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QTL mapping reveals malt barley quality improvement in two dryland environments associated with extended grain fill and seminal root traits

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Abstract

To achieve malt grade and receive full price, barley (*Hordeum vulgare* L.) crops must meet standards for certain quality traits including percent plump and protein. Terminal drought stress reduces quality and is projected to worsen in barley cultivation areas, underscoring the need for varieties that maintain good malt production with unreliable precipitation. The stay-green trait extends the grain fill phase between heading and maturity and has been linked to stable quality under dry conditions. However, this relationship can be inconsistent and is not well understood. To effectively leverage a longer grain fill phenotype for drought adaptation, a better grasp of its genetics and environmental interaction is needed. Stay-green root system differences have been observed and could be at play. We performed correlation and quantitative trait locus (QTL) analysis on grain fill duration, grain quality, and seminal root traits using a recombinant inbred line (RIL) population segregating for stay-green. Agronomic data were collected in four field trials at two distinct semi-arid locations, and roots were measured in a greenhouse assay. Earlier heading and later maturity led to improved quality in both locations and more consistent quality between locations. Earlier heading had a greater influence on quality in the drier environment, while later maturity was more impactful in the less dry environment. We observed co-locations of seminal root trait QTLs with grain fill duration and grain quality. These QTLs lay the groundwork for further investigation into root phenotypes associated with stay-green and the deployment of these traits in breeding for drought adaptation.

Abbreviations: ANOVA, analysis of variance; BLUP, best linear unbiased prediction; CIM, composite interval mapping; DH, doubled haploid; KASP, competitive allele-specific PCR; LOD, logarithm of odds; MSU, Montana State University; NIL, near-isogenic line; QTL, quantitative trait locus; RIL, recombinant inbred line.

1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal crop in the United States and globally. End-use products for barley grain include animal feed, human food, and malt. Malt is used for brewing, for distilling, and as a food additive and is usually the most profitable end use for a barley grower. From 2012 to 2022 in the United States, the price received for malt barley averaged 47% higher than the price for feed barley (USDA NASS, 2023). For a barley crop to be sold at this higher malt price, it must meet a series of strict quality requirements including low grain protein content (<13.0%) and high percent plump grain (>90% on a $^{6}/_{64}$-inch sieve) (AMBA, 2021). Thus, these crop traits are at least as important a consideration as yield in malt barley cultivation and breeding (McVay et al., 2017). In recent years, most barley in the United States has been grown in Montana, North Dakota, and Idaho (USDA NASS, 2023). A survey of Montana growers ranked malt quality as the second most important trait to consider when choosing a barley variety (USDA NASS, 2022). Drought tolerance was the highest ranked trait.

The states where most US barley is grown regularly experience terminal drought stress (NOAA NCEI, 2023), where a reduction in precipitation occurs during the latter phases of crop development. In barley, the grain fill phase when the plant is accumulating starch and protein in the seed endosperm begins with heading and ends with maturity (Alqudah & Schnurbush, 2017). While barley has a reputation as a relatively drought-tolerant crop, terminal drought conditions coinciding with grain fill negatively impact yield and malt quality parameters due to decreased starch accumulation, resulting in higher protein content and thinner grains (Gordon et al., 2020; Tarawneh et al., 2020). Many malt growers mitigate a lack of precipitation during grain fill with irrigation. However, the usefulness of irrigation as a risk management tool is likewise curtailed by limited availability of water and irrigable acres. Increasing temperatures and shifting precipitation patterns threaten the reliability of water supplies and are predicted to exacerbate the terminal drought hazard (Conant et al., 2018). Additionally, malting, brewing, and distilling businesses are increasingly concerned with sustainability. There is mounting pressure to conserve water, and the drought adaptation of barley needs to be improved in order to maintain high-quality malt production.

Stay-green is a trait that has been observed in several plant species (Thomas & Smart, 1993), including barley, and has received attention as an adaptation to drought. Functional stay-green is longer retention of photosynthetic leaf area due to delayed initiation of senescence and/or a slower rate of senescence (Thomas & Ougham, 2014). This results in a prolonged grain fill period. We have also observed further extension of the grain fill period in stay-green barley lines via earlier heading in a previous study (Williams et al., 2022). Stay-green has been connected to yield stability in water-limited environments in wheat (*Triticum aestivum* L.) (Christopher et al., 2008), sorghum (*Sorghum bicolor* L. Moench) (Borrell et al., 2014), and maize (*Zea mays* L.) (Trachsel et al., 2016). This trait has been studied less in barley than other cereals (Kamal et al., 2019) but has benefits for grain quality. A thorough examination of barley grain components in a controlled environment experiment by Gous et al. (2013) showed that a stay-green cultivar maintained low protein content and stable starch structure under water stress, while these qualities were sensitive to water stress in a non-stay-green cultivar. Shirdelmoghanloo et al. (2022) observed instances of plumper barley grains correlating with longer green leaf area retention and grain fill duration in heat-stressed field trials. However, expression of the stay-green phenotype and its agronomic advantages can be inconsistent across environments and genotypes, and the genetic and physiological basis of stay-green is not fully understood.

Core Ideas

- Extended grain fill improved and stabilized barley plump and protein quality for malting.
- Grain quality was more affected by heading in the drier location and by maturity where there was more moisture.
- Extended grain fill, early root development, and grain quality co-located at multiple QTLs.
- Seminal root-to-shoot length ratio co-located with a gene (*HvNAM1*) that affects protein, plump, and maturity.

Stay-green is a complex trait that exhibits environmental plasticity, highlighting the need for further genetic dissection and field examination of the phenotype. In a glasshouse study of a doubled haploid (DH) barley population segregating for stay-green, different quantitative trait loci (QTLs) were identified for measures of leaf greenness depending on whether the plants had been subjected to water stress or heat stress (Gous et al., 2016). Emebiri (2013) found different QTLs for green color retention depending on the genetic source of stay-green in field trials of two different barley DH populations. Their measure of stay-green was also found to be either positively or negatively correlated with flowering time and grain plumpness depending on the population. In order to appropriately utilize a protracted grain fill period to enhance drought adaptation, the genetics of this phenotype and its relation to other agronomically significant traits need to be examined in the given genetic background. It is also important to test crop performance in the target production environment. A strategy that helps a plant maintain yield and quality in one drought-stressed environment may not work in another (Palta & Turner, 2019). Yet, consistent expression of key phenotypes across locations is important to plant breeders striving to produce reliable varieties (Bernardo, 2010), so genotype-by-environment interactions need to be understood. In a multi-environment study of a stay-green wheat line, Christopher et al. (2008) observed expression of the stay-green phenotype and its associated yield benefit in environments with deep soil moisture, but they observed neither in an environment where deep moisture was lacking. Access to stored water during grain fill could be part of the mechanism behind the stay-green trait.

As the site of water acquisition, root systems are a logical focal point for investigations into the source of a putative drought tolerance trait. Indeed, multiple studies have uncovered evidence suggesting a relationship between roots and stay-green. Our previous study (Williams et al., 2022) examined roots in the field with the use of minirhizotrons and found that stay-green barley lines with longer grain fill duration had a greater percentage of their roots deeper in the soil and prolonged root growth compared to non-stay-green lines. Stay-green lines were also found to differ from non-stay-green in that study for traits measured in a greenhouse assay such as the length and number of seminal roots and the number of initiated lateral roots at the one-leaf stage. Furthermore, some of these seminal root phenotypes correlated with root traits measured in the field later in development. Other researchers have also drawn connections between stay-green and root attributes in controlled environment experiments. For example, a stay-green barley line was found to have a wider seminal root angle and lower root number than a non-stay-green line in a seedling assay (Robinson et al., 2016). Manschadi et al. (2006) found a stay-green wheat variety to have increased deep rooting compared to a non-stay-green variety in a root growth observation chamber. In QTL mapping studies, traits measured in root assays have been found to be co-located with stay-green QTLs in barley (Gous et al., 2016), wheat (Christopher et al., 2018), and sorghum (Mace et al., 2012). Observation of root systems in the field poses many challenges, so ex situ root examinations are useful especially for large populations. Roots are also understudied compared to more readily observed aboveground structures and thus represent a largely untapped resource for potential crop improvement.

The goal of this study was to genetically dissect seminal root traits, the stay-green trait, and agronomic and grain quality traits in two distinct semiarid environments with different precipitation patterns to discern the relationships between these phenotypes and their environmental interactions. We performed genetic linkage analysis on 168 recombinant inbred lines (RILs), which were derived from a cross between two-rowed barley lines—MT124118, a Montana State University (MSU) experimental line, and ND19119, a line from North Dakota State University regarded as stay-green due to its longer grain fill duration and known for large kernel size (Frankowiak et al., 2007, 2010). We performed QTL mapping for grain fill duration and grain quality parameters assessed in field trials, and seminal root traits measured in a greenhouse seedling assay. The locations used for our field trials allowed us to compare performance in environments differing for precipitation levels, soil nitrogen availability, and other factors. In situ examination of mature root systems was not practical for a population of this size, but significant correlations were previously observed between seminal root traits measured by this greenhouse method and root traits measured in the field (Williams et al., 2022). Here, we present genetic loci associated with length of grain fill, grain quality, and seminal root traits, as well as relationships between these phenotypes and with their environments.

2 | MATERIALS AND METHODS

2.1 | Plant material

To generate the biparental mapping population used in this study, female parent MT124118 (Hockett/MT070174) from MSU was crossed to pollen donor ND19119 (ND15403.3/ND15368//ND16453) from North Dakota State University. Both are spring two-rowed barley lines developed for malt end use. ND19119 is characterized as having long grain fill and large kernels (Frankowiak et al., 2007), and a close descendant of this line has been shown to have prolonged retention of green leaf area under water stress (Gous et al., 2016). MT124118 exhibits comparatively shorter grain fill duration and lower percent plump (Williams et al., 2022). F_1 progeny resulting from this cross were advanced through single seed descent for six generations to produce 168 sibling RILs with approximately 98% homozygosity. This F_7 generation was grown in 0.3-m-long single rows at the Post Agronomy Farm in 2018 to ensure that there was segregation within the population for grain fill duration. Seed from this preliminary field trial was used for multi-row field trials in 2019, which in turn provided seed for 2020 field trials. The two parent lines were used as checks in the experimental design, along with the common malt barley varieties Craft (BETZES/DOMEN/BARONESE) and Hockett (Bearpaw/ND7593).

2.2 | Field experiments

2.2.1 | Site descriptions

A total of four field trials were conducted in 2019 and 2020 at two locations: the MSU Arthur H. Post Agronomy Farm (45.6°N, 111.2°W) in Bozeman, Montana, and the MSU Northern Agricultural Research Center (NARC) (48.5°N, 109.8°W) in Havre, Montana. Soil at the Bozeman location

TABLE 1 Environmental conditions for field trials conducted at the two locations.

Field trial condition	Bozeman 2019	Bozeman 2020	Bozeman Historic mean	Havre 2019	Havre 2020	Havre Historic mean
Total precipitation (mm)						
Preseason	235	230	218	118	147	134
Planting to heading	115	115	141	115	99	119
Heading to harvest	110	31	67	48	28	62
Total	460	375	426	281	274	315
Mean temperature (˚C)						
Planting to heading	13	13	13	12	15	14
Heading to harvest	18	20	19	20	20	21
Fertility						
Yield goal (lbs·ac^{-1})	100	100	–	60	60	–
Total available nitrogen (lbs·ac^{-1})	145	148	–	29	34	–
Percent of recommended nitrogen available	121	123	–	40	47	–

Note: Total precipitation and mean temperature are given for each location-year for the previous September to planting date, planting date to mean heading date for the population, and from heading date to harvest date. Historic precipitation and temperature means are given for each site from 1966 to 2020, using mean dates from the current field trials to determine time periods. Yield goals and soil nitrogen levels for the top 24 inches are also reported for each location-year.

is an Amsterdam-Quagle silt loam, and at the Havre location it is a mix of Joplin, Scobey, and Telstad clay loams (USDA, 1998). Daily precipitation and temperature data were obtained from NOAA climatological summaries collected at the two study locations (Table 1). Both sites typically experience reduced precipitation during the second half of the growing season, but this is usually more pronounced in Havre where there is also less precipitation during the winter between growing seasons.

2.2.2 | Experimental design and management

An augmented randomized complete block design (Federer, 1956) was used for the field study such that within each location-year trial, each RIL appeared once, and four replicated check lines (MT124118, ND19119, Craft, and Hockett) appeared at randomized positions in each of eight blocks. The replicated checks allowed for the calculation of best linear unbiased predictions (BLUPs) of RIL phenotypes to account for environmental variation within the field.

Field preparation differed between the two locations but was consistent with locations between seasons. In Bozeman, the soil was tilled and fertilized, while soil was not tilled or fertilized at the Havre location, which has been under no-till management for over 20 years in an effort to conserve soil moisture. Experience at these farms has shown that yields are typically lower in Havre due to drier conditions and thus recommended fertility rates are lower. The nitrogen levels in soil tests for our field trials were below the recommended rate in Havre and above the recommended rate in Bozeman (Table 1).

Trials were seeded during the last week of April for each of the four location-years except for, 2019 which was seeded on May 8th due to weather constraints. Seed was treated prior to planting with CruiserMaxx Vibrance Cereals (Syngenta) to protect against early season pests and pathogens. Plots were three rows each with 0.3-m spacing and were 5.5-m long at the Bozeman location and 6.4-m long in Havre. All trials were rainfed and maintained weed free. Grain was harvested after drying to approximately 15% moisture content or less in the field.

2.2.3 | Agronomic data collection

Heading date, stage 59 on the Zadoks scale for the growth stages of cereals (Zadoks et al., 1974), was visually determined for each plot when 50% of the seed heads in the plot had emerged from the boot. Maturity date (Zadoks 89) was visually determined for each plot when 50% of the seed heads in the plot were no longer green. For the 2019 trial in Havre, Zadoks stage was assessed for each plot on 3 days around the time of heading and 3 days around the time of maturity, then heading and maturity dates were extrapolated from these observations. Heading and maturity dates were reported in Julian days using a continuous counting of days from the beginning of the respective year. Heading date was subtracted from maturity date to determine the duration of the grain fill period. Planting date was subtracted from maturity date to determine days to maturity. The Field Book app was used for data collection in the field (Rife & Poland, 2014).

After maturity, a measuring stick was used to find the heights of two plants per plot from the ground to the top of the seed head excluding awns, and the mean of these two measurements was reported for plant height. Grain was harvested with a plot combine (Wintersteiger). Gross weight of harvested grain for each plot and measured individual plot lengths were used to calculate grain yield. Seed was threshed and cleaned after harvest. Test weight was measured using a Dickey-John Grain Analysis Computer 2500-UGMA (Corn Belt Testing Inc.). Grain protein content was measured by near-infrared spectroscopy (NIR) (Infratec NOVA, FOSS) following the protocol from the American Society of Brewing Chemists (ASBC, 1992). Percent plump grain was considered to be the percentage of a grain sample remaining on top of a $^{6}/_{64} \times {}^{3}/_{4}$-inch sieve after sieving as defined by the United States Standards for Barley (CFR, 2023).

2.3 | Seedling root roll-up assay

A high-throughput seedling assay called root roll-ups was conducted in a greenhouse at MSU, Bozeman, to observe seminal root traits at the one-leaf stage (Zadoks 11). This method is described in Williams et al. (2022) and summarized here with modifications noted. Artificial light exposure and temperature were controlled in the greenhouse. The assay was conducted once running from December 2019 to February 2020, and again from June to August of 2020, for two total replications with rolls grown in successive groups over each period. To be able to account for possible variation due to seasonal differences in natural daylength and climatic fluctuations within the greenhouse from week to week, the same augmented design and check varieties used in the field trials were used in this assay.

Four seeds of a single barley genotype were placed along the short edge of a 25.4 × 38.1-cm sheet of germination paper (Anchor Paper Co.), which was then rolled up from long edge to long edge. Rolls for the different barley lines were then wetted, placed together in a bucket with 2–3 cm of standing water, and held upright with the seeds at the tops of the rolls. Following germination, the seminal roots (those originating in the seed embryo [Mankse & Vlek, 2002]) grew down into the rolls of paper, while the shoots grew up out of the rolls. A roll was removed from the bucket when a second leaf tip was just visible in two out of its four seedlings.

Rolls were then unrolled for data collection. The numbers of seminal roots and initiated lateral roots were counted. The lengths of the longest and shortest seminal roots were measured with a ruler as was the length of the shoot. Root length range was determined by subtracting the length of the shortest root from the length of the longest root, then standardized by dividing that difference by the mean of those two root lengths. Root-to-shoot length ratio was defined as the length of the longest root divided by the length of the shoot. In this text, the term root length refers to the length of the longest root unless specified as referring to the length of the shortest root. The mean of the measurements for the four seedlings was reported as the value for a single roll.

2.4 | Statistical analysis

Field trials at the Bozeman and Havre farms were analyzed separately to facilitate comparison of the two distinct environments. Initial explorations of the data indicated that results were generally consistent between years within locations, so these were analyzed together to be concise. Trait ranges, means, and standard deviations were calculated for the RIL population and each parental line across seasons and within locations for the field trials, and across replications for the root roll-up assay. The two parental lines were compared by two-sample unpaired t-test for each trait. Analyses were performed using R Statistical Software (R Core Team, 2022).

Environmental variation in the RIL data was accounted for as follows. Utilizing the augmented randomized complete block design, BLUPs were calculated for each trait as described by Kehel et al. (2010). This was done using the “lmerTest” package (Kuznetsova et al., 2017) with restricted maximum likelihood (REML) estimation and the following mixed linear models.

$$Y_{ijkl} = \mu + Checks_i + Block_j + Year_k + Genotype_l + \varepsilon_{ijkl}$$

was used for field trial traits where $Checks_i$ was a fixed effect of the replicated check varieties, and $Block_j$, $Year_k$, and RIL $Genotype_l$ were random effects.

$$Y_{ijkl} = \mu + Checks_i + Block_j + Rep_k + Genotype_l + \varepsilon_{ijkl}$$

was used for root roll-up traits where $Checks_i$ was again fixed, and $Block_j$, Rep_k, and RIL $Genotype_l$ were random effects. BLUPs given by the genotype coefficient for each RIL were then centered around the raw population mean for each trait to provide RIL phenotypic values adjusted for spatial and temporal heterogeneity.

The same package and mixed linear models were used to calculate heritability for each trait as described by Holland et al. (2003). Variance due to RIL genotype was divided by the total variance due to all random effects and residuals.

In order to assess how consistent RILs were in their expression of phenotypes between the contrasting field environments, percent difference was calculated for yield and quality traits. For each RIL, the absolute difference between the Bozeman BLUP and the Havre BLUP was calculated. This difference was then divided by the mean of those BLUPs, so that the percent difference would be an indication of stability across locations regardless of whether the raw yield or quality measurements were high or low. The effect of location was also examined for each field trait via analysis of variance (ANOVA) on data from all four field trials. For the RIL population, the same mixed linear models were used as for BLUPs and heritability with the addition of location as a fixed effect. Parent lines were analyzed separately with location as a fixed effect, and year and block as random effects.

Pearson correlation tests using the R package "Hmisc" (Harrell, 2022) facilitated comparison of traits. BLUPs of heading date, days to maturity, and grain fill duration were tested for correlation with BLUPs of all other field trial traits within locations. The percent differences of traits that were calculated between locations were tested for correlation with heading, maturity, and grain fill means across locations for the RILs. Correlation analysis was also performed to compare all field trial traits with all root roll-up assay traits.

Figures were made using "R/QTL" (Broman et al., 2003), "ggplot2" (Wickham, 2016), and "ggpubr" (Kassambara, 2023).

2.5 | Genotyping and genetic mapping

Genotyping of the RILs and two parental lines was done by the USDA North Central Small Grains Genotyping Lab (Fargo, ND) using the barley 50K Illumina SNP microarray. Previous work at MSU had indicated that the parent lines to the RIL population differed in allelic identity for the *HvNAM1* NAC transcription factor gene (Burcu Alptekin, personal communication, 2018), which significantly impacts protein content, percent plump grain, and time to maturity (Alptekin et al., 2021)—traits of interest in the present study. Thus, it was decided to additionally genotype the population for this polymorphism. Genomic DNA extractions were performed as described by Alptekin et al. (2021). A competitive allele-specific PCR (KASP) assay was run at MSU, using primers designed to differentiate between the two parental alleles for *HvNAM1*. KASP primers (Table S1) were created by LGC, Biosearch Technologies' KASP on Demand system based on the *uhb6* primer sequences in Distelfeld et al. (2008). The 61–55°C touchdown PCR protocol was run on 5-µL reaction volumes of DNA from the RILs and the parents.

For the construction of a genetic linkage map based on the genotyping data, markers were eliminated if the parents were not polymorphic, if the parents were heterozygous, if minor allele frequency in the population was less than 30%, or if more than 30% of the population was missing call data or heterozygous. The genotyping data were then imported into the program QTL IciMapping (Wang et al., 2019) to build a linkage map. The program's binning algorithm was used to remove redundant markers. Markers were then grouped by logarithm of odds (LOD) score using an LOD threshold of 3. Ordering was done using k-Optimality by LOD. Rippling was done by LOD with a window size of 8. The map was further refined by dropping 23 markers that substantially increased the size of the map. The resulting map consisted of 1316 markers covering 1153 centimorgans as summarized in Table S2.

QTL mapping of the phenotype BLUPs was done using "R/QTL" (Broman et al., 2003). Composite interval mapping (CIM) was performed for each trait using the Haley–Knott regression method and an infinite window size. LOD score thresholds for QTL significance were determined for each trait by permutation test ($n = 1000$). The genetic positions of QTLs identified by CIM with an LOD above the $\alpha = 0.2$ threshold were used to create an initial model. This model was used to check for interactions between QTLs. No QTL interactions above the $\alpha = 0.2$ LOD threshold were found in this study. Multiple interval mapping was then performed to refine QTL positions and search for additional QTLs. These were added to the model if their LOD scores were above the $\alpha = 0.2$ significance cutoff determined for the trait. This iterative process was repeated until no additional QTLs were identified, thus arriving at the final model describing the positions, LOD scores, and percent of phenotypic variation explained for each QTL.

3 | RESULTS

3.1 | Phenotypic variation and heritability

Transgressive segregation was observed for all traits in the field trials, as the distributions for the RIL population extended both above and below the ranges of the two parents (Table 2). This suggests that both positive and negative alleles were inherited from each parent. Consistent with expectations (Franckowiak et al., 2007, 2010; Williams et al., 2022), ND19119, the stay-green parent, displayed earlier heading and later maturity than MT124118 in both locations and thus a longer grain fill period (Table 2). ND19119 had greater percent plump grain and grain protein than MT124118 in both locations. The parent lines did not significantly differ for yield and test weight.

A wide range of heritability estimates was observed for field trial traits, and these varied between the two locations (Table 2). Heritability of heading date and days to maturity was about 0.4 in Bozeman but lower in Havre, particularly for days to maturity. Duration of grain fill was moderately heritable at about 0.2 in both locations. Grain plumpness was also moderately heritable (0.3), while grain protein had high heritability in Bozeman (0.872) but low heritability in Havre (0.185). Yield and test weight had low heritability in both locations.

Location had a large impact on all traits for the RILs and each parent as indicated in ANOVAs, excluding protein for ND19119 and test weight throughout (Table S3). Overall, grain fill was shorter and occurred earlier in the season in Havre (Table 2). Plant height, yield, and grain plumpness were

TABLE 2 Summary statistics for traits measured in the field trials and the root roll-up assay on the recombinant inbred line (RIL) population and each of the two parents.

Field trait	RIL population					MT124118 parent					ND19119 parent			
Location	**Min**	**Mean**	**Max**	**SD**	H^2	**Min**	**Mean**	**Max**	**SD**	**Parent *t*-test**	**Min**	**Mean**	**Max**	**SD**
Heading date (Julian)														
Bozeman	178	184.7	192	3.2	0.413	183	185.1	187	1.7	**	179	182.4	186	3.0
Havre	172	178.6	189	4.0	0.258	176	180.9	185	2.4	***	172	175.3	181	3.2
Grain fill duration (days)														
Bozeman	30	40.2	52	4.5	0.246	33	37.1	41	3.0	***	39	43.8	48	2.7
Havre	18	30.5	40	4.5	0.247	21	25.3	30	2.6	***	28	33.7	40	4.4
Days to maturity														
Bozeman	96	101.4	108	2.2	0.422	96	98.8	100	1.1	***	100	102.6	105	1.5
Havre	84	92.1	102	3.7	0.059	85	89.2	95	2.5	*	87	91.9	98	3.7
Plant height (cm)														
Bozeman	73.0	89.2	101.5	5.4	0.693	79.0	84.1	89.5	2.6	***	86.0	94.5	102.5	4.0
Havre	51.7	70.0	86.0	6.7	0.264	61.0	65.9	73.5	3.8	**	60.8	71.6	80.5	5.9
Yield (kg·ha^{-1})														
Bozeman	4000	6237	8255	746	0.076	5725	6840	7810	600	ns	5086	6507	7611	557
Havre	1552	3017	4397	520	0.198	2494	3248	4159	453	ns	2183	2974	3792	428
Test weight (kg·hL^{-1})														
Bozeman	62.0	69.0	75.8	3.7	0.026	66.5	70.0	74.1	3.1	ns	64.6	68.4	74.6	3.3
Havre	59.2	68.8	75.0	3.4	0.052	65.4	69.3	72.8	2.9	#	65.6	69.5	73.9	2.8
Percent plump grain														
Bozeman	88.9	97.6	99.3	1.4	0.331	90.4	94.5	97.3	2.0	***	98.3	99.0	99.3	0.2
Havre	74.0	94.4	99.2	3.6	0.349	80.8	86.1	91.6	3.0	***	93.5	97.2	99.2	1.4
Percent grain protein														
Bozeman	9.6	12.0	14.9	1.0	0.875	10.8	11.5	12.0	0.3	***	11.6	12.1	12.5	0.3
Havre	8.7	11.5	14.2	1.2	0.189	9.2	10.8	12.4	1.2	**	10.4	11.9	13.3	0.8
Root roll-up trait														
Days to one-leaf	9	12.6	18	1.8	0.430	9	11.6	15	1.8	**	11	13.7	16	1.5
Shoot length (cm)	4.6	9.7	16.3	2.1	0.435	5.9	8.3	11.4	1.4	***	7.0	10.7	14.0	2.1
Longest root length (cm)	6.9	30.5	42.4	6.6	0.309	18.6	27.5	36.3	5.1	#	14.2	31.6	39.2	6.3
Root-to-shoot length ratio	1.2	3.3	6.3	0.7	0.447	2.6	3.4	4.1	0.5	#	1.9	3.1	4.0	0.5
Shortest root length (cm)	0.8	9.1	18.4	3.4	0.072	2.7	8.8	14.6	3.4	ns	1.4	7.3	14.6	3.3
Root length range (cm)	4.7	20.5	34.2	5.7	0.346	8.3	16.4	27.7	5.4	***	12.8	24.2	30.5	4.8
Seminal root count	4	6.0	8	0.4	0.430	5	5.6	6	0.4	***	6	6.3	7	0.3
Lateral root count	0	24.2	189	30.2	0.176	0	18.7	75	22.9	ns	1	27.2	87	25.0

Note: The two seasons are combined within locations for the field traits. Heritability estimates for the population are listed, along with significance levels from *t*-tests comparing trait means of the parental lines: ns, $p \geq 0.1$; #$p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

higher in Bozeman. Protein content was slightly higher in Bozeman, and test weight barely differed between locations.

Transgressive segregation and a range of heritability estimates were also observed for all traits measured in the root roll-up seedling assay (Table 2). ND19119 had a longer shoot and root than MT124118 (Table 2). However, a smaller root-to-shoot length ratio indicated ND19119 had a shorter root relative to the shoot. This line also reached the one-leaf stage more slowly, had more seminal roots, and had a larger root length range. The parents did not differ in *t*-tests for lateral root count or length of the shortest root.

3.2 | Correlation of traits

Pearson correlation tests revealed relationships between some traits. Longer grain fill duration was generally correlated with improved quality, although whether earlier heading or later

TABLE 3 Pearson correlation coefficients for tests comparing recombinant inbred line (RIL) population best linear unbiased predictions (BLUPs) for heading date, grain fill duration, and days to maturity with agronomic traits within each location.

	Heading date		Grain fill duration		Days to maturity	
Agronomic trait	**Bozeman**	**Havre**	**Bozeman**	**Havre**	**Bozeman**	**Havre**
Plant height	0.361***	0.007ns	−0.304***	−0.013ns	0.012ns	−0.011ns
Yield	0.257***	0.015ns	−0.174**	−0.159**	0.080ns	−0.226***
Test weight	−0.118ns	−0.333***	0.272***	0.398***	0.284***	0.166**
Plump	−0.040ns	−0.323***	0.287***	0.510***	0.421***	0.355***
Protein	−0.192**	−0.431***	−0.130$^{\#}$	0.236***	−0.494***	−0.213***
Yield percent difference	0.004ns		0.072ns		0.092ns	
Test weight percent difference	0.072ns		−0.068ns		−0.004ns	
Plump percent difference	0.340***		−0.480***		−0.288***	
Protein percent difference	0.022ns		−0.256***		−0.384***	

Note: The bottom four rows show correlations of mean RIL heading date, grain fill duration, and days to maturity for combined locations with the percent difference in agronomic traits between locations. ns, $p \geq 0.1$; #$p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

maturity was more correlated varied between environments (Table 3). Correlations with grain quality were stronger with days to maturity than with heading date in Bozeman, and the opposite was observed in Havre. Percent plump and test weight were both positively correlated with a longer grain fill duration in both locations. Conversely, lower percent protein was associated with a grain fill period that was shifted later in the season in both locations, as indicated by negative correlations with both heading and maturity. There were slight negative correlations between grain fill duration and grain yield in both locations.

Correlation analysis comparing heading, maturity, and grain fill BLUPs across locations to the percent difference in yield and quality traits between locations indicated association of more consistent percent plump with earlier heading and later maturity. Percent protein was more consistent between locations when maturity was later, but there was not a relationship with heading date. Consistency of test weight and yield between locations were not correlated with grain fill timing.

A pattern emerged when comparing the traits from the root roll-up seedling assay to the Bozeman and Havre field trial traits. A smaller root-to-shoot length ratio was correlated with later maturity ($r = -0.539$, $p < 0.001$ in Bozeman, −0.369, $p < 0.001$ in Havre), greater test weight ($r = -0.340$, $p < 0.001$ in Bozeman, −0.343, $p < 0.001$ in Havre), and lower percent grain protein ($r = 0.454$, $p < 0.001$ in Bozeman, 0.359, $p < 0.001$ in Havre). The full set of correlations between field traits and seedling assay traits is presented in Table S4.

3.3 | Identification of QTL

Genetic mapping revealed a total of 79 QTL associations from the field study and 32 from the root roll-up assay. These are listed in Table S5. There were six main loci displaying co-segregation between grain fill timing and duration, grain quality, and seedling root traits (Table 4). In some cases, QTLs were observed in both environments, although with different effect sizes but never opposite effects. In other cases, the association was only observed in one environment. On chromosome 2H, the allele from the ND19119 parent (ND allele) at QGFhd-2H was associated with earlier heading, higher grain protein, and shorter plants in both locations, and plumper grain in Havre. There were also associations with this locus for lateral root count and shortest root length in the root roll-up assay. Nearby, QLN-2H contained the highest number of QTLs for seminal root traits, with the ND allele extending the length of the longest and shortest roots and the root length range. Grain plumpness and plant height in Bozeman were the only traits from the field trials to map to this locus. QDD-5H contained smaller effect QTLs where the ND allele delayed development at the one-leaf stage, heading, and maturity, and increased longest root length, root length range, and shoot length. At QGFmt-6H, the ND allele was strongly associated with later maturity and lower protein in both field locations, as well as a smaller root to shoot length ratio in the root roll-ups. The QGFmt-6H ND allele was also associated with plumper grain and higher test weight in both locations. This locus overlapped the position of the HvNAM1 KASP marker (Table 4). At QGFhd-7H, it was the allele from the MT124118 parent (MT allele) that was associated with earlier heading and this was much stronger in Bozeman. The ND allele at QGF-7H was associated with longer grain fill in both locations, but this was stronger in Havre where it was also associated with greater percent plump.

The relationship between traits at one locus was not consistent across QTLs (Table 4). The timing of developmental stages (one-leaf, heading, and or maturity) co-segregated with the same direction of allelic effects at *QDD-5H* and

TABLE 4 Highlighted quantitative trait loci (QTLs) identified for traits from the field trials and the root roll-up assay.

QTL Trait	Position (cM)	LOD	Percent of variance	Effect of ND allele	Co-locating trait and reference
QGFhd-2H					
Longest root length, RRU	79.8	3.8	6.1	–	Flowering time: Alqudah et al., 2014 Flowering time: Dang et al., 2022 Multiple malt quality measures, plump, test weight, plant height, and heading date: Pauli et al., 2015 Seedling root depth: Jia et al., 2019 Multiple seedling root traits: Khodaeiaminjan et al., 2023
Plump, HV	81.7	7.6	11.5	+	
Plump percent difference	81.7	7.9	14.4	–	
Shortest root length, RRU	84.6	4.1	10.7	–	
Lateral root count, RRU	85.5	6.8	13.9	–	
Heading date, HV	85.5	22.4	40.3	–	
Heading date, BZ	85.5	47.2	47.7	–	
Grain fill, HV	85.8	18.9	25.9	+	
Plant height, HV	85.8	8.7	12.7	–	
Protein, HV	85.8	10.7	13.3	+	
Plant height, BZ	86.0	15.8	15.5	–	
Protein, BZ	86.0	15.6	17.7	+	
Grain fill, BZ	86.8	26.4	22.0	+	
Days to one-leaf, RRU	87.4	4.5	5.9	–	
QLN-2H					
Shortest root length, RRU	143.0	3.1	6.5	+	Seedling root trait: Khodaeiaminjan et al., 2023 Plump: Pauli et al., 2014
Longest root length, RRU	145.6	12.3	22.6	+	
Shoot length, RRU	145.6	11.1	13.7	+	
Plant height, BZ	147.8	4.5	3.8	+	
Plump, BZ	147.8	5.8	6.3	+	
Days to one-leaf, RRU	148.0	12.5	18.5	+	
Root length range, RRU	149.0	7.9	14.6	+	
QDD-5H					
Days to maturity, BZ	174.3	5.4	7.8	+	Flowering time: Alqudah et al., 2014 Multiple malt quality measures: Pauli et al., 2015
Heading date, BZ	175.0	4.1	1.9	+	
Days to one-leaf, RRU	178.7	6.1	8.2	+	
Root length range, RRU	181.2	4.7	8.4	+	
Longest root length, RRU	182.2	3.9	6.4	+	
Days to maturity, HV	185.0	6.7	11.3	+	
Shoot length, RRU	187.2	7.2	8.5	+	
QGFmt-6H					
Test weight, HV	52.0	6.1	11.5	+	Protein: Distelfeld et al., 2008; See et al., 2002 Days to maturity, protein, plump, and test weight: Alptekin et al., 2021
Plump, BZ	54.0	5.5	6.0	+	
Plump, HV	58.8	6.2	9.3	+	
Plump percent difference	59.0	4.4	7.6	–	
Shoot length, RRU	59.0	3.0	3.3	+	
Protein, HV	66.0	22.3	32.6	–	
Protein, BZ	66.0	34.4	51.9	–	
Grain fill, BZ	66.8	21.0	16.1	+	
Longest root-to-shoot ratio, RRU	67.0	14.1	29.3	–	
Protein percent difference	67.4	14.5	30.3	–	
Longest root length, RRU	68.4	4.6	7.5	–	
Test weight, BZ	68.4	8.8	14.0	+	
Days to maturity, BZ	69.0	18.5	32.2	+	

(Continues)

TABLE 4 (Continued)

QTL Trait	Position (cM)	LOD	Percent of variance	Effect of ND allele	Co-locating trait and reference
Grain fill, HV	70.0	6.6	6.6	+	
Days to maturity, HV	70.2	10.2	18.0	+	
Days to one-leaf, RRU	72.4	3.0	3.9	–	
QGFhd-7H					
Protein percent difference	39.0	4.2	7.5	+	Flowering time: Alqudah et al., 2014 Flowering time: Dang et al., 2022 Plant height: Pauli et al., 2014
Grain fill, BZ	41.5	4.2	2.5	–	
Heading date, BZ	41.5	32.1	25.5	+	
Plant height, BZ	41.5	6.1	5.2	+	
Heading date, HV	44.0	4.1	5.6	+	
Grain fill, HV	46.0	4.4	4.4	–	
QGF-7H					
Test weight, BZ	72.9	4.0	5.9	+	Flowering time: Alqudah et al., 2014 Multiple seed size measures: Gordon et al., 2020 Heading date, plant height, test weight, and plump: Pauli et al., 2015
Heading date, HV	76.8	5.0	7.0	–	
Plump, HV	76.8	8.0	12.2	+	
Plump percent difference	76.8	6.0	10.6	–	
Days to maturity, HV	78.0	3.3	5.2	+	
Days to one-leaf, RRU	79.9	5.5	7.3	+	
Grain fill, HV	80.8	10.7	10.7	+	
Heading date, BZ	80.8	6.0	3.2	–	
Shoot length, RRU	80.8	5.2	6.0	+	
Grain fill, BZ	81.1	4.9	3.0	+	
Plant height, HV	85.0	4.5	6.1	+	

Note: Chromosomes are indicated by the end of QTL names. LODs for all QTLs are above the $\alpha = 0.05$ LOD significance threshold determined for that trait. A BZ suffix in the trait name indicates a trait from the Bozeman field trials, HV for Havre, and RRU for the root roll-up assay.
Abbreviations: LOD, logarithm of odds.

QGFhd-2H, but there were opposite allelic effects on developmental timing at *QGFmt-6H* and *QGF-7H*, where the ND allele was associated with earlier heading but later maturity and one-leaf stage. Likewise, there were opposite directions of allelic effects for grain fill duration and plant height at *QGFhd-2H* and *QGF-7H*, but effects for these traits were in the same direction at *QGF-7H*. The lengths of both the shoot and the root in the root roll-up assay were increased by the ND allele at *QLN-2H* and *QDD-5H*, but at *QGFmt-6H*, the shoot length was increased although the root length was decreased.

Several QTLs (*QGFhd-2H*, *QGFmt-6H*, *QGFhd-7H*, and *QGF-7H*) were associated with quality consistency indicated by the percent difference in plump, protein, or both between the Bozeman and Havre locations (Table 4). A smaller percent difference in quality indicates more stable trait expression between locations, or in other words more resistance to environmental heterogeneity. Figure 1 shows that the alleles associated with longer grain fill were also associated with smaller percent differences in plump and or protein between locations due to quality improvement with respect to requirements for malt.

4 | DISCUSSION

In the Western United States, cultivating malt barley without irrigation is becoming increasingly risky for growers as lack of water during grain fill decreases plumps and increases protein (Tarawneh et al., 2020), negatively impacting quality such that grain is rejected for malting. In this study, through genetic dissection, we identified QTLs related to seminal root traits, plant development, and grain quality that may help to stabilize malt quality especially under dryland conditions. Our work supports and is supported by the findings of previous mapping analyses of these same and related traits in barley (Table 4).

4.1 | Earlier heading

Grain fill duration was impacted by heading date at *QGFhd-2H*, *QGFhd-7H*, and *QGF-7H*, and earlier heading appeared to benefit crop performance in our work. Negative correlations between plumpness and timing of development around

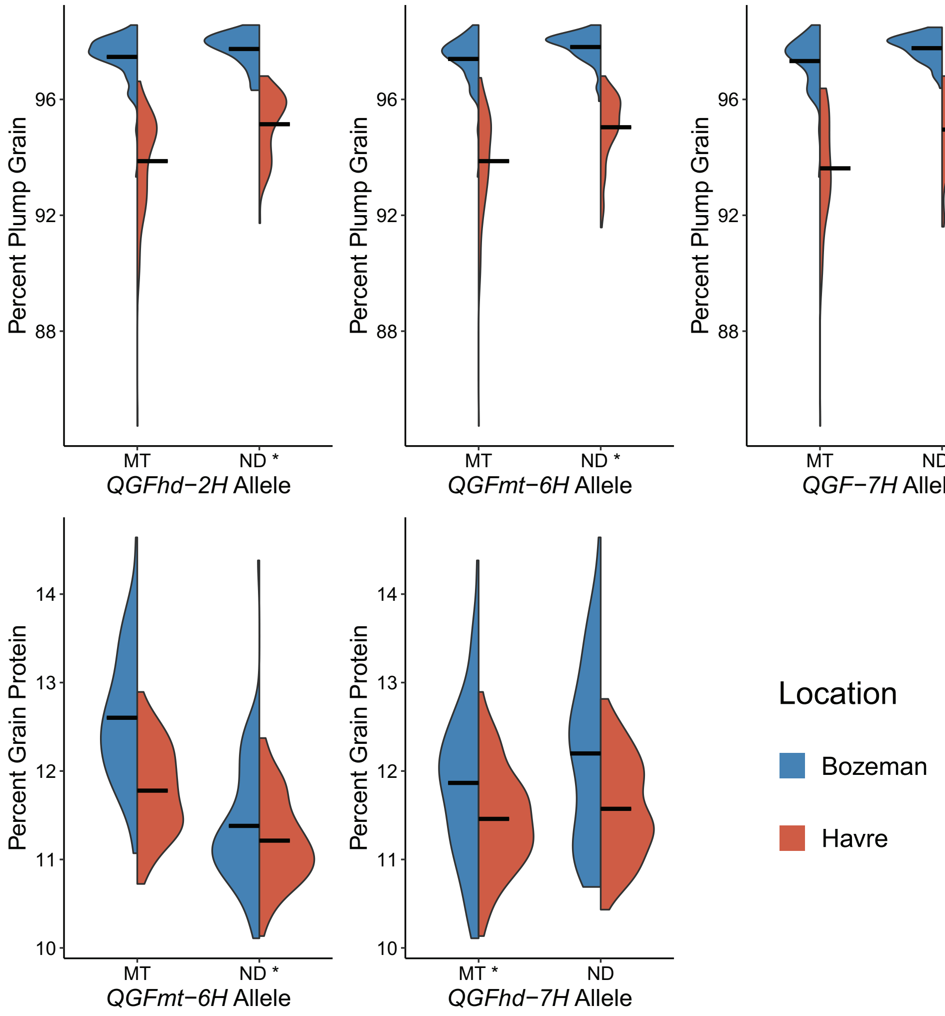

FIGURE 1 Split violin plots of percent plump grain and percent grain protein in Bozeman and Havre field trials at quantitative trait locus (QTL) positions for the percent differences of those traits between locations. The parental allele associated with a smaller difference or more consistent trait expression (as well as longer grain fill) is indicated with an asterisk. Horizontal bars represent trait means by location and allelic group.

heading have been documented before (Shirdelmoghanloo et al., 2022), and here we observed not only greater plumps with earlier heading (Table 3), but also more stable plumps due to plump improvement in drier environment at *QGFhd-2H* and *QGF-7H* (Figure 1). Dang et al. (2022) mapped flowering time in barley near *QGFhd-2H*, and Alqudah et al. (2014) mapped flowering time near both *QGFhd-2H* and *QGF-7H*, as did Pauli et al. (2015) for heading date. The Pauli study also mapped plump and test weight near both loci, as well as multiple direct measures of malt quality such as beta-glucan content, free amino nitrogen, and malt extract near *QGFhd-2H*. Gordon et al. (2020) identified QTLs near the *QGF-7H* locus as being associated with measures of barley seed size under terminal drought conditions. An early start to the grain fill period could be particularly beneficial for starch accumulation in environments such as Havre that depend on finite stores of soil moisture.

The earlier heading *QGFhd-2H* ND allele was associated with greater plumps in Havre and higher protein levels in both locations (Table 4). This runs counter to the idea that grain size and protein content are inversely related, but research has shown that high soil nitrogen levels may be required for the inverse relationship (Magliano et al., 2014). Soil fertility at our Havre location was quite low (Table 1), so it is not entirely unexpected that both plump and protein were associated in the same direction with heading there. While lower percent protein content is generally better for malt quality, there is a lower bound to the ideal range of about 9.0% (Kumar et al., 2013). The protein minimums for the RIL population in both of our field locations were approaching that limit (Table 2). Earlier heading was correlated with greater percent protein and greater percent plump in Havre, but these correlations were weak or absent in Bozeman (Table 3) where soil nitrogen levels were much higher (Table 1). It could be that earlier heading enabled use of additional nitrogen for grain production relative to lines with later heading dates in Havre where nitrogen was more limited. It would be interesting to investigate *QGFhd-2H* further for potential improvement of nitrogen-use efficiency. Grain quality was more affected by heading in the drier location and by maturity where there was more moisture.

4.2 | Later maturity

The timing of maturity also has important impacts on grain quality in different environments. Grain fill duration was affected by days to maturity at *QGFmt-6H* and *QGF-7H* in our study. Correlations and QTL co-segregations connected later maturity to better malt quality at both of our field study locations (Tables 3 and 4). Longer retention of green leaf area has benefitted grain plumpness in other studies, although this dynamic can change depending on plant genotype (Emebiri, 2013). More time to maturity was also correlated with more consistent plump and protein between our locations (Table 3). The ND allele at *QGF-7H* was associated with later maturity and plumper grain in Havre, as well as a smaller percent difference in plump between locations due to this quality improvement in the drier environment (Table 4; Figure 1). It appears that a longer grain fill period may protect plumps from environmental variability.

Low heritability values for days to maturity and percent protein at our Havre location (Table 2) could call into question the QTL results for those traits. Heritability values are reduced by large environmental impact, which was likely pronounced in Havre with the low precipitation and fertility rates (Tables 1 and S3). Large population sizes with low replication of genotypes can also result in lower heritability values (Zila et al., 2014), and our model for heritability calculation depended on many unreplicated RIL genotypes in our augmented design. The model for QTL mapping, however, considered alleles that are abundantly replicated among the RIL population. Other instances have been observed in RIL research where traits exhibit low heritability but robust QTL mapping results (Zhang et al., 2017). Furthermore, alignment of QTLs for maturity and protein in Havre with results from Bozeman where heritabilities for those traits were higher (Table 2), as well as the fact that other studies have also mapped maturity and protein to loci at or near *QGFmt-6H* and *QGF-7H* (Table 4), gives us confidence in these QTLs.

The *QGFmt-6H* ND allele was even more strongly associated than *QGF-7H* with delayed maturity in both locations, and with greater test weight, plumper grain, lower protein, and smaller plump and protein differences between locations via quality improvement (Table 4; Figure 1). Our KASP assay placed the marker for the *HvNAM1* NAC transcription factor gene within this chromosome 6H locus (Table 4). Previous work (Alptekin et al., 2021; Distelfeld et al., 2008; See et al., 2002) demonstrated that an allele for *HvNAM1* found in the barley variety Karl was associated with delayed senescence, lower grain protein content, and increased plumps and test weight compared to the allele found in most barley varieties. The ND19119 parent to our RIL population carries the Karl allele for *HvNAM1* and presents these corresponding phenotypes compared to the MT124118 parent, which carries the more common allele (Table 2). NAC transcription factors are also involved in stress responses and senescence processes in several species (Nuruzzaman et al., 2013; Thomas & Ougham, 2014), so it is not surprising that one would be involved in the timing of maturity and grain quality in our dryland field trials.

4.3 | Seedling root traits

Allocating energy to roots versus aerial structures is a balancing act for plants between capturing resources aboveground (sunlight) or belowground (water and nutrients). This balance can shift over the course of a plant's life in response to developmental and environmental cues especially abiotic stress (Koevoets et al., 2016). In a previous study (Williams et al., 2022), we used minirhizotrons to examine the root systems of barley lines varying for grain fill duration in field trials at the same Havre location and performed the root roll-up assay on those same lines. Correlations between root roll-up traits, field root traits during grain fill, and agronomic traits, while circumstantial, motivated the genetic dissection of root traits in a population segregating for grain fill duration in the current study. However, resource limitation prohibited minirhizotron examination of the large mapping population, so root observations were limited to the root roll-up traits as tentative proxies. Multiple correlation and co-segregation relationships between seedling root traits and agronomic traits were observed in this study. QTLs are large genetic regions with respect to gene sequences, and while co-segregation of traits may indicate pleiotropic effects of individual genes, it is perhaps more likely that the relationship is due to linkage of different genetic elements. Correlation and co-segregation are not indicative of causation, but do suggest hypotheses to investigate with future work.

A shorter root length compared to shoot length in the root roll-ups correlated with deeper and more prolonged root proliferation during grain fill in the previous minirhizotron study. In the present study, this smaller root-to-shoot length ratio correlated with later maturity and better grain quality (Table S4) and strongly co-located with these phenotypes at the *QGFmt-6H*/*HvNAM1* locus (Table 4). Involvement in roots has been shown for other NAC transcription factors in barley (Christiansen et al., 2011), but this is the first suggestion of an association between *HvNAM1* and root growth, which we hope to investigate further. Slower belowground growth versus faster aboveground growth at early stages may facilitate accumulation of photosynthetically derived energy stores that could be used to support deeper root growth under dry conditions later in development. Along these lines, when comparing two spring wheat genotypes under drought conditions, Reynolds et al. (2007) found that the genotype with higher yield and biomass distributed more of its root system to deeper soil depths, but had less root mass overall and a smaller root-to-shoot ratio shortly after anthesis. If the dynamic of slower root growth compared to shoot growth at the one-leaf stage were to continue through later vegetative stages, it may help to conserve soil moisture for use later in the season by reducing uptake by the roots. Early vigor of young shoots in cereals can also prevent soil moisture loss by shading the soil surface and preventing weed competition (López-Castañeda et al., 1996), and connections have been made between early vigor and the stay-green trait under drought stress in maize (Trachsel et al., 2016). Considering how root and shoot growth rates compare to each other at early developmental stages could be more informative for understanding subsequent root system architecture and crop performance than considering either in isolation.

Seedling root traits also mapped to *QGFhd-2H* and *QLN-2H* (Table 4). The involvement of these loci in root traits is supported by co-locations with other QTL studies. Jia et al. (2019) mapped barley seminal root system depth in rhizoboxes (similar to the seminal root length trait in our study) near *QGFhd-2H*. Khodaeiaminjan et al. (2023) examined the roots of 3-week-old barley seedlings in paper pouches under osmotic stress and mapped root number and root system area near *QGFhd-2H*, and root system length near *QLN-2H*, further suggesting that these loci could be involved in root phenotypes contributing to drought adaptation. Of course, the one-leaf stage is distant from reproductive and maturity stages, and connections drawn between seedling assay and field traits are speculative. However, correlations and co-locations between seminal root traits and agronomic traits have been presented before (Ali et al., 2015; Robinson et al., 2018) and have laid the groundwork for subsequent research that has identified causal genes behind the connections (Feng et al., 2022; Kirschner et al., 2021). More research is required to discover how seminal root trait loci might associate with root phenotypes throughout development in the field and how such phenotypes could impact crop performance and environmental adaptation.

4.4 | Utility of alleles in different environments

Although we did not observe any opposite effects of alleles in our study, there were differences in the presence and strength of effects, suggesting different alleles may be preferrable for breeding depending on the target environment. Shorter roots in our seedling assay were related to better quality in both locations at *QGFmt-6H* (Table 4), but at *QGFhd-2H*, shorter roots only associated with better quality at the Havre location, and at *QLN-2H*, longer seedling root length was related to better quality in Bozeman. Havre was the drier of the two locations (Table 1), so having small roots early on may be more important in environments with less stored soil moisture. Havre was also the location where earlier heading had a more positive impact on grain quality, and it has been suggested (Voss-Fels et al., 2018) that when plants depend on water deep in the soil profile to withstand terminal drought, an early flowering time combined with deeper roots is an effective strategy for maintaining crop performance. Under such conditions, it could be beneficial to start putting water toward starch accumulation sooner, considering water extracted during grain fill is directed more toward grain production than water extracted during vegetative phases (Vadez, 2014). In support of this, Carter et al. (2019) found that barley varieties adapted to water stress flowered earlier, had more roots deeper in the soil at maturity, and used more water after rather than before anthesis in their field study. If water stores are sufficient and root systems can access them, then delayed maturity could further promote quality grain production. This may have been the case in the Bozeman location where precipitation levels are higher overall but still low during grain fill (Table 1), and better grain quality was more tightly correlated with later maturity than with heading (Table 3).

Grain plumpness was increased by ND alleles for *QGFhd-2H*, *QGFmt-6H*, and *QGF-7H*, and the MT allele for *QGFhd-7H* in both environments. However, the utility of the same QTLs to improve protein depends on the end use and soil fertility conditions. For example, the ND allele at *QGFmt-6H* decreases grain protein, perhaps even below acceptable levels in low-nitrogen environments, but could potentially be deployed with higher fertility to maintain low protein for malt while yields are increased. Conversely, the ND allele for *QGFhd-2H* increases protein and so could be deployed in low-nitrogen environments and to increase protein levels for feed, forage, and food barley. Of course, to more fully understand how these alleles function and might behave in a wider variety of environments and genetic backgrounds, more research is needed.

Overall, longer grain fill led to better, more consistent crop performance with respect to malt quality indicator traits in our study; however, the effect on yield was neutral. While the stay-green trait can provide higher yield under dry conditions for sorghum (Borrell et al., 2014), maize (Trachsel et al., 2016), and wheat (Christopher et al., 2008), mixed or neutral relationships between yield and stay-green indicators have been observed in barley (Emebiri, 2013) and rice (*Oryza sativa* L.) (Kamal et al., 2019), so our yield results are not without precedent. Yield is a highly complex trait. Although the parent varieties in this study differed for development and grain quality, they did not differ phenotypically for grain yield. They thus may not have differed for yield genetically, resulting in low heritability of this trait (Table 2) and lack of correlation and QTL results for yield in our work. However, since a much higher price is paid for a barley crop if it receives the malt designation (USDA NASS, 2023) that is largely based on plump and protein (AMBA, 2021), a neutral or even slightly negative impact on yield would be acceptable if these malt parameters were reliably and sufficiently improved.

5 | CONCLUSION

Here, we have demonstrated a relationship between extended grain fill duration and improved and consistent grain quality for malting and have identified associated QTLs. The relationships between traits observed in our correlation and QTL analyses in connection with the results from our field study of roots (Williams et al., 2022) suggest a plant strategy for producing high-quality barley grain under terminal drought conditions. Slower growth belowground compared to faster growth aboveground early in the season could gather energy to support the growth of a deeper mature root system and/or save soil moisture to be available for filling grain. An earlier start to grain fill and deeper root system would allow more resources to be put toward seeds than other plant structures. Delayed senescence aboveground and belowground could further enhance quality grain production if water stores lasted.

Understanding the genetic component in these interactions is important for breeding malt barley varieties suited to different cultivation areas and conditions. The present study lays the groundwork for this but has limitations. QTLs are large genetic regions with respect to actual genes, and the relationships between traits and with genetic loci presented in this study are not causative or fully elucidated. Similarly, a greenhouse seedling assay is far removed in developmental time and environmental condition from field trials on farms, and the connections we have drawn between traits from these different experiments are speculative. We plan to address these remaining knowledge gaps in future work with near-isogenic lines (NILs) we have developed for the key QTLs identified here. These will allow us to confirm QTLs, narrow their genetic regions with fine mapping, and identify gene candidates. We also plan to directly examine the impact of these alleles on root systems in the field throughout development with the use of these NILs and minirhizotrons. We hope this work will be helpful to scientists developing the barley varieties of the future.

AUTHOR CONTRIBUTIONS

Jessica Williams: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing—original draft; review and editing. **Peggy Lamb**: Funding acquisition; investigation; project administration; resources. **Greg Lutgen**: Investigation; project administration; resources. **Jennifer Lachowiec**: Formal analysis; writing—review and editing. **Jason Cook**: Formal analysis; writing—review and editing. **Jospeh Jensen**: Investigation; writing—review and editing. **Maryse Bourgault**: Conceptualization; funding acquisition; investigation; methodology; supervision; writing—review and editing. **Jamie Sherman**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Complete data are available upon request from the corresponding author.

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