GENETIC DISSECTION OF MALT QUALITY IN BARLEY: AN EXAMINATION OF HYDRATION INDEX AND METABOLITE QTL

by

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DEDICATION

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ABSTRACT

Malting barley is a high value crop for American farmers. Through the malting process grain is turned into malt which is primarily used in brewing but can also be a flavor and nutritional additive for the food industry. During the malting process hydration of the endosperm is a rate limiting step resulting in increased time and water usage if a third water immersion is needed during steeping. To better understand the genetics of endosperm hydration we used a genome wide association (GWAS) population to map quantitative trait loci (QTL) for malt quality, hydration index (endosperm hydration), and seed morphology traits. We found six hydration index QTL with three related to seed size (qHYI1H, qHYI3H.a, and qHYI3H.b), two improving malt quality (qHYI2H and qHYI6H), and the last (qHYI7H) wasn't related to any other traits. We then wanted to see the relationship between hydration index and dormancy because dormancy is needed to prevent preharvest sprouting. Preharvest sprouting in malt barley results in extreme price reductions for producers however the dormancy genes needed to prevent this negatively impact malt quality. Using a biparental mapping population we were able to map two hydration index QTL and relate them to the dormancy gene SD2. Our results confirmed SD2 negatively impacts malt quality but when the positive alleles for hydration index are present, they negate dormancy's negative effects on quality. The results from these two studies show hydration index can benefit growers and maltsters with barley and malt production. Our third study looked at metabolite variation in the GWAS population to see if we could identify QTL related to malt flavor. This would help craft malt houses and breweries which are usually in search of unique flavors. We were able to identify 827 metabolite QTL however the most impactful to flavor were amino acid, saccharide, and maltol QTL which totaled 39. The three maltol QTL which are directly related to flavor all co-located with maltol precursors (amino acids and saccharides) showing these QTL would be good targets for marker assisted selection to create maltol variation.

CHAPTER ONE

LITERATURE REVIEW

Barley History

Barley (*Hordeum vulgare L.*) is considered a founder crop of early agriculture domesticated around 8000 B.C. from its wild relative *Hordeum spontaneum* K. Koch (Zohary and Hopf 2000). Compared to its wild, relative modern barley has shorter stems and awns, broader leaves, shorter and thicker spikes, easier threshing, and increased kernel size (Zohary 1969). *Hordeum spontaneum* can still be found growing on Mediterranean maquis, empty fields, and roadsides in its native habitat the Fertile Crescent (Harlan and Zohary 1966). Barley's centers of domestication have been observed in the Himalayas, Ethiopia, and Morocco (Aberg 1938; Molina-Cano et al. 1987). However, barley was not domesticated to be free threshing so to utilize this grain early people had to develop a process called malting (Badr et al. 2000).

Malting

The modern malt process consists of three main stages 1) steeping - grains are submerged in water with air rest to raise total percent moisture from about 10 percent to around 45 percent through a process called imbibition, 2) germination – imbibed grains are maintained in a humid environment allowing endosperm degradation and embryo growth, and 3) kilning – germinated seeds are heated to kill the embryo, stop growth and preserve enzymatic capacity and nutrients. The main goal of malting is to make nutritionally unavailable biopolymers such as cell-wall polysaccharides, proteins, and starch available for human use. The extent these compounds become available through malting is described as the level of modification of the barley

endosperm, which is indicated by several malt quality parameters (as described below). The most common use for malt is the brewing and distilling industries, using over 67% of US barley. However, malted barley is added to many foods to increase nutrition and add flavor, using about four percent of the supply. Malt extract is a natural sweetener, with unique flavors and provides important nutrients, including an abundance of antioxidants (five times the amount of broccoli) as well as essential amino acids, vitamins, minerals, and soluble fiber.

Grain Composition

Better understanding of the malting process arises from knowledge of barley grain anatomy, composition, and biochemistry. A barley seed consists of the embryo, endosperm, aleurone layer, and seed covering. The embryo is the living tissue that will grow into a barley plant. While the grain waits to germinate, it survives on a limited amount of starch, lipids, and protein not tied up in the endosperm (Fox 2009). The endosperm consists of starch granules surrounded by a protein matrix all packaged in cell walls primarily made up of β-glucan. The starch in the endosperm accounts for approximately 60% of the total grain weight (Jaeger et al. 2021). The aleurone layer is a thin layer of living cells that surrounds the endosperm. When hydrated this structure releases enzymes critical to endosperm degradation (Jacobsen et al. 1971). The seed covering is made up of three parts: seed coat, pericarp, and husk (Lynch et al. 2016). All three of these structures function as protective layers for the seed with the seed coat and pericarp protecting the embryo and endosperm (Arendt and Zannini 2013). The husk or hull is the final protective layer of the seed primarily made up of cellulose with a small amount of polyphenols and terpenoids to defend against predation. The husk is useful in the brewing

process as an extra source of filtration (Fox 2009). Malting is primarily driven by the degradation of the endosperm first affecting cell walls.

The cell wall structure of barley endosperm is primarily made up of β-glucan (about 75%) with the remainder consisting of arabinoxylans (about 20%) (Fox 2009). β-glucan comprises 2 to 10% of total grain weight with variation in these levels being caused by variety, heat stress, and water availability (Zhang et al. 2001). Models of the malting process indicate that the endosperm cell wall's outer arabinoxylan layer needs to complete enzymatic hydrolysis before the inner β-glucan layers can be hydrated and degraded. Thickness of the arabinoxylan layer impacts hydration and β-glucan degradation (Bamforth and Kanauchi 2001). It takes about four days for the majority of β-glucan to be degraded (Henry 1986), but once this structure begins to break down, degradation of the protein matrix begins.

The protein matrix of modern malting barley varieties comprises 8 to 13% of the grain weight (Fox 2009). The ideal range of grain protein is between 10 and 12%. If grain protein is too high, it can lead to low sugar extract. If it is too low, then there aren’t enough amino acids to feed yeast or enzymes to convert starch to sugars (Fox 2009; Eagles et al. 1995; Howard et al. 1996). Grain protein variation is influenced by soil fertility, water availability, and genetic variation across barley varieties, effects of which have all been thoroughly studied. Nitrogen availability plays a major role in grain protein with too much nitrogen leading to too high of protein, while too little nitrogen will not yield enough protein (Eagles et al. 1995). Water availability also changes how much nitrogen is needed to reach the desired protein range for malt barley. Environments with increased temperatures and reduced rainfall need less nitrogen since these drought-stressed conditions lead to less starch and more protein in the seed (Howard et al.

1996). However high precipitation or irrigated environments need more nitrogen but if too much precipitation is present reductions in endosperm development have been observed (Zhang et al. 2001). Genetic regulation of grain protein content has also been shown to negate some of the negative effects the environment can have on grain protein (Pauli et al. 2014; Alptekin et al. 2022; Shewry 2007).

Grain protein in barley is made up of three different groups of storage proteins. Hordeins account for 30 to 50% of the proteins in barley. They contain high concentrations of glutamine and proline but depending on variation of other amino acids they fall into four groups: B-hordeins, C-hordeins, D-hordeins, and γ-hordeins (Qi et al. 2006; Howard et al. 1996; Baxter 1981). B-hordeins are sulfur-rich and the most abundant hordein making up 70 to 90% of the total hordein proteins and are further divided into B1, B2, and B3 subunits based on electrophoretic capabilities (Baxter 1981; Gupta et al. 2010). C-hordeins make up 10 to 20% of the total hordeins and are sulfur-poor. D and γ-hordeins make up less than 5% of the total hordeins with D-hordeins having high molecular weights, while γ-hordeins are sulfur rich (Shewry et al. 1985). The genes that regulate the hordein proteins are Hor2 (B-hordeins), Hor1 (C-hordeins), Hor3 (D-hordeins), and Hor5 (γ-hordeins) with all four genes located on chromosome 1H (Shewry and Miflin 1982; Shewry et al. 1985). The other primary storage proteins are glutelin and protein Z which make up 35 to 45% and 5% of the barley storage proteins respectively (Zhao et al. 2011; Klose et al. 2010). Glutelin is made up of high levels of glutamine, proline, and glycine and is a good emulsifier (Wang et al. 2010). Protein Z is very heat stable and resistant to enzymatic degradation causing it to be the primary protein in finished beer that impacts foam stability (Klose et al. 2010; Niu et al. 2018).

Starch makes up most of the endosperm. It is comprised of two polymers, amylose and amylopectin. Amylose is made up of a linear chain of glucose molecules linked via α-(1-4) glucosidic bonds while amylopectin consists of a larger branching structure with α-(1-4) and α-(1-6) glucosidic bonds (Hough 1985). The ratio of amylopectin to amylose in barley is about 3 to 1 (Palmer 1989). Amylose and amylopectin are found as starch granules in the barley endosperm with large granules (Type A) containing 70 to 80% amylopectin while small granules (Type B) contain 40 to 80% amylose (Evers et al. 1999). The compounds responsible for the degradation of starch into sugars are α-amylase and β-amylase (Osman 1996). The goal of malting is to free these starch granules and produce the enzymes needed to degrade them. Starch degradation results in reduced sugar extract for brewing and there for a less desirable malt.

Endosperm hydration

With a clear understanding of grain structure, the next thing to understand is how the endosperm hydrates during steeping. As the grain is submerged in water the first thing to hydrate is the seed coat followed by diffusion of the water into the rest of the grain (Seefeldt et al. 2007). The change in moisture for hydrating grain tends to follow exponential decay, where hydration begins rapidly and then slows until equilibrium moisture content is reached (Miano et al. 2018). In malt houses, to ensure adequate hydration, and in research studies, to understand genetic variation, it is common to measure the moisture uptake through a percent change in seed weight at the end of steeping (Cu et al. 2016a; Holopainen et al. 2014; Montanuci et al. 2013). However, this testing does not provide a clear picture of the hydration level of the endosperm, which is necessary to ensure adequate degradation of β-glucan and protein (Turner et al. 2019). The Chapon test provides a more detailed image of endosperm hydration by boiling seeds at the end

of steeping (steep out) to gelatinize the hydrated starch in the endosperm. Seeds can then be cut and scored based on the amount of endosperm that has gelatinized providing a Chapon score or hydration index (Chapon 1959; Molina-Cano et al. 2002). Variation in hydration index could be caused by any of the following: physical differences (seed size, seed hardness, cell wall barriers), hormonal differences (gibberellic acid [GA], abscisic acid [ABA]), metabolic activity, and enzymatic differences. However, the genetics of endosperm hydration are still largely unknown (Miano and Augusto 2018).

Seed Morphology

Several seed morphology traits are directly related to the rate of barley imbibition. In the malting industry, it has been recommended since 1933 to sort samples by seed size since malting seeds of a similar size produces a more uniform malt (Pollock 1962). It has also been reported that while larger seeds have more extract they also take longer to hydrate during steeping (Fox 2009). Softer grains also imbibe and modify faster than harder grains however it is unclear if this is a result of the composition of β-glucan, protein, starch, or all three in the grain (Gamlath et al. 2008; Psota et al. 2007). Although gross seed morphology explains some differences in endosperm hydration, morphology does not fully capture the variation in endosperm hydration.

Dormancy

Dormancy and the hormones that regulate it could also impact the hydration of the endosperm. Grain has dormancy when seed germination is suppressed for a time after maturation. The hormones abscisic acid and gibberellin are the two primary hormones that regulate dormancy. Abscisic acid holds plants in a dormant state while gibberellin encourages germination (Rodríguez et al. 2015). Dormancy is broken over time and with cold which signals

plants to start producing more gibberellin. Breeding for malt barley naturally selects against dormancy with short turnaround windows between harvesting and replanting seeds. Dormancy is not popular with malt houses. Dormancy delays malting, and when dormancy is finally broken, it can negatively impact malt quality however the mechanisms behind this are still unclear (Sweeney et al. 2022; Rooney et al. 2023). QTL for dormancy have been found across all seven chromosomes but most of the variation is usually explained by SD1 (Qsd1) and SD2 (Qsd2) (Hori et al. 2007; Ullrich et al. 1992). The gene regulating SD1 has been identified as an alanine aminotransferase (AlaAT) (Sato et al. 2016) and SD2 is regulated by a mitogen-activated protein kinase kinase 3 (MKK3) (Nakamura et al. 2016). Two mutations on the MKK3 gene have been identified to produce three phenotypes for dormancy consisting of no dormancy, slightly dormant, and dormant (Rooney et al. 2023). While the connection between dormancy and hydration index has not been thoroughly investigated it could be negatively impacting hydration index which in turn results in poor malt quality.

Malt Quality

Endosperm hydration is a rate-limiting step in the malting process. Hydration of the endosperm starts the germination process, releasing enzymes that are responsible for the degradation of cell walls, storage proteins, and stored starch. Enzymes like β-glucanase, endopeptidase, and α-amylase are initiated in the aleurone layer from GA signaling initiated when embryo takes up water (Bamforth 2006). These enzymes are then dependent on water for mobility through the endosperm (Kuusela et al. 2004). Cell wall degradation is necessary for further hydration, so genetic variation in β-glucanase activity and/or cell wall thickness can both impact hydration of the endosperm (McEntyre et al. 1998; Bamforth 2006). Temperature and

time also play an important role in β-glucanase activity (Bourne and Wheeler 1984). As the cell wall is degraded proteases and peptidases can travel through the hydrated endosperm and degrade the protein matrix (Osman et al. 2002). This frees up starch granules but before the starch can be heavily degraded, the process is stopped with the kilning phase. Kilning uses heat to dry out the grain and kill the embryo, thereby stopping all enzymes from performing further degradation of the endosperm.

Malt quality measures how well the malting process has degraded storage structures and produced enzymes. Eight traits are commonly measured to determine malt quality. These traits are all measured on wort which is made by grinding the malt into a flour, suspending it in water, and then subjecting it to heating in a process called a congress mash (Evans et al. 2011). The amount of β-glucan in the wort tells you how well the cell walls were degraded. The goal is to have less than 100 ppm of β-glucan in the final malt product. Degradation of protein is measured from soluble protein, free amino nitrogen (FAN), and the ratio of soluble protein to total grain or malt protein (S/T) present in the wort (Fox 2009). The desired soluble protein and S/T levels range from 4.8 to 5.6% for soluble protein and 38 to 47% for S/T. FAN is more dependent on the style of beer with adjunct brewers, using corn, rice, and malt, needing more (>210 mg/L) while all-malt brewers, using just malt, need less (140-190 mg/L). This is because FAN is an important energy source for yeast. Having too little FAN starves yeast, while residual FAN supports microbes responsible for staling and off flavors (Ferreira and Guido 2018). Extract measures the amount of sugar available in the wort with the goal of having it at 81% or higher. Enzyme levels are measured with α-amylase and diastatic power. Desired enzyme levels again depend on how the malt will be used. Adjunct brewers require more than 50 DU of α-amylase and diastatic

power above 140 °ASBC, while all-malt brewers want α-amylase around 40 to 70 DU and diastatic power ranging from 110 to 150 °ASBC. The malt quality standards for these traits are decided by members of the American Malt Barley Association (AMBA) (https://ambainc.org/), while methods for measuring each of these quality traits are published by the American Society of Brewing Chemists (ASBC) (https://www.asbcnet.org/).

Another important part of the malting process is the creation of flavor metabolites. These are of particular interest since different varieties have been identified as having different flavor profiles (Herb et al. 2017a). Most flavor metabolites are created during kilning where heat converts lipids into esters (Holt et al. 2019) and is an important part of Maillard reactions and Strecker degradation (Schwarz and Li 2011). Maillard reactions occur optimally between 140 and 165 °C while kilning temperatures can range from 104 to 204 °C depending on the recipe (Perez-Locas and Yaylayan 2010). This heat range causes reactions between saccharides and amino acids to produce melanoidins that are responsible for flavors associated with browned foods. Amino acids are also important flavor metabolites and flavor precursors for beer production. They are absorbed by yeast at different rates and it is suggested they can impact some of the flavor metabolites yeast produce (Ferreira and Guido 2018). Excess amino acids in beer are also a concern because they negatively impact the shelf life of beer.

Research Objectives

Many studies have looked at the genetic regulation of malt quality using linkage mapping and genome wide association (GWAS). The goal of this research was to use these tools to identify QTL for malt quality, hydration index, dormancy, and metabolites to better understand how all of these traits interact with each other. A GWAS population was first used to identify

hydration index QTL and relate them to malt quality and seed morphology traits. Then a bi-parental mapping population was used to identify more hydration index QTL. This population also allowed us to identify different haplotypes for hydration index and dormancy QTL to see how they impacted malt quality. Last we revisited the GWAS population to identify QTL for metabolite variation. Particular QTL of interest included those impacting amino acids, sugars, and maltol which co-located with FAN, hydration index, kernel hardness, extract, grain fill, and other metabolite QTL.

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CHAPTER TWO

GENETIC DISSECTION OF ENDOSPERM HYDRATION IN MALTING BARLEY (HARDUEM VULGARE)

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Manuscript Information

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Abstract

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

Introduction

Barley (Hordeum vulgare L.) is a major cereal crop necessary to the malting and brewing industry. Unlike the results of wheat domestication, barley is not free-threshing. To utilize nutritional resources from barley despite the undigestible hull, early people developed a process of controlled germination and preservation called malting (Badr et al. 2000). The main goal of malting is to make nutritionally unavailable biopolymers such as cell-wall polysaccharides, proteins, and starch available for humans through a process called modification. In the 1870s in current Czechia, early malt barley selections were made from landraces (Psota et al. 2009). Early

in the 1900s, European breeders also identified landraces with high malt quality. These landraces were incorporated into breeding programs across Europe, resulting in a lack of genetic diversity in European malt lines (Fischbeck 1992; Melchinger et al. 1994). Similarly, genetically narrow germplasm has been reported in North American Malt barley (Martin et al. 1991) and a recent focus on 2-row malt has potentially narrowed the malt germplasm further. Although a lack of genetic diversity has been observed, there is still variation in malt quality. The burgeoning craft brewing industry requires diversity and expresses a preference for heritage malt (C. Swersey, Brewers Association, personal communication, March 2016), believing it has unique malt quality and flavor traits.

Malting begins with imbibition, where water enters the seed and eventually moves through the storage tissues and is a rate-limiting step in germination. However, little is known about the genetic control of imbibition, although many industrialized food processes like cooking, extraction, fermentation, and germination all rely on adequately hydrated grain (Miano and Augusto 2018). Most imbibition or hydration studies measure moisture uptake through the gross change in seed weight over time (Cu et al. 2016a; Holopainen et al. 2014; Montanuci et al. 2013). While this measurement is fast and easy to collect, it does not distinguish the level of endosperm hydration, which is crucial to ensuring high-quality malt (Turner et al. 2019). The lag of endosperm hydration to moisture uptake has also been demonstrated through non-invasive MRI approaches (Yin 2021) but is cost prohibitive for genetic mapping studies. The Chapon technique provides an inexpensive and detailed view of endosperm hydration by determining hydration index (HYI) (Chapon 1959; Molina-Cano et al. 2002). Variation in HYI could be due to physical differences (seed size, seed hardness, cell wall barriers), hormonal differences

(gibberellins and abscisic acid), metabolic activity, and/or enzymatic differences (Miano and Augusto 2018). Hydration index at the end of steeping (steep out) also impacts malt quality traits (Turner et al. 2019).

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

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

Post-harvest dormancy inhibits germination until broken. In climates where winter temperatures can kill a developing seedling, seed dormancy protects from premature germination. Modern breeding for malt barley has selected for lines without significant dormancy, since breeders shorten generation times and select for lines with the immediate ability

to malt. However, the cost of this selection is the increased likelihood of barley germinating in the field before harvest in a process called pre-harvest sprouting, resulting in loss of malt quality. The economic importance of seed dormancy in barley has resulted in many studies to identify the genetic controls of this trait (Bonnardeaux et al. 2008; Gong et al. 2014; Hickey et al. 2012; Nakamura et al. 2017; Ullrich et al. 2009). Dormancy quantitative trait loci (QTL) occur on all seven barley chromosomes (Bonnardeaux et al. 2008; Ullrich et al. 2009; Hickey et al. 2012; Gong et al. 2014; Nakamura et al. 2017), but the consensus is that seed dormancy QTLs SD1 (Qsd1) and SD2 (Qsd2) located near the centromeric region and distal end of the long arm of 5H control the majority of dormancy in barley (Hori et al. 2007).

To identify and utilize the genetic controls of malt traits, we performed a Genome-Wide Association Study (GWAS) on a subset of spring 2-row lines with a malting history (Munoz-Amatriain et al. 2014). By focusing strictly on malting lines, we hoped to identify new malt quality QTLs by eliminating the impact of already mapped loci with large effects (e.g., 2-row versus 6-row and lax versus dense). Through this evaluation, it became clear that endosperm hydration impacted malt quality, but its genetic regulation was not understood (Miano and Augusto 2018). Therefore, a second goal was to identify hydration index QTLs and their potential impact on malt quality.

Methods

Germplasm

A subset of the NSGC Barley Core Panel using 169 malting lines was utilized for this study (Munoz-Amatriain et al. 2014). Lines were selected based on row type (2-row) and evidence of a malting background to ensure identification of malt quality QTLs that may be

undetected when larger effect loci are present (e.g., 2-row vs 6-row). The included lines originated from 42 countries with 152 named lines, 13 landraces, and 4 derived from mutations. Lines were ordered from the Germplasm Resources Information Network (GRIN) and increased in the field in short rows in 2017.

Genotyping

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

Agronomics

We grew material at the Bozeman Post Farm in 2018 under irrigated conditions and in 2019 under dryland conditions. Plots were 5 m2 with seeding rates of 40g per 5m2 in 2018 and 30g per 5m2 in 2019. The trial was planted in an augmented block design both years. The 2018 trial consisted of 4 blocks with 3 checks replicated 3 times for a total of 9 checks in each block. This trial had a total of 36 check plots and 210 experimental lines. The malting lines used for check plots were Craft, Hockett, and Genie. Some lines were lost to lodging in 2018, so the 210 experimental lines were reduced to 169. In 2019, we opted to use a more powerful and efficient design to fit the lower number of lines consisting of 6 blocks with 4 checks for a total of 24 check plots and 169 experimental lines. The checks consisted of the malt lines Craft, Hockett, Merit 57, and Metcalfe.

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

Hydration Index

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

Dormancy

In 2019, germination was determined using a modified ASBC method, of Barley-3 Germination. Twenty-four days post-harvest (%Germ@24) 100 seeds from each line were placed in a petri dish with two sheets of Whatman #1 filter paper and 4 mL of water and held constant at 20°C in a germination chamber. Germinated seeds were counted and removed at 24, 48, and 72 hours. After 72 hours ungerminated seeds were suspended in a 0.75% hydrogen peroxide solution and left for 48 hours. Any seeds that still had not germinated were noted as dead and not

included in total germination potential. Since 95% germination is required for malting, we repeated the germination tests each week until all lines reached 95% germination to determine the days required to reach this threshold (DT95).

Malt Quality

Once dormancy was broken in all lines, the grain was malted as described in Turner et al. (2019) with the following changes (Table 2.1). The 120g grain sample was not sorted by size, therefore unplumped, to capture the full phenotypic variation in the population. Malt quality was determined following ASBC methods with modifications as noted in Turner et al. (2019).

Statistical Analyses/GWAS

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

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

where Y_{ijkl} represents the traits for each line, block, year, and line type combination. Checki was modeled as a fixed factor representing the replicated check varieties. $Block_j$, $Year_k$, and $Entry_l$ were modeled as random factors, following N(0, σ2). Variation from malt tank to malt tank and day-to-day testing was monitored with control lines.

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

GAPIT's farmCPU method was used to analyze the data without the compression process (Wang and Zhang 2021). To run GAPIT the following packages were also loaded: multtest (Pollard et al. 2004), gplots (Warnes et al. 2021b), LDheatmap (Shin et al. 2006), genetics (Warnes et al. 2021a), ape (Paradis and Schliep 2019), EMMREML (Godfrey 2015), compiler (Team 2021), and scatterplot3d (Ligges and Mächler 2003). The appropriate number of PCs to correct for the population structure of each trait was determined by running the model on 0, 1, 2, and 3 PCs to compare QQ plots. The QQ plots showed that the kinship matrix was sufficient for corrections; therefore, the PC correction was set to 0. The kinship matrix was also calculated in GAPIT and a dendrogram was produced from this output to help understand the genetic relationships between lines. To calculate significance thresholds for QTLs we used GAPIT's Bonferroni correction where the negative log of alpha (0.01) was divided by the number of markers (5716) to get a significance threshold of about 5.8 (Wang and Zhang 2021). Any SNP above this threshold was recorded in Supplemental Table 2.1.

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

To assess further relatedness of traits to the HYI QTLs we took a generalist approach where we separated the lines based on their major and minor allele data from the most significant SNP for each HYI QTL. Then we ran two-sample unequal variances t-tests on all measured traits. Any trait with a p-value less than 0.05 was considered related to the HYI QTL being tested. Effects were evaluated by comparing the means of minor alleles between traits.

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

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

where μ is the baseline mean, QTL1 is the i^{th} HYI QTL (i = qHYI1H, qHYI2H, qHYI3H.a, qHYI3H.b, qHYI6H, and qHYI7H), and QTL2 is the k^{th} HYI QTL that is not i. All interactions are reported in Supplemental Table 2.2. Pirate plots made with yarrr (Phillips 2017) show their effects (Supplemental Figure 2.1).

Results

Population

The 169 lines, with origins from around the world, were reported as being malting lines for their end-use quality. Of those, 152 were named varieties, for which 138 pedigrees were determined and 31 pedigrees unknown. Assessing the GAPIT PCA output no clustering or grouping was observed even when geographic origin was included, so PCA structure corrections were not used for association mapping. Evaluation of a line's country of origin and malting quality using PCA analysis suggests no association (Supplemental Figure 2.2). Where possible we investigated the pedigrees further. Six of the 14 highest-quality lines came from Czechia, with Hanna, Gotland, and Valticky in the backgrounds of most. Diamant, an X-ray mutant of Valticky is also prevalent in high-quality lines. Table 2.2 reports the high-quality lines along with their pedigrees and country of origin.

Heritability calculations for HYI showed that the trait was highly heritable (0.688). These calculations also showed that β-glucan (0.757) was the most heritable trait and soluble protein (0.233) was the least heritable (Table 2.3). Heritability was not calculated for %Germ@24, DT95, Diameter, and Hardness because they were only measured for one year. Our PCA analysis of phenotypes indicated that traits related to modification were primarily explained by PC1 while variation in seed size was described by PC2. Some traits like enzymatic activity (AA and DP) along with protein traits (Soluble protein and FAN) were partially explained by both PCs, unsurprisingly suggesting seed size and modification impact their final concentrations (Supplemental Figure 2.2).

GWAS

Phenotypic averages from the 2018 and 2019 trials were used for GWAS (Table 2.3), since Pearson correlations between the trials indicated similar environments. GWAS indicated 61 associations for all traits measured except SOM and AA. (Supplemental Table 2.1).

Hydration Index (HYI)

Six HYI QTLs were found on 5 chromosomes - 1H (qHYI1H), 2H (qHYI2H), 3H (qHYI3H.a and qHYI3H.b), 6H (qHYI6H), and 7H (qHYI7H) (Table 2.4). The effect of each QTL varied from about 1.7 to 2.9 points on a range of 25-100. The HYI QTLs appear to behave additively where on average there is a 10-point difference in HYI between lines with 4 or 1 HYI positive QTLs (Figure 2.1). Of the QTLs shown evidence of non-additive interactions were also observed. We detected an interaction between qHYI1H and qHYI3H.a (p-value = 0.020578), where the three lines with both fast alleles have a decrease in HYI. There was also weak evidence (p-value = 0.05504) of an interaction between qHYI6H and qHYI7H where lines with

both fast alleles for qHYI6H and qHYI7H had a similar mean HYI to lines carrying a single fast allele (Supplemental Table 2.2).

Relationship of Hydration Index to Other Traits

GWAS identified two malt quality traits, BG on 2H and extract on 6H, that co-segregated with HYI QTLs. In both cases higher HYI co-segregated with improved malt quality (lower BG and higher extract). To interrogate further the relationship between HYI and other traits, we compared the trait data of allelic groups for each HYI SNP (Table 2.5). The analysis indicates the expected relationship between steep out moisture and HYI at qHYI1H, qHYI2H, and qHYI3H.b (Table 2.5), where HYI increased with percent moisture. Although several seed morphology QTLS were identified through GWAS, none co-segregated with HYI (Supplemental Table 2.1). However, when observing allelic differences (Table 2.5), HYI increased with a decrease in test weight and percent plumps at qHYI1H, HYI increased with increasing percent plumps and decreasing kernel hardness at qHYI3H.a, while HYI increased with decreasing kernel diameter at qHYI3H.b. Endosperm hydration was also found to impact malt quality beyond the co-segregated traits. Allelic differences that increased HYI improved α-amylase while decreasing β-glucan at qHYI2H, while at qHYI6H, an increase in HYI was associated with an increase in extract and α-amylase levels while decreasing β-glucan levels and grain protein (Table 2.5).

Dormancy was mapped as %Germ@24 and DT95. For %Germ@24, we found QTLs on 2H, 4H, 5H, and 6H (Supplemental Table 2.1). For DT95 we observed 5 QTLs on 1H, 2H, 5H, and 7H (Supplemental Table 2.1). The 2H and 5H QTLs co-segregate for %Germ@24 and DT95. Of the six HYI QTLs, none coincided with DT95 or %Germ@24 QTLs. The closest dormancy QTL to qHYI2H is 6.5 Mb downstream while the closest dormancy QTL to qHYI6H

is 33.7 Mb upstream. However, when comparing allelic patterns, at qHYI2H and qHYI6H HYI increased with less dormancy (Table 2.5).

Discussion

Population

When examining this population, we found minimal evidence for population structure. The population is a subset of a larger population also used for GWAS (Munoz-Amatriain et al. 2014). The lines selected from the larger population were all 2-row lines with a malting background. Most of these lines (125) fell into subgroup 3, while the remainder (44) were outside of subgroup 3 of Munoz-Amatriain et al. (2014) findings. Interestingly, the pedigrees of the panel indicated shared parents across breeding programs. For example, the named varieties Gull and Binder are parents of Dutch, British, Irish, French, Scandinavian, and German varieties (Aufhammer et al. 1968). Gull is a selection from the Gotland landrace and Binder is a selection from Hanna. In fact, the presence of a few parents is pervasive across this population demonstrating the exchange of malt lines between breeding programs and explaining the lack of need for PCA corrections.

Evaluating the kinship matrix provided further perspective about the population structure. While we did see groupings, they were very deep in the relationships among lines (Supplemental Figure 2.3), consistent with the pedigrees indicating shared parents throughout all the branches. We did notice the first branch lines were being separated based on occurrence in subgroup 3 from Munoz-Amatriain et al. (2014), but again shared parents throughout branches indicated relatedness. While the panel was genetically similar, we did see variation in malt quality. Although not exclusively, we noticed that lines of Czechia origin were present in many pedigrees

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

Endosperm Hydration

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

Through further interrogation of traits based on HYI allelic differences, (Table 2.5), we were able to categorize each HYI QTL as related to grain morphology and malt quality to aid our understanding of these QTLs and identify the most beneficial. The HYI QTLs related to grain size are qHYI1H and qHYI3H.b where smaller seed has higher HYI. Since larger seeds are

preferred by maltsters, these QTLs are unfavorable for breeding. However, at qHYI3H.a large seeds are associated with high HYI due to soft texture. The HYI QTLs qHYI2H and qHYI6H increase HYI and improve malt quality, but also may increase PHS risk. Interestingly, although qHYI7H has one of the largest impacts on HYI, it does not significantly relate to any other trait and therefore may be a candidate to improve endosperm hydration without negative impact, warranting further investigation. Where allelic variation at HYI QTLs correlates with variation in another trait, it is unclear whether that variation is due to pleiotropy or linkage. However, this examination helps determine that qHYI2H, qHYI3H.a, qHYI6H and qHYI7H are worth further investigation.

Although HYI QTLs appear to act additively, there is a large amount of variation in HYI for lines with 2 or 3 QTLs (Figure 2.1). Epistatic interactions indicate that qHYI1H and qHYI3H.a decreased HYI when both positive HYI alleles were present. Although qHYI3H.a's important effect is likely related to seed hardness, the two fast alleles at qHYI1H and qHYI3H.a had opposite effects on seed size perhaps resulting in this negative interaction. The interaction between qHYI6H and qHYI7H did not increase HYI when both positive alleles were present (Supplemental Figure 2.2). Although further study is needed to understand these interactions, they do explain the low HYI of some lines with two high HYI alleles, but it does not explain why some lines with only two QTLs have such high HYI values. Our ability to observe epistasis was likely limited by the low frequencies of some two gene combinations. Also, other alleles could occur with such minor frequency that a significant association was not observed. This is further supported by our heritability calculations which show that HYI is heritable (H2=0.688), but the genetic variation explained by the HYI QTLs was only about fourteen percent. To better

understand variation in HYI, we are developing bi-parental mapping populations to identify any unmapped HYI QTLs.

Steep Out Moisture

When gauging water uptake other studies have used SOM (Cu et al. 2016a; Holopainen et al. 2014; Montanuci et al. 2013), identifying QTLs on 4H, 5H, and 7H in a set of double haploid lines (Cu et al. 2016a). Although we did not identify any SOM QTLs through GWAS, allelic comparisons associated SOM with HYI at qHYI1H, qHYI2H, and qHYI3H.b suggest some connection between the traits (Table 2.3). However, the lack of a relationship between all 6 HYI QTLs and SOM QTLs from other studies emphasizes variation in the genetic control of the two measurements. Turner et al. (2019) came to similar conclusions that these traits are related, but not perfectly correlated.

Dormancy

Dormancy is another important trait for malting barley and has likely been selected against by the modern breeding process of advancing generations as quickly as possible. QTLs for dormancy have been identified on all 7 barley chromosomes (Bonnardeaux et al. 2008; Gong et al. 2014; Hickey et al. 2012; Ullrich et al. 2009; Nakamura et al. 2017) but SD1 and SD2 on 5H consistently have the greatest effects on dormancy (Hori et al. 2007). For our two traits that assessed dormancy (%Germ@24 and DT95), we found associations near SD2. We also found an association near the QTLs reported in Gong et al. (2014) on 1H, 2H, 4H, and 6H for both traits. Although no dormancy QTLs co-segregated with HYI QTLs through GWAS, there were some relationships between hydration and dormancy traits at qHYI2H and qHYI6H. Importantly, for

the most part, QTLs for malt quality and dormancy do not overlap. Providing the opportunity to increase dormancy, while also improving malt performance with alleles for increased HYI.

Morphology

Since grain size, structure, and composition impact hydration pathways (Miano and Augusto 2018), the malting industry has historically sorted grain by size to ensure even hydration (Pollock 1962). Also, softer grains are preferred since harder grains imbibe slower than softer grains (Gamlath et al. 2008; Psota et al. 2007). The kernel size QTL on 6H (Supplemental Table 2.1) matches the findings of Wang et al. (2019a) while grain hardness association on 4H and 5H matches Walker et al. (2013) and Fox et al. (2007b) findings, respectively. While none of these morphological QTLs co-segregate with the HYI QTLs, allelic comparisons indicate seed size and hardness have some impact on endosperm hydration. qHYI1H, qHYI3H.a, and qHYI3H.b were all related to seed size and hardness traits, confirming previous findings, although results of qHYI3H.a indicate that larger seeds can have a higher HYI with softer kernels. Importantly, HYI QTLs on 2H, 6H, and 7H were not associated with seed size or hardness traits suggesting a deeper level of complexity for this trait.

Protein content and protein structure are one piece of a very complicated interaction between grain size, hardness, porosity, starch, and fat content that all impact hydration (Miano et al. 2018). QTLs related to grain protein have been mapped to all seven chromosomes except 3H (Emebiri et al. 2005; Fan et al. 2017). NAC transcription factors on 6H (HvNAM1) and 2H (HvNAM2) have been related to delayed senescence, larger seed, lower grain protein and malt quality (Alptekin et al. 2022). We mapped QTLs for grain protein to the same six chromosomes, but none co-segregated with HYI QTLs. Examination of allelic variation indicates that qHYI6H

has some relation with grain protein; however, this population is fixed for the functional allele of HvNAM1 on 6H suggesting qHYI6H is controlled by another gene. Although this population does segregate for HvNAM2 on 2H, qHYI2H localized 400 Mb from this gene.

Malt Quality

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

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

Breeding Impacts

The relatedness and lack of structure for these historic malting barley lines pose a unique problem for breeders and have been reported by others (Martin et al. 1991; Fischbeck 1992; Melchinger et al. 1994). Limited genetic variation could limit the ability to improve malt quality. However, we observed variation in malt quality that could in part be explained by variation in endosperm hydration. We are currently evaluating traits in this population not evaluated during

malting quality analysis including metabolite differences that might be used to further improve malt.

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

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

which is roughly 8 gallons of water per bushel (Yin 2021). Lines with higher HYI could ensure fewer steeps saving a malt house time and money.

Tables

Table 2.1 MSU Pale base malt regime

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

Table 2.2 The top 14 malting lines, pedigrees, and country of origin. Interbreeding between barley cultivars indicated by superscript with Valticky (1), Hanna (2), and Gull (3). Lines without superscript either did not have one of the three main parents or could not be traced back to one of them.

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

Table 2.3 Summary table of average values of mapped traits for the population during the 2018 and 2019 growing seasons. All values are calculated from the average values of the two years except for %Germ@24 and DT95, which were only collected in 2019 and Diameter and Hardness which were only collected in 2018. H2 shows the broad-sense heritability of each trait for the population and could not be calculated for traits with only a single location year of data. * SOM: percent moisture of the grain at steep out; %Germ@24: percent germination at 24 hours; DT95: days to 95% germination; S/T: soluble divided by total protein or the Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: diastatic power

	Hydration Index	SOM	%Germ@24	DT95	Diameter	Hardness	Test Weight	Plump	Protein	β-Glucan	Soluble Protein	S/T	FAN	Extract	AA	DP
Mean	46.0266	0.4432	87.89	32.57	2.84	47.98	53.89	91.42	13.60	660.45	4.131	31.28	179.79	77.19	53.64	137.24
SD	10.2231	0.0145	18.20	13.33	0.08	6.66	1.67	6.46	1.12	359.7	0.6511	4.57	26.31	1.65	14.65	32.76
CV	0.2221	0.0327	0.2071	0.4093	0.0282	0.1388	0.031	0.0707	0.0822	0.5446	0.1576	0.1462	0.1464	0.0213	0.273	0.2387
Min	26	0.3622	9.32	24	2.64	29.79	46.3	64.20	11	54.68	2.6901	20.87	113.35	72.35	17.29	21.15
Max	78	0.4926	100	87	3.06	67.95	57.2	99.42	17.36	1683.49	6.758	49.18	284.68	82.31	134.79	270.06
H^2	0.688	0.239	NA	NA	NA	NA	0.520	0.458	0.620	0.757	0.233	0.521	0.694	0.656	0.405	0.469

Table 2.4 Hydration index (HYI) QTLs, with co-segregating traits and their effects. The most significant SNP for each QTL as indicated by low p-values of FDR, with chromosome and base pair positions posted. The effect and frequency of the minor allele (MAF) are reported for each QTL. Effects of co-segregating traits are also reported. *BG: β-Glucan

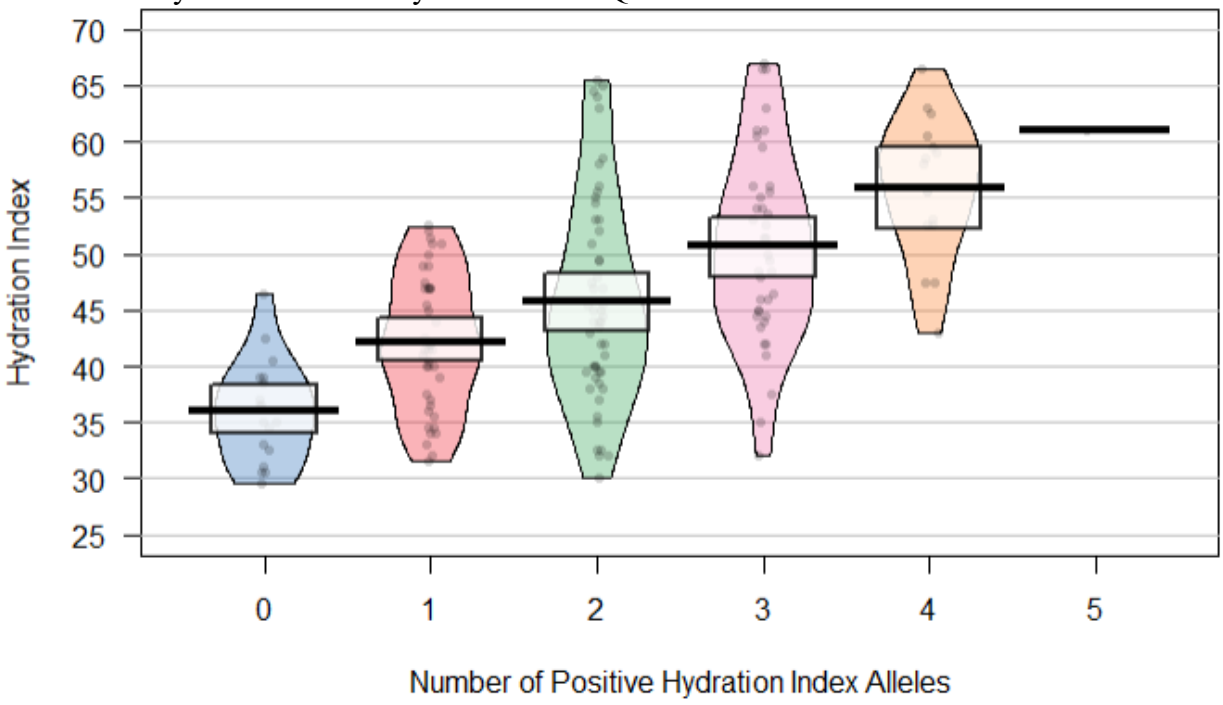
QTL Designation	SNP	Chromosome	Position	MAF	FDR Adjusted P-values	effect	Co-Segregating traits with effects
qHYI1H	11_11293	1H	76294035	0.16568	5.65E-04	2.578	
qHYI2H	SCRI_RS_219333	2H	2238515	0.23669	1.22E-05	2.9319	BG -73.22
qHYI3H.a	SCRI_RS_97417	3H	10631754	0.15385	1.67E-03	2.1132	
qHYI3H.b	SCRI_RS_1435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

Table 2.5 The impact of the Hydration Index (HYI) QTLs on other traits. HYI QTLs are designated as as qHYI1H (1H 11_11293), qHYI2H (2H SCRI_RS_219333), qHYI3H.a (3H SCRI_RS_97417), qHYI3H.b (3H SCRI_RS_1435), qHYI6H (6H SCRI_RS_167845), and qHYI7H (7H SCRI_RS_189107). HYI reports the effects of the minor allele on hydration index. For the remaining traits the difference between the mean of the lines with minor and major alleles is reported. Results from T-tests indicate if the differences are meaningful. * Indicates where major and minor alleles are not equal with p-values equal to * < 0.05, ** < 0.01, and ***<0.0001. * SOM: percent moisture of the grain at steep out; %Germ@24: percent germination at 24 hours; DT95: days to 95% germination; S/T: soluble divided by total protein or the Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: diastatic power

QTLs	HYI	SOM	%Germ@24	DT95	Diameter	Hardness	Test Weight	Plump	β-Glucan	Protein	Soluble Protein	S/T	FAN	Extract	AA	DP
qHYI1H	2.59	0.0094**	-0.81	-0.65	-0.0221	1.47	-0.89**	-2.87*	-75.99	0.14	0.1583	1.14	1.77	-0.1288	4.43	2.47
qHYI2H	2.93	0.0076**	6.91***	-6.41**	-0.0161	0.81	-0.47	-1.54	-219.85***	-0.24	0.0954	1.47*	6.42	0.2754	5.19*	3.60
qHYI3H.a	2.11	0.0022	4.98	-3.77	0.0314	-3.18**	0.54	2.75**	-113.15	-0.28	0.0539	0.99	-0.28	0.2415	-0.36	-4.29
qHYI3H.b	-1.69	-0.0079***	-1.76	0.35	0.0321**	-0.43	0.39	1.26	66.84	0.26	0.0788	-0.02	4.29	0.1219	-0.03	5.37
qHYI6H	-2.43	-0.0029	-7.48*	4.97*	0.0097	1.01	-0.42	-1.38	173.99**	0.53**	-0.0653	-1.66**	-1.98	-0.7976**	-3.69*	7.81
qHYI7H	2.26	0.0011	-2.33	1.29	-0.0118	0.99	0.26	0.03	-41.43	-0.07	0.0422	0.40	2.90	0.4915	0.47	3.13

Figures

Figure 2.1 A pirate plot showing the hydration index vs the number of positive alleles. The gray dots represent each line tested. The bold line represents the mean Hydration Index for that number of positive alleles and the white band shows the confidence interval around the mean. Each bean functions as a smoothed density curve showing the distribution of the data points. There was only one line with 5 hydration index QTLs and 0 with all 6.



Supplementals

Supplemental table 2.1: Summary of all associations identified in this study.

Traits	SNP	Chromosome	Position	Effect
DT95	SCRI_RS_120059	1H	653064	2.83
β-Glucan	SCRI_RS_165811	1H	30837602	102
Protein	12_30592	1H	74052398	0.226
Test Weight	12_30592	1H	74052398	-0.331
Hydration Index	11_11293	1H	76294035	2.58
Extract	12_31208	1H	1.48E+08	-0.471
FAN	SCRI_RS_147203	2H	313674	15.1
β-Glucan	SCRI_RS_219333	2H	2238515	-73.2
Hydration Index	SCRI_RS_219333	2H	2238515	2.93
DT95	SCRI_RS_196276	2H	8761644	3.74
Protein	BK_14	2H	22953027	0.308
β-Glucan	11_21261	2H	29185036	69.8
FAN	SCRI_RS_215080	2H	40064925	-5
%Germ@24	11_10436	2H	5E+08	6.67
%Germ@24	SCRI_RS_156871	2H	5.46E+08	-6.31
DT95	SCRI_RS_156871	2H	5.46E+08	9.1
%Germ@24	11_10287	2H	5.53E+08	-5.3
DP	11_10128	2H	5.79E+08	7.63
FAN	11_10990	2H	5.8E+08	-8
Hydration Index	SCRI_RS_97417	3H	10631754	2.11
β-Glucan	11_10276	3H	4.34E+08	148
DP	12_31238	3H	5.11E+08	8.77
Hydration Index	SCRI_RS_1435	3H	5.51E+08	-1.7
Protein	SCRI_RS_163112	4H	82008	0.179
Soluble Protein	SCRI_RS_127657	4H	13198440	0.0996
FAN	12_30793	4H	14844591	8.3
DP	SCRI_RS_204804	4H	2E+08	-6.21
DP	12_30824	4H	3.48E+08	-7.29
%Germ@24	SCRI_RS_189180	4H	4.19E+08	4.32
β-Glucan	SCRI_RS_25685	4H	5.1E+08	112
DP	SCRI_RS_119390	4H	5.37E+08	6.36
Hardness	SCRI_RS_31797	5H	2492193	3.14
DT95	12_30080	5H	3.6E+08	3.16
%Germ@24	11_20736	5H	4.22E+08	-7.11

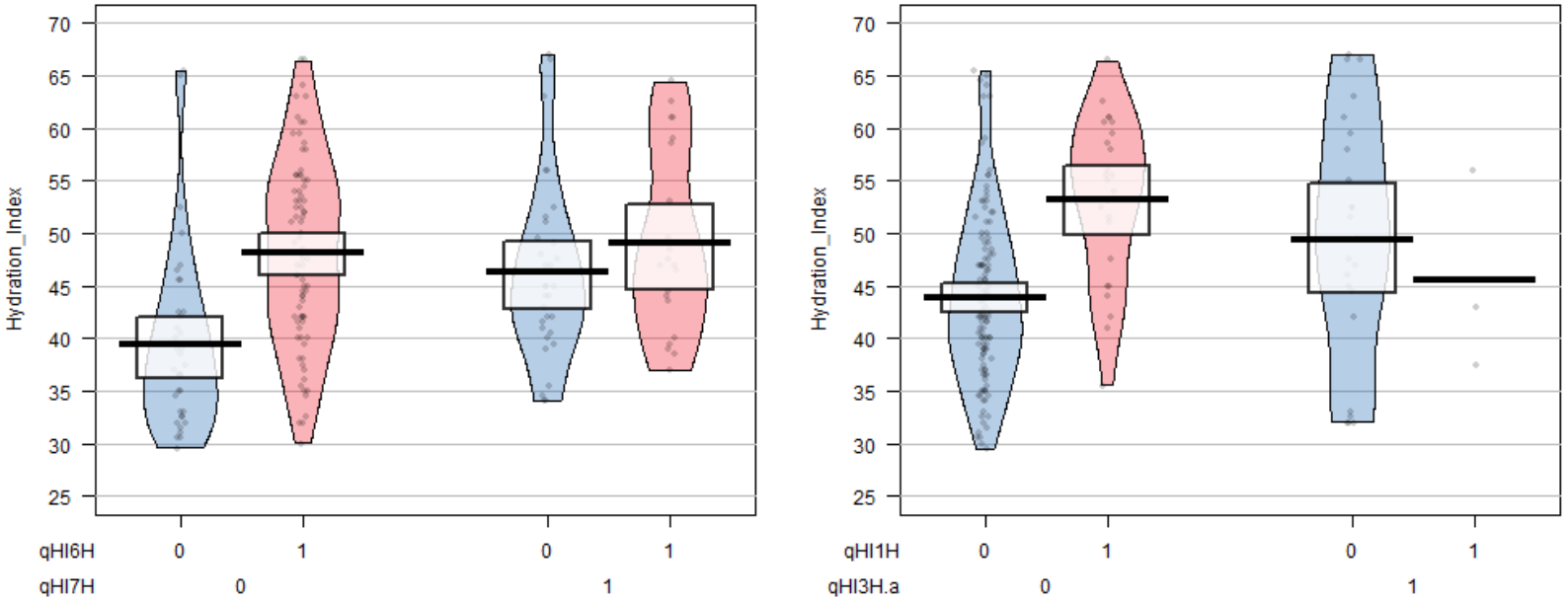
Supplemental table 2.1 Continued

Traits	SNP	Chromosome	Position	Effect
Extract	12_30580	5H	5.13E+08	0.377
Soluble Protein	SCRI_RS_155688	5H	5.26E+08	0.059
%Germ@24	SCRI_RS_221599	5H	5.31E+08	-8.37
Protein	12_31239	5H	5.44E+08	-0.193
Extract	SCRI_RS_203036	6H	9496882	0.382
Soluble Protein	SCRI_RS_201251	6H	16263422	-0.0967
Protein	SCRI_RS_124850	6H	2.49E+08	-0.234
Test Weight	SCRI_RS_143415	6H	2.77E+08	0.352
FAN	SCRI_RS_139937	6H	4.43E+08	4.43
%Germ@24	11_11349	6H	4.65E+08	3.97
Hydration Index	SCRI_RS_167845	6H	4.98E+08	-2.43
Extract	SCRI_RS_167845	6H	4.98E+08	-0.215
Test Weight	SCRI_RS_151574	6H	5.12E+08	-0.29
Diameter	SCRI_RS_138295	6H	5.29E+08	0.0323
Extract	SCRI_RS_138887	6H	5.29E+08	0.294
Protein	12_31173	7H	8026234	-0.266
Protein	11_10025	7H	21034181	0.297
Test Weight	SCRI_RS_230487	7H	67038413	0.301
DT95	12_11492	7H	1.63E+08	10.5
Plump	12_10897	7H	5.43E+08	-2.24
Hydration Index	SCRI_RS_189107	7H	5.63E+08	2.26
%Germ@24	11_21001	UNK	3800	-7.13
DT95	11_21001	UNK	3800	7.59
β-Glucan	11_21381	UNK	4480	-147
Hydration Index	11_21397	UNK	4540	2.33
β-Glucan	SCRI_RS_137824	UNK	7650	-70.3
Protein	SCRI_RS_161476	UNK	10310	-0.197

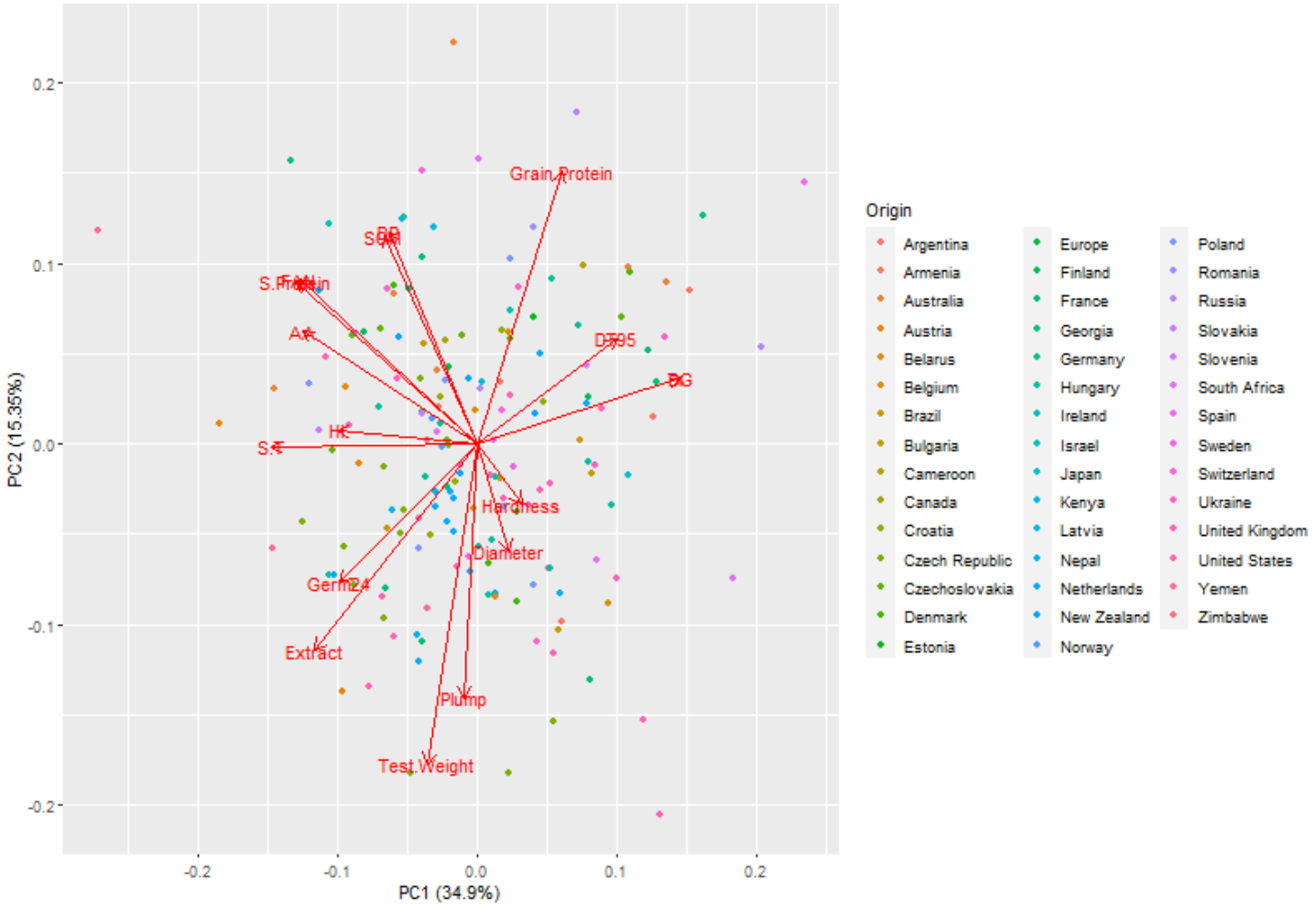
Supplemental table 2.2: Epistatic interactions between hydration index QTLs.

Interaction	Sum Sq	Df	F value	Pr(>F)
qHI1H:qHI2H	112	1	1.595	0.20839
qHI1H:qHI3H.a	417	1	5.467	**0.020578**
qHI1H:qHI3H.b	24	1	0.3244	0.5697428
qHI1H:qHI6H	0	1	0.0006	0.980173
qHI1H:qHI7H	27	1	0.3372	0.56227
qHI2H:qHI3H.a	6	1	0.0854	0.77053
qHI2H:qHI3H.b	9	1	0.1211	0.728309
qHI2H:qHI6H	108	1	1.542	0.2160802
qHI2H:qHI7H	46	1	0.649	0.421646
qHI3H.a:qHI3H.b	51	1	0.6389	0.4253
qHI3H.a:qHI6H	79	1	0.9985	0.319126
qHI3H.b:qHI6H	65	1	0.8571	0.355903
qHI3H.b:qHI7H	4	1	0.0536	0.81716
qHI6H:qHI7H	286	1	3.7338	**0.05504**

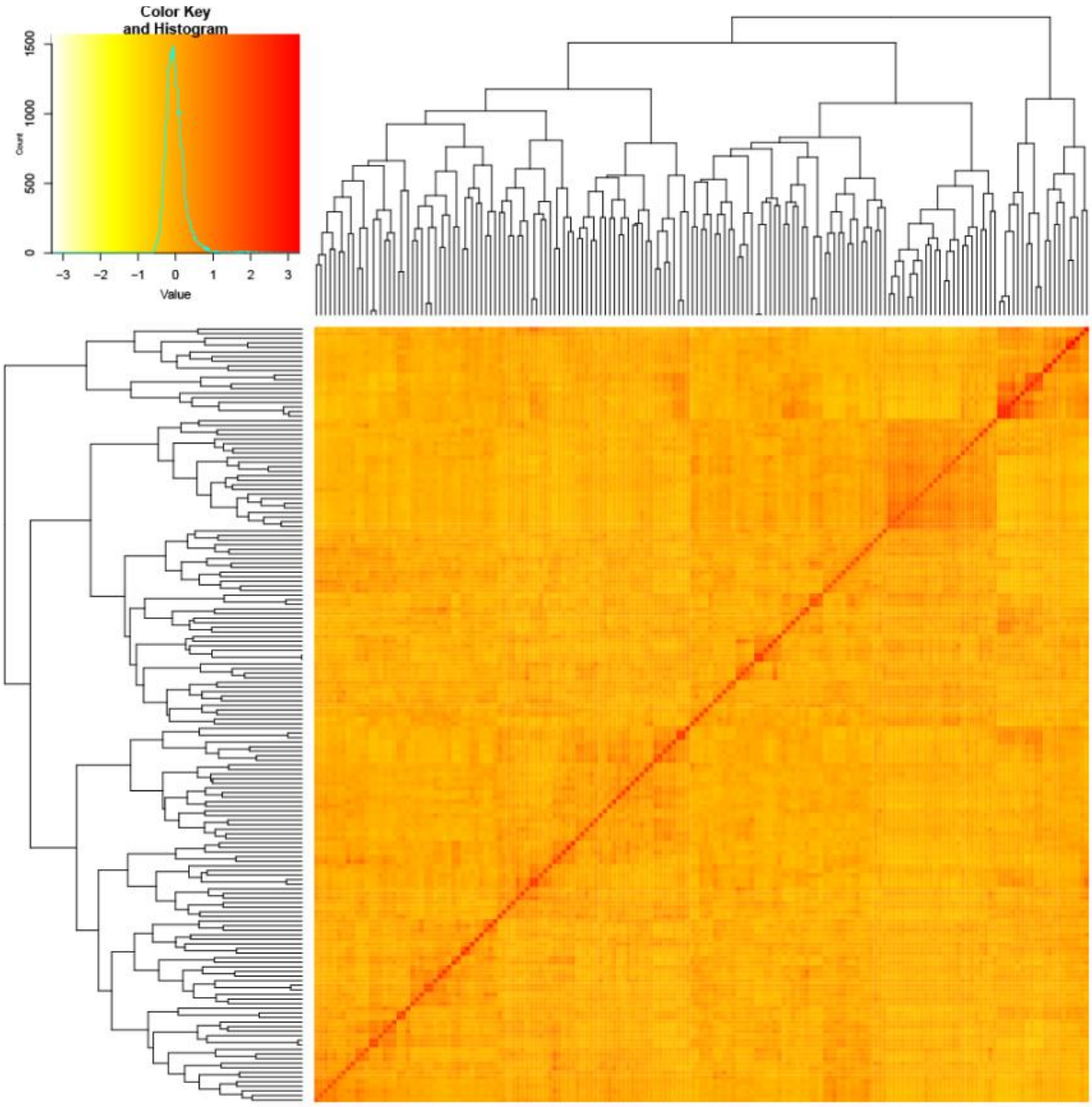
Supplemental figure 2.1: Pirate plots of the two significant interactions between QTLs. The left figure shows the qHYI6H*qHYI7H interaction, and the right figure shows the qHYI1H*qHYI3H.a interaction.



Supplemental figure 2.2: PCA of all lines plotted based on traits measured in this study. Each point is a line, and they are colored based on their origin.



Supplemental figure 2.3: The Kinship matrix generated from GAPIT shows the separation of lines not found in subgroup 3 from Munoz-Amatriain (2014) to be the lines on the right of the first branch.



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CHAPTER THREE

GENETIC IMPROVEMENT OF MALT QULAITY IN BARLEY WITH DORMANCY (Hordeum vulgare)

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Abstract

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

Introduction

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

The quality of malt is determined by the degree of degradation, referred to as modification, achieved during the malting process. As germination begins, hormones and some

enzymes are activated in the embryo and move through the endosperm by the movement of water, signaling β-glucanase production to hydrolyze endosperm cell walls (Bamforth 2006). Water-activated transaminases and peptidases enter degraded endosperm cell walls and break down the protein matrix (Bourne and Wheeler 1984). The now soluble protein can then be digested further into free amino nitrogen (FAN). As the protein structures are degraded, starch granules are exposed to digesting enzymes, but the malting process is stopped mid-modification through kilning to preserve starch to instead be acted on by enzymes during brewing. Measuring the degree of modification includes examining the levels of β glucan (ppm), soluble protein (%) and FAN (ppm), as well as the amount of potential fermentable sugar (% extract) and available enzymes (α-amylase and diastatic power). Malting is a balance, where if under modified then the full potential of sugars and enzymes in the malt is not reached, while if over modified then fermentable sugars are decreased having been used by the developing embryo.

Several studies have interrogated the hydration kinetics of water movement during germination, with change in percent moisture serving as a common proxy (Miano and Augusto 2018). These studies have identified several morphological traits related to endosperm hydration, including seed hardness and seed size. Softer kernels have been shown to have increased imbibition rates compared to harder kernels, impart due to greater porosity of endosperm (Gamlath et al. 2008; Psota et al. 2007). The malting industry has long recognized the impact of seed size on endosperm hydration, with the standard practice since the 1930s of sorting malt samples by seed size (Pollock 1962). Several genetic mapping studies have used percent moisture as a proxy for endosperm hydration, identifying QTLs on 4H, 5H, and 7H (Cu et al. 2016b; Holopainen et al. 2014; Montanuci et al. 2013). However, in a recent GWAS paper

identifying 6 endosperm hydration QTLs, there was no overlap with percent moisture QTLs (Jensen et al. 2023).

The malt process requires living grain that will germinate upon exposure to moisture. However, in temperate regions many grains are dormant upon maturation to protect the new plant from freezing temperatures. The seed lies dormant over the winter until dormancy is broken, and germination can occur in the spring. Lack of dormancy in barley has been selected, eliminating the required delay between harvest and malting. This selection is due to dormancy's potential negatively impact on malt quality even after it is broken (Rooney et al. 2023). Unfortunately, lines without dormancy can prematurely germinate, known as pre-harvest sprouting (PHS), when exposed to moisture post maturation, which negatively impacts malt quality. Due to the importance of dormancy on seeds in general and malt barley quality in particular, the genetic control over dormancy has been thoroughly investigated (Wang et al. 2019a). Although dormancy QTLs have been found on all 7 chromosomes, there are two that control most of the genetic variation in barley (SD1 and 2) (Hori et al. 2007; Ullrich et al. 1992). Both QTLs are located on the long arm of 5H, with SD1 (Qsd1) near the centromeric region and SD2 (Qsd2) more distal. The SD1 recessive dormant allele is most commonly found in wild barley but can also be found in some historic malting lines (e.g. Golden Promise) (Sato et al. 2016). The SD2 recessive dormant allele can be found in cultivars domesticated in East Asia, where dormancy was needed to prevent pre-harvest sprouting (Nakamura et al. 2016). Of the minor dormancy QTLs, Qsdw-4H and Qsdw-5H appear to have the greatest impact on dormancy (Nakamura et al. 2017). However due to selection against dormancy these two QTLs may have been lost from modern malting germplasm (Wang et al. 2019b).

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

Methods

Population

The bi-parental mapping population was made by crossing two Montana State University experimental lines MT124128 (Hocket/*2 MT0101740) and MT124148 (Craft/ MT010174). Hockett has been a successful malt line in Montana because of plump seed and stable yields in rainfed environments. In 2022 Hockett was grown on 118,024 acres in Montana (NASS). However, Hockett has some disadvantages as a malt variety, including higher grain protein and extended malting times. Craft was created with the hope of acceptance by the craft brewing industry, but due to quality issues was not accepted by maltsters. MT010174 (Hockett//*3Lewis/Karl) resulted from backcrossing the low protein allele of HvNam 1 from Karl into Hockett (Alptekin et al. 2022) to reduce grain protein. The two experimental lines were created as part of the breeding program and varied for dormancy, length of grain fill, yield, seed

size, kernel hardness, grain protein, plumps, β-glucan, extract, and FAN. The progeny from this cross were selfed to F6 through single seed descent. Recombinant inbred lines (RILs) were derived from the population totaling 139 lines.

Genotyping

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

Agronomics

This material was grown at the Bozeman Post Farm in 2018, 2019, and 2020 under dryland conditions. It was also grown under irrigated conditions in Bozeman in 2019 and at the Southern Agricultural Research Center (SARC) in Huntley Montana in 2019 and 2020. Plots at Bozeman were 5 m2 with seeding rates of 30g per 5 m2 in dryland and 40g per 5m2 in irrigated. Plots at SARC were 5.2 m2 with seeding rates of 40g per 5.2 m2. The 2019 irrigated trial in Bozeman was hit by a significant rain event that resulted in PHS in some lines. The trial was grown in an augmented block design during all location years, with 7 blocks containing 4 checks and 20 experimental lines each. The checks for this experiment were MT124128, MT124148, Craft, and Hockett.

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

PHS: α-Amylase SD Test

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

Dormancy

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

Hydration Index

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

Malting and Malt Quality

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

Statistical Analyses

Corrections due to field variation were performed using best linear unbiased predictors (BLUPs). The lme4 package (Bates et al. 2014) was used to run the model below for these

corrections. Broad sense heritability was determined with this BLUPs model and the R code described by (Bates et al. 2014).

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

where Y_{ijkl} represents the traits for each line, block, location year, and line type combination. Checki was modeled as a fixed factor representing the replicated check varieties. $Block_j$, Location $Year_k$, and $Entry_l$ were modeled as random factors, following N(0, σ2). Malt tank variation and day-to-day testing were monitored with control lines.

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

The R qtl package (Broman et al. 2003) was used to perform QTL analysis on this population. Single interval mapping (SIM) was performed with the scanone function. Peaks identified from SIM were then assessed as potential covariates for composite interval mapping (CIM). The cim function was used to perform CIM with reading windows of 10 cM, 20 cM, and 40 cM along with the covariates from SIM. QTLs from CIM were then placed into a model for Multiple Interval Mapping (MIM). Model assessment was then performed to see if terms needed to be added or removed as well as searched for interactions between the terms using fitqtl, refineqtl, addint, and addqtl functions. To confirm the results of manual model selection, an automated model selection method was also performed using the stepwiseqtl function in the qtl package. Significance thresholds for all QTL analysis methods were determined using 1,000 permutations. Haley-Knott regression was used for all QTL analysis methods.

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

Results

Population

MT124128 and MT124148 are half-siblings each sharing a paternal parent, MT010174, with the female parent being Hockett or Craft, respectively. As a result of backcrossing, MT010174 is about 88% homologous to Hockett, while MT124128 is about 97% homologous to Hockett. MT124148 consists of about 50% Craft and 50% MT0110174. Although MT124128 and MT124148 are closely related, they have complementary phenotypic variation, suggesting a cross could result in an improved line (Table 3.1). MT124148 was the most dormant of the two parents, with the lowest percent germination at 3 days post-harvest and the longest time to 95 percent germination, and the least PHS. However, it also had the highest hydration index. MT124128 had earlier heading resulting in longer grain fill and shorter stature. MT124128 also has lower grain protein and plumper seed, while MT124148 is higher yielding, with smaller and harder seeds. MT124128 outperforms MT124148 in every malt quality trait except BG and DP. The resulting F6 population varied with line means between the parental means for all traits except PHS, test weight, grain protein, and DP, indicating transgressive segregation (Table 3.1).

When evaluating year-to-year variation within this population each trait was correlated between location years and BLUP corrected average. Therefore, the BLUP corrected averages were used in QTL mapping. As expected, endosperm hydration, as measured by hydration index,

was negatively correlated with the three indicators of seed size (diameter, plumps and test weight). Hydration index was highly correlated with heading date (positively) and grain fill (negatively), likely due to the positive correlation between length of grain fill and seed size (Table 3.2). However, unlike other studies, kernel hardness was positively correlated with hydration index, likely due to a relationship with seed size, since we observed that harder seed was correlated with smaller seed in this population. Of the malt quality traits, β glucan was most highly correlated with hydration index, with β glucan decreasing with increasing hydration index (Table 3.2). We also observed a negative correlation between percent extract and hydration index, but one would expect more starch (extract) to be made available with higher hydration. However, since the malt recipe was consistent across genotypes and not adjusted for faster hydration, those samples with higher hydration index could have been over modified, resulting in reduced extract. There has been a belief in the industry that dormancy reduces malt quality even after dormancy is broken. Therefore, we delayed malting until dormancy was broken and 95 % germination achieved. Even so, we observe a negative correlation between dormancy and most malt quality traits. However, hydration index is not highly correlated with dormancy or PHS, indicating endosperm hydration and thereby malt quality could be improved, while maintaining resistance to PHS, encouraging this mapping study.

QTL Mapping

The MT124128/MT124148 population map included 779 informative SNP markers, creating 6 linkage groups (Table 3.3). The population was fixed for all 4H markers, resulting in no 4H linkage group. Of the remaining 6 chromosomes, 2H was the longest while 6H was the shortest chromosome, and 1H contained the fewest SNPs. The average and maximum spacing

between SNPs suggest regions of homology occurred across the genome, as well as on 4H (Table 3.3).

QTL analysis yielded 71 significant QTLs across all 22 measured traits (Supplemental Table 3.1). Of these, 27 were related to agronomic characteristics, 4 to dormancy, and 40 to malt quality traits. Many of these QTL were reported previously in similar locations as summarized in Supplemental Table 3.1. Four QTLs (Table 3.4) were associated with hydration index, three being new and one (qHYI3H) in a similar position to a previously mapped QTL on 3H (Jensen et al. 2023). Three of the HYI QTLs co-segregated with other traits (Table 3.4). The HYI QTL on 1H not only had the largest effect on hydration index but also on plant phenology and seed traits, explaining the most variation in heading date, maturity and grain fill as well as seed diameter and hardness. The allele from MT124148 contributed shorter grain fill due to later heading and earlier maturity, resulting in smaller, harder seed with faster hydration. The MT124148 allele at 1H was also negatively associated with malt quality traits, including lower extract and S/T but higher DP. The 2H MT124148 allele increased hydration index and was associated with earlier heading and was positively associated with modification, thereby increasing AA, FAN, soluble protein and DP while lowering BG. The MT124148 allele at 5H decreased hydration index, and malt quality as measured by α-amylase, extract, FAN, soluble protein and S/T, while increasing dormancy (DT95 and %germ), PHS. In this study, the positive allele for hydration index 3H originated from MT124128 and was not associated with any other trait. Because 1H and 5H QTLs are likely impacting HYI pleiotropically, they were designated qSS1H and qD5H for seed size and dormancy respectively. The HYI QTL on 2H and 3H appear to be having a more direct effect on HYI and so are named qHYI2H and qHYI3H.

Allele Combinations

The impact of seed size and dormancy on malt quality was further highlighted by PCA (Figure 3.1). We first examined all the genotypes used in the study, comparing those carrying both the large and small seed alleles for qSS1H (Figure 3.1A). PC1 explained seed size and most of the malt quality traits, while PC2 coincided with dormancy. To better reveal the variation explained by dormancy, PCA was next performed on the population after removing the large seeded qSS1H genotypes, since no large seeded dormant lines were in the population (Figure 3.1B). PC1 corresponded to dormancy and malt quality traits often related to modification, extract, BG, FAN AA, soluble protein and S/T. PC2 explained variation in grain protein and several agronomic and developmental traits clustering based on HvNAM1 allele.

Hydration index is in opposition to both seed size and dormancy (Figure 3.1) and we hypothesize that the two HYI QTLs could help improve the negative effects of seed size and dormancy on malt. Figure 3.2 reports the interaction of dormancy and HYI QTLs excluding the large seeded qSS1H genotypes. Hydration index increased with each positive allele in both non-dormant (AA) and dormant (BB) genotypes, resulting in equivalent hydration index between non-dormant and dormant genotypes. When carrying both positive HYI alleles an improvement of malt quality traits particularly BG, FAN, and AA are observed however we do not see improvements in extract. Importantly, increased positive HYI alleles do not increase PHS (Figure 3.2). Unfortunately, only one allelic combination with the large-seeded allele was recovered preventing us from interrogating the differential impact of the HYI QTL alleles on seed size and so requires further study.

Discussion

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

This study confirms the industry knowledge that higher hydration index correlates with overall better malt quality. Here we observe that the level of endosperm hydration at steep out is partially under genetic control, meaning that genetic improvement of hydration index can improve malt quality. Endosperm hydration, as determined by HYI, correlated negatively with seed size and dormancy, but positively with several malt quality traits as has been reported in other studies (Jensen et al. 2023). A difference here is that HYI has been reported to be negatively correlated with hardness (Gamlath et al. 2005; Psota et al. 2007) where here it was positively correlated, likely due to the correlation of hard seed with small seed.

Many of the QTLs reported here were reported previously (Supplemental Table 3.1), supporting the validity of our results. Three of the four QTLs associated with HYI (qSS1H, qHYI2H and qD5H) also map with malt quality traits here and in other studies (Fang et al. 2019; Panozzo et al. 2007; Pauli et al. 2014; Beattie et al. 2010; Mohammadi et al. 2015; Nice et al. 2019; Sweeney et al. 2022; Rooney et al. 2023). Wang et al. (2019a) identified a seed size QTL at a similar position on 1H and several studies have mapped malt quality traits to this region

(Supplemental Table 3.1). The dormancy QTL (qD5H) was previously mapped, and the gene identified (MKK3) (Ullrich et al. 1992; Nakamura et al. 2016), as well as negatively associated with malt quality (Sweeney et al. 2022; Rooney et al. 2023) as summarized in Supplemental Table 3.1. Albeit, qHYI2H has not been previously mapped, the region on 2H is a hotspot for malt quality reported by a number of studies (Pauli et al. 2015; Fang et al. 2019; Beattie et al. 2010), suggesting the cause of this improved quality others have reported is increased endosperm hydration. qHYI3H was not tightly linked with malt quality traits in this study, however, a hydration index QTL was mapped to a similar location by Jensen et al. (2023). Also, the qHYI3H did impact malt quality traits in the allelic combination comparisons.

The lack of genetic diversity in this population limits the findings to only the HYI QTLs for which these two parents vary. Another study of a GWAS population with higher genetic diversity identified 6 HYI QTLs, with only one (qHYI3H) overlapping between this study and the association panel (Jensen et al. 2023). Here as in Jensen et al. (2023) there is little overlap between steep out moisture (SOM) and HYI, with SOM only co-segregating with HYI at 1H in the current study. The heritability of SOM was lower than HYI in this study like in Jensen et al. (2023). The lack of correlation between SOM and HYI could explain the lack of overlap between this study and those previous studies mapping SOM (Cu et al. 2016a; Holopainen et al. 2014; Montanuci et al. 2013). However, the lack of overlap could also be due to lack of genetic diversity, especially considering one QTL for SOM previously mapped to 4H, which lacked polymorphisms in this study.

Importantly, this population varied for seed dormancy and qD5H mapped near the well described dormancy gene MKK3 (Ullrich et al. 1992; Nakamura et al. 2016). Our findings match

what others have reported where the dormant allele has a negative effect on quality (Sweeney et al. 2022; Rooney et al. 2023). We also saw poor correlation between HYI and rate of germination or germination index which Sweeney et al. (2022) suggested could impact malt quality. Here is the first indication that a dormancy allele can reduce hydration of the endosperm even after dormancy is broken, suggesting a reason dormant lines have poor malt quality. This, in turn, can explain why we see improvements in quality for the dormant lines through the addition of positive HYI alleles (Supplemental Table 3.2). For some traits, the addition of two higher HYI alleles made dormant and non-dormant malt quality equal. The cross involving MT124128 x MT124148 was first initiated due to a request from all malt brewers for lower FAN malt. FAN unused by yeast as a nitrogen source and remaining in finished beer, can cause off-flavors. The largest QTL for FAN in this population co-segregated with the dormancy QTL, explaining about 54% of the variation with dormant lines having lower FAN. Adding both positive HYI QTLs increases FAN but on average dormant lines even with both HYI QTLs have lower FAN (Figure 3.2). Further study is needed, to explore the impact on quality and the interactions of the HYI positive alleles identified in this and previous work.

Dormancy at harvest provides resistance to PHS but has been selected against due to the negative correlation with malt quality. Fortuitously, in 2019, rain during harvest exposed this population to PHS. Since this population segregated for dormancy, this provided the opportunity to evaluate the effectiveness of dormancy and the impact of high hydration index on resistance to PHS. Traditional methods of measuring PHS, falling number, are highly labor intensive. Therefore, we tested the utility of a proxy for PHS with the amount of α-amylase in sprouted grain, where a score of approximately 0.129 was similar to a falling number of 300, the industry

cut off for sound grain, and a score between 0.129 and 0.824 (Falling number of 300 to 125) meets the industry standard for moderate pre-germination with some risk of loss if not immediately malted and/or stored properly under cold dry conditions (Mangan et al. 2016). This test, in our study, detected one QTL for PHS that mapped to the same SNP as qD5H, confirming the known relationship between dormancy and reduced PHS damage. The qHYI2H and qHYI3H alleles that increased hydration index improved the malt quality of dormant lines but did not impact resistance to PHS. This finding makes it possible to deploy PHS resistance in high quality malt lines.

Hydration of the endosperm is impacted by seed size, with larger seed having reduced hydration due to greater area to be hydrated (Miano and Augusto 2018). The QTL with the largest impact on seed size in this population was qSS1H. The other two QTLs identified in this population impacting seed size have about half the estimated effect and were not associated with changes in hydration index. Wang et al. (2019a) also identify this region as impacting seed size with the candidate genes HvCO9 regulating flowering time in barley and HvSMOS1 producing small grains in rice. Although the big seed allele for qSS1H correlates with low hydration index, it is associated with high malt quality, most importantly high extract. Therefore, it may be important not to select against seed size to improve hydration index because of the decrease in extract. Instead, a breeder could select for larger seed combined with other QTLs that increase hydration index. In fact, in this population all the lines with the large-seeded allele also carried the two fast hydration alleles and lacked dormancy. Selections were not intentionally made in the creation of this population. However, the haplotypes that would slow germination further (i.e., dormancy and lower hydration index alleles) were likely selected against when associated with

the largest seed by the inbreeding process. To complete three generations of inbreeding in a year in the greenhouse, we dry seed and then place it in a cold chamber for several days to break dormancy, but time is limited between harvest and planting. F2 and F3 seed are placed in flats where the slower to germinate lines could be shaded out by faster lines, potentially eliminating slower lines from the population. Since this study lacked haplotypes of big-seeded lines varying in dormancy and HYI QTLs, we cannot establish the interactions as we have with dormancy. In future work, we hope to create a big seeded, dormant line with additional HYI QTLs identified in Jensen et al. (2023), to determine if we could have a dormant, large-seeded, fast hydrating malt barley. We are currently developing markers to empower this selection.

HvNAM1 increases grain fill by impacting senescence resulting in increased plumpness and reduced protein while also improving malt quality stability (Alptekin et al. 2022). Our population varied for HvNAM1 with the desirable Karl allele coming from MT124128. Evaluation of this gene shows that even with the complex traits of grain fill explained by PC2 (Figure 3.1B), HvNAM1 plays a major role for these traits in this population and matches what Alptekin et al. (2022) observed. Also, dormancy and quality traits (PC1) see little interaction with HvNAM1 in this figure. This is further supported when looking at haplotype combinations in Supplemental Table 3.1 where we see that haplotypes with the positive HvNAM1 allele do not overcome the negative effects of dormancy unless paired with positive HYI alleles. These interactions likely underline why dormancy has been selected against, since not all regions that improve quality balance the negative effects of dormancy, further emphasizing the importance of HYI QTLs for further breeding efforts if dormancy is desired.

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

Tables

Table 3.1 Summary statistics for all traits measured and mapped. The population means as well as the means for the two parents are reported in the first two rows. The standard deviation (SD), CV, Minimum value, Maximum value, and broad sense heritability (H2) are reported for the mapping population. *SOM: steep out moisture, %Germ@3: percent germination 3 days post-harvest; DT95: days to 95% germination; PHS: pre-harvest sprout; S/T: soluble/total protein or Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: Diastatic power

Trait	MT124128	MT124148	Population Mean	SD	CV	Min	Max	H2
Hydration Index (points)	47.9	57.9	54.3	9.9	0.18	30	85	0.507
SOM (%)	40.9	42.6	41.9	2.3	0.06	36	50	0.12
%Germ@3	95	68	90	14	0.16	27	100	0.259
DT95	5	20	9	9.7	0.86	3	47	0.48
PHS (U/g)	0.45	0.30	0.47	0.56	1.2	0.04	4.08	NA
Heading (Julian days)	173	181	178	8.58	0.04	157	192	0.18
Maturity (Julian days)	217	216	217	10.97	0.05	192	236	0.01
Length of Grain Fill (days)	44	35	38	6.22	0.16	20	57	0.521
Height (cm)	82.9	87.6	85.2	7.59	0.09	65.5	102	0.118
Yield (bushels/acre)	126.6	141.8	132.7	23.22	0.17	61.6	193.3	0.054
Kernal Diameter (mm)	3.02	2.83	2.92	0.1	0.037	2.62	3.25	0.518
Kernal Hardness (HI)	37.1	45.4	41.1	5.55	0.13	31.3	58.9	0.761
Test Weight (lbs/bushel)	53.7	53.5	53.9	2.17	0.04	47.1	58.1	0.012
Plump (%)	97.2	90.1	94.8	5.5	0.05	59.3	99.8	0.184
Grain Protein (%)	10.5	11.3	11.3	0.97	0.08	9.4	14.3	0.483

Table 3.2: Continued

Trait	MT124128	MT124148	Population Mean	SD	CV	Min	Max	H2
β-Glucan (ppm)	444	212	363	193	0.533	35	987	0.381
Soluble Protein (%)	5.33	4.37	5.03	0.44	0.08	3.49	5.96	0.667
S/T (%)	50.7	39.3	45.2	5.9	0.13	28.1	59.3	0.596
FAN (ppm)	230	188	221	25	0.11	157	301	0.586
Extract (%)	81.7	78	79.5	1.9	0.02	73.7	83.9	0.744
AA (DU)	66	56	57	13	0.23	23	100	0.396
DP (ASBC)	119	122	134	22	0.17	87	210	0.545

Table 3.2 Correlation matrix of the BLUP corrected averages for each trait measured. *SOM: steep out moisture, %Germ@3: percent germination 3 days post-harvest; DT95: days to 95% germination; PHS: pre-harvest sprout; S/T: soluble/total protein or Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: Diastatic power

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

Table 3.3 Summary statistics for each chromosome. The parent lines were monomorphic for 4H markers so no map of 4H.

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

Table 3.4: Hydration index QTLs and all other QTLs that mapped to the same region. Summary statistics for LOD score, percent variance explained (%Var), P-values based on F statistics, estimated effects of MT124148, and the standard error of the effect (SE) all being reported. *SOM: steep out moisture, %Germ@3: percent germination 3 days post-harvest; DT95: days to 95% germination; PHS: pre-harvest sprout; S/T: soluble/total protein or Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: Diastatic power

QTL Name	Trait	Chromosome	Position (cM)	LOD	%Var	P-value (F)	Estimated Effect	SE
qSS1H	Hydration Index	1H	67	21.04	33.84	< 2e-16	15.59	1.33
	SOM	1H	67	5.00	15.58	1.99071E-06	1.35	0.27
	Kernel Hardness	1H	67	29.02	65.91	<2e-16	2.74	1.49
	Kernel Diameter	1H	67	11.66	19.77	6.38E-13	-0.081	0.01
	Height	1H	67	5.49	10.23	0.000000843	5.83	0.58
	Heading	1H	67	63.60	35.55	< 2e-16	7.34	0.23
	Maturity	1H	67	3.40	4.63	0.000105	-1.82	0.20
	Length of Grain Fill	1H	67	74.07	60.88	< 2e-16	-8.82	0.23
	Grain Protein	1H	67	33.32	43.50	< 2e-16	1.29	0.077
	S/T	1H	67	22.92	17.71	< 2e-16	-0.93	0.075
	Extract	1H	67	22.89	15.64	< 2e-16	-2.02	0.16
	DP	1H	67	7.22	7.77	2.67E-08	19.83	3.34
qHYI2H	AA	2H	155	5.53	7.06	0.00000077	5.74	1.11
	FAN	2H	155	10.08	6.87	4.97E-11	11.79	1.64
	Heading	2H	157	7.55	1.36	8.65E-09	-1.14	0.185
	S. Protein	2H	159	8.05	8.78	2.46E-09	0.246	0.038
	Hydration Index	2H	160	5.61	6.81	0.000000646	4.60	0.879
	BG	2H	160	17.59	22.98	< 2e-16	-104.82	13.74

Table 3.4: Continued

QTL Name	Trait	Chromosome	Position (cM)	LOD	%Var	P-value (F)	Estimated Effect	SE
qHYI2H	DP	2H	160	11.14	12.85	3.96E-11	9.25	2.099
qHYI3H	Hydration Index	3H	53	7.56	9.50	7.54E-09	-7.32	1.18
qD5H	Hydration Index	5H	95	5.73	6.98	0.000000482	-5.84	1.10
	PHS	5H	95	4.40	13.12	8.79E-06	-0.443	0.08
	%Germ@3	5H	95	33.87	68.21	< 2e-16	-14.22	0.85
	DT95	5H	95	29.55	63.20	< 2e-16	15.32	1.01
	Extract	5H	95	17.50	10.80	< 2e-16	-1.49	0.145
	S. Protein	5H	95	27.63	43.38	< 2e-16	-0.59	0.04
	S/T	5H	95	27.71	23.48	< 2e-16	-0.97	0.068
	FAN	5H	95	42.34	53.95	< 2e-16	-36.59	1.83
	AA	5H	95	12.48	18.03	1.11E-13	-9.81	1.18

Figures

Figure 3.1 A: PC analysis of all genotypes and all measured traits. Red points indicate lines with the MT124128 (big seed) allele for qSS1H, while blue points indicate the MT124148 (small seed). Note PC1 is primarily explained by variation in qSS1H. PC2 is primarily explained by dormancy. 1B: PC analysis after removing lines with 128 allele at qSS1H. Triangles indicate lines with dormant allele at qD5H, while circles indicate non-dormant lines. Blue shapes indicate the 128 allele (low protein) at *HvNAM1*, while red indicate 148 (high protein) allele. Note PC1 is primarily explained by dormancy and accounts for much of the malt quality variation, while PC2 is partially explained by variation in HvNAM1 and accounting for length of grain fill and grain protein. Perpendicular relationship between PC 1 and 2 indicate lack of interaction between *HvNAM1* and dormancy, such that *HvNAM1* cannot improve poor malt quality due to dormancy. *SOM: steep out moisture, %Germ@3: percent germination 3 days post-harvest; DT95: days to 95% germination; PHS: pre-harvest sprout; S/T: soluble/total protein or Kolbach index; FAN: free amino nitrogen; AA: α-amylase; DP: Diastatic power

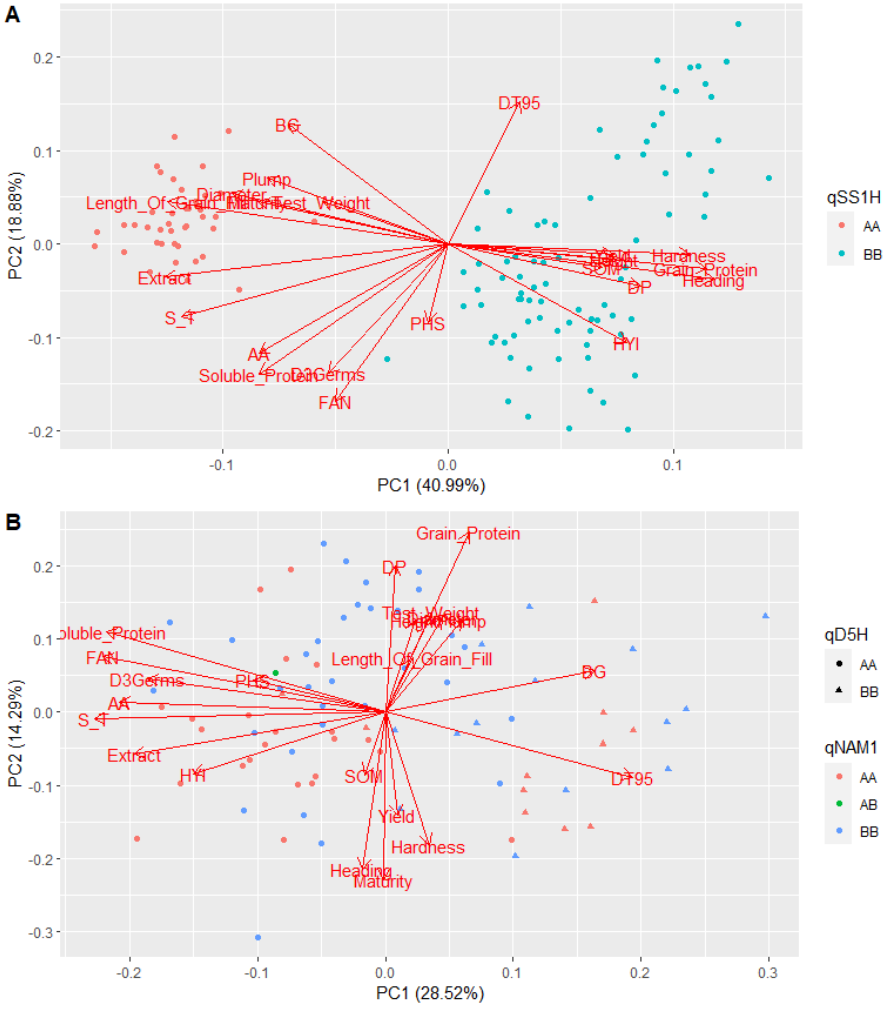
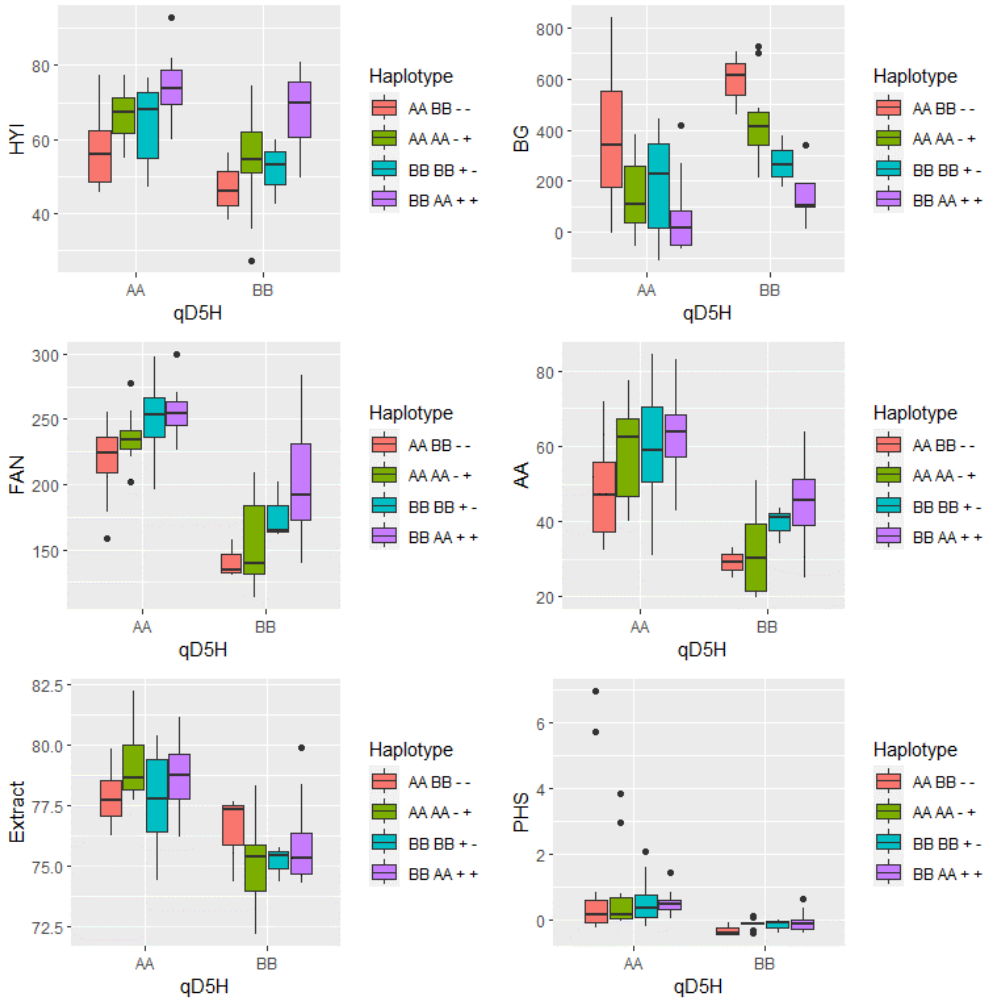


Figure 3.2: Evaluation of haplotypes for the subset of the population with the small seed allele (BB) for qSS1H. BLUP corrected averages of traits are reported on the Y axis while the X-axis is reporting dormant (BB) or non-dormant (AA) alleles with different combinations of the HYI alleles. The qHYI2H allele is indicated first in the legend and qHYI3H second. In the case of qHYI2H AA reduces (-) HYI while BB increases (+) HYI but for qHYI3H AA increase (+) HYI while BB reduces (-)HYI and the whisker plots are distributed with increasing number of HYI alleles. *HYI: hydration index; BG: β-glucan; FAN: free amino nitrogen; AA: α-amylase; PHS: Preharvest Sprout damage from α-amylase SD test



Supplementals

Supplemental Table 3.1: All identified QTLs are reported with their chromosome, position, and summary statistics. References are also reported where other QTLs have been reported for the same trait.

Trait	Chromosome	Position (cM)	LOD	%Var	Estimated Effect	Reference
Hydration Index	1H	67	21.041	33.841	15.5898	
Kernel Hardness	1H	67	29.024	65.913	2.7419	Walker 2013 and Cu 2016
Kernel Diameter	1H	67	11.661	19.77	-0.081244	Wang 2019 and Wang 2021
SOM	1H	67	5.000012	15.57513	1.3536	
DP	1H	67	7.221	7.772	19.827	Fang 2019
Length of Grain Fill	1H	67	74.065	60.877	-8.815	
Extract	1H	67	22.887	15.6352	-2.0188	Panozzo 2007
Grain Protein	1H	67	33.323	43.497	1.28793	Pauli 2014
Heading	1H	67	63.5951	35.5457	7.3363	Schmalenbach 2009
Height	1H	67	5.489	10.217	5.8264	Pauli 2014
Maturity	1H	67	3.403	4.633	-1.8181	
S/T	1H	67	22.924	17.706	-0.93339	Fang 2019
BG	2H	13	8.715	9.688	-78.43	
Extract	2H	16	3.017	1.4368	0.4214	Fang 2019
Height	2H	38	6.573	12.47	-1.7673	Schmalenbach 2009
Plumps	2H	38	10.22	16.387	-2.3571	
Kernel Diameter	2H	38	6.89	10.621	-0.043015	
FAN	2H	48	14.133	10.365	-16.878	
Test Weight	2H	48	5.107	12.78	-0.2236	Mohammadi 2015
Plumps	2H	62	10.093	16.146	-2.246	Pauli 2015

FAN	2H	66	10.39	7.121	14.652	Pauli 2015
AA	2H	66	5.615	7.178	5.758	Beattie 2010
BG	2H	66	7.828	8.566	-113.78	Pauli 2015 and Fang 2019
BG	2H	77	2.924	2.938	68.84	Pauli 2015
Heading	2H	77	7.0086	1.2504	-1.0785	Pauli 2015 and Nice 2019
Maturity	2H	83	5.512	7.783	-0.8249	
AA	2H	101	16.778	26.222	-12.802	Beattie 2010
DT95	2H	104	4.912	6.648	-5.083	Gong 2014
DP	2H	109	2.802	2.793	10.33	Beattie 2010
FAN	2H	110	3.334	2.018	8.62	Beattie 2010
AA	2H	155	5.529	7.058	5.741	Beattie 2010
FAN	2H	155	10.081	6.871	11.793	
Heading	2H	157	7.5532	1.3605	-1.1426	
S. Protein	2H	159	8.049	8.775	0.24561	
BG	2H	160	17.592	22.982	-104.82	
DP	2H	160	11.139	12.854	9.246	Pauli 2015 and Fang 2019
Hydration Index	2H	160	5.605	6.807	4.6028	
S. Protein	3H	9	3.257	3.265	-0.14182	
DP	3H	11	3.477	3.506	-8.484	
Kernel Diameter	3H	11	5.663	8.522	0.038656	Wang 2019
Extract	3H	16	1.969	0.9207	-0.4103	Nice 2019
FAN	3H	31	8.465	5.606	-10.673	
Hydration Index	3H	53	7.556	9.496	-7.3204	Jensen 2022
Height	5H	0	5.602	10.449	-1.8787	Pauli 2014
Maturity	5H	0	2.971	4.015	0.6145	
FAN	5H	35	4.541	2.807	-8.815	

Kernel Hardness	5H	35	6.224	8.767	0.4768	Walker 2013
Hydration Index	5H	95	5.733	6.978	-5.8429	
DT95	5H	95	29.554	63.199	15.315	Ullrich 1992, Hori 2007, Nakamura 2017, Sweeney 2022, and Rooney 2023
AA	5H	95	12.482	18.031	-9.809	Beattie 2010, Mohammadi 2015, Nice 2019, Sweeney 2022, and Rooney 2023
PHS	5H	95	4.403	13.116	-0.4426	Rooney 2023
%Germ@3	5H	95	33.87	68.206	-14.2236	Ullrich 1992, Hori 2007, Nakamura 2017, Sweeney 2022, and Rooney 2023
Extract	5H	95	17.495	10.7965	-1.4884	Beattie 2010, Mohammadi 2015, and Fang 2019

FAN	5H	95	42.343	53.95	-36.586	Pauli 2015, Fang 2019, Nice 2019, Sweeney 2022, and Rooney 2023
S. Protein	5H	95	27.633	43.382	-0.59429	Beattie 2010, Mohammadi 2015, Fang 2019, and Nice 2019
S/T	5H	95	27.711	23.478	-0.96592	Pauli 2015, Mohammadi 2015, Nice 2019, Sweeney 2022, and Rooney 2023
DP	6H	32	5.533	5.781	-14.376	
DP	6H	47	4.827	4.981	10.926	Mohammadi 2015
Extract	6H	48	4.067	1.9723	-0.5899	Pauli 2015
S/T	6H	48	3.116	1.679	-0.24171	Pauli 2015 and Fang 2019
Grain Protein	6H	52	4.732	3.616	0.34657	Pauli 2015, Mohammadi 2015, and Nice 2019
Test Weight	6H	64	6.412	16.42	-0.26076	Cu 2016
S. Protein	7H	34	6.446	6.832	-0.2817	
%Germ@3	7H	70	3.957	4.552	3.7043	Gong 2014

Plumps	7H	92	4.438	6.427	-1.4784	Pauli 2014
BG	7H	95	11.005	12.745	-83.06	Mohammadi 2015
Length of Grain Fill	7H	97	7.924	1.661	-1.5697	
Heading	7H	97	30.7002	8.5334	3.0456	Schmalenbach 2009
DP	7H	98	12.793	15.21	-11.842	Fang 2019
Grain Protein	7H	99	2.795	2.065	-0.2766	Pauli 2015 and Nice 2019
Maturity	7H	99	5.257	7.391	0.8449	

Supplemental Table 3.2: Evaluation of haplotypes for qD5H, qSS1H, qHYI2H, qHYI3H, and Nam1 where the number of each combination of alleles are reported along with their BLUP corrected mean values for dormancy, seed size, and malt quality traits. P-values and Tukey's test results are reported below each trait and as super scrips.

Alleles	Number	HI	D3Germs	DT95	PHS	Diameter	Hardness	Gprotein	BG	S_Protein	S_T	FAN	Extract	AA	DP
AA:AA:AA:AA:AA	46	41.99^{c}	96.60^{abc}	7.631^{b}	0.4217^{b}	3.0045^{a}	36.53^{b}	9.65^{b}	551.11^{a}	5.494^{ab}	0.5054^{a}	234.79^{abc}	83.49^{a}	70.46^{a}	105.14^{b}
AA:BB:AA:BB:BB	10	57.86^{bc}	99.21^{abc}	1.648^{b}	0.2762^{b}	2.9017^{ab}	42.27^{ab}	12.21^{a}	470.66^{a}	4.799^{bcd}	0.4232^{cd}	217.13^{bc}	77.84^{b}	44.31^{abc}	134.37^{ab}
AA:BB:AA:BB:AA	5	56.78^{bc}	94.94^{abc}	3.735^{b}	2.5659^{a}	2.8142^{ab}	46.02^{a}	12.09^{a}	195.59^{ab}	4.881^{abcd}	0.4330^{bc}	222.26^{abc}	78.01^{b}	55.86^{ab}	147.63^{ab}
AA:BB:AA:AA:BB	2	64.23^{abc}	101.03^{ab}	-0.585^{b}	0.2618^{b}	2.9407^{ab}	42.84^{ab}	12.61^{a}	77.08^{ab}	5.125^{abcd}	0.4305^{bcd}	227.06^{abc}	78.59^{b}	67.71^{ab}	148.97^{ab}
AA:BB:AA:AA:AA	10	64.72^{abc}	100.64^{ab}	0.379^{b}	0.8686^{ab}	2.8334^{ab}	43.53^{ab}	11.89^{a}	196.82^{ab}	5.166^{abcd}	0.4484^{bc}	233.27^{abc}	78.93^{b}	53.99^{ab}	134.56^{ab}
AA:BB:BB:BB:BB	16	61.98^{abc}	98.45^{abc}	0.969^{b}	0.7186^{b}	2.8708^{ab}	43.54^{ab}	12.72^{a}	234.35^{ab}	5.299^{abc}	0.4429^{bc}	254.05^{a}	77.42^{b}	57.22^{ab}	174.95^{a}
AA:BB:BB:BB:AA	5	69.49^{abc}	99.93^{abc}	-1.042^{b}	0.9378^{ab}	2.7785^{ab}	44.12^{ab}	11.91^{a}	94.65^{ab}	5.579^{ab}	0.4707^{ab}	253.37^{ab}	79.17^{b}	64.19^{ab}	152.49^{ab}
AA:BB:BB:BB:AB	1	71.14^{abc}	100.50^{abc}	-0.565^{b}	0.5013^{b}	3.0492^{a}	44.77^{ab}	11.62^{ab}	220.46^{ab}	5.225^{abcd}	0.4619^{abc}	235.45^{abc}	79.73^{ab}	71.66^{a}	103.57^{b}
AA:BB:BB:AA:BB	7	75.40^{a}	102.06^{a}	-0.763^{b}	0.4422^{b}	2.8719^{ab}	46.04^{a}	12.38^{a}	94.96^{ab}	5.341^{abc}	0.4493^{bc}	253.84^{ab}	78.06^{b}	63.79^{ab}	154.21^{ab}

AA:BB:BB:AA:AA	6	72.55^{ab}	100.94^{ab}	-0.813^{b}	0.5406^{b}	2.8879^{ab}	44.35^{ab}	11.89^{a}	16.35^{b}	5.719^{a}	0.4784^{ab}	261.63^{a}	79.34^{b}	60.74^{ab}	155.59^{ab}
BB:BB:AA:BB:BB	1	46.12^{c}	86.87^{abcd}	40.009^{a}	-0.4511^{b}	2.9097^{ab}	51.06^{a}	13.23^{a}	707.45^{a}	3.648^{d}	0.3561^{d}	134.64^{c}	74.37^{b}	33.14^{abc}	167.77^{ab}
BB:BB:AA:BB:AA	4	40.68^{c}	54.22^{e}	39.645^{a}	-0.3492^{b}	2.8169^{ab}	44.15^{ab}	12.13^{a}	547.34^{a}	3.806^{d}	0.3811^{cd}	154.93^{c}	76.47^{b}	27.01^{ac}	128.37^{ab}
BB:BB:AA:AA:BB	7	54.26^{c}	75.35^{cd}	27.795^{ab}	-0.1493^{b}	2.8930^{ab}	43.03^{ab}	12.85^{a}	462.21^{a}	3.851^{d}	0.3733^{d}	157.42^{c}	74.56^{b}	31.23^{ac}	152.54^{ab}
BB:BB:AA:AA:AA	4	55.75^{bc}	80.50^{bcd}	29.888^{a}	-0.0851^{b}	2.7709^{ab}	47.31^{a}	11.63^{a}	444.03^{a}	3.758^{d}	0.3876^{cd}	151.26^{c}	75.49^{b}	30.37^{ac}	131.49^{ab}
BB:BB:BB:BB:BB	2	51.21^{c}	81.99^{abcd}	23.691^{ab}	-0.2423^{b}	2.7959^{ab}	49.87^{a}	12.18^{a}	219.05^{ab}	4.221^{d}	0.4029^{cd}	183.42^{c}	75.59^{b}	42.19^{abc}	148.77^{ab}
BB:BB:BB:BB:AA	1	53.09^{c}	72.95^{cde}	29.627^{ab}	0.0049^{b}	2.9281^{ab}	40.84^{ab}	13.82^{a}	376.22^{ab}	4.094^{d}	0.3706^{d}	161.95^{c}	74.32^{b}	34.20^{abc}	225.77^{a}
BB:BB:BB:AA:BB	4	64.44^{abc}	65.90^{de}	26.645^{ab}	-0.2705^{b}	2.8700^{ab}	49.97^{a}	13.10^{a}	103.35^{ab}	4.539^{cd}	0.4045^{cd}	209.07^{bc}	75.40^{b}	45.32^{abc}	176.08^{a}
BB:BB:BB:AA:AA	5	70.42^{abc}	69.34^{de}	26.233^{ab}	0.1029^{b}	2.8578^{ab}	48.53^{a}	11.91^{a}	153.90^{ab}	4.372^{d}	0.4127^{cd}	196.95^{c}	76.65^{b}	44.91^{abc}	148.38^{ab}
p-value		< 2.2e-16 ***	< 2.2e-16 ***	<2e-16 ***	0.0004538 ***	3.769e-11 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	< 2.2e-16 ***	1.019e-13 ***

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CHAPTER FOUR

IDENTIFYING QTL RELATED TO AMINO ACID AND FLAVOR METABOLITES

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

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Abstract

Barley is a key ingredient in beer production; however, there is still a considerable knowledge gap in the genetic regulation of malt flavor. We performed ultra-high performance liquid chromatography mass spectrometry (UHPLC-MS) on a GWAS population of 169 lines with a history of malting. Our goal was to identify QTL responsible for metabolic variation and flavor metabolites. We identified 827 metabolite QTL across two years. The majority of these QTL were for lipids (596); however, we were able to detect QTL for amino acids (33), saccharides (3), and maltol (3), all of which affect malt and beer flavor. The amino acid QTL were found across all seven chromosomes with four mapping near hordein genes. We also identified eight candidate genes near the amino acid QTL regulating seed development and maturation, protease inhibitors, peptidases, and amino acid biosynthesis. The three maltol QTL all mapped with maltol precursors suggesting increased modification increases maltol. All these QTL provide good targets for future metabolite research while the maltol QTL could also be used in marker assisted selection when breeding for flavor variation.

Introduction

Barley (Hordeum vulgare L.) is a major cereal crop cultivated around the world. Its primary uses include human and livestock feed and forage, but in the US malt barley has the highest value and is part of the value-added chain of the food and beverage industry. Barley is used for malt because of its grain composition and enzyme production. Sixty percent of barley grain weight is starch, which is stored in the endosperm surrounded by a protein matrix and inside cell walls, which are composed primarily of β-glucans (Fox 2009). During malting,

enzymes are produced that degrade cell walls and the protein matrix to expose starch granules for extraction during the initial stage of brewing, called mashing. Mashing makes sugars, proteins, and other metabolites available to yeast during fermentation.

Malting involves the controlled germination and killing of the grain across three steps: steeping, germination, and kilning. Steeping begins by submerging grain in water followed by short air rests to hydrate the endosperm. This allows for enzymes (e.g. β-glucanases, proteases, lipases, and amylases) to move from the embryo and aleurone layers to degrade cell walls and storage compounds (Celus et al. 2006; Osman et al. 2002). Once hydrated, the grain is left to germinate in a humid environment. In nature, the degradation of storage compounds during germination provides the germinating embryo with nutrition to grow (Hayes et al. 2003). However, the degradation process is stopped during malting before starch degradation begins through the kilning process which kills the embryo using heat. Kilning also creates new classes of metabolites that impact flavor. Some examples are esters created from lipids (Holt et al. 2019) and Maillard reaction and Strecker degradation products, created from amino acids interacting with saccharides (Schwarz and Li 2011). Maillard reactions occur optimally between 140 and 165 °C, causing reactions between saccharides and amino acids to produce melanoidins, which are responsible for desirable flavors in browned foods (Perez-Locas and Yaylayan 2010). Therefore, malting forms many metabolites, which either produce flavor or flavor precursors important to foods and brewing.

Malt quality measures the degree of cell wall and protein matrix degradation while also describing the availability of starch. This is done by simulating the mashing process, where malt is ground into flour, suspended in water and heated in a process called a congress mash. During

mashing enzymes created during malting break down starch and further degrade proteins (Evans et al. 2011). Heating while mashing not only aids in extraction of starch but also allows for more Maillard reaction products to be made (Schwarz and Li 2011). At the end of mashing the wort is filtered, resulting in a suspension of sugars, solubilized protein, free amino nitrogen, β-glucan, and flavor metabolites that can be used for brewing, but is also evaluated for malt quality. Methods of malt quality analysis are well established in the US by ASBC (American Society of Brewing Chemists) and industry standards for acceptable malt quality are available at AMBA (American Malting Barley Association) (https://ambainc.org/). Key malt quality traits include β-glucan, extract, protein (soluble and total), free amino nitrogen (FAN), α-amylase, and diastatic power.

Protein-related traits are of interest to malt houses and breweries. Malt barley grain has been bred for lower grain protein, varying from approximately 8% to 13% because if protein is too high it interferes with the malting process. The primary barley storage proteins, called hordeins (Fox 2009), belong to the prolamin protein group and contain large quantities of glutamine and proline (Baxter 1981; Qi et al. 2006). Depending on amino acid composition, hordein proteins can further be divided up into B, C, D, and γ hordeins (Gupta et al. 2010). Each of these storage proteins are coded for by a different gene, Hor2 (B-hordeins), Hor1 (C-hordeins), Hor3 (D-hordeins), and Hor5 (γ-hordeins), all of which are located on chromosome 1H (Shewry and Miflin 1982; Shewry et al. 1985). The other two important storage proteins in barley are glutelin and protein Z, which are coded for by genes on 1H and 4H respectively (Ladogina et al. 1988; Kaneko et al. 1999). Glutelin, the second most abundant storage protein, is rich in glutamine, proline, and glycine (Zhao et al. 2011). Protein Z is a serpin protein and the

least abundant of the three types of storage proteins. It is also very heat-stable and plays an important role in foam stability of beer (Niu et al. 2018).

During the malting process protein structures are broken down by proteases and peptidases. Proteases are responsible for digesting the larger hordein, glutelin, and protein Z structures into peptides. Peptidases then convert peptides into free amino acids (Osman et al. 2002). Of the three storage proteins hordeins and glutelin are the most easily degraded by proteases (Osman et al. 2002). The Maillard reaction during mashing is reliant on FAN levels to produce desired flavors and colors (Steiner et al. 2012). Importantly, some of the storage protein is removed from the wort by the filtration process after mashing, with some proteins more likely to be removed than others (Jaeger et al. 2021). The resulting wort contains FAN, which is an important nutritional source of amino acids for yeast (Briggs 2004). However, yeast does not absorb all amino acids at the same rate. Researchers have divided amino acids into four groups based on yeast uptake varying from readily used, e.g. threonine, to not used, proline (Ferreira and Guido 2018). If all the FAN in the wort is not used by the yeast during brewing, then the resulting beer can have off flavors and staling (Narziss and Back 2012).

Determining the genetic regulation of metabolites in barley malt can inform breeding for flavor. In other plants the integration of genetics and metabolomics data others have identified metabolic QTL for Arabidopsis, tomato, maize, and rice (Lisec et al. 2009; Matsuda et al. 2012; Jin et al. 2017; Schauer et al. 2006). In barley, metabolic QTL related to drought and heat stress (Templer et al. 2017; Piasecka et al. 2017), disease resistance (Bollina et al. 2011), and nitrogen remobilization (Mickelson et al. 2003) have been identified, as well as metabolic QTLs for malt quality. One study identified QTL related to sugar metabolism in a wild barley NAM population,

which provided new targets and germplasm for sugar selection (Gemmer et al. 2021). Another group was able to identify QTL and candidate genes related to beer flavor and metabolites impacting beer flavor (Sayre-Chavez et al. 2022). Other non-mapping studies indicate genetic variation for flavor metabolites in malting barley (Bettenhausen et al. 2018; Bettenhausen et al. 2020; Herb et al. 2017a).

To interrogate the genetic regulation of metabolites, we screened a 169 line population with a history of malting and origins from around the world using GWAS. The population varied for malt quality and endosperm hydration (Jensen et al. 2023). Since modification impacts metabolic profile (Herb et al. 2017a) we hypothesized we would be able to detect variation in metabolite QTL that could be used for breeding.

Methods

Germplasm

The NSGC Barley Core Panel subset, genotyping data, and alignments as described in Jensen et al. (2023) were used in this study. The population consisted of 169 two-rowed lines with a history of malting and origins from around the world. The Morex 2012, IBSC physical map was used to align 5716 SNPs obtained from T3 for this population's genetic map (Blake et al. 2012).

Plant Material

As described in Jensen et al. (2023) the population was grown for two years (2018 and 2019) at the Montana State University Arthur H. Post Research Farm, Bozeman, Montana, in an augmented block design. In 2018 the trial was irrigated and in 2019 rain fed but due to high

precipitation in 2019 moisture levels were comparable. Agronomic, malt quality, hydration index, dormancy, and seed size data were all collected and BLUP corrections were performed as described in Jensen et al. (2023). The only traits that were not collected across both years were kernel hardness and dormancy traits.

The grain was malted by the MSU Malt Quality Lab using a three-steep pale base malt recipe described in Jensen et al. (2023). Quality analysis was also performed using ASBC methods on wort extracted through a congress mash as described in Turner et al. (2019). The malting recipe and quality analysis did not change between years.

Metabolomics

Extraction of grain non-volatile metabolites was performed at the Colorado State University Proteomics and Metabolomics Facility on ground malted barley from both years. A 100 mg subsample of ground malt was transferred to a VWR 2 Dram glass vial with a phenolic cap where 1 mL of MTBE/MeOH solution was added. The solution was vortexed for 1 hour in a cold fridge and then centrifuged at 4 °C for 15 minutes at 3500 rpm. Following centrifuging 200 ul of cold LC grade water was added and the sample was centrifuged again under the same conditions for 25 minutes. The organic and aqueous layers were then removed with the organic layer being stored in autosampler vials. The aqueous layer was vortexed and then placed in the centrifuge at 3500 rpm for 10 minutes before also being sampled and stored in autosampler vials. Analysis was performed using reverse phase ultra-high performance liquid chromatography mass spectrometry (UHPLC-MS) as described in Bettenhausen et al. (2018) to detect semi-polar and nonpolar metabolites.

Quality control for analytical variation was performed through replicated injections (n=2) of each sample. Coefficient of variance (CV) of replicates for all metabolites was as less than 25%, which is considered acceptable variation for these platforms (Schiffman et al. 2019). Mass spectra from the three metabolomics platforms were converted to .cdf files and processed using the workflows described in Bettenhausen et al. (2018). Metabolite quantities were normalized to total ion current and relative abundance since each molecular feature was determined by the mean area of the chromatographic peaks among replicate injections. Spectral matching using RamSearch software (Broeckling et al. 2014) with an in-house database of approximately 1,500 compounds, MSFinder software (Tsugawa et al. 2016; Lai et al. 2018), and external databases NIST v14 (http://www.nist.gov), Metlin (Tautenhahn et al. 2012), Human Metabolome Database (HMDB) (Wishart et al. 2012), and FooDB (FooDB 2017) were all used to identify non-volatile metabolites. Chemical ontologies were established using HMDB and ClassyFire (Djoumbou Feunang et al. 2016) in R.

Statistics

GAPIT's farmCPU method (Wang and Zhang 2021) was used to perform genome wide association studies (GWAS) on all measured metabolites. The farmCPU mapping method requires normal distribution of data so all metabolomics data was Z transformed in R to correct for skews in the data. Once transformed the metabolomics data was mapped without compression, and PC structure corrections set to zero based on Jensen et al. (2023) previous findings for this population's structure. QQ plots were also used to confirm that PC structure corrections were not needed. Significance thresholds were calculated using GAPIT's default Bonferroni correction taking the negative log of alpha (0.01) and dividing by the number of

markers (5716) to get a significance threshold of 5.8 (Wang and Zhang 2021). All significant SNPs are reported in Supplemental Table 4.1.

Metabolites were divided into chemical classes and principal component analysis (PCA) was performed for each year on transformed data. Principal components (PCs) were calculated for each chemical class in R using the prcomp function with scaling and centering (Team 2021). PCs were then plotted with vectors for each metabolite to see how the classes varied between 2018 and 2019.

Network analysis was performed on transformed data for each year. Pearson correlations were made using the cor function in base R (Team 2021) with covariance computation set to "pairwise.complete.obs". Then using the package igraph (Csárdi and Nepusz 2006) edge files were made. Correlation minimums were set to 0.6 when observing all metabolites and 0.5 when looking at malt quality traits, and all lipid metabolites were removed for both years to easily visualize the interactions of all other metabolites.

FAN and amino acids QTL did not map to similar regions so using a generalist approach we sorted lines based on alleles of SNPs for each FAN QTL. Then we ran two-sample unequal variances t-tests on all the amino acids. Any amino acids with a p-value less than 0.05 were considered related to the FAN QTL.

GAPIT's calculation of linkage disequilibrium (LD) was used to establish a window for possible candidate genes. SNP base pair positions for the Morax v3 physical map were searched using T3 barley (Blake et al. 2012; Mascher et al. 2021). Then using GrainGenes gene browser (Yao et al. 2022), all genes within a 1 Mb window centered on the SNP of interest were evaluated. Predicted genes in the grain genes data base that were suggested to impact amino

acids were noted and the candidate genes predicted functions were confirmed using Pfam and Ensembl Plants.

Results

Agronomics and Malt Quality

We examined the consistency of the data collected from the NSGC Barley Core Panel subset between the two years. Although agronomic and malt quality traits were highly correlated as reported in Jensen et al. (2023), the phenotypic population means varied for some traits between the two years. The population grown in 2019 was grown on dryland and had lower plumps and higher protein, resulting in overall lower malt quality with lower extract, and higher β-glucan than the population under irrigation (Table 4.1). However, the steep out moisture was higher in 2019 as was S/T and enzymes α-amylase and diastatic power.

Metabolomics

The small variation in agronomic and malt quality between 2018 and 2019 coincided with a large difference in metabolites between the two years. We detected 301 metabolites in 2018 and 441 metabolites in 2019. Metabolites within the chemical classes of alkaloids, benzenoids, lipids, nucleosides, organic acids, organoheterocyclic compounds, organonitrogen compounds, organooxygen compounds, and phenylpropanoids were all detected (Table 4.2). Lipids were detected more than any other metabolite class in both years and more lipids were detected in 2019 than 2018. Organic acids were the second largest chemical class detected. This class included amino acids, which had the most co-occurring metabolites in the two years. Organoheterocyclic compounds, organooxygen compounds, and phenylpropanoids were all

detected at similar levels; however, saccharides, classified as organooxygen compounds, were not detected in 2019. Only a few alkaloids, benzenoids, and organonitrogen compounds were detected across both years with the same number of alkaloids being detected in 2018 and 2019, more benzenoids being detected in 2019, and more organonitrogen compounds being detected in 2018 (Table 4.2).

A PCA explaining amino acid variation in the population indicates differences between the two years in amino acids and FAN (Figure 4.1). In 2018, amino acid vectors varied with both PC1 and 2. In contrast, most of the amino acid variation is explained by PC1 in 2019, with amino acid vectors primarily pointing to the right (Figure 4.1). This pattern holds for the rest of the metabolic classes but is the most pronounced in the amino acids. In 2018, variation in FAN is primarily explained by PC2 along with arginine, l-threonine, valine, and leucine. While FAN in 2019, also primarily explained by PC2, correlates with alanine and histidine (Figure 4.1). Short vectors also show us tyrosine and lysine are poorly explained by PC1 and 2 in both years while proline is only poorly explained by the first two PCs in 2018.

Although 2018 and 2019 malt quality data was correlated (Jensen et al. 2023), network analysis for metabolites, excluding lipids, indicated little overlap between the years (Figure 4.2). A large cluster of metabolites were correlated in 2018, while the 2019 metabolites formed smaller correlation clusters. A few metabolites correlate between 2018 and 2019 thereby connecting the metabolite groups (Figure 4.2). Malt quality from both years correlated more with 2019 than 2018 metabolites. Most of the correlations between the metabolites were positive, indicating most were forming due to degradation processes.

GWAS

Since the metabolites were different in 2018 and 2019, each year's data was separately mapped, resulting in 215 QTL detected for 74 metabolites in 2018 and 612 QTL detected for 192 metabolites in 2019. Here, we map both mean malt quality across years and for each year separately to determine if metabolite differences between the years could be explained by malt quality differences, unlike in Jensen et al. (2023) where only the mean across years was mapped. Although more malt quality QTLs were observed in 2019 (56) versus 2018 (24), for the most part, the additional QTLs did not co-localize with metabolite QTL. An exception is the new qAMA5H.a hydration index QTL on 5H co-segregated with methionine and kernel hardness. QTLs were identified for metabolites in every chemical group observed in both years (Table 4.2). Lipids were the chemical group with both the most metabolites detected and the group with the most QTLs (Table 4.2). Maltol is reported separately in Table 4.2 due to its singular importance in malt flavor. The complete list of metabolites observed and QTLs mapped is reported in Supplemental Table 4.1, while Figure 4.3 summarizes the genomic distribution of metabolic and quality QTL for both years.

We observed QTL related to protein anabolism or catabolism. Thirty-three amino acid QTL were identified across both location years with more amino acid QTL identified in 2019 (Table 4.2). These QTL were present across all seven chromosomes with other metabolite QTL collocating to these regions (Table 4.3). Most amino acid QTL (21) co-located with another metabolite, although a few (12), with histidine, methionine, and phenylalanine most commonly, mapped singularly. While six FAN QTL were mapped, only one co-located with an amino acid, l-threonine, and two co-located with metabolites. However, exploring FAN QTLs further, we grouped lines by allele for each QTL and observed variation in a number of amino acids at each

FAN QTL (Table 4.3). Other malt quality QTLs also co-located with metabolites. Hydration index co-locates with metabolites in two regions. The first is a collocation with glucose and maltol where a higher hydration index increases the level of these two metabolites. The second hydration index collocation is with methionine and kernel hardness where a harder kernel increases methionine and reduces hydration index. We also see extract co-locate with an arginine QTL where less extract correlates with more arginine. A grain fill QTL also co-locates with metabolites, where increased grain fill positively correlates with threonine and furaneol 4-glucoside and negatively correlates with benzenoid (Table 4.3).

Maltol, a known flavor compound in malt, was detected in both years but only mapped in 2018 to 3 QTLs. Maltol co-located with serine on 5H, protein degradation products and sugars on 6H, and hydration index and glucose on 2H.

The amount of protein, amino acids, and other protein related degradation products results from a complex set of processes and interactions occurring throughout development and malting. Therefore, the genes involved could impact both anabolism and catabolism. The Morex v3 genome sequence was examined for potential candidate genes at each QTL. *HvNAM2* cosegregated with L-threonine. Hydration index and kernel hardness cosegregated with methionine and a candidate gene for seed texture (*HORVU.MOREX.r3.5HG0420160*). QTL for amino acid variation on 1H, qAMA1H.a, qAMA1H.c, qAMA1H.d, and qAMA1H.e, mapped near the B, C, and γ Hordein storage proteins. However, qAMA1H.a and qAMA1H.c also mapped near *HORVU.MOREX.r3.1HG0000360.1*, encoding a carboyl-therminal peptidase and *HORVU.MOREX.r3.1HG0064780.1*, encoding a cysteine proteinase inhibitor. Three candidates for genes encoding the protein family Bifunc_inhib/LTP/seed_store were identified at qFAN2H.a

while three candidate genes were identified at qFAN4H related to amino acid biosynthesis. Of the thirty-three QTL for variation in protein degradation products, only eight were near candidate genes that clearly impacted amnio acid variation. These candidate genes consisted of protease inhibitors (qAMA1H.c), peptidases (qAMA1H.a, qAMA2H.a, and qAMA2H.g), amino acid biosynthesis (qAMA1H.b and qAMA2H.b), (Table 4.3).

Discussion

Agronomics and Malt Quality

The NSGC Barley Core Panel subset varied between variety and between year across traits, empowering the mapping of agronomic, malt quality, and metabolite QTL. While strong correlations in malt quality between years justified mapping averages in Jensen et al. (2023), the differences in malt quality between years appear magnified when comparing the metabolic profile in each year. The malt quality and metabolomics data suggest, even though the two years were malted by the same recipe, that variation in environment impacted grain protein and plumps, resulting in different levels of modification, which in turn impacted the metabolites observed. The increased protein observed in 2019, may have resulted in greater variation observed in protein metabolites (i.e., amino acids), which we suspect allowed more protein related QTL to be identified in 2019 (Table 4.2). Conversely, we detected more saccharides in 2018 suggesting that starch degradation had proceeded further compared to 2019. Therefore, the data from the two years metabolites likely represent two different modification snapshots of the metabolites produced during malting. Differences in malt modification has been observed previously to impact metabolites (Herb et al. 2017a). Our results can be interpreted based on the

known progression of modification, with proteins tending to be degraded first making starch available to enzyme action during mashing.

Network analysis indicated that malt quality data overlapped between the two years. On the other hand, metabolites correlated within years but did not correlate much between years, supporting the possibility of the two years representing two different modification time points. Also, there was not much correlation between malt quality traits from either year with metabolites (Figure 4.2). Here, as in previous studies, metabolites were extracted from malted grain while malt quality analysis was performed on wort (Guo et al. 2020). Therefore, the metabolomics data is missing the impact of mashing and filtering, which further degrades starch and proteins and forms other metabolites (Evans et al. 2011). Instead, the metabolomics data represents the variation between genotypes due to malting through the kilning phase.

Other QTL studies in barley also detected a wide range of variation in metabolites across populations. Some researchers mapped the metabolites that varied between two treatments (Piasecka et al. 2017), while others mapped all metabolites varying in a population (Gemmer et al. 2021; Templer et al. 2017). However, in all of these studies, metabolites representing all classes were mapped to QTL across all seven chromosomes. We also observed metabolic QTL across seven chromosomes for each class of compounds (Figure 4.3).

In this study we genetically examined maltol, an important malt flavor compound. Maltol is made from the reaction of a sugar and an amino acid and produces a sweet caramel or malty flavor (Pittet et al. 1970). Different lines have different flavor profiles and maltol is a desirable metabolite that is a part of this variation (Herb et al. 2017b; Nobis et al. 2019). The QTLs we identified for maltol all co-segregated with QTL for amino acids or sugars. Variation in maltol

and the amino acid serine co-segregated at qAMA5H.b. The maltol QTL on 3H co-located with glucose and the qHYI3H.b QTL identified in Jensen et al. (2023). They also found that at qHYI3H.b smaller seed was related to an increase in hydration. Therefore, this region likely impacts the availability of starch for degradation through seed size. The other maltol QTL was identified on 6H (Table 4.3) and co-located with glucose-6-phosphate and sucrose. Since glucose 6 phosphate is a key part of sugar metabolism this QTL could be impacting starch degradation during germination allowing for the increased production of maltol. All three of these maltol QTL were only observed in 2018 where we believe modification of the malt was more advanced. We hypothesize that these QTL will be very helpful in breeding for variation in malt flavor.

Protein metabolism was genetically variable in our population not only resulting in malt quality differences, but also differences in protein related metabolites. Identification of QTL for protein metabolism related to germination could provide targets for malt and beer quality and flavor (Narziss and Back 2012; Steiner et al. 2012). The protein products available for brewing are impacted by a number of interacting processes throughout development. First, the health of the plant dictates its ability to make seed so the availability of sunlight, rain, nutrients, and pests can all impact seed protein (Zhang et al. 2001; Howard et al. 1996). Under ideal conditions the plant grows and stores starch and protein in the grain. Once enough nutrients have been accumulated to produce viable seeds hexokinases will signal the start of senescence (Swartzberg et al. 2011). This process activates a suite of genes that are responsible for senescence and nutrient remobilization in the seeds (Parrott et al. 2007). Genetic variation in these genes in turn impact grain yield, protein, and starch levels (Distelfeld et al. 2014). Some of the most heavily studied genes that impact protein are the Hordein genes which all vary in amino acid

composition (Qi et al. 2006; Howard et al. 1996; Baxter 1981). All these genes and environmental factors, as well as genetic and environmental interactions establish the protein available in the seed, which carries over to the malt. The malt process then acts on the available protein, degrading it through the activity of proteases and peptidases (Osman et al. 2002; Akeroyd et al. 2016). Amino acids and other modifying byproducts are then involved in biosynthesis pathways which can change amino acid levels (Umbarger 1978; Wendisch 2007). All of these compounds can then be up or down-regulated by the malting process (Fox 2009). All of this variation in protein products impacts the amino acids available to yeast during brewing. The metabolite QTL we mapped at the end of malting could be controlled by any of these listed systems making them a challenge to use in selection without candidate genes to describe their functions.

Malt houses and breweries rely on FAN levels to determine available free amino nitrogen to feed yeast during brewing. FAN is a measure of all the amino groups made available by the malt process. Degradation of the storage proteins, including amino acids, ammonia, and end-group y-amino nitrogen in peptides and proteins are all measured with FAN (ASBC FAN wort-12). In this population, we were able to detect six FAN QTL mapping near previously identified QTLs (Pauli et al. 2015; Fang et al. 2019; Cu et al. 2016a) of which three co-located with metabolites. While we detected 33 amino acid QTL, only one co-located with FAN, although other protein related metabolites co-locate with FAN (Table 4.3). Also, allelic groupings of lines at each of the six FAN QTL were different ($p < 0.05$) for amino acids, where an increase in FAN was associated with an increase in amino acids even though the specific amino acids involved varied. This is not surprising since FAN measures more than amino acids, and in some cases,

protein degradation may not have proceeded to the extent of measuring single amino acids. Amino acid QTLs co-located with a wide range of traits including grain fill, test weight, dormancy, hydration index, kernel hardness, and extract while also collocating with a wide range of metabolites (Table 4.3).

To better understand the genetics behind the protein products in malt and the likely stage of impact we evaluated each QTL for possible candidate genes. Although we could not identify candidate genes for a number of QTL, candidate genes involved at a number of stages in development were observed (Table 4.3).

Seed Development and Maturation

Although none of the FAN QTL mapped to 1H, five amino acid QTL did, collocating to regions near hordein genes, which code for storage proteins in barley. Of the four hordein genes, four amino acid QTL mapped near three of them. First, variation in valine at qAMA1H.a maps near *HOR2* (B-hordein) and *HOR5* (γ-hordein) (Shewry and Miflin 1982; Shewry et al. 1985). The QTL for phenylalanine, leucine, and histidine all mapped at qAMA1H.c near *HOR3* (D-hordein) (Shewry and Miflin 1982). Therefore, the amino acid QTLs mapping to 1H may be due to genetic differences related to the hordein coding genes. Amino acid differences in hordein proteins have long been an area of interest due to their impacts on grain protein and malt quality (Gozukirmizi and Karlik 2017); however, the protein variation at hordeins is not yet available for the population studied.

qAMA5H.a co-located with a hydration index QTL not previously identified by Jensen et al. (2023) along with a kernel hardness QTL matching other findings (Fox et al. 2007a). The candidate gene *HORVU.MOREX.r3.5HG0420160* has been identified as a grain softness protein

according to grain genes and Ensembl plant (Yao et al. 2022; Yates et al. 2022). This particular QTL suggests that a harder kernel results in more amino acid so the grain softness protein could be impacting protein packing or the amount of protein in the grain. Others have reported that harder kernels have more starch-protein bindings resulting in a continuous protein matrix around starch granules (Nair et al. 2011). This could explain why we are seeing more amino acids with a harder kernel.

HvNAM2 has been shown to impact grain protein with the Karl allele decreasing protein while the Lewis allele increased protein (Alptekin et al. 2022). We were able to map qAMA2H.d to a marker for *HvNAM2* (Alptekin et al. 2022). *HvNAM2* is a NAC transcription factor and a member of the NAC gene family which is responsible for regulating plant development and abiotic stress (Jensen and Skriver 2014). Malt quality QTL have been mapped near *HvNAM2* (Pauli et al. 2015) and it has been confirmed that the Karl allele that decreases protein improves malt quality (Alptekin et al. 2022). In this population, the desirable Karl allele also increased l-threonine which falls into the fast absorption group of amino acids (Ferreira and Guido 2018).

Malt Process

Candidate genes impacting amino acid variation during malting fell into three groups consisting of protease inhibitors, peptidases, and amino acid biosynthesis. qFAN2H.a had three candidate genes all of which are a part of the Bifunc_inhib/LTP/seed_store protein family. This protein family has been identified in *Eleusine coracana* (Indian finger millet) to be a trypsin/α-amylase inhibitor (Strobl et al. 1998). It has also been identified in *Zea mays* (maize) to be a Hageman factor/amylase inhibitor (Behnke et al. 1998). Unfortunately, this protein family has not been thoroughly studied in barley, but based on findings in other plant species it can inhibit

the degradation of the storage proteins and starch in the grain which would be undesirable for the malting process. qAMA1H.c was near *HOR3* and a protease inhibitor from the SQAPI protein family, which is involved in the inhibition of aspartic proteases (Galleschi et al. 1993). These proteases are water catalyzed and degrade larger proteins into peptides (Conner 2004). Both of these candidate genes could be undesirable for the malting process since they inhibit proteases from breaking down large proteins into peptides.

There were three QTL with peptidase candidate genes. qAMA1H.a was near *HOR2* and *HOR5* but the QTL also contained a candidate gene that encodes a carboxyl-terminal peptidase and part of the Neprosin protein family. These peptidases are non-specific in the length of the proteins they cleave while most peptidases are more limited (Rey et al. 2016). The qAMA2H.a candidate gene was a part of the Peptidase S8 family while qAMA2H.g's candidate gene belonged to the Peptidase C54 family both of which are involved in peptide degradation. Understanding the regulation of these peptidases ensures peptides are broken down into amino acids and selection for them could help ensure lines are within desirable FAN ranges.

During the malting process amino acids can be modified through biosynthetic pathways. qFAN4H occurs near three candidate genes all a part of amino acid biosynthesis. The first candidate gene, *HORVU.MOREX.r3.4HG0336360*, is responsible for producing 3-phosphoshikimate 1-carboxyvinyltransferase (EPSP) which is a part of aromatic amino acid biosynthesis. EPSP has been shown to be inhibited by glyphosate and glyphosate tolerant EPSP synthase has been studied for its uses in glyphosate resistance (Stallings et al. 1991). *HORVU.MOREX.r3.4HG0336380* encodes for 2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase which is a part of L-methionine salvage (Xu et al. 2007) and

HORVU.MOREX.r3.4HG0336490 encodes for a cysteine synthase which converts serine into cysteine (Hatzfeld et al. 2000). While it is not clear which of these candidate genes qFAN4H is mapping to due to our large LD values the potential of this QTL impacting amino acid biosynthesis seems quite likely. It is also worth noting these are just candidate genes and our calculated LD is quite large with many genes to select from, but it provides a good starting point for further investigation. qAMA1H.b did not map near any hordein encoding genes but has a potential candidate that impacts histidine biosynthesis. The protein product 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase has been identified as being a part of histidine biosynthesis (Papaleo et al. 2010). qAMA2H.b's candidate gene is a part of the Trimeric LpxA-like superfamily which includes proteins involved in lipid biosynthesis (Raetz and Roderick 1995) and lysine biosynthesis (Beaman et al. 2002). While it is unclear if these QTL are impacting amino acid biosynthesis during grain fill or during malting the potential for impacting amino acid variation in the malting process through conversion of undesirable amino acids to desirable ones could have major impacts on beer flavor and shelf life (Ferreira and Guido 2018).

The candidate genes for protease inhibition, peptidases, and amino acid biosynthesis show the challenges of breeding for FAN variation and amino acid variation in malt. Further understanding of the genetic regulation of proteases could ensure protein degradation during malting since poor degradation of larger storage proteins means peptidases lack peptides to further degrade (Osman et al. 2002). Poor degradation of these large storage proteins also leads to reduced modification and bad malt. Since peptidases are responsible for degrading peptides into amino acids (Luoto et al. 2012), the QTL regulating them could be used to impact FAN

levels and flavor. Amino acids play an important role in beer flavor as precursors to other flavor metabolites (Ferreira and Guido 2018). They also serve as food for yeast and can impact the flavors they produce. Excess amino acids can also result in off-flavors in the form of food for other microbes (Ferreira and Guido 2018) so a better understanding of the genetic regulation of amino acids in malt can improve flavor predictions. This can all be further complicated by amino acid biosynthesis during malting. When amino acids are present in excess they can be converted into other needed amino acids through these biosynthesis pathways. While well studied in model systems (Umbarger 1978; Wendisch 2007), further understanding of amino acid biosynthesis could allow for selection or upregulation of more desirable amino acids for yeast in malt. However, considerably more work needs to be done to further understand the regulation of proteases, peptidases, and amino acid biosynthesis before we can think about selecting for increased levels of desired amino acids.

This population was very useful in identifying an abundance of metabolite QTL. We were able to detect QTL across all nine chemical classes we measured. The most impactful flavor QTL we detected was for maltol. These QTL could be used for marker-assisted selection to produce lines with a range of maltol levels providing malt houses and breweries with unique flavor variations to work with. We also detected FAN and amino acid QTL which had candidate genes responsible for seed development and maturation, protease inhibition, peptidase activity, and amino acid biosynthesis. These QTL and candidate genes show the complex nature of protein storage and degradation in barley grain. However, continuing to better understand this process could be a boon to breeders, growers, maltsters, and brewers.

Tables

Table 4.1: Phenotypic averages for the 2018 and 2019 growing season. Mean, standard deviation (Std), minimum (min), and maximum (max) along with a p-value comparing the two years are reported.

Trait Year	Mean	Std	Min	Max	Year comparison p-value
Hydration Index					0.3052
2018	45.45562	9.457901	26	71	
2019	46.59763	10.93341	27	78	
Steep Out					1.70E-08
2018	0.438819	0.014886	0.404876	0.485143	
2019	0.447525	0.012662	0.362171	0.492589	
Test Weight					0.0627
2018	53.72308	2.062707	46.3	57.2	
2019	54.06154	1.137771	49.9	56.7	
Plump					5.88E-06
2018	92.98886	5.176132	76.37663	99.42136	
2019	89.84438	7.21333	64.2	98.8	
Protein					8.78E-10
2018	13.23728	0.999092	11	15.3	
2019	13.96414	1.11534	11.79	17.36	
β-Glucan					2.51E-05
2018	578.8918	374.5521	60.90526	1563.761	
2019	742.0005	325.3725	54.67711	1683.485	
Soluble Protein					<2.2E-16
2018	3.667204	0.437426	2.69013	5.60043	
2019	4.594756	0.475618	3.44605	6.75797	
S/T					5.11E-14
2018	29.47617	4.290227	20.88662	47.49487	
2019	33.08284	4.126577	25.29854	49.18464	
FAN					1.20E-05
2018	173.5954	27.09307	113.3459	284.6801	
2019	185.9765	24.0351	123.272	270.2437	
Extract					4.75E-07
2018	77.62925	1.505947	73.37212	82.31155	
2019	76.74195	1.665724	72.34625	81.45758	
AA					5.74E-10
2018	48.83261	12.81054	17.28588	104.6693	
2019	58.45202	14.81967	23.15816	134.7992	

Table 4.1 continued

Trait Year	Mean	Std	Min	Max	Year comparison p-value
DP					1.42E-09
2018	126.72	32.53812	21.1503	270.0598	
2019	147.7608	29.51645	59.39651	264.1995	

Table 4.2: Metabolite classes and number of QTL detected for both location-years. The metabolite column reports the total number of metabolites observed for each class in each year while the QTL column show the total number of QTL detected for each class and each year. Amino acids are reported as a subclass of organic acids, while saccharides are reported as a subclass of organooxygen compounds. Maltol variation is also reported.

Metabolite class	Metabolites		QTL	
	2018	2019	2018	2019
Alkaloids	2	2	0	4
Benzenoids	5	12	6	16
Lipids	199	341	159	437
Nucleosides	2	1	0	7
Organic Acids	35	34	17	62
Amino acids	16	19	7	26
Organoheterocyclic Compounds	19	16	18	31
Maltol	1	1	3	0
Organonitrogen Compounds	8	3	2	5
Organooxygen Compounds	15	15	9	18
Saccharides	6	0	3	0
Phenylpropanoids	16	17	4	32
Total	301	441	215	612

Table 4.3: Protein metabolite QTL from both years. FAN, amino acid, and maltol QTL and their co-located QTL are reported along with each QTL year, SNP, chromosome, base pair position, effect, cosegregating amino acids through less stringent assessments, and candidate genes. QTL reported as average were found using BLUP corrected averages. All metabolite effects are based on Z-transformed relative abundance. Less stringent cosegregation assessments were performed using t-tests for each FAN marker.

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qAMA1H.a	Valine	2018	SCRI_RS_120059	1	653064	0.362		HORVU.MOREX.r3.1HG0000360.1
	DT95	average	SCRI_RS_120059	1	653064	2.826		
qAMA1H.b	Anethole	2019	12_31208	1	148023288	-0.464		HORVU.MOREX.r3.1HG0049420.1
	N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	12_31208	1	148023288	-0.359		
	Hordenine	2019	12_31208	1	148023288	-0.495		
	Extract	average	12_31208	1	148023288	-0.471		
	Arginine	2019	SCRI_RS_155439	1	148156867	0.293		
qAMA1H.c	Phenylalanine	2019	SCRI_RS_151894	1	342735416	0.24		HORVU.MOREX.r3.1HG0064780.1
qAMA1H.d	Leucine	2019	12_10166	1	364575709	-0.447		
qAMA1H.e	Histidine	2019	11_20990	1	388893493	-0.357		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qFAN2H.a	L-threonine	2018	SCRI_RS_147203	2	313674	0.668	L-threonine, Alanine, Glycine, Aspartic acid, Tryptophan, and Glutamic acid	HORVU.MOREX.r3.2HG0193790, HORVU.MOREX.r3.2HG0193820, and HORVU.MOREX.r3.2HG0193840
	FAN	average	SCRI_RS_147203	2	313674	15.070		
	(-)-Salsoline	2019	SCRI_RS_147203	2	313674	-0.626		
qAMA2H.a	arginine	2019	SCRI_RS_238263	2	7748598	-0.343		HORVU.MOREX.r3.2HG0099030.1
	4-Hydroxycinnamoylagmatine	2019	SCRI_RS_238263	2	7748598	-0.449		
qFAN2H.b	TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_174935	2	39977500	0.238	Serine, Arginine, Threonine, and Leucine	
	FAN	average	SCRI_RS_215080	2	40064925	-5.004		
	N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	SCRI_RS_165427	2	40520273			
qAMA2H.b	benzenoid	2018	12_31127	2	80676114	-0.548		HORVU.MOREX.r3.2HG0119500.1
	Threonine	2019	12_31127	2	80676114	0.664		
	Furaneol 4-glucoside	2019	12_31127	2	80676114	1.144		
	grain fill	average	12_31127	2	80676114	1.798		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qAMA2H.c	Phenylalanine	2019	SCRI_RS_199897	2	106008567	-0.227		
qAMA2H.d	L-threonine	2018	HVNAM2	2	190163256	0.692		HVNAM2
qAMA2H.e	Histidine	2019	SCRI_RS_30973	2	411560941	0.202		
qFAN2H.c	DP	average	11_10128	2	579471612	7.635	Valine, Leucine, Glycine, Arginine, Isoleucine, and Tryptophan	
	DP	2019	11_10128	2	579471612	8.183		
	FAN	average	11_10990	2	579747942	-8.000		
qAMA2H.f	Cytidine	2019	SCRI_RS_610	2	581007862	-0.445		
	L-threonine	2018	SCRI_RS_156220	2	581446086	-0.632		
	(-)-Salsoline	2019	SCRI_RS_207327	2	583638330	0.457		
qAMA2H.g	3-(2,3-dihydroxyphenyl)propanoic acid	2019	11_10566	2	606908872	0.282		HORVU.MOREX.r3.2HG0213380
	N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_226193	2	606925099	0.545		
	(-)-Salsoline	2019	SCRI_RS_226193	2	606925099	0.539		
	Histidine	2019	SCRI_RS_114673	2	609222527	-0.46		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qAMA2H.g	Furaneol 4-glucoside	2019	SCRI_RS_114673	2	609222527	-0.513		HORVU.MOREX.r3.2HG0213380
qAMA3H.a	3-(2,3-dihydroxyphenyl)propanoic acid	2019	12_11322	3	477449286	-0.468		
	arginine	2019	SCRI_RS_166119	3	478686508	0.434		
qAMA3H.b	Serine	2018	SCRI_RS_198594	3	502023919	0.462		
	leu-tyr	2018	SCRI_RS_198594	3	502023919	0.535		
	hypoxanthine	2018	SCRI_RS_198594	3	502023919	0.365		
	leu-tyr	2018	SCRI_RS_159125	3	502053245	0.535		
	d-pantothenoyl-l-cysteine	2018	SCRI_RS_192360	3	503631370	0.329		
qHYI3H.b	Hydration Index	2019	SCRI_RS_1435	3	550749370	-2.15		
	Hydration Index	average	SCRI_RS_1435	3	550749370	-1.698		
	Glucose	2018	SCRI_RS_192891	3	552141738	-0.313		
	Maltol	2018	SCRI_RS_203164	3	555914016	-0.269		
qAMA4H	Phenylalanine	2019	SCRI_RS_146989	4	8976337	0.303		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qFAN4H	Soluble Protein	average	SCRI_RS_127657	4	13198440	0.100	Serine, Asparagine, L-threonine, Alanine, Histidine, and Threonine	HORVU.MOREX.r3.4HG0336380, HORVU.MOREX.r3.4HG0336360, and HORVU.MOREX.r3.4HG0336490
	FAN	average	12_30793	4	14844591	8.299		
	FAN	2019	12_30793	4	14844591	8.746		
	S_Protein	2019	12_30793	4	14844591	0.150		
qAMA5H.a	Hydration Index	2019	SCRI_RS_88710	5	1070915	-2.996		HORVU.MOREX.r3.5HG0420160
	Methionine	2019	12_30976	5	2378070	0.346		
	Kernel Hardness	average	SCRI_RS_31797	5	2492193	3.136		
qAMA5H.b	Maltol	2018	SCRI_RS_176142	5	46107344	-0.594		
	Serine	2018	SCRI_RS_145348	5	46450909	0.4		
qAMA5H.c	Alanine	2018	11_21061	5	464172004	0.397		
	succinic acid	2019	12_30848	5	464543429	0.262		
qAMA5H.d	Phenylalanine	2019	12_30556	5	506407644	-0.354		
qAMA5H.e	alanyl-tyrosine	2018	12_31206	5	517434588	0.788		
	Furaneol 4-glucoside	2019	12_31206	5	517434588	-0.808		
	N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_182353	5	517543545	0.347		
	Threonine	2019	SCRI_RS_161614	5	517568728	0.319		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qAMA5H.e	2-furoic acid	2019	11_20791	5	517610241	-0.472		
qAMA5H.f	Glutathione	2019	SCRI_RS_224854	5	556238395	0.489		
	Methionine	2019	12_30502	5	559311588	0.413		
qAMA6H	Leucine	2019	11_20212	6	4572863	-0.387		
	3-(2,3-dihydroxyphenyl)propanoic acid	2019	11_20212	6	4572863	-0.618		
	Test Weight	2019	SCRI_RS_194023	6	4859440	0.3		
qFAN6H	S_Protein	2019	SCRI_RS_182648	6	415546434	-0.143	Isoleucine, Phenylalanine, Alanine, Glycine, Aspartic acid, Cysteine, and Tryptophan	
	FAN	average	SCRI_RS_139937	6	442755740	4.431		
	FAN	2019	SCRI_RS_139937	6	442755740	5.878		
	N-(1-Deoxy-1-fructosyl)phenylalanine	2019	SCRI_RS_139937	6	442755740	0.137		
	Maltol	2018	11_10496	6	504498512	-0.452		
	G6P	2018	12_30626	6	504788309	-0.503		
	Sucrose	2018	11_11294	6	505690213	-0.355		

Table 4.3: Continued

QTL name	Trait	Year	SNP	Chromosome	Position	Effect	Amino acid cosegregation	Candidate gene
qFAN7H	FAN	2019	SCRI_RS_152931	7	10772499	13.420	Glutamine, Alanine, and Serine	
qAMA7H.a	Methionine	2019	SCRI_RS_162708	7	65473816	-0.309		
qAMA7H.b	Histidine	2019	SCRI_RS_157035	7	291013740	1.002		
qAMA7H.c	Histidine	2019	11_11461	7	331923108	-0.544		
qAMA7H.d	Methionine	2019	SCRI_RS_200021	7	520408967	0.23		

Figures

Figure 4.1: Principal component analysis (PCA) of amino acids and FAN for 2018 and 2019. Variation explained by each PC is reported. Each point represents a variety in the population while vectors indicate how the varieties vary for amino acids and FAN levels.

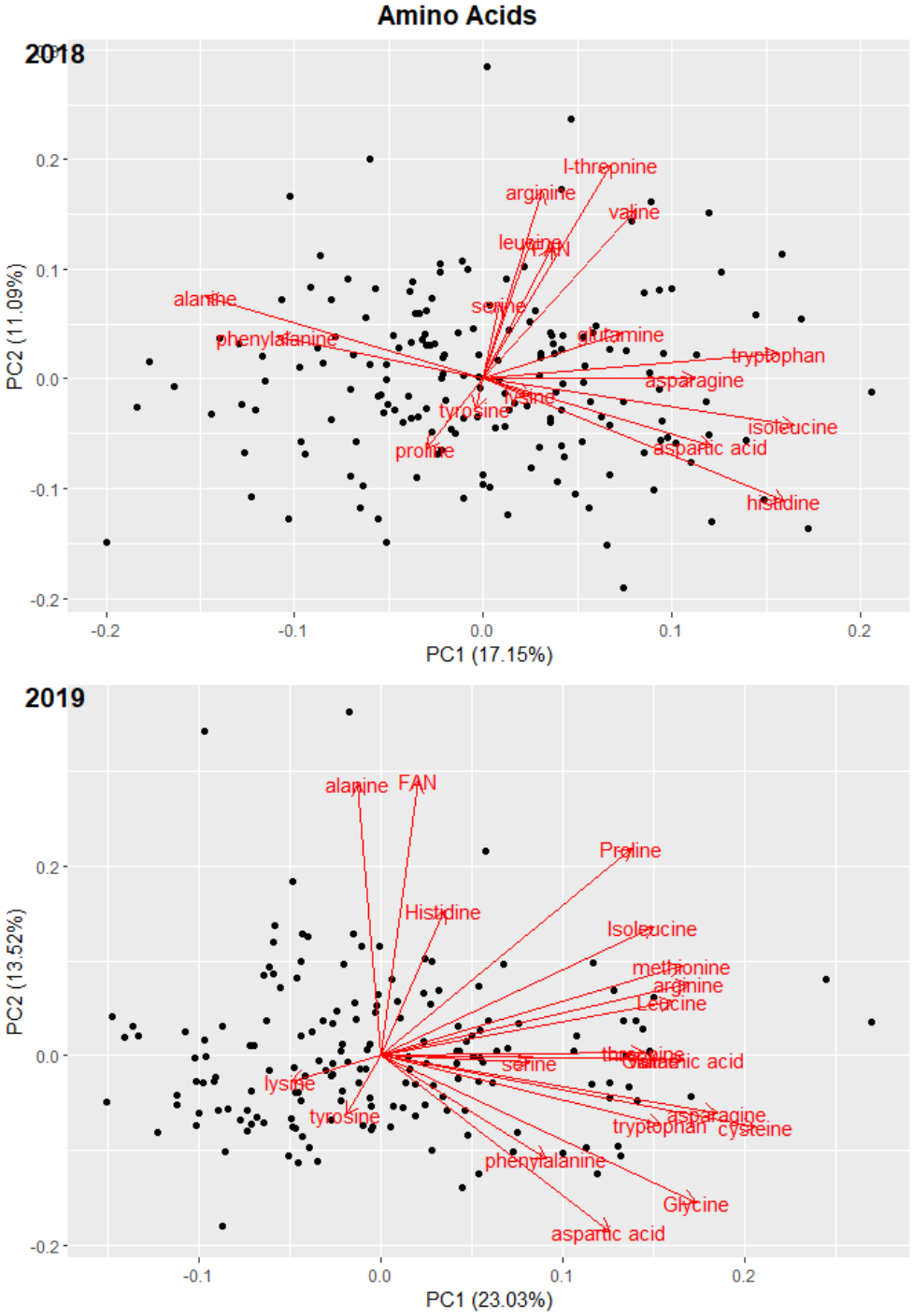

Figure 4.2: Network analysis of 2018 and 2019 malt quality and metabolite data excluding lipids. Edges are drawn between points with correlations of 0.5 or higher. Blue edges indicate a positive correlation while red edges indicate a negative correlation. Squares indicate data from 2018 while circles show data from 2019.

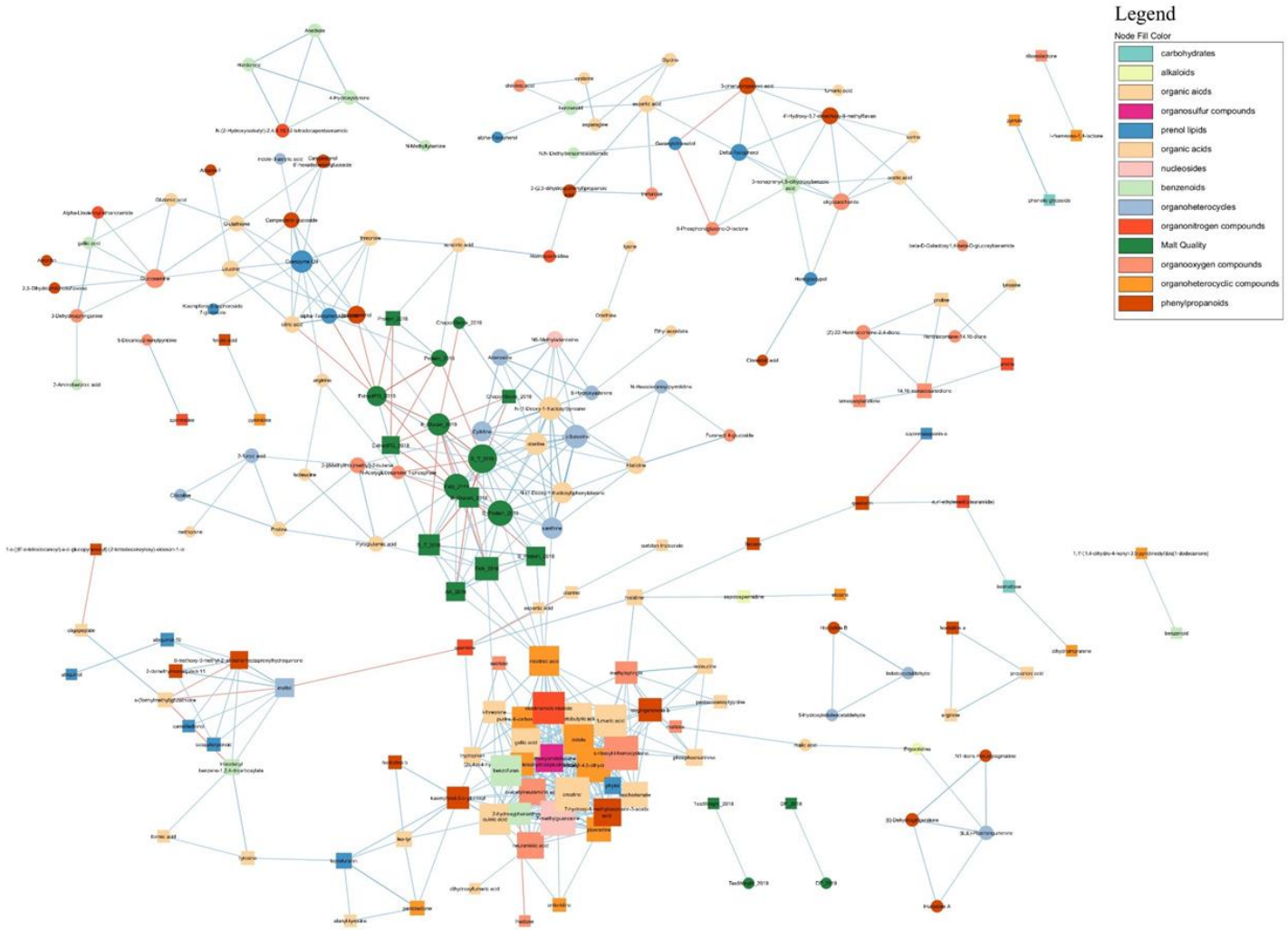

Figure 4.3: Metabolite and malt quality QTL for both years collected by metabolic groups. Each point shows a significant QTL. Amino acids and saccharides are regrouped as subgroups due to their impact on flavor. The x-axis reports the base pair position of each QTL while the y-axis shows the -$\log_{10}$(p-value) for each QTL. Chromosomes are differentiated by color.

Supplementals

Supplemental Table 4.1: All significant QTL identified in 2018 and 2019. Information about each QTL includes location years, SNP, chromosome, and base pair position.

Traits	Year	SNP	Chromosome	Position (BP)
DG(18:2(9Z,12Z)/20:1(11Z)/0:0)	2019	SCRI_RS_154700	1	3376
5-Hydroxy-14,16-hentriacontanedione	2019	SCRI_RS_154700	1	3376
malic acid	2019	11_20373	1	282538
Campesterol glucoside	2019	11_20373	1	282538
DT95	average	SCRI_RS_120059	1	653064
valine	2018	SCRI_RS_120059	1	653064
GLYCerOL 1-(9Z-OCTADECENOATE) 2-TETRADECANOATE 3-PHOSPHATE	2018	12_30715	1	883565
PE(15:0/18:2(9Z,12Z))	2018	SCRI_RS_161137	1	2259862
TG(14:0/16:1(9Z)/16:1(9Z))	2019	12_30933	1	4331267
4-Hydroxystyrene	2019	SCRI_RS_198544	1	4373895
LysoPC(14:0)	2018	11_21226	1	5641203
TG(20:4(5Z,8Z,11Z,14Z)/18:2(9Z,12Z)/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_224686	1	6645890
4-Hydroxy-16,18-tritriacontanedione	2019	SCRI_RS_224686	1	6645890
hypoxanthine	2018	SCRI_RS_224686	1	6645890
TG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/O-18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_200512	1	7047004
DG(16:1(9Z)/16:1(9Z)/0:0)	2019	SCRI_RS_148733	1	10781923
4-Hydroxy-16,18-tritriacontanedione	2019	SCRI_RS_130592	1	10955747
DG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0)	2019	SCRI_RS_189465	1	12316759
TG(15:0/18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	2019	11_20371	1	12889306

DG(16:0/16:0/0:0)	2018	SCRI_RS_14227	1	18155915
PC(18:2(9Z,12Z)/18:1(9Z))	2019	SCRI_RS_155382	1	19691025
PE(18:1(9Z)/18:2(9Z,12Z))	2019	SCRI_RS_155382	1	19691025
LysoPC(18:1(9Z))	2019	SCRI_RS_155382	1	19691025
DG(18:2(9Z,12Z)/20:1(11Z)/0:0)	2019	11_10757	1	20558168
Plump	2019	11_10757	1	20558168
BG	average	SCRI_RS_165811	1	30837602
N-Methyltyramine	2019	12_30817	1	36467747
TG(16:0/16:1(9Z)/16:1(9Z))	2019	12_30817	1	36467747
PE(16:0/18:1(11Z))	2018	SCRI_RS_126734	1	41663740
Alpha-Linolenic acid	2019	11_21134	1	60282262
sesquiterpenoid	2018	11_21134	1	60282262
citric acid	2019	11_10259	1	60841560
DG(18:2(9Z,12Z)/18:2(9Z,12Z)/0:0)	2019	11_10526	1	60969607
DG(16:0/18:2(9Z,12Z)/0:0)	2019	11_10526	1	60969607
glyCerol trinonadecanoate	2018	11_10294	1	61752648
beta-Sitosterol 3-O-beta-D-galactopyranoside	2019	12_30592	1	74052398
DG(14:0/20:3(8Z,11Z,14Z)/0:0)	2019	12_30592	1	74052398
Grain Protein	average	12_30592	1	74052398
Test Weight	average	12_30592	1	74052398
Coenzyme Q10	2019	12_30592	1	74052398
DG(15:0/20:1(11Z)/0:0)	2019	SCRI_RS_222748	1	74533594
Hydration Index	average	11_11293	1	76294035
sesquiterpenoid	2018	12_10235	1	108042404
DG(18:1(9Z)/22:2(13Z,16Z)/0:0)	2019	12_11301	1	147143136
Anethole	2019	12_31208	1	148023288
Hordenine	2019	12_31208	1	148023288
Extract	average	12_31208	1	148023288

N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	12_31208	1	148023288
arginine	2019	SCRI_RS_155439	1	148156867
PE(16:1(9Z)/16:1(9Z))	2018	SCRI_RS_118168	1	156295500
TG(20:4(5Z,8Z,11Z,14Z)/18:2(9Z,12Z)/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_189248	1	156504710
TG(14:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/o-18:0)	2019	SCRI_RS_189248	1	156504710
Cer(d18:2/18:1)	2019	11_11064	1	169102385
Stearidonic acid	2019	12_10506	1	170862950
BG	2019	12_10506	1	170862950
PE(14:0/18:2(9Z,12Z))	2019	SCRI_RS_151874	1	187171712
Plump	2019	SCRI_RS_151874	1	187171712
22-methyl-5z,9z-triacontadienoic acid	2018	SCRI_RS_148600	1	275442606
acetic acid	2019	SCRI_RS_198546	1	278348102
PA(O-16:0/22:0)	2019	12_30499	1	278381241
1,2-di-(9Z,12Z-octadecadienoyl)-3-(beta-D-galactosyl)-sn-glycerol	2019	SCRI_RS_145336	1	334653981
1,2-dioleoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_145336	1	334653981
1-18:2-2-16:0-monogalactosyldiacylglycerol	2019	SCRI_RS_145336	1	334653981
Height	average	SCRI_RS_145336	1	334653981
phenylalanine	2019	SCRI_RS_151894	1	342735416
DG(18:0/18:1(9Z)/0:0)	2019	12_31464	1	350981662
succinic acid	2019	12_31464	1	350981662
DG(16:0/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_120605	1	359793981
DG(18:1(9Z)/20:1(11Z)/0:0)	2019	SCRI_RS_120605	1	359793981
N-(2R-Hydroxytricosanoyl)-2S-amino-1,3S,4R-octadecanetriol	2019	SCRI_RS_120605	1	359793981
Cohibin C	2019	SCRI_RS_120605	1	359793981
Extract	2019	SCRI_RS_175487	1	361528416
Cer(d18:2/20:1)	2019	SCRI_RS_183588	1	363085015

LysoPC(22:0)	2019	12_10166	1	364575709
Leucine	2019	12_10166	1	364575709
DG(18:4(6Z,9Z,12Z,15Z)/18:1(9Z)/0:0)	2019	SCRI_RS_188360	1	380169600
20-Carboxy-leukotriene B6	2019	SCRI_RS_181239	1	384784187
(4S)-N-(2,3-dihydroxytetracosanoyl)-4-hydroxysphinganine	2019	SCRI_RS_181239	1	384784187
Histidine	2019	11_20990	1	388893493
N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	SCRI_RS_166168	1	412266002
TG(18:2(9Z,12Z)/18:2(9Z,12Z)/20:1(11Z))	2019	12_31319	1	419157263
TG(16:0/20:4(5Z,8Z,11Z,14Z)/20:4(5Z,8Z,11Z,14Z))	2018	SCRI_RS_197337	1	430320726
TG(18:2(9Z,12Z)/14:0/18:3(9Z,12Z,15Z))	2019	11_20844	1	430490473
PC(18:4(6Z,9Z,12Z,15Z)/16:1(9Z))	2018	12_10149	1	430923865
altrans-3,4-Didehydroretinoate	2019	SCRI_RS_173813	1	431011435
PA(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z))	2019	SCRI_RS_153450	1	435297408
Cer(D18:0/16:0)	2018	12_31511	1	441228305
TG(24:1(15Z)/20:2N6/O-18:0)	2018	SCRI_RS_127646	1	444606999
PG(18:0/18:0)	2018	SCRI_RS_201865	1	447280561
erucic acid	2018	SCRI_RS_201865	1	447280561
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	SCRI_RS_165886	1	456102025
Eriodictyol	2019	11_11105	1	458285252
DG(15:0/20:1(11Z)/0:0)	2019	SCRI_RS_141322	1	461025960
N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_4928	1	463020814
FAN	average	SCRI_RS_147203	2	313674
l-threonine	2018	SCRI_RS_147203	2	313674
(-)-Salsoline	2019	SCRI_RS_147203	2	313674
PG(18:0/18:0)	2018	SCRI_RS_175550	2	2119953
Ergocristine	2019	12_31446	2	2218132
PS(15:0/22:0)	2018	SCRI_RS_219333	2	2238515
BG	2019	SCRI_RS_219333	2	2238515

BG	average	SCRI_RS_219333	2	2238515
Hydration Index	average	SCRI_RS_219333	2	2238515
1-[(9Z)-hexadecenoyl]-2-acetysn-glycero-3-phosphocholine	2019	SCRI_RS_225720	2	2240079
Hydration Index	2019	SCRI_RS_225720	2	2240079
N1-trans-Feruloylagmatine	2019	SCRI_RS_174552	2	4611012
PC(20:0/24:1(15Z))	2018	SCRI_RS_186847	2	4637383
panaxynol	average	SCRI_RS_186847	2	4637383
PG(18:0/18:1(9Z))	2018	SCRI_RS_10642	2	4826489
Ergocristine	2019	12_30132	2	6841778
malic acid	2019	12_30132	2	6841778
4-Hydroxy-16,18-tritriacontanedione	2019	SCRI_RS_146936	2	7529743
TG(13:0/17:2(9Z,12Z)/17:2(9Z,12Z))[iso3]	2019	SCRI_RS_146936	2	7529743
n-tetradecanoyl-15-methylhexadecasphinganine	2018	SCRI_RS_184899	2	7593585
5-hydroxy-14,16-hentriacontanedione	2018	SCRI_RS_184899	2	7593585
lumequoylacetone	2018	SCRI_RS_184899	2	7593585
sesquiterpenoid	2018	SCRI_RS_184899	2	7593585
1-18:2-2-16:0-monogalactosyldiacylglycerol	2019	SCRI_RS_167823	2	7594390
DG(24:0/0:0/18:2n6)	2019	SCRI_RS_167823	2	7594390
1-amino-3-((24-[(3-amino-2-hydroxypropyl)amino]tetracosa-3,6,18,21-tetraen-1-yl)amino)propan-2-ol	2019	SCRI_RS_167823	2	7594390
arginine	2019	SCRI_RS_238263	2	7748598
4-Hydroxycinnamoylagmatine	2019	SCRI_RS_238263	2	7748598
TG(22:2(13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_168604	2	7777193
DT95	average	SCRI_RS_196276	2	8761644
3-decaprenyl-4,5-dihydroxybenzoate	2018	SCRI_RS_231057	2	9726690
Test Weight	2019	12_31497	2	10046815

2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	12_10777	2	22156317
TG(18:3(9Z,12Z,15Z)/16:0/18:3(9Z,12Z,15Z))	2019	SCRI_RS_205712	2	22437917
p-Mentha-1,3,8-triene	2019	SCRI_RS_205712	2	22437917
1,2-di-(9Z,12Z-octadecadienoyl)-3-(beta-D-galactosyl)-sn-glycerol	2019	BK_15	2	22952634
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	BK_15	2	22952634
Acetylcarnitine	2019	BK_14	2	22953027
Grain Protein	average	BK_14	2	22953027
Heading Date	average	BK_14	2	22953027
Height	average	BK_14	2	22953027
Maturity Date	average	BK_14	2	22953027
4-Pyridoxic acid	2019	BK_14	2	22953027
N-(2-hydroxytetracosanoyl)phytosphingosine	2019	11_21015	2	26072210
LysoPC(20:1(11Z))	2019	11_21015	2	26072210
Glucosylceramide (d18:1/9Z-18:1)	2019	SCRI_RS_170337	2	27081972
Cer(d18:2/18:1)	2019	SCRI_RS_170337	2	27081972
BG	2019	11_21261	2	29185036
BG	average	11_21261	2	29185036
Glutathione	2019	SCRI_RS_147371	2	30863157
TG(15:0/20:0/22:4(7Z,10Z,13Z,16Z))	2019	12_30748	2	38367726
2-Phenylethyl acetate	2019	11_10525	2	39974055
TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_174935	2	39977500
FAN	average	SCRI_RS_215080	2	40064925
N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	SCRI_RS_165427	2	40520273
Alpha-galactosylceramide	2019	11_10837	2	46100258
PC(22:0/24:1(15Z))	2018	SCRI_RS_171029	2	53287267

TG(21:0/17:0/17:0)	2018	SCRI_RS_171029	2	53287267
N6-Methyladenosine	2019	SCRI_RS_239231	2	72148870
benzenoid	2018	12_31127	2	80676114
(2E,11Z)-5-[5-(Methylthio)-4-penten-2-ynyl]-2-furanacrolein	2019	12_31127	2	80676114
grain fill	average	12_31127	2	80676114
threonine	2019	12_31127	2	80676114
Furaneol 4-glucoside	2019	12_31127	2	80676114
DG(16:0/18:3(9Z,12Z,15Z)/0:0)	2019	SCRI_RS_175065	2	88833828
TG(20:4(5Z,8Z,11Z,14Z)/18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[ISO6]	2018	11_10638	2	98344242
PS(O-18:0/20:3(8Z,11Z,14Z))	2019	SCRI_RS_199897	2	106008567
phenylalanine	2019	SCRI_RS_199897	2	106008567
TG(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_155546	2	115042186
PS(18:0/18:1(9Z))	2019	SCRI_RS_155546	2	115042186
Campesterol glucoside	2019	SCRI_RS_186443	2	116382055
LysoPC(24:0)	2019	SCRI_RS_206492	2	142369352
PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/21:0)	2018	HVNAM2	2	190163256
l-threonine	2018	HVNAM2	2	190163256
PA(O-20:0/19:0)	2019	11_20251	2	372375351
Plump	2019	11_20251	2	372375351
Albanin F	2019	SCRI_RS_192676	2	391028056
Heading Date	average	SCRI_RS_237688	2	394331986
Histidine	2019	SCRI_RS_30973	2	411560941
(4S)-N-(2,3-dihydroxytetracosanoyl)-4-hydroxysphinganine	2019	12_20861	2	435637430
Ergosterol	2019	12_20861	2	435637430
PGP(18:0/18:0)	2018	SCRI_RS_195938	2	438973590
PA(O-16:0/22:0)	2019	12_30224	2	448119774

ferulic acid	2018	SCRI_RS_175300	2	482357394
Test Weight	2019	12_30042	2	495293795
DG(18:4(6Z,9Z,12Z,15Z)/18:1(9Z)/0:0)	2019	11_11430	2	496133758
Acetylcarnitine	2019	11_11430	2	496133758
1-[(9Z)-hexadecenoyl]-2-acetysn-glycero-3-phosphocholine	2019	11_11430	2	496133758
Height	average	11_11430	2	496133758
4-Pyridoxic acid	2019	11_11430	2	496133758
Albanin F	2019	11_11430	2	496133758
PG(18:0/18:0)	2018	11_10436	2	499856846
%Germ@24	average	11_10436	2	499856846
Glycerol 2-(9Z,12Z-octadecadienoate) 1-hexadecanoate 3-O-[alpha-D-galactopyranosy(1->6)-beta-D-galactopyranoside]	2019	SCRI_RS_144776	2	508217323
DG(14:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	2019	12_11121	2	517832677
2-furoic acid	2019	SCRI_RS_162700	2	519165384
PC(14:0/16:0)	2018	SCRI_RS_12492	2	527221662
hexanoic acid	2019	SCRI_RS_198848	2	544510866
LysoPC(24:0)	2019	12_31021	2	544687358
grain fill	average	12_31021	2	544687358
1-18:2-2-16:0-monogalactosyldiacylglycerol	2019	12_10719	2	545230783
D-galactosyN-hexadecanoylsphinganine	2019	12_10719	2	545230783
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	12_10719	2	545230783
DG(18:4(6Z,9Z,12Z,15Z)/18:1(9Z)/0:0)	2019	SCRI_RS_156871	2	545903858
DG(14:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	2019	SCRI_RS_156871	2	545903858
%Germ@24	average	SCRI_RS_156871	2	545903858
DT95	average	SCRI_RS_156871	2	545903858
PC(14:0/14:0)	2018	SCRI_RS_16995	2	547322869
PE(16:1(9Z)/16:1(9Z))	2018	SCRI_RS_219568	2	548519980
DG(16:1(9Z)/16:1(9Z)/0:0)	2019	SCRI_RS_172667	2	548606944

DG(15:0/20:1(11Z)/0:0)	2019	SCRI_RS_207237	2	549468809
%Germ@24	average	11_10287	2	553315685
PC(16:0/14:0)	2019	SCRI_RS_159484	2	560967517
LysoPC(24:0)	2019	SCRI_RS_221763	2	560990132
1-o-linoleoyl-3-o-beta-d-galactopyranosyl-syn-glyCerol	2018	SCRI_RS_209622	2	561361042
fatty acyl	2018	SCRI_RS_135248	2	561510360
Palmitoleoyl Ethanolamide	2019	11_10263	2	574574101
TG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	12_31402	2	578937547
DP	average	11_10128	2	579471612
DP	2019	11_10128	2	579471612
FAN	average	11_10990	2	579747942
TG(13:0/17:2(9Z,12Z)/17:2(9Z,12Z))[iso3]	2019	SCRI_RS_15537	2	579988318
Cytidine	2019	SCRI_RS_610	2	581007862
l-threonine	2018	SCRI_RS_156220	2	581446086
n-(2-hydroxypentacosanoyl)-4-hydroxy-15-methylhexadecasphinganine-1-phosphocholine	2018	12_10739	2	582272018
(-)-Salsoline	2019	SCRI_RS_207327	2	583638330
dihydroimpranine	2018	11_10780	2	585204653
Cohibin A	2019	SCRI_RS_174214	2	585706440
TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_200033	2	588265648
malic acid	2019	SCRI_RS_162820	2	588630313
(E,E)-Piperlonguminine	2019	SCRI_RS_12934	2	589545779
N1-trans-Feruloylagmatine	2019	SCRI_RS_12934	2	589545779
PS(O-18:0/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_208901	2	589618904
Coenzyme Q9	2019	SCRI_RS_153811	2	589657722
PC(20:2(11Z,14Z)/24:1(15Z))	2018	SCRI_RS_201870	2	589699991

Ergocristine	2019	12_30310	2	590117175
Cer(d18:2/18:1)	2019	12_30310	2	590117175
TG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_116226	2	590118377
Campesterol 6'-hexadecanoylglucoside	2019	SCRI_RS_185506	2	590781115
TG(20:0/19:0/16:0)	2019	11_20366	2	592597275
PG(18:0/18:1(9Z))	2018	SCRI_RS_165373	2	592609313
altrans-3,4-Didehydroretinoate	2019	12_30351	2	595669919
PC(14:0/16:0)	2018	SCRI_RS_215471	2	603669339
3-(2,3-dihydroxyphenyl)propanoic acid	2019	11_10566	2	606908872
N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_226193	2	606925099
(-)-Salsoline	2019	SCRI_RS_226193	2	606925099
Histidine	2019	SCRI_RS_114673	2	609222527
Furaneol 4-glucoside	2019	SCRI_RS_114673	2	609222527
TG(18:2(9Z,12Z)/18:2(9Z,12Z)/20:1(11Z))	2019	SCRI_RS_188579	2	609408952
PC(20:2(11Z,14Z)/24:1(15Z))	2018	11_11380	2	610008174
DG(14:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	2019	SCRI_RS_161636	2	610510724
anileridine	2018	SCRI_RS_161636	2	610510724
altrans-3,4-Didehydroretinoate	2019	SCRI_RS_4691	2	613471142
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	12_31506	2	616374314
S. Protein	2019	12_31506	2	616374314
N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_109266	2	616638860
hexanoic acid	2019	SCRI_RS_236521	2	621377346
1,2-dioleoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_195051	2	621444548
DG(16:0/18:3(9Z,12Z,15Z)/0:0)	2019	SCRI_RS_195051	2	621444548
PE(16:0/18:1(11Z))	2018	SCRI_RS_231015	2	623527290
1-(sn-Glycero-3-phospho)-1D-myo-inositol	2019	SCRI_RS_161538	2	623547317

DG(14:0/22:5(7Z,10Z,13Z,16Z,19Z)/0:0)	2019	SCRI_RS_206020	2	623997597
DG(16:0/16:0/0:0)	2018	12_30378	2	625519439
1-18:2-2-16:0-monogalactosyldiacylglycerol	2019	12_30378	2	625519439
PC(P-18:1(11Z)/18:0)	2019	12_10103	3	2451580
TG(16:0/16:1(9Z)/16:1(9Z))	2019	12_31448	3	3345079
n-arachidonoylsphinganine	2018	11_21027	3	7608606
n-docosanoyl-4-hydroxysphinganine	2018	SCRI_RS_6682	3	8052637
Hydration Index	average	SCRI_RS_97417	3	10631754
DG(14:0/20:3(8Z,11Z,14Z)/0:0)	2019	SCRI_RS_197216	3	13538349
TG(14:0/16:1(9Z)/16:1(9Z))	2019	SCRI_RS_9614	3	14525926
TG(22:5(4Z,7Z,10Z,13Z,16Z)/O-18:0/22:5(7Z,10Z,13Z,16Z,19Z))	2018	11_20968	3	18472770
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	11_20968	3	18472770
DG(20:1(11Z)/22:4(7Z,10Z,13Z,16Z)/0:0)	2018	11_20794	3	18493771
22-methyl-5z,9z-triacontadienoic acid	2018	SCRI_RS_177084	3	19239390
N-(octadecanoyl)-4-hydroxyeicosasphinganine	2019	SCRI_RS_177084	3	19239390
PS(O-16:0/22:2(13Z,16Z))	2019	SCRI_RS_204148	3	20456724
PE(20:5(5Z,8Z,11Z,14Z,17Z)/16:0)	2019	SCRI_RS_204148	3	20456724
LysoPC(24:0)	2019	SCRI_RS_186444	3	20561744
LysoPC(22:0)	2019	SCRI_RS_186444	3	20561744
PG(18:0/18:0)	2018	11_10672	3	27352670
ferulic acid	2018	11_10672	3	27352670
Ceramide (d18:1/9Z-18:1)	2019	SCRI_RS_102837	3	29092420
1,2-dioleoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_170626	3	29117786
1-O-beta-D-Glucopyranosy2,3-di-O-palmitoylglycerol	2019	SCRI_RS_152682	3	29194466
TG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_11012	3	29431963
TG(14:0/14:1(9Z)/20:3n6)	2019	SCRI_RS_11012	3	29431963

PC(14:0/16:0)	2018	12_30953	3	30009622
TG(22:2(13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	12_30953	3	30009622
TG(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)/22:5(7Z,10Z,13Z,16Z,19Z))	2018	12_30953	3	30009622
TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	12_30953	3	30009622
TG(22:5(4Z,7Z,10Z,13Z,16Z)/O-18:0/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_154769	3	36348427
TG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/O-18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_154769	3	36348427
TG(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_154769	3	36348427
PC(14:0/16:0)	2018	11_10601	3	48244690
PE(18:0/18:2(9Z,12Z))	2018	11_10601	3	48244690
TG(19:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso3]	2019	11_10601	3	48244690
PC(16:0/14:0)	2019	11_10601	3	48244690
Cer(D18:0/16:0)	2018	SCRI_RS_204057	3	128957964
PE(15:0/18:2(9Z,12Z))	2018	11_20866	3	141790290
PC(18:2(9Z,12Z)/18:1(9Z))	2019	11_20866	3	141790290
PE(18:1(9Z)/18:1(9Z))	2019	11_20866	3	141790290
PA(O-20:0/19:0)	2019	11_20866	3	141790290
PE(18:1(9Z)/18:2(9Z,12Z))	2019	11_20866	3	141790290
LysoPC(20:1(11Z))	2019	11_20866	3	141790290
PC(14:1(9Z)/16:1(9Z))	2019	11_20866	3	141790290
LysoPC(20:2(11Z,14Z))	2019	11_20866	3	141790290
Height	average	SCRI_RS_128706	3	166101988
alanyl-tyrosine	2018	11_21062	3	207479427

PG(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2019	SCRI_RS_164254	3	268116654
panaxynol	average	11_10620	3	284171686
PS(18:0/16:0)	2018	SCRI_RS_238114	3	351449809
LysoPC(18:3(9Z,12Z,15Z))	2019	SCRI_RS_103211	3	351875014
LysoPC(20:1(11Z))	2019	SCRI_RS_103211	3	351875014
1-palmitoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_103211	3	351875014
LysoPC(20:2(11Z,14Z))	2019	SCRI_RS_103211	3	351875014
Cohibin A	2019	SCRI_RS_184523	3	353753949
Cer(D18:1/9Z-18:1)	2018	SCRI_RS_8664	3	360790868
PE(16:0/18:1(11Z))	2018	SCRI_RS_190876	3	366914110
N1-Acetylspermine	2019	SCRI_RS_190876	3	366914110
DG(18:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_205949	3	406778101
Glycerol 2-(9Z,12Z-octadecadienoate) 1-hexadecanoate 3-O-[alpha-D-galactopyranosy(1->6)-beta-D-galactopyranoside]	2019	SCRI_RS_205949	3	406778101
DG(16:0/18:1(9Z)/0:0)	2019	SCRI_RS_205949	3	406778101
DG(18:1(9Z)/18:1(9Z)/0:0)	2019	SCRI_RS_205949	3	406778101
succinic acid	2019	SCRI_RS_205949	3	406778101
Cer(D16:2(4E,6E)/20:0(2OH))	2018	11_11016	3	408666250
Cer(D18:0/16:0)	2018	SCRI_RS_173348	3	409933961
N6-Methyladenosine	2019	11_10005	3	418585785
PI(19:1(9Z)/21:0)	2019	11_10373	3	422460551
TG(16:0/20:4(5Z,8Z,11Z,14Z)/20:4(5Z,8Z,11Z,14Z))	2018	11_21502	3	425731450
fatty acyl	2018	SCRI_RS_232895	3	428742419
DG(14:0/22:5(7Z,10Z,13Z,16Z,19Z)/0:0)	2019	SCRI_RS_204491	3	431513217
3-decaprenyl-4,5-dihydroxybenzoate	2018	SCRI_RS_152472	3	432860936
BG	average	11_10276	3	434382534
PS(18:0/16:0)	2018	SCRI_RS_187887	3	434399946
Cohibin A	2019	SCRI_RS_221981	3	439078457

TG(18:2(9Z,12Z)/14:0/18:3(9Z,12Z,15Z))	2019	11_20362	3	441683549
TG(18:1(9Z)/24:1(15Z)/20:2n6)	2019	SCRI_RS_191130	3	444358378
anileridine	2018	SCRI_RS_149160	3	444658014
n-arachidonoylsphinganine	2018	11_11276	3	445413256
Phytosphingosine	2019	11_11276	3	445413256
PGP(18:0/18:0)	2018	12_30278	3	453392521
2-furoic acid	2019	SCRI_RS_234564	3	476967738
PS(18:0/20:4(5Z,8Z,11Z,14Z))	2019	12_11322	3	477449286
3-(2,3-dihydroxyphenyl)propanoic acid	2019	12_11322	3	477449286
arginine	2019	SCRI_RS_166119	3	478686508
Cer(D18:0/16:0)	2018	12_30640	3	484339567
TG(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)/20:5(5Z,8Z,11Z,14Z,17Z))	2019	12_30640	3	484339567
N-arachidonoylsphinganine	2019	SCRI_RS_120503	3	486677093
(4S)-N-(2,3-dihydroxytetracosanoyl)-4-hydroxysphinganine	2019	SCRI_RS_120503	3	486677093
Cer(d14:1(4E)/22:1(13Z)(2OH))	2019	SCRI_RS_120503	3	486677093
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	11_21513	3	487794674
TG(16:0/16:1(9Z)/16:1(9Z))	2019	11_11503	3	499054747
1-18:3-2-16:0-monogalactosyldiacylglycerol	2019	12_10100	3	499340339
TG(16:1(9Z)/16:1(9Z)/18:2(9Z,12Z))	2019	SCRI_RS_13434	3	499558301
TG(14:0/16:1(9Z)/16:1(9Z))	2019	SCRI_RS_13434	3	499558301
PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/21:0)	2018	SCRI_RS_166189	3	500075940
TG(13:0/17:2(9Z,12Z)/17:2(9Z,12Z))[iso3]	2019	SCRI_RS_166189	3	500075940
PS(15:0/22:0)	2018	SCRI_RS_198594	3	502023919
hypoxanthine	2018	SCRI_RS_198594	3	502023919
leu-tyr	2018	SCRI_RS_198594	3	502023919
serine	2018	SCRI_RS_198594	3	502023919
leu-tyr	2018	SCRI_RS_159125	3	502053245

d-pantothenoyl-l-cysteine	2018	SCRI_RS_192360	3	503631370
PE(15:0/18:2(9Z,12Z))	2018	12_30926	3	503705171
N1-Acetylspermine	2019	SCRI_RS_181071	3	507907871
1-O-beta-D-Glucopyranosy2,3-di-O-palmitoylglycerol	2019	SCRI_RS_153414	3	507936503
Isoproterenol	2019	SCRI_RS_165334	3	509048832
DG(16:0/16:0/0:0)	2018	11_10867	3	509179435
DP	average	12_31238	3	510562766
LysoPC(22:0)	2019	SCRI_RS_237846	3	517282199
fatty acyl	2018	12_30973	3	521726856
1-(2E,6E,10E-phytatrienyl)-2-(2E,6E10E-phytatrienyl)-sn-glycero-3-phosphocholine	2019	SCRI_RS_175220	3	535392908
n-(2-hydroxyhexacosanoyl)-1-o-beta-d-glucosyl-15-methylhexadecasphing-4-enine	2018	11_21272	3	537926944
3-Dehydrosphinganine	2019	SCRI_RS_184593	3	537931027
1-O-[(E)-hexadecen-1-yl]-sn-glycerol	2019	SCRI_RS_135156	3	537988822
Cytidine	2019	SCRI_RS_135156	3	537988822
Acetylcarnitine	2019	12_31500	3	541484316
cholesteryl 6-O-palmitoybeta-D-galactoside	2019	12_30921	3	542919332
3-Dehydrosphinganine	2019	SCRI_RS_157113	3	543178437
PC(16:0/16:1(9Z))	2019	SCRI_RS_110995	3	543238196
N-hexacosanoylphytosphingosine	2019	SCRI_RS_154449	3	543253427
Hydration Index	average	SCRI_RS_1435	3	550749370
Hydration Index	2019	SCRI_RS_1435	3	550749370
glucose	2018	SCRI_RS_192891	3	552141738
maltol	2018	SCRI_RS_203164	3	555914016
acetic acid	2019	SCRI_RS_220221	3	557997849
PC(P-18:1(11Z)/18:0)	2019	SCRI_RS_160338	3	558823263
PI(20:3(8Z,11Z,14Z)/15:1(9Z))	2019	SCRI_RS_153022	3	559148664

Grain Protein	average	SCRI_RS_163112	4	82008
hypoxanthine	2018	SCRI_RS_164381	4	226252
TG(24:1(15Z)/20:2N6/O-18:0)	2018	SCRI_RS_13428	4	255886
phenylalanine	2019	SCRI_RS_146989	4	8976337
Cer(D16:2(4E,6E)/20:0(2OH))	2018	11_11136	4	12995896
S. Protein	average	SCRI_RS_127657	4	13198440
FAN	average	12_30793	4	14844591
FAN	2019	12_30793	4	14844591
S. Protein	2019	12_30793	4	14844591
N6-Methyladenosine	2019	SCRI_RS_125487	4	16444779
Cytidine	2019	SCRI_RS_125487	4	16444779
Phytosphingosine	2019	12_10860	4	35410588
N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	SCRI_RS_6956	4	35818039
Acetylcarnitine	2019	SCRI_RS_183399	4	38700940
fatty acyl	2018	SCRI_RS_233908	4	135946120
Cer(D18:1/9Z-18:1)	2018	SCRI_RS_10216	4	154963325
Cer(D18:0/16:0)	2018	SCRI_RS_194316	4	199165374
PG(O-20:0/18:1(9Z))	2019	SCRI_RS_194316	4	199165374
DP	average	SCRI_RS_204804	4	200413120
Campesterol glucoside	2019	11_10261	4	243711464
(-)-Salsoline	2019	SCRI_RS_208732	4	244116264
PGP(18:0/18:0)	2018	11_20135	4	245152706
PA(22:4(7Z,10Z,13Z,16Z)/21:0)	2019	SCRI_RS_144983	4	256111106
PE(18:0/18:2(9Z,12Z))	2018	12_30684	4	310285254
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	11_11042	4	345138902
Furaneol 4-glucoside	2019	11_11042	4	345138902
TG(15:0/20:0/22:4(7Z,10Z,13Z,16Z))	2019	SCRI_RS_188329	4	347888657

DP	average	12_30824	4	347980358
DP	2019	12_30824	4	347980358
alanyl-tyrosine	2018	11_11114	4	349971038
N-Hexadecanoylpyrrolidine	2019	11_11114	4	349971038
benzenoid	2018	SCRI_RS_208828	4	403145342
LysoPC(22:0)	2019	11_21481	4	405868724
DG(16:0/18:1(9Z)/0:0)	2019	12_31462	4	406057681
DG(18:1(9Z)/18:1(9Z)/0:0)	2019	12_31462	4	406057681
PS(18:0/20:4(5Z,8Z,11Z,14Z))	2019	12_31462	4	406057681
succinic acid	2019	12_31462	4	406057681
Glucosylceramide (d18:1/9Z-18:1)	2019	SCRI_RS_113574	4	419028092
TG(18:2(9Z,12Z)/18:2(9Z,12Z)/20:1(11Z))	2019	SCRI_RS_189180	4	419345932
%Germ@24	average	SCRI_RS_189180	4	419345932
Cytidine	2019	SCRI_RS_235738	4	428122652
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	11_21273	4	428189476
1,2-dioleoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_195216	4	428271516
2-O-oleoy3-O-palmitoy1-O-alpha-D-galactosysn-glycerol	2019	SCRI_RS_195216	4	428271516
acetic acid	2019	SCRI_RS_195216	4	428271516
glucose	2018	11_20906	4	440804399
PS(O-18:0/20:3(8Z,11Z,14Z))	2019	SCRI_RS_136648	4	445694674
n-docosanoyl-4-hydroxysphinganine	2018	11_11224	4	449541604
N-Palmitoylsphingosine	2019	12_30693	4	467446220
PS(18:0/20:4(5Z,8Z,11Z,14Z))	2019	SCRI_RS_195017	4	474276352
beta-D-glucosyN-(octadecanoyl)sphinganine	2019	SCRI_RS_106435	4	481545219
Citicoline	2019	11_10829	4	481668270
3-Dehydrosphinganine	2019	SCRI_RS_179438	4	490098533
Alpha-Linolenic acid	2019	SCRI_RS_141214	4	503381662
TG(13:0/17:2(9Z,12Z)/17:2(9Z,12Z))[iso3]	2019	SCRI_RS_172072	4	505609010

BG	2019	SCRI_RS_25685	4	510238845
BG	average	SCRI_RS_25685	4	510238845
LysoPC(18:2(9Z,12Z))	2019	12_10666	4	520944734
TG(18:2(9Z,12Z)/18:2(9Z,12Z)/20:1(11Z))	2019	11_20454	4	521654181
LysoPC(16:0)	2019	12_30988	4	521981872
panaxynol	average	SCRI_RS_216855	4	533451076
Ceramide (d18:1/9Z-18:1)	2019	SCRI_RS_198507	4	534364397
TG(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_142792	4	536282693
Ergosterol	2019	SCRI_RS_149609	4	536442590
Glycerol 2-(9Z,12Z-octadecadienoate) 1-hexadecanoate 3-O-[alpha-D-galactopyranosy(1->6)-beta-D-galactopyranoside]	2019	SCRI_RS_85607	4	536633757
2-O-oleoy3-O-palmitoy1-O-alpha-D-galactosysn-glycerol	2019	SCRI_RS_85607	4	536633757
ferulic acid	2018	SCRI_RS_85607	4	536633757
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	12_31422	4	536788334
N-(1-Deoxy-1-fructosyl)phenylalanine	2019	12_31422	4	536788334
DP	average	SCRI_RS_119390	4	536919346
TG(21:0/17:0/17:0)	2018	SCRI_RS_145343	4	537052956
4-Pyridoxic acid	2019	11_10269	4	540213561
Glucosylceramide (d18:1/9Z-18:1)	2019	SCRI_RS_88710	5	1070915
Hydration Index	2019	SCRI_RS_88710	5	1070915
methionine	2019	12_30976	5	2378070
Kernel Hardness	average	SCRI_RS_31797	5	2492193
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_236640	5	3208280
PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/21:0)	2018	SCRI_RS_7720	5	13203937
PC(22:0/24:1(15Z))	2018	SCRI_RS_221631	5	13364102
N-(2R-Hydroxypentacosanoyl)-2S-amino-1,3S,4R-octadecanetriol	2019	SCRI_RS_221631	5	13364102

Hydration Index	2019	SCRI_RS_85089	5	13384391
Alpha-galactosylceramide	2019	11_11048	5	13385212
PS(O-18:0/20:3(8Z,11Z,14Z))	2019	11_11048	5	13385212
2-furoic acid	2019	11_21324	5	16084450
PS(18:0/16:0)	2018	SCRI_RS_156016	5	20006513
PC(20:2(11Z,14Z)/24:1(15Z))	2018	SCRI_RS_144042	5	20029194
LysoPC(14:0)	2018	SCRI_RS_220165	5	22038083
n-(2-hydroxynonadecanoyl)-1-o-beta-d-glucosyl-4-hydroxy-15-methylhexadecasphinganine	2018	SCRI_RS_220165	5	22038083
(4S)-N-(2,3-dihydroxytetracosanoyl)-4-hydroxysphinganine	2019	12_30410	5	22698931
PS(15:0/22:0)	2018	11_20987	5	42136556
PC(20:2(11Z,14Z)/24:1(15Z))	2018	SCRI_RS_176142	5	46107344
maltol	2018	SCRI_RS_176142	5	46107344
serine	2018	SCRI_RS_145348	5	46450909
TG(14:0/14:1(9Z)/20:3n6)	2019	SCRI_RS_91468	5	72234091
TG(14:0/16:1(9Z)/16:1(9Z))	2019	SCRI_RS_91468	5	72234091
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	12_30709	5	77199535
1-[(9Z)-hexadecenoyl]-2-acetysn-glycero-3-phosphocholine	2019	SCRI_RS_238123	5	78996326
DG(16:0/18:3(9Z,12Z,15Z)/0:0)	2019	SCRI_RS_238123	5	78996326
benzenoid	2018	SCRI_RS_232700	5	83491889
N-Palmitoylsphingosine	2019	12_31517	5	107508885
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	SCRI_RS_148870	5	121677020
PC(16:1(9Z)/16:1(9Z))	2019	12_31423	5	124241382
acetic acid	2019	11_10856	5	124481462
Cohibin A	2019	SCRI_RS_13395	5	179237212
1,1'-(1,4-dihydro-4-nonyl-3,5-pyridinediyl)bis[1-dodecanone]	2018	11_11128	5	180004496
PS(18:0/18:1(9Z))	2019	SCRI_RS_13353	5	299736048
N6-Methyladenosine	2019	SCRI_RS_231244	5	300297889

DT95	average	12_30080	5	360321650
BG	2019	11_20441	5	360532441
1-(2E,6E,10E-phytatrienyl)-2-(2E,6E10E-phytatrienyl)-sn-glycero-3-phosphocholine	2019	SCRI_RS_223699	5	389968124
TG(22:0/20:2n6/18:2(9Z,12Z))	2019	SCRI_RS_7112	5	390014023
PA(22:4(7Z,10Z,13Z,16Z)/21:0)	2019	SCRI_RS_221999	5	399797033
PS(18:0/18:1(9Z))	2019	SCRI_RS_221999	5	399797033
PI-Cer(d20:1/16:0)	2019	SCRI_RS_166296	5	419360784
DG(18:1(9Z)/18:2(9Z,12Z)/0:0)	2019	11_20736	5	421668932
DG(16:0/18:1(9Z)/0:0)	2019	11_20736	5	421668932
DG(18:1(9Z)/18:1(9Z)/0:0)	2019	11_20736	5	421668932
TG(15:0/20:1(11Z)/20:3n6)	2019	11_20736	5	421668932
%Germ@24	average	11_20736	5	421668932
Cer(D16:2(4E,6E)/20:0(2OH))	2018	SCRI_RS_196566	5	435528704
tryptamine	2018	11_10578	5	440387169
5-Hydroxy-14,16-hentriacontanedione	2019	SCRI_RS_153937	5	443589127
Test Weight	2019	11_11473	5	446234350
4-Guanidinobutanoic acid	2019	12_31279	5	456757795
Hydration Index	2019	SCRI_RS_143508	5	460732040
N-Hexadecanoylpyrrolidine	2019	SCRI_RS_143508	5	460732040
alanine	2018	11_21061	5	464172004
DG(18:1(9Z)/18:1(9Z)/0:0)	2019	12_30848	5	464543429
20-Carboxy-leukotriene B7	2019	12_30848	5	464543429
succinic acid	2019	12_30848	5	464543429
PI(20:3(8Z,11Z,14Z)/15:1(9Z))	2019	11_20805	5	465928813
DG(16:1(9Z)/16:1(9Z)/0:0)	2019	SCRI_RS_142217	5	470800813
N-(1-Deoxy-1-fructosyl)tyrosine	2019	12_30456	5	472292808
xanthine	2019	12_30456	5	472292808

S. Protein	2019	SCRI_RS_172024	5	473857019
Cohibin A	2019	SCRI_RS_13766	5	478661720
TG(18:1(9Z)/24:1(15Z)/20:2n6)	2019	SCRI_RS_240005	5	480875877
Palmitoleoyl Ethanolamide	2019	SCRI_RS_240005	5	480875877
Alpha-galactosylceramide	2019	SCRI_RS_136812	5	483668804
DG(18:1(9Z)/20:1(11Z)/0:0)	2019	SCRI_RS_212515	5	484176635
PE(20:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2019	SCRI_RS_182540	5	484354652
Extract	2019	SCRI_RS_236782	5	484533523
tryptamine	2018	11_20629	5	485915800
BG	2019	SCRI_RS_162590	5	489392206
TG(22:2(13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_167563	5	489642942
1-Octen-3-yl primeveroside	2019	11_20003	5	490226506
1-18:2-2-16:0-monogalactosyldiacylglycerol	2019	SCRI_RS_189371	5	490254358
N1-Acetylspermine	2019	SCRI_RS_189371	5	490254358
Isoproterenol	2019	SCRI_RS_189371	5	490254358
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	SCRI_RS_1501	5	492266452
Eriodictyol	2019	SCRI_RS_148402	5	497654292
TG(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_201248	5	497666412
TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_133602	5	498866453
TG(20:4(5Z,8Z,11Z,14Z)/18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[ISO6]	2018	SCRI_RS_161471	5	500888726
TG(18:2(9Z,12Z)/18:2(9Z,12Z)/20:1(11Z))	2019	SCRI_RS_175672	5	504810469
phenylalanine	2019	12_30556	5	506407644
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_168544	5	506604145

2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_175848	5	506701484
TG(22:5(4Z,7Z,10Z,13Z,16Z)/O-18:0/22:5(7Z,10Z,13Z,16Z,19Z))	2018	12_30833	5	507598408
Campesterol glucoside	2019	SCRI_RS_9182	5	509906132
Oleamide	2019	SCRI_RS_6203	5	510419962
Oleamide	2019	SCRI_RS_188572	5	510452072
N6-Methyladenosine	2019	SCRI_RS_105701	5	510647025
20-Carboxy-leukotriene B5	2019	SCRI_RS_236610	5	511810526
Extract	average	12_30580	5	512594107
PS(18:0/16:0)	2018	12_30400	5	515895168
alanyl-tyrosine	2018	12_31206	5	517434588
Furaneol 4-glucoside	2019	12_31206	5	517434588
N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_182353	5	517543545
DG(18:2(9Z,12Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_161614	5	517568728
threonine	2019	SCRI_RS_161614	5	517568728
Oleamide	2019	11_20791	5	517610241
2-furoic acid	2019	11_20791	5	517610241
TG(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_193063	5	518161778
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_197361	5	518440943
Plump	2019	12_30642	5	523522386
Cer(D16:2(4E,6E)/20:0(2OH))	2018	SCRI_RS_230034	5	524074090
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	11_10536	5	525665263
S. Protein	average	SCRI_RS_155688	5	526164378
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	11_20560	5	528199546

%Germ@24	average	SCRI_RS_221599	5	531398201
Cer(d14:2(4E,6E)/20:1(11Z))	2019	12_30162	5	532059355
Cer(d18:2/20:1)	2019	SCRI_RS_160975	5	535086892
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_132308	5	535122244
Alpha-Linolenic acid	2019	SCRI_RS_157557	5	536704582
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_206883	5	536827509
4-Hydroxystyrene	2019	SCRI_RS_220005	5	537006650
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_220002	5	537006655
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_354	5	537009559
N-Hexadecanoylpyrrolidine	2019	SCRI_RS_354	5	537009559
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_12491	5	537010780
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_236958	5	537122694
PE(16:0/18:1(11Z))	2018	11_20546	5	537634679
PS(18:0/18:1(9Z))	2019	12_10769	5	538638758
PI-Cer(d20:1/16:0)	2019	12_10769	5	538638758
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	12_20867	5	539759052
Glutathione	2019	12_20867	5	539759052
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	12_21290	5	539893392
2,2-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)decanedioic acid	2019	SCRI_RS_165290	5	540646361
TG(18:2(9Z,12Z)/14:0/18:3(9Z,12Z,15Z))	2019	SCRI_RS_4965	5	541695642
TG(16:1(9Z)/16:1(9Z)/18:2(9Z,12Z))	2019	SCRI_RS_4965	5	541695642

TG(14:0/14:1(9Z)/20:3n6)	2019	SCRI_RS_4965	5	541695642
TG(14:0/16:1(9Z)/16:1(9Z))	2019	SCRI_RS_4965	5	541695642
TG(14:0/18:4(6Z,9Z,12Z,15Z)/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_4965	5	541695642
n-(2-hydroxypentacosanoyl)-4-hydroxy-15-methylhexadecasphinganine-1-phosphocholine	2018	SCRI_RS_186984	5	543308641
Campesterol 6'-hexadecanoylglucoside	2019	SCRI_RS_235652	5	543465028
Grain Protein	average	12_31239	5	543653338
Campesterol 6'-hexadecanoylglucoside	2019	12_31239	5	543653338
DG(14:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_145394	5	546494056
n-tetradecanoyl-15-methylhexadecasphinganine	2018	SCRI_RS_198007	5	550956088
[6]-Dehydrogingerdione	2019	SCRI_RS_157762	5	551046712
1-amino-3-((24-[(3-amino-2-hydroxypropyl)amino]tetracosa-3,6,18,21-tetraen-1-yl)amino)propan-2-ol	2019	SCRI_RS_216726	5	551181390
TG(13:0/17:2(9Z,12Z)/17:2(9Z,12Z))[iso3]	2019	SCRI_RS_4753	5	551346503
PS(O-18:0/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_4753	5	551346503
N-(octadecanoyl)-4-hydroxyeicosasphinganine	2019	SCRI_RS_224854	5	556238395
Glutathione	2019	SCRI_RS_224854	5	556238395
TG(16:0/16:1(9Z)/16:1(9Z))	2019	12_30382	5	556254899
methionine	2019	12_30502	5	559311588
PI(19:1(9Z)/21:0)	2019	SCRI_RS_169941	6	166441
PA(20:2(11Z,14Z)/22:0)	2019	SCRI_RS_123065	6	249993
TG(21:0/17:0/17:0)	2018	SCRI_RS_162771	6	277368
PI(20:3(8Z,11Z,14Z)/15:1(9Z))	2019	SCRI_RS_162771	6	277368
DG(16:0/18:3(9Z,12Z,15Z)/0:0)	2019	11_10635	6	4164080
Leucine	2019	11_20212	6	4572863
3-(2,3-dihydroxyphenyl)propanoic acid	2019	11_20212	6	4572863
Test Weight	2019	SCRI_RS_194023	6	4859440
Extract	average	SCRI_RS_203036	6	9496882

4-Pyridoxic acid	2019	SCRI_RS_231372	6	15750639
TG(14:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/o-18:0)	2019	SCRI_RS_189122	6	15826773
TG(22:5(4Z,7Z,10Z,13Z,16Z)/O-18:0/22:5(7Z,10Z,13Z,16Z,19Z))	2018	SCRI_RS_201251	6	16263422
S. Protein	average	SCRI_RS_201251	6	16263422
1,1'-(1,4-dihydro-4-nonyl-3,5-pyridinediyl)bis[1-dodecanone]	2018	SCRI_RS_157552	6	24914066
DG(14:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_147090	6	24924157
PA(22:4(7Z,10Z,13Z,16Z)/21:0)	2019	SCRI_RS_154121	6	27049467
PI(20:3(8Z,11Z,14Z)/15:1(9Z))	2019	11_10494	6	36052487
1-18:1-2-16:0-digalactosyldiacylglycerol	2019	SCRI_RS_210025	6	52091868
Glucosylceramide (d18:1/9Z-18:1)	2019	SCRI_RS_236452	6	52408805
acetic acid	2019	SCRI_RS_151282	6	58557976
TG(19:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[iso6]	2019	SCRI_RS_169829	6	61849314
PG(18:0/18:1(9Z))	2018	SCRI_RS_140158	6	61942571
PC(14:0/18:3(9Z,12Z,15Z))	2019	11_10355	6	62199877
1-[4-[[methy[(1-methylimidazo2-yl)methyl]amino]methyl]piperidino]propan-2-ol	2019	11_10003	6	64424020
PE(22:0/20:0)	2019	SCRI_RS_147342	6	77453664
cholesteryl 6-O-palmitoybeta-D-galactoside	2019	SCRI_RS_130605	6	110797359
citric acid	2019	SCRI_RS_130605	6	110797359
1-[(9Z)-hexadecenoyl]-2-acetysn-glycero-3-phosphocholine	2019	SCRI_RS_159136	6	121565309
20:0-Glc-Sitosterol	2019	SCRI_RS_236545	6	167469750
2-Phenylethyl acetate	2019	SCRI_RS_11588	6	167587124
Grain Protein	average	SCRI_RS_124850	6	248553523
TG(21:0/17:0/17:0)	2018	SCRI_RS_237887	6	269181459
TG(12:0/13:0/20:5(5Z,8Z,11Z,14Z,17Z))[iso6]	2019	SCRI_RS_195914	6	275319471
DG(16:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_195914	6	275319471

DG(14:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	2019	SCRI_RS_195914	6	275319471
DG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0)	2019	SCRI_RS_195914	6	275319471
Test Weight	average	SCRI_RS_143415	6	276564176
DG(14:0/22:5(7Z,10Z,13Z,16Z,19Z)/0:0)	2019	SCRI_RS_168964	6	294633391
Cer(d18:2/18:1)	2019	SCRI_RS_168964	6	294633391
TG(14:0/18:4(6Z,9Z,12Z,15Z)/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_137464	6	302469243
hypoxanthine	2018	SCRI_RS_138001	6	303025304
DG(16:0/18:2(9Z,12Z)/0:0)	2019	11_10513	6	331945677
TG(20:4(5Z,8Z,11Z,14Z)/20:4(5Z,8Z,11Z,14Z)/21:0)[iso3]	2019	11_10189	6	363744451
DG(14:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0)	2019	SCRI_RS_4697	6	366080889
TG(18:1(9Z)/24:1(15Z)/20:2n6)	2019	11_20058	6	405412438
2-Phenylethyl acetate	2019	12_30346	6	405535548
TG(22:0/20:2n6/18:2(9Z,12Z))	2019	11_21310	6	408107840
S. Protein	2019	SCRI_RS_182648	6	415546434
FAN	average	SCRI_RS_139937	6	442755740
FAN	2019	SCRI_RS_139937	6	442755740
N-(1-Deoxy-1-fructosyl)phenylalanine	2019	SCRI_RS_139937	6	442755740
1,1'-(1,4-dihydro-4-nonyl-3,5-pyridinediyl)bis[1-dodecanone]	2018	SCRI_RS_133948	6	446564518
%Germ@24	average	11_11349	6	464515091
PC(18:0/18:1(11Z))	2019	12_30908	6	469899818
TG(16:0/16:1(9Z)/16:1(9Z))	2019	SCRI_RS_205971	6	473495581
18:3-Glc-Stigmasterol	2019	SCRI_RS_153435	6	474975324
Campesterol glucoside	2019	11_20746	6	475280396
N-(2-hydroxytetracosanoyl)phytosphingosine	2019	11_11458	6	475821139
TG(15:0/18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	2019	11_11458	6	475821139
Test Weight	2019	11_11458	6	475821139
beta-D-Galactosy1,4-beta-D-glucosylceramide	2019	SCRI_RS_206976	6	484841173

n-(2-hydroxyhexacosanoyl)-1-o-beta-d-glucosyl-15-methylhexadecasphing-4-enine	2018	SCRI_RS_207284	6	485256998
Citicoline	2019	SCRI_RS_170674	6	494998053
1-(2E,6E,10E-phytatrienyl)-2-(2E,6E10E-phytatrienyl)-sn-glycero-3-phosphocholine	2019	SCRI_RS_170674	6	494998053
Cer(t18:0/16:0)	2019	SCRI_RS_167845	6	498173284
Extract	average	SCRI_RS_167845	6	498173284
Hydration Index	average	SCRI_RS_167845	6	498173284
Hydration Index	2019	SCRI_RS_167845	6	498173284
PE(15:0/18:2(9Z,12Z))	2018	SCRI_RS_200907	6	500176281
maltol	2018	11_10496	6	504498512
G6P	2018	12_30626	6	504788309
PC(22:0/24:1(15Z))	2018	SCRI_RS_190647	6	505188917
MGDG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	2019	11_20996	6	505676547
sucrose	2018	11_11294	6	505690213
fatty acyl	2018	SCRI_RS_131929	6	508165432
PS(18:0/18:1(9Z))	2019	SCRI_RS_124224	6	508832820
PA(O-16:0/22:0)	2019	SCRI_RS_95857	6	508945787
Test Weight	average	SCRI_RS_151574	6	511783718
1-(2E,6E,10E-phytatrienyl)-2-(2E,6E10E-phytatrienyl)-sn-glycero-3-phosphocholine	2019	11_20467	6	517956165
1-18:1-2-16:0-digalactosyldiacylglycerol	2019	12_30734	6	523493069
benzenoid	2018	SCRI_RS_124549	6	524155278
Acetylcarnitine	2019	12_31053	6	526044552
Citicoline	2019	12_31053	6	526044552
PGP(18:0/18:0)	2018	SCRI_RS_138295	6	529202031
Kernel Diameter	average	SCRI_RS_138295	6	529202031
Extract	average	SCRI_RS_138887	6	529290986

PE(18:0/18:1(9Z))	2019	SCRI_RS_238352	6	530550125
Cer(d18:2/18:1)	2019	SCRI_RS_203457	6	531431137
Cohibin A	2019	SCRI_RS_152414	6	532278322
hexanoic acid	2019	SCRI_RS_10811	6	537953359
PS(18:0/20:4(5Z,8Z,11Z,14Z))	2019	12_30956	6	538736718
Cohibin A	2019	12_30956	6	538736718
n-hexacosanoylsphinganine-1-phosphocholine	2018	SCRI_RS_99965	7	219427
PS(18:0/18:1(9Z))	2019	SCRI_RS_156125	7	2086390
PG(19:0/20:0)	2019	SCRI_RS_14174	7	2755480
GLYCerOL 1-(9Z-OCTADECENOATE) 2-TETRADECANOATE 3-PHOSPHATE	2018	SCRI_RS_8079	7	3200298
2-Phenylethyl acetate	2019	11_21307	7	4641071
DG(16:0/16:0/0:0)	2018	SCRI_RS_207095	7	5055208
Grain Protein	average	12_31173	7	8026234
methyl 5-(cyanomethoxy)-2-methy1-benzofuran-3-carboxylate	2019	SCRI_RS_230149	7	8620900
Anethole	2019	SCRI_RS_159196	7	9191677
1-palmitoy3-alpha-D-galactosysn-glycerol	2019	11_11495	7	9195323
5-hydroxy-14,16-hentriacontanedione	2018	SCRI_RS_156158	7	9905672
FAN	2019	SCRI_RS_152931	7	10772499
N6-Methyladenosine	2019	11_20225	7	13749931
N-(2R-Hydroxypentacosanoyl)-2S-amino-1,3S,4R-octadecanetriol	2019	SCRI_RS_139563	7	15555365
PC(20:2(11Z,14Z)/18:1(11Z))	2019	SCRI_RS_174720	7	15980246
LysoPC(18:1(9Z))	2019	SCRI_RS_194866	7	16109088
LysoPC(20:1(11Z))	2019	SCRI_RS_126944	7	16322156
LysoPC(18:1(9Z))	2019	11_21050	7	20607446
Grain Protein	average	11_10025	7	21034181
Plump	2019	11_10025	7	21034181

oligosaccharide	2019	SCRI_RS_182092	7	27581567
n-(2-hydroxypentacosanoyl)-4-hydroxy-15-methylhexadecasphinganine-1-phosphocholine	2018	SCRI_RS_47235	7	27582843
oligosaccharide	2019	12_30329	7	28053284
Plump	2019	SCRI_RS_203781	7	28793381
DG(18:2(9Z,12Z)/20:1(11Z)/0:0)	2019	SCRI_RS_202811	7	33674327
DG(16:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_202811	7	33674327
N-Palmitoylsphingosine	2019	SCRI_RS_154007	7	33715863
PC(20:0/24:1(15Z))	2018	SCRI_RS_114631	7	33775957
Cer(d18:2/20:1)	2019	SCRI_RS_114631	7	33775957
Alpha-Linolenic acid	2019	SCRI_RS_114631	7	33775957
Test Weight	2019	12_30702	7	34210385
3-Dehydrosphinganine	2019	SCRI_RS_175424	7	35544855
TG(14:1(9Z)/22:5(7Z,10Z,13Z,16Z,19Z)/o-18:0)	2019	SCRI_RS_106456	7	37004609
PC(18:0/18:1(11Z))	2019	12_30065	7	50627857
Maturity Date	average	SCRI_RS_223017	7	52278917
TG(18:3(9Z,12Z,15Z)/16:0/18:3(9Z,12Z,15Z))	2019	SCRI_RS_155795	7	53565826
1-amino-3-((24-[(3-amino-2-hydroxypropyl)amino]tetracosa-3,6,18,21-tetraen-1-yl)amino)propan-2-ol	2019	SCRI_RS_188133	7	61769654
N-Hexadecanoylpyrrolidine	2019	SCRI_RS_188133	7	61769654
GLYCerOL 1-(9Z-OCTADECENOATE) 2-TETRADECANOATE 3-PHOSPHATE	2018	SCRI_RS_7797	7	65469742
TG(14:0/14:1(9Z)/20:3n6)	2019	SCRI_RS_162708	7	65473816
methionine	2019	SCRI_RS_162708	7	65473816
Test Weight	average	SCRI_RS_230487	7	67038413
Cer(d18:2/18:1)	2019	SCRI_RS_202653	7	67348822
beta-Sitosterol 3-O-beta-D-galactopyranoside	2019	SCRI_RS_138111	7	74611988
erucic acid	2018	SCRI_RS_158126	7	163047477

PS(18:0/16:0)	2018	12_11492	7	163407418
DG(16:0/18:2(9Z,12Z)/0:0)	2019	12_11492	7	163407418
DT95	average	12_11492	7	163407418
anileridine	2018	12_11492	7	163407418
Isoproterenol	2019	12_11492	7	163407418
5-Hydroxy-14,16-hentriacontanedione	2019	SCRI_RS_219081	7	164423442
Albanin F	2019	SCRI_RS_108830	7	165590991
PE(16:1(9Z)/16:1(9Z))	2018	SCRI_RS_169639	7	189038722
(2E,11Z)-5-[5-(Methylthio)-4-penten-2-ynyl]-2-furanacrolein	2019	SCRI_RS_168543	7	249862865
N-(1-Deoxy-1-fructosyl)tyrosine	2019	SCRI_RS_168543	7	249862865
(-)-Salsoline	2019	SCRI_RS_168543	7	249862865
xanthine	2019	SCRI_RS_168543	7	249862865
Cer(d18:2/20:1)	2019	SCRI_RS_132879	7	250105521
LysoPC(20:1(11Z))	2019	SCRI_RS_164251	7	255118703
Histidine	2019	SCRI_RS_157035	7	291013740
N-(2-hydroxytetracosanoyl)phytosphingosine	2019	SCRI_RS_131323	7	310541440
PG(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2019	SCRI_RS_131323	7	310541440
N-(1-Deoxy-1-fructosyl)tyrosine	2019	11_11461	7	331923108
Histidine	2019	11_11461	7	331923108
PG(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2019	SCRI_RS_175164	7	416924299
TG(24:1(15Z)/20:2N6/O-18:0)	2018	11_20880	7	419934775
PE(16:0/18:1(11Z))	2018	11_20205	7	457591831
xanthine	2019	12_30565	7	471969965
Cer(D16:2(4E,6E)/20:0(2OH))	2018	SCRI_RS_194085	7	474994662
glyCerol trinonadecanoate	2018	SCRI_RS_219581	7	485724943
DG(16:1(9Z)/16:1(9Z)/0:0)	2019	SCRI_RS_181727	7	488536392
1-(sn-Glycero-3-phospho)-1D-myo-inositol	2019	SCRI_RS_181727	7	488536392
PS(O-18:0/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_194841	7	515456696

20-Carboxy-leukotriene B4	2019	SCRI_RS_134640	7	519404099
N-(2-hydroxytetracosanoyl)phytosphingosine	2019	SCRI_RS_134640	7	519404099
succinic acid	2019	SCRI_RS_134640	7	519404099
methionine	2019	SCRI_RS_200021	7	520408967
PGP(18:0/18:0)	2018	SCRI_RS_146640	7	525202350
Plump	average	12_10897	7	542561236
Plump	2019	12_10897	7	542561236
BG	2019	SCRI_RS_219260	7	555969465
Phytosphingosine	2019	SCRI_RS_205991	7	558272440
Cer(d18:2/20:1)	2019	SCRI_RS_205991	7	558272440
N-(2-hydroxytetracosanoyl)phytosphingosine	2019	SCRI_RS_205991	7	558272440
PA(O-20:0/19:0)	2019	SCRI_RS_205991	7	558272440
N-hexacosanoylphytosphingosine	2019	SCRI_RS_205991	7	558272440
PC(14:0/18:3(9Z,12Z,15Z))	2019	SCRI_RS_205991	7	558272440
3-Dehydrosphinganine	2019	SCRI_RS_205991	7	558272440
Albanin F	2019	12_31254	7	562507741
Hydration Index	average	SCRI_RS_189107	7	562630313
Hydration Index	2019	SCRI_RS_189107	7	562630313
PS(O-18:0/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_149573	7	570542954
Cer(d14:1(4E)/22:1(13Z)(2OH))	2019	SCRI_RS_4520	7	575311772
4-Hydroxycinnamoylagmatine	2019	SCRI_RS_235853	7	576600971
1-palmitoy3-alpha-D-galactosysn-glycerol	2019	SCRI_RS_193330	7	583247871
Pfaffic acid	2019	SCRI_RS_134057	7	583484206
DG(18:2(9Z,12Z)/20:1(11Z)/0:0)	2019	12_10973	7	583738054
2-furoic acid	2019	12_10973	7	583738054
TG(22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)/20:5(5Z,8Z,11Z,14Z,17Z))	2019	SCRI_RS_162972	7	583927265
DG(14:1(9Z)/18:2(9Z,12Z)/0:0)	2019	SCRI_RS_138598	7	586929539

TG(22:5(7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2018	11_11440	7	587312711
22-methyl-5z,9z-triacontadienoic acid	2018	SCRI_RS_200086	7	590414367
N-(2-Hydroxyisobutyl)-2,4,8,10,12-tetradecapentaenamide	2019	11_20586	7	595266320
Hordenine	2019	12_30974	7	595557735
Ergosterol	2019	SCRI_RS_175551	7	596756289
TG(20:4(5Z,8Z,11Z,14Z)/18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))[ISO6]	2018	SCRI_RS_193505	7	599252522
PC(18:0/18:1(11Z))	2019	SCRI_RS_157208	7	599255356
PG(18:0/18:1(9Z))	2018	SCRI_RS_167617	7	600219184
1,2-di-(9Z,12Z-octadecadienoyl)-3-(beta-D-galactosyl)-sn-glycerol	2019	SCRI_RS_200397	7	600292675
PC(24:0/24:0)	2018	11_10174	7	600567366

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CHAPTER FIVE

CONCLUSION

Barley grown for malt is a high-value crop for American farmers. While breeders have been successful in selecting for improved malt quality and quality stability across environments, screening for these traits is cost-prohibitive and only reserved for elite germplasm. To aid in breeding efforts many research groups have identified malt quality QTL for marker assisted selection. However, there is a lack of knowledge and QTL for traits impacting hydration of the endosperm and metabolic profiles.

To bridge this knowledge gap, we first used a GWAS population to map malt quality and hydration index QTL as reported in chapter two. This allowed us to identify six QTL related to hydration index and separate them into two groups, impacting malt quality or impacted by seed size. For two of the hydration index QTL (qHYI1H and qHYI3H.a) increased hydration index was associated with decreased seed size, which is undesirable for malt houses because it reduces the amount of available starch in the grain. At the third seed size QTL (qHYI3H.b) a larger softer seed improved hydration index and is worth further investigation to see how it impacts extract levels. Two other hydration index QTL (qHYI2H and qHYI6H) co-located with quality traits, where increasing hydration index improved malt quality. The last hydration index QTL (qHYI7H) didn't map with any other traits. qHYI2H, qHYI6H, and qHYI7H all have the potential to improve malt quality and increase the sustainability of the malting process by ensuring only two water immersions are needed and reducing the water used.

To further interrogate the genetics of endosperm hydration a bi-parental mapping population was evaluated for interactions between hydration index and dormancy to determine if

increased endosperm hydration QTL can overcome the negative effects of dormancy on malt quality. Our findings indicate that the dormant allele from SD2 reduced hydration index and malt quality. However, haplotypes with varying levels of positive hydration index alleles indicate malt quality of dormant lines to be acceptable. This information will allow breeders to release dormant lines that are resistant to pre-harvest sprouting and meet malt quality standards. These varieties will in turn help producers by reducing the risk of sprout damage which can turn their high-value malt barley crop ($7/bu) into a feed barley crop ($3/bu).

There has also been interest from malt houses and breeders to understand the genetics of malt flavor. In chapter four, metabolic analysis of metabolites in a GWAS population resulted in the identification of metabolite QTL. While we were able to map 827 QTL across two years some of the flavor metabolites of note are amino acids, saccharides, and maltol. Amino acids are flavor precursors and an important food source for yeast. It was surprising that only a few amino acid QTL mapped near the hordein genes which code for most of the storage protein in barley. Some of the amino acid QTL that did not map with the hordein genes had candidate genes for protease inhibitors, peptidases, amino acid biosynthesis, and agronomic traits. All of the saccharide and maltol QTL mapped to QTL associated with increased modification. Importantly, maltol also mapped with its precursor metabolites (sugars and amino acids) providing good targets for breeders to increase maltol levels in malt. All these metabolite QTL could be useful in further research to better understand metabolite variability during germination but also show the complexities of breeding for malt flavor with most of the metabolite QTL not collocating with malt quality QTL.

Collectively this work provides breeders with new tools to improve malting barley. Selection for hydration of the endosperm will not only ensure consistent quality but could help reduce the amount of water needed in malting. When positive hydration index alleles are stacked with dormant alleles malt quality standards can be met with resistance to preharvest sprouting, reducing the risk of growing malt barley for producers and ensuring high-quality grain is available for malt houses. The amino acids, which are made available through degradation of the endosperm storage proteins, are critical to beer quality, impacting yeast nutrition and the production of flavor and flavor stability. Amino acid QTLs can empower understanding of the protein degradation process and be deployed to improve beer quality. Malt barley breeders will also deploy the maltol QTLs to impact flavor.

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