |
| Spring | DNN | .290 (.151) | .324 (.178) | .173 (.147) | .267 (.165) | -0.012 (.027) |
| Spring | DDD | -0.214 (0.23) | -0.283 (.271) | -0.72 (.213) | -0.014 (.228) | .013 (.044) |
| Spring | NDD | .032 (.108) | -0.079 (.128) | -.197 (.112) | .211 (.123) | -0.013 (.020) |
| Spring | NDN | .302 (.137) | .285 (.163) | 0.149 (.131) | .219 (.145) | -0.019 (.024) |
| Spring | DDN | .534 (.284) | .519 (.352) | 0.089 (.261) | .248 (.236) | 0.014 (.056) |
| Winter | NNN | .486 (.232) | .51 (.265) | .359 (.276) | .542 (.374) | -0.095 (.083) |
| Winter | DNN | .386 (.24) | .608 (.296) | .62 (.296) | -0.043 (.333) | .153 (.05) |
| Winter | DDD | .098 (.203) | .121 (.237) | .107 (.247) | .079 (.294) | -0.082 (.046) |
| Winter | NDD | .297 (.183) | .443 (.208) | .418 (.203) | -0.039 (.268) | .106 (.041) |

Table 7: Primer sequences for KASP markers (**this will need to be horizontal or rearranged**)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker | Chr | Position (Morex v1) | Position (Morex v2) | Gene | Target gene ID | Allele-1 forward primer | Allele-2 forward primer | Universal reverse primer | **Non-dormant**/dormant allele | Part of 50K chip? | Causal SNP? |
| HvQsd1 | 5H | 489071876 | 442161820 | *HvAlaAT1* | HORVU5Hr1G062990 | GATTTTCGAAGTAAAGAGGTGCTT**G** | GATTTTCGAAGTAAAGAGGTGCTT**C** | CACGAACAGTCAAACCTGCG | **G**/C | no | Likely |
| SCRI\_RS\_121526 | 5H | 665575557 | 595256155 | *HvGA20ox1* | HORVU5Hr1G124120 | AGCATAGACTGTCAGGCTCC**A** | AGCATAGACTGTCAGGCTCC**C** | AAATGCATAAATCATGGCAGCAA | **A**/C | yes | No |
| JHI-Hv50k-2016-367342 | 5H | 668430624 | 596729543 | *HvMKK3* | HORVU5Hr1G125290 | GTGATTCCTCGCTGCTTGGT**A** | GTGATTCCTCGCTGCTTGGT**G** | AGTGAGTAATAATGAGCCCAGCC | **A**/G | yes | No |

Figure 1: Average monthly May-August temperature and rainfall for Ithaca, NY from 1990-2020. Blue bars indicate years used in this study. **This is likely supplement, might not be useful at all. Will probably omit.**  **Need to convert to C and cm if kept.**

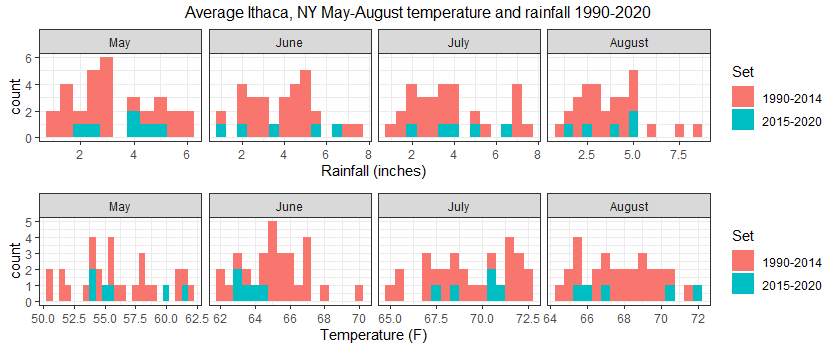


Figure 2: Spring barley QTL by precipitation interactions during grain fill and deviations due to background polygenic entry effects. Black lines indicate the main haplotype intercept and QTL by precipitation interaction and gray lines indicate entry deviations from the main haplotype intercept and slope. Haplotypes are defined as allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele.

A picture containing calendar

Description automatically generated

Figure 3: Spring barley QTL by average high temperature interactions during grain fill and deviations due to background polygenic entry effects. Black lines indicate the main haplotype intercept and QTL by average high temperature interaction and gray lines indicate entry deviations from the main haplotype intercept and slope. Haplotypes are defined as allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele.

A picture containing chart

Description automatically generated

Figure 4: Winter barley QTL by temperature range interactions during grain fill and deviations due to background polygenic entry effects. Black lines indicate the main haplotype intercept and QTL by temperature range interaction and gray lines indicate entry deviations from the main haplotype intercept and slope. Haplotypes are defined as allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele.

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Figure 5: Winter barley QTL by average high temperature interactions during grain fill and deviations due to background polygenic entry effects. Black lines indicate the main haplotype intercept and QTL by average high temperature interaction and gray lines indicate entry deviations from the main haplotype intercept and slope. Haplotypes are defined as allelic state at *HvAlaAT1*, *HvGA20ox1*, and *HvMKK3,* respectively, where N specifies a non-dormant allele and D specifies a dormant allele.

A picture containing chart

Description automatically generated