EFFECTS OF A BARLEY CHROMOSOME 6H GRAIN PROTEIN QTL ON
AGRONOMIC TRAITS, MALT QUALITY TRAITS, AND STOMATAL CONTROL
UNDER TWO IRRIGATION AND NITROGEN FERTILIZATION REGIMES

by

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Cereal grain protein concentration (GPC) is an important quality parameter, with high GPC desirable when grain is used for food or feed, while low (typically below 12-13%) GPC is needed when barley grain is used for malting. A major QTL controlling grain protein has previously been identified on barley chromosome 6H. Based on the comparison with a co-linear region on wheat chromosome 6B, the functional status of a gene coding for a NAC transcription factor (HvNAM-1) is responsible for controlling whole-plant senescence, nutrient remobilization from leaves to developing grains, and grain protein concentration. In this context, the purpose of this study was the analysis of the influence of a low- vs. a high-grain protein allele at this locus on barley agronomic and malting quality parameters in different genetic backgrounds. Integration of the low-protein allele stably lowered GPC by 1.6-1.8%, in both years and across all combinations of genetic backgrounds and management practices. Lines with the low-protein allele matured 1.4 to 2.5 days later, dependent on management, and had stably lower malt protein and diastatic power. Effects on additional agronomic and malt quality parameters including yield, test weight, percentage of plump kernels, free amino nitrogen and α-amylase activity were subtler and depended on the genetic background tested. Our data also provide important information on the influence of soil N and water availability on malt quality characteristics. Overall, use of the chromosome 6H low-grain protein allele stably lowered grain and malt protein levels without important negative influences on any of the tested agronomic and malt quality parameters, indicating its usefulness in the development of new malting barley germplasm.

The studied chromosome 6H GPC QTL also controls expression of a gene coding for a glycine-rich RNA-binding protein (HvGR-RBP1) that may improve drought tolerance. Stomatal conductance and canopy thermal imaging assays did not show consistent effects of the allelic state of the GPC QTL on stomatal control. However, our data showed important correlations between stomatal conductance, canopy temperature depression and agronomic parameters including yield, demonstrating the value of the performed assays for variety selection and breeding.
CHAPTER ONE

LITERATURE REVIEW

Introduction

Barley, *Hordeum vulgare* L., is one of the oldest and most important field crops, likely because barley is adapted to cool and short growing seasons, drought and salinity tolerance as well as marginal soil production (Zohary, Hopf, & Weiss, 2012). Another strength of barley is its long history as the preferred grain for malt production.

Barley is currently the 5\textsuperscript{th} most important crop worldwide in terms of acreage planted (FAOSTAT, accessed at http://faostat.fao.org/site/291/default.aspx). However, the quality of land used for barley production is often marginal in comparison with land used for higher producing crops.

In the United States, barley is grown on over 3,000,000 acres annually. Montana alone makes up about 30\% of national barley acres (USDA, 2016). Though barley’s field acreage is substantial and growing in Montana, its production occurs increasingly under dryland conditions where it is subject to harsher summers and under increased drought stress. Aside from decreased yield, this is not a problem for barley produced for feed or human consumption. Though barley is produced for feed and human consumption in many places around the world, the US grows barley primarily for use as malt for alcohol production. In Montana’s 2014 field season, 66.7\% of barley was intended to be used as malt, 13.5\% was planted for forage, 14.8\% for feed, and 5\% was planted for other purposes ("Montana Barley Varieties 2014," 2014). Growing competition for premium
malt barley contracts drives the science behind making more reliable, and higher quality barley cultivars.

**Origins of Barley as a Crop**

Barley (*Hordeum vulgare* L.) is a short-season self-pollinating diploid with 2n=14 chromosomes. Barley is in the family *Poaceae* and the subfamily *Pooideae*. It was domesticated from its wild relative, *H. spontaneum*, approximately 10,000 years ago in the central Fertile Crescent Israel-Jordan region (Badr et al., 2000).

Barley’s importance in the U.S. has been overshadowed by other crops in recent years, but researchers have long been interested in its protein characteristics and use for malt.

**Barley Grain Composition**

**Overview of Grain Composition**

Evers and Millar (2002) provide a useful breakdown of the barley caryopsis. They describe a starchy endosperm as the primary seed component making up 76.3% of the total caryopsis mass, a living aleurone layer that makes up 4.8% of the mass, an embryo consisting of an embryonic axis and scutellum that contribute 1.7% and 1.3%, respectively. The grain is surrounded by a pericarp and testa which are 2.9% of the weight and the hull which is 13% of the weight. Barley’s cell walls are also rich in mixed-linkage β-glucans (Fincher & Stone, 1986). These values are subject to change
with barley improvement; however, it is generally accepted that barley extraction recovers much of the endosperm component during malting.

**Starch Storage**

The barley endosperm component serves as an energy source for the growing embryo. The endosperm is a non-living component of which the primary constituents are granulated starch, followed by hordein storage proteins, which provides nitrogen and sulfur during germination (Holopainen-Mantila, 2015).

Barley undergoes a double fertilization like all angiosperms. Mogensen (1982) describes this as two male gametes entering the embryo sac through the pollen tube into the intercellular space between the egg apparatus and the central cell; one fusing with the egg while the other fuses with the two central cell nuclei to form the first (triploid) endosperm nucleus. Barley rarely outcrosses as fertilization occurs while the ear is still protected in the stem/boot.

The starchy endosperm begins development after pollination and divides into ~70,000 endosperm cells by approximately 14 days after pollination when cell divisions cease (Brown, Lemmon, & Olsen, 1994; Brunori, Forino, Frediani, & Ruberti, 1993; Cochrane & Duffus, 1981). Once these cells are formed accumulation of starch granules will occur until maturity at approximately 40 days past anthesis (Bosnes, Weideman, & Olsen, 1992).
Proteins

Barley storage proteins can be broken down into two primary groups based on the extraction fractioning of Osborne (1895), called the globulins and the prolamins.

The barley globulin protein fraction, is found primarily in the barley aleurone layer (Yupsanis, Burgess, Jackson, & Shewry, 1990). Globulins are soluble in dilute salt solutions (P. R. Shewry & Halford, 2002).

The primary storage proteins in barley are called the hordeins, which are in the prolamin group (Kreis, Forde, Rahman, Miflin, & Shewry, 1985). The hordein fraction of barley makes up 30 to 50% of the total grain protein and is found in the endosperm (Kirkman, Shewry, & Miflin, 1982; Qi, Zhang, & Zhou, 2006; P. R. Shewry, Franklin, Parmar, Smith, & Miflin, 1983; Yupsanis et al., 1990). Hordeins are again subset into groups: ‘γ’, ‘B’, ‘C’ and ‘D’ based on SDS-PAGE separation (Køie, Ingversen, Andersen, Doll, & Eggum, 1976; P. Shewry, Kreis, Parmar, Lew, & Kasarda, 1985). The ‘B’ hordeins are the largest subcomponent followed by the ‘C’ hordeins making up 70-90% and 10-30% respectively (P. Shewry et al., 1985). High nitrogen increases the C hordeins disproportionately in comparison to the other fractions (Kirkman et al., 1982).

Hordeins are synthesized in the rough endoplasmic reticulum accumulate as protein bodies in the endosperm during mid to late grain fill (Matthews & Miflin, 1980; Qi et al., 2006; Rahman, Shewry, & Miflin, 1982). The monomeric protein β-amylase has a molecular weight in the same range as ‘C’ hordeins, and their accumulation rates are linked (Giese, Andersen, & Doll, 1983; Giese & Hejgaard, 1984; Qi et al., 2006; Ziegler,
The enzymatic activity of β-amylase in combination with α-amylase contribute to the malting parameter ‘diastatic power’.

**Malt Production**

Barley has a long history of malt production. The process has one simple outcome which is the conversion of the endosperm into sugars which are extractable. The sugars are made available through a process called malting. The malting process is a three-part process consisting of steeping, germinating, and kilning (outlined by Burger and LaBerge (1985)).

The steeping step consists of imbibing the grain to bring it up to a suitable moisture content so germination can begin. This is a multiday process in which malt is often submerged and aerated, or submerged and drained many times to allow oxygenation of the imbibing grain.

The germination step is conducted to activate the seeds’ metabolism, to break down storage proteins in the endosperm and prepare the grain for starch extraction. This is done by aerating the imbibed grain and maintaining its moisture content in a controlled environment for about four days. During this process, hydrolytic enzymes including amylases, proteases and glucanases are secreted by the scutellum and aleurone layers of the now metabolically active caryopsis; these enzymes partially degrade the endosperm cell walls, releasing β-glucans, and (partially) degrade storage proteins and starch (Burger & LaBerge, 1985). This natural process which has been used by humans, is how a germinating seedling prepares to access its stored energy to begin growth. This is where
the process is then stopped to prevent the growing embryo from utilizing too much endosperm reserves, the developing seedling is killed in the final step of the malting process.

Kilning is a heating process in which the endosperm is killed preserving most of the starch within the caryopsis for extraction. This process is done by drying the grain with warm dry aeration at temperatures which slowly increase to ~85 °C, over a 24-hour period. This process brings the grain moisture down to 3-5% and the dried malt can be stored or used for starch extraction.

Barley’s wide use in malting comes from the ease with which this process can quickly and evenly prepare the endosperm for conversion into sugars. The final process outlined by Burger and LaBerge (1985) in extraction of the starch are mashing and lautering. The mashing process consists of steeping the ground malt, called mash, to activate enzymes and extract the starch. The mash is heated to ~69 °C to encourage enzymes to continue starch conversion. After the starch has been sufficiently converted, the mash is then heated to 80 °C to inactivate enzymes.

The final step is lautering, in which the malt is filtered through the hulls of the malt grain. The final product is called the wort and is used in beer production.

Barley Chromosome 6H Grain Protein Concentration QTL

The barley chromosome 6H grain protein concentration (GPC) QTL was identified in the 6-row type barley cultivar ‘Karl’. The variety Karl was developed by
USDA-ARS and the Idaho Agricultural Experiment Station through a cross of ‘Traill’//‘Good Delta’/‘Everest’/3/Trail (Wesenberg et al., 1976).

The newly released 6-row barley variety, Karl, was quickly recognized for its low and stable grain protein levels and its improved malt extract levels (Burger, Wesenberg, Carden, & Pawlisch, 1979). It was also pointed out by Burger that none of Karl’s parents, Good Delta, Everest or Traill, carried the low protein phenotype and that it was most likely caused by outcrossing, transgressive segregation, a mutation, or an error in the pedigree (Burger et al., 1979).

In the following decade, Dailey, Peterson, and Osborn (1988) reported that Karl was deficient in hordeins, but had normal levels of other endosperm protein fractions. Hordeins, named after the genus, are the primary endosperm storage proteins in barley. Through protein rebalancing, Karl’s lack of hordeins component high in proline and glutamine, is replaced by storage proteins which have higher lysine levels; therefore, the remaining grain protein is higher in lysine. Dailey et al. (1988) concluded that Karl’s “low-hordein character” was due to the low levels of its hordein mRNA pool, caused by transcriptional or post-transcriptional controls.

In the early 1990’s, Weston et. al., (1993) compared two low-protein cultivars that had uncommonly low protein levels, a trait which they suspected came from the parent Karl, and two commonly grown malting cultivars. Their study focused on evaluating primarily the differences in grain protein content, yield, and malt characteristics across five fertility levels and two planting dates. They reported no significant effects caused by planting dates, but that the Karl type low-GPC lines
maintained lower grain protein levels across nitrogen treatments. They also suggested that Karl-type lines had a slightly diminished yield as compared to their control cultivars (Weston, Horsley, Schwarz, & Goos, 1993).

In the late 1990’s researchers began to map QTLs for protein in malting barley. These studies reported QTLs for protein and grain nitrogen concentration on every barley chromosome (Bezant, Laurie, Pratchett, Chojecki, & Kearsey, 1997; Oziel, Hayes, Chen, & Jones, 1996; Powell et al., 1997; See, Kanazin, Kephart, & Blake, 2002). The barley chromosome 6H QTL being analyzed in this research was identified from a GPC mapping population at Montana State University (Mickelson et al., 2003; See et al., 2002). Specifically, a 146-member population of recombinant inbred lines was derived from a cross between ‘Karl’ and variety ‘Lewis’, a commonly used two-row malting variety with higher grain protein levels (Hockett et al., 1985), and grain protein QTLs were identified through molecular marker analysis and mapping (See et al., 2002). This study found that lines carrying a chromosome 6H allele, linked to the markers HVM74 and ABG458, from the Karl parent had on average 1.3% less GPC than lines with the Lewis allele. Investigating the locus further, Jukanti et al. (2008) generated near-isogenic lines from the mapping population in both the Karl and Lewis backgrounds. They found that the expression of many genes was controlled by the region including the chromosome 6H QTL in the Karl and Lewis backgrounds. Several of them were strongly (>10-fold) differentially expressed in near-isogenic lines carrying the Karl- vs. Lewis-type allele at the studied locus.
These genes included a glycine-rich RNA-binding protein (HvGR-RBP1) that shares approximately 65% sequence identity with the Arabidopsis GRP7 protein (Lacerenza, Parrott, & Fischer, 2010). HvGR-RBP1 expression levels are increased approximately >45-fold in the high-grain protein germplasm (Jukanti et al., 2008; Tripet et al., 2014).

**HvNAM-1, Whole-Plant Senescence and Grain Protein Concentration**

Karl’s low-grain protein trait is most likely due to variation in the HvNAM-1 gene, which was shown to be orthologous to durum wheat TiNAM-B1 (Assaf Distelfeld et al., 2008). NAM-B1 has been shown to impact senescence timing in wheat (Uauy, Brevis, & Dubcovsky, 2006) and verified through RNA interference (RNAi) in hexaploid wheat by reducing the transcript levels of all NAM copies, which resulted in lines reaching maturity more than 30 days after the control lines (Uauy, Distelfeld, Fahima, Blechl, & Dubcovsky, 2006). The NAM genes are thought to act as transcriptional regulators of multiple processes during leaf senescence (Uauy, Distelfeld, et al., 2006).

The Gpc-B1 allele from wild emmer was also introgressed into modern wheat varieties, which have a non-functioning allele. Grain protein levels were increased in the resulting germplasm, while grain yield was not effected (Brevis & Dubcovsky, 2010).

Previous work from the authors’ laboratory has demonstrated that lines containing the low-GPC allele from Karl on barley chromosome 6H GPC QTL are later-senescing when compared to their NILs, a similar phenotype to NAM knockdowns in wheat. They also showed slower degradation of leaf proteins (including Rubisco) and of chlorophylls,
and decreased amino acid levels in the leaves (Heidlebaugh et al., 2008). Lines containing the low-GPC allele are also slower to begin storage protein accumulation in developing kernels and ultimately have lower GPC in mature kernels (Heidlebaugh et al., 2008; Jukanti & Fischer, 2008; Jukanti et al., 2008).

**HvGR-RBP1 and Drought Tolerance**

An additional gene of interest at the chromosome 6H protein QTL codes for Glycine-Rich RNA-Binding Protein 1 (HvGR-RBP1; see above). The Barley Genome Sequencing Project (Mascher et al., 2017) has demonstrated that *HvNAM-1* and *HvGR-RBP1* are linked, with a distance of ~5.4 cM between the two genes. This finding most likely explains expression of *HvGR-RBP1* linked to the allelic state of the GPC locus (*HvNAM-1*), with high expression of *HvGR-RBP1* associated with high grain protein and early senescence in germplasm previously investigated by the authors’ lab (Jukanti et al., 2008).

Research in *Arabidopsis* has shown that *Arabidopsis* glycine-rich RNA-binding proteins (*AtGRP7*) plays an important role in regulating floral transition, and that its expression is upregulated during whole-plant senescence (Gepstein et al., 2003; Streitner et al., 2008). In previous studies, rice plants expressing *AtGRP7* have been shown to have improved recovery rates after a drought stress and enhanced grain yields after recovering from a drought stress (Yang et al., 2014). Importantly, *AtGRP7* is abundantly expressed in stomata (guard cells), and has been shown to influence stomatal opening and closing in response to stress treatments (Kim et al., 2008). Based on these findings, germplasm with
enhanced expression of AtGRP7 or its orthologs in other species may be more drought-tolerant, a particularly valuable trait for crops growing under dryland conditions.

Barley Management

Management decisions also impact grain quality traits like protein and yield. Nitrogen is a highly managed nutrient in field crops. Early use centered around increasing grain yield, but it was quickly linked to increases in grain protein concentration in malting barley (Lauer & Partridge, 1990; Lejeune & Parker, 1954). Increases in grain yield however pushed research for a greater understanding of the interactions of nitrogen and malt quality.

Reisenauer and Dickson (1961) showed that while increased nitrogen did increase yield, the kernel size decreased, while also increasing malt and wort nitrogen. Increased nitrogen rates also increased GPC, yield, soluble wort nitrogen, diastatic power and α-amylase activity, and decreased kernel weight, kernel plumpness, and fine-grind malt extract (Weston et al., 1993). Oscarsson, Andersson, Aman, Olofsson, and Jonsson (1998) also evaluated the effect of nitrogen fertilizer treatments on starch content, protein and β-glucan; their results indicated that increased nitrogen caused an increase in yield and grain protein, in addition to an increase in β-glucan during one of their two trial years. These results indicate that excessive nitrogen can easily lead to unacceptable malt quality.
The goals for malt barley are different from typical yield-optimized field crops. There are specific quality requirements for the grain and the crop is sensitive to both varietal selection and field management. This is important as producers facing rejection for barley malt quality experience economic losses.

A novel allele originally identified in the barley variety ‘Karl’ may help to optimize some malt quality parameters. The \textit{HvNAM-1} gene has been shown to influence maturity date and grain protein content, with later maturity (caused by the Karl-type allele) associated with lower grain protein, as needed for malting variety development. The \textit{HvGR-RBP1} gene could potentially improve barley drought tolerance. Both traits are highly important, and need thorough evaluation under field conditions, including varied nitrogen and water availability.
CHAPTER TWO

EFFECT OF A BARLEY CHROMOSOME 6H GRAIN PROTEIN QTL ON AGRONOMIC AND MALT QUALITY PARAMETERS UNDER TWO IRRIGATION AND NITROGEN FERTILIZATION REGIMES

Introduction

Barley (*Hordeum vulgare* L.) has long been recognized as the preferred grain for malt production. This is due in part to the ease by which its starchy endosperm can be converted into sugars. In this process, starch becomes more accessible due to degradation of the endosperm through the action of amylases (Burger & LaBerge, 1985). The resulting malt sugars are then extracted through a mashing process so they can be used by yeast during the production of alcoholic beverages.

In barley, the primary component of the endosperm is granulated starch, followed by hordein storage proteins for use as reserve nitrogen and sulfur in germination (Holopainen-Mantila, 2015). Unfortunately, grain is often unfit for malt production when some of these non-starch constituents exceed acceptable levels. Rejected malt grain is sold primarily as feed, with a substantial loss to farmers. For this reason, growing malt constitutes a risk compared to other grain crops. Improved germplasm and crop management could mitigate growers risk by providing more stable results under varying environments.
The American Malting Barley Association (AMBA) and the USDA Cereal Crop Research Unit provide guidance on acceptable grain quality characteristics. Their guidance focuses on 19 factors in the categories: (1) Barley Agronomic Factors, (2) Malt Factors, (3) Measures of Malt Modification, (4) Congress Wort, and (5) Malt Enzymes ("Malting Barley Breeding Guidelines Ideal Commercial Malt Criteria," 2017). For this research, we have focused on the barley agronomic factors: grain protein content (GPC), maturity date, grain yield, grain test weight, grain plumpness, tiller count, plant height, harvest index, and the malt traits: malt protein content, malt color, soluble wort protein, grain fine grind extract, turbidity, free amino nitrogen (FAN), α-amylase, β-glucan levels, and diastatic power (DP).

The primary trait for which malting barley is often rejected at the grain elevator is GPC. AMBA requirements for two-row malt stipulate GPC should be under 13% for adjunct brewers and under 12% for all malt brewers. For this reason, there has been interest in utilizing genetics which lead to lower protein levels. Management is also an important factor in understanding how to optimally farm malting barley and a lot of research has focused on fertilizer use and management (Oscarsson et al., 1998; Reisenauer & Dickson, 1961).

To address high rejection levels, previous research has identified a chromosome 6H quantitative trait locus (QTL) which controls grain protein levels under different environmental and management conditions called HvNAM-1 (Emebiri, 2015; See et al., 2002).
Previous work from this research group has demonstrated that lines containing the chromosome 6H GPC allele from the variety ‘Karl’ are lower in GPC and later-senescing when compared to near-isogenic lines (NIL) carrying the ‘Lewis’ type allele (Heidlebaugh et al., 2008; Jukanti & Fischer, 2008; Jukanti et al., 2008); they also showed slower degradation of leaf proteins and chlorophylls, and decreased leaf amino acid levels, leading to slower protein accumulation in developing kernels (Heidlebaugh et al., 2008).

In addition, a previous study found that the Karl-type barley was deficient in hordeins, the main storage protein fraction, and that the deficiency was caused by a decreased hordein mRNA pool which could be controlled by transcriptional and/or posttranscriptional regulation (Dailey et al., 1988).

While the *HvNAM-1* impact on GPC has been widely studied, and the QTL’s impact on some malt characteristics has been evaluated, an exhaustive malt quality study has not been performed to evaluate the impact of the QTL on all malt quality characteristics. In addition, this QTL’s effect on malt quality has not been tested under both dryland and irrigated conditions.

This research aims to show the effect of this chromosome 6H QTL on the broader spectrum of malt quality traits with continued interest in testing nitrogen fertilization levels under both dryland and irrigated locations.
Materials and Methods

Plant Material

To analyze the influence of the chromosome 6H grain protein QTL on agronomic and malt quality parameters, this study used two pairs of near-isogenic barley varieties/lines designated ‘Lewis’/‘21_7, and ‘Karl’/’10_11. These near-isogenic lines (NILs), in the Lewis and Karl backgrounds, were created by backcrossing the high-GPC locus from the Lewis background into the Karl background creating the line 10_11, and backcrossing the Karl low-GPC locus into the Lewis background creating the line 21_7 four times (Jukanti et al., 2008).

However, neither Lewis or Karl are current malt lines in Montana. Therefore, we also tested two additional families from the ‘Hockett’ and ‘Amsterdam’ backgrounds were also used because they varied at the QTL of interest. The variety Hockett contains the high-GPC allele and the related lines MT124673, MT124128, MT090182, and MT090190 contain the low-GPC allele. The variety Amsterdam contains the high-GPC allele and the related lines MT124027, and MT124071 contain the low-GPC allele.

Thus, the varieties and lines Amsterdam, Hockett, 10_11, and Lewis, all contained the chromosome 6H high-protein allele. These lines will henceforth be referred to as the high-GPC lines. The varieties/lines MT090182, MT090190, MT124027, MT124071, MT124673, MT124128, Karl, and 21_7 all contained the low-GPC allele. These lines will henceforth be referred to as the low-GPC lines. The pedigree of lines included is shown in Figure 2.1.
Figure 2.1. Plant material pedigree indicating estimated percent of pedigree obtained from each parent.

Experimental Design

The experiment was planted at the Arthur Post Research Farm in Bozeman, Montana, United States (45°40’40.78 N, 111°09’07.14 W) during the summers of 2016 and 2017, and at the Western Triangle Agricultural Research Station in Conrad, Montana, United States (48°18’26.05 N, 111°55’29.24 W) during the 2017 field season. Post Farm field experiments were planted on May 6th in 2016 and May 4th in 2017. The Western Triangle Field experiments were planted on May 3rd for dryland and May 11th for irrigated experiments.

At the Post Farm field experiments, irrigated and dryland plots were grown 0.2 km from each other in 2016 and 0.02 km (20 m) in the 2017 season. Both years included
a nitrogen fertilizer treatment split-plot randomized complete block design for both locations in 2017 where the main plots were Nitrogen treatment and the subplots were the genotypes. The dryland and irrigated locations at the Western Triangle station were planted approximately 0.4 km from each other. The 2016 dry and irrigated experiments were plant as randomized complete blocks with separate but adjacent experiments for the Nitrogen treatments.

In each season, field soil samples were analyzed for total nitrogen content by AGVISE Laboratories (Benson, MN) on a field level in 2016 and a replication level in 2017. Nitrogen application was performed in each individual split-plot to increase field nitrogen to the desired level. Nitrogen fertilizer, in the form of urea (46-0-0), was top dressed after planting but before a rainfall or irrigation event to ensure that nitrogen was not lost to the atmosphere, to achieve the desired soil nitrogen treatment.

The nitrogen treatment consisted of two levels, a normal and a high nitrogen level which was recommended rate plus 56 kg/ha. The normal nitrogen application was calculated using the Montana Barley Production Guide (McVay, Burrows, Jones, Wanner, & Menalled, 2009) calculation for malting barley nitrogen application

\[
\frac{(61.77 \text{ grams } N \times \text{ Expected Yield in kg})}{\text{hectare}}
\]

At the 2016 Post Farm experiment, the estimated rate was 112 kg/ha for the normal treatment and 168 kg/ha for the high treatment for both the dryland and irrigated treatment. The 2017 Post Farm experiment used a normal rate of 97.5 kg/ha, and a high rate of 168 kg/ha for both the dryland and irrigated treatment. The 2017 Western Triangle experiment used a different rate for dryland and irrigated locations. The dryland plots were applied with 56 kg/ha for
the normal rate and 112 kg/ha for the high rate. The irrigated plots were applied with 112 kg/ha for the normal rate and 168 kg/ha for the high rate.

The experiments were planted in 3-row plots (1.2x3.65 m) in the 2016 field season and in 4-row plots (1.2x5.48 m) during the 2017 field season. The seeding rate used was 5.9 g/m² in the dryland plots and was 7.9 g/m² in the irrigated plots.

Post Farm plots collected 119 mm of rainfall from May to July during the 2016 season and during the same time in 2017 the plots received 124.5 mm. Rainfall at the Western Triangle location amounted to 89.9 mm (from May to July 2017) (Table 2.1). All plots had adequate pre-season rainfall to reach soil field capacity.

Approximately 150 mm of irrigation was added to the irrigated plots over three events throughout the season to ensure that plots did not dry below the barley wilting point. Irrigation was stopped in the first week of July. The 2016 Post Farm fields were harvested on 08/30/2016. The 2017 Post Farm fields were harvested on 09/07/2017. The 2017 Western Triangle irrigated field was harvest on 09/01/2017, and the dryland field was harvested on 08/21/2017.

Table 2.1. Rainfall of field study locations for pre-plant months and individual monthly rates during the growing season.

<table>
<thead>
<tr>
<th>Rainfall (mm)</th>
<th>January - April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Farm 2016</td>
<td>79.4</td>
<td>68.9</td>
<td>19.5</td>
<td>30.7</td>
<td>22.1</td>
<td>220.6</td>
</tr>
<tr>
<td>Post Farm 2017</td>
<td>132.8</td>
<td>65.1</td>
<td>56.6</td>
<td>2.8</td>
<td>14.1</td>
<td>271.4</td>
</tr>
<tr>
<td>Western Triangle 2017</td>
<td>128.5</td>
<td>36.6</td>
<td>53.3</td>
<td>0</td>
<td>1.3</td>
<td>219.7</td>
</tr>
</tbody>
</table>
Agronomic Trait Measurement

Grain protein and moisture contents were quantified after harvest using a Foss Grain Analyzer (Foss Infratec 1241 Grain Analyzer, Foss North America, Eden Prairie, MN, USA), and reported as percent of grain on a dry matter basis. Seed plumpness was measured in accordance with the American Malting Barley Association guidelines with a 2.38 mm (6/64\textsuperscript{th} inch) screen. It is reported as the percent of seed which did not pass through the screen. Plant height was determined by extending the tip of the barley ear upward and recording the maximum height including awns (two measurements per plot; averages were recorded). Plant heights were measured at maturity. Tiller count was measured at maturity by counting the number of productive tillers found in a 30.5 cm length of the row (two measurements per plot; averages were recorded). Anthesis was recorded when approximately 50% of plants in each plot had reached Zadoks growth stage 49 (first awns visible) (Zadoks, Chang, & Konzak, 1974). Maturity dates were recorded only at the Post Farm experiments as the date at which approximately 50% of barley ears had reached Zadoks stage 92, as indicated by the inability to dent the dry caryopsis with a thumb nail (Zadoks et al., 1974). Harvest index was calculated at the Post Farm experiments in 2016 and 2017. Harvest index was calculated by harvesting one row of each plot, determining the weight of all aboveground plant material, followed by threshing and determining grain weight. Harvest index was then calculated by dividing the grain weight by the total dry biomass \((\text{grain weight/total row biomass weight})\). Test weights were determined post season using a grain analysis computer (GAC2500-
UGMA, Dickey-john Corporation, Auburn, IL). Variety ‘Karl’ and its NIL ‘10_11’ were not planted into the high nitrogen treatment in 2016 due to seed shortage.

**Malt Quality Analysis**

A full malt quality analysis was performed by the laboratory on grain from the 2017 Post Farm experiment and the 2017 Western Triangle experiment. This analysis only included lines from the Hockett, Amsterdam and Lewis family backgrounds (Figure 2.1). The laboratory also conducted a partial analysis on the 2016 Post Farm experiment. Traits measured included malt protein (mg g\(^{-1}\)), malt extract (fine grind dry basis), turbidity (Nephelometric Turbidity Units (NTU)), soluble wort protein (mg g\(^{-1}\)), kernel color, Free Amino Nitrogen (FAN, ppm), β-glucan (ppm), α-amylase (DU), and Diastatic Power (ºASBC).

**Malting Procedure.** For the 2017 Post Farm and Western Triangle locations, barley was malted with the following method. Grain was screened for plumpness on a 2.18 mm (5.5/64\(^{th}\) inch) screen. All three replications from each treatment combination were pooled and analyses were performed on the combined samples. Barley samples from each pool (120g) from the 8 low-GPC lines and 4 high-GPC varieties/lines were steeped until the grain reached an average of 45% moisture, germinated and kilned in a CLP micro-malter (SGK ‘Combi’ and SG Steep Germinator, CLP, Milton Keynes, UK).

The steeping regime used was 2 days of steeping and air cycles at 15 °C with the intervals: 10-hour steep, 18-hour rest, 6-hour steep, 10-hour rest, and 4-hour steep. The grain was germinated over 96 hours at 15°C with the containerized samples being rotated.
5 minutes out of every 30 minutes and aeration for 1 minute out of every 10 minutes. The grain was then kilned for 24 hours with the temperature gradually increasing from 60 °C to 85 °C, with 12 hours at 60 °C, 6 hours at 65 °C, 2 hours at 75 °C, and 4 hours at 85 °C to kill and dry the grain down to 3-5% moisture. Chit was then mechanically separated from the malt. Malt was ground on a mill to 0.2 mm for fine grind or 1 mm for coarse grind (DFLU Laboratory Disc Mill, Buhler Scientific, Lake Bluff, Illinois).

The 2016 Post Farm location was malted using the same method; however, smaller (5.6 g) samples of all three replications for each treatment combination were individually malted inside tea infuser balls which acted as infusion cages (Winco STB-5 Tea Infuser Ball 2”, Lodi, New Jersey). Because of the small sample size, greater care was taken to ensure that broken or skinned seed was removed before obtaining the sample. Malt kilning was also modified to more gently kiln the malt. The grain was kilned for 24 hours with the temperature gradually increasing from 45 °C to 85 °C, with 6 hours at 45 °C, 6 hours at 55 °C, 3 hours at 60 °C, 3 hours at 68 °C, 3 hours at 80 °C and 3 hours at 85 °C.

**Malt Quality Assays.** Malt protein (in percent) was measured post malting using a grain analyzer (Foss Infratec 1241 Grain Analyzer, Foss North America, Eden Prairie, MN, USA). The following measures of malt quality were determined using American Society of Brewing Chemists protocols: Malt 4, Malt 6, Wort 17, and Wort 18. (*ASBC Methods of Analysis, Online*, 2003; *ASBC Methods of Analysis, Online*, 2010a; *ASBC Methods of Analysis, Online*, 2010b; *ASBC Methods of Analysis, Online*, 2011). Percent malt extract was measured on a fine grind dry basis for all samples using the ASBC Malt-
procedure, using 40 g of grain where the protocol calls for 50 g. Density of the filtrates was measured in Degrees Plato with an Anton-Paar density meter (DMA 5000 M, Ashland, VA 23005). Degrees Plato and grain moisture, calculated with the Malt-4 Extract procedure, were then used to calculate percent malt extract. Turbidity was measured using a tungsten lamp turbidimeter (TL2300, Hach, Loveland, Colorado).

Soluble protein, diastatic power, α-amylase, free amino nitrogen, and β-glucan levels were measured using the Gallery Basic analyzer (Thermo Fischer Scientific, Waltham, MA) and Fisher Scientific established protocols ("Alpha-Amylase in malt," 2016; "Beta-Glucan," 2016; "Diastatic Power," 2016; "TON (Total Oxidized Nitrogen) as N and Nitrate by calculation (TON-Nitrite)," 2014; "Total Protein (Biuret)," 2011).

The 2016 Post Farm experiment was analyzed as above with the following exceptions: (1) Enzyme gravity filtration was replaced with a centrifuge filtration where samples were loaded into 96 well fritted deep-well plates and spun for 5 minutes at 715 g. (2) Wort gravity filtration was replaced with a centrifuge filtration where samples within 50 mL falcon tubes were spun for 9 minutes at 14,600 g. (3) Malt protein concentration, turbidity and coarse grind extract were omitted from the 2016 data as malt sample sizes were too small.

Malt kernel color was analyzed by the U.S. Department of Agriculture Malt Laboratory, for the Post Farm 2016 environments only. Kernel color is reported on a scale of 1 to 100 with 1 being bright and un-weathered kernels and 100 as dark or stained barley.
QTL Marker Analysis

The allelic state (at the chromosome 6H GPC locus) was confirmed or determined using the three cleaved amplified polymorphic sequence (CAPS) markers; *uhb71, uhb6*, and *uhb7* (Assaf Distelfeld et al., 2008), and allowing assignment to the high- or low-GPC groups shown in Fig. 2.1.

Plant tissue was collected from barley seedlings (at the two-leaf stage). Tissue was then flash-frozen in liquid nitrogen and stored at -80 ºC. Tissue was then placed into a 96-well plate with each well filled to 25% with silica beads. The plate was shaken in a “Mini-BeadBeater” (Biospec Products Inc., Bartlesville, OK) for 2 minutes to break up tissue.

Genomic DNA isolation was completed using the method described by Riede and Anderson (1996) with the following exceptions: (1) Sodium bisulfite was not added to the extraction buffer. (2) The protein and pigment removal step was omitted.

PCR was carried out as described in Distelfeld et. al., (2008) with the following exceptions: (1) the reaction size was increased from 20 µg to 25 µg. (2) conditions were extended to 1 denaturation cycle at 94 ºC for 4 min; 35 cycles of 94 ºC for 1 min, 58 ºC for 1 min, 72 ºC for 1.2 min, and a final extension cycle of 72 ºC for 7 min.

DNA was stained with GelRed nucleic acid gel stain (Biotium, Fremont, CA) and separated in a 16% acrylamide gel.

Statistical Analysis

Statistical analysis was conducted to determine the effects of the chromosome 6H grain protein QTL on important agronomic and malt characteristics under two nitrogen
regimes, and four environments in the 2017 season, two irrigated and two rainfed, and
two environments in the 2016 season, one irrigated and one dryland, using mixed models
for each quality trait. The 2017 trials were analyzed according to a randomized split plot
model which was combined over the locations (Post farm and Western triangle) and the
irrigation treatment (no irrigation and irrigation) making four location by irrigation
combinations. The model included irrigation location Nitrogen and genotype as fixed
effects and block nested within irrigation by location combinations and block by Nitrogen
nested within location by irrigation combinations as random effects. This was
accomplished using the lme4 package (Bates, Sarkar, and Matrix, 2017) in R version
3.5.4 (R Core Development Team, 2017). The 2016 trials were analyzed as separate
randomized block experiments. The difference between high-GPC and low-GPC alleles
was tested for each near isoline family background using the lsmeans package (Lenth,
2017) in R.

Results

Agronomic Parameters

Barley varieties and lines analyzed in this study varied for the allelic state of the
chromosome 6H grain protein concentration (GPC) locus (Figure 2.1). Across all
germplasm and treatment combinations in the 2016 season, lines carrying the low-GPC
allele had an average of 17.63 mg/g less grain protein than related lines containing the
alternate allele in the normal nitrogen treatment (p-value < 0.0001) (Figure 2.2). When
the Karl family (6-rows) (‘Karl’ and line ‘10_11’) was excluded, the low-GPC lines had
13.67 mg/g less protein than related lines carrying the high-protein allele. In the high nitrogen treatment, with the Karl family excluded, due to seed shortage, protein was 13.34 mg/g less than in related lines. Soil nitrogen levels also impacted grain protein across all treatments by an average 5.55 mg/g increase in the high nitrogen treatment compared to the normal nitrogen treatment for the Lewis, Amsterdam and Hockett families (p-value = 0.082). Across all families and treatment combinations in the 2017 season the lines carrying the low-GPC allele had a mean 15.163 mg/g less grain protein than lines containing the alternate allele (p-value < 0.0001) (Figure 2.2). Nitrogen also impacted grain protein across all treatments by a mean 3.33 mg/g increase in the high nitrogen treatment compared to the normal nitrogen treatment (p-value = 0.0164).

In 2016, the low-GPC lines matured an average of 1.40 days later than related lines with the high-GPC allele in the dryland environments (p-value < 0.001) and 1.53 days later in the irrigated environments (p-value < 0.0001) (Figure 2.3). There was no evidence that nitrogen level impacted maturity date. In 2017, we observed strong evidence that the lines containing the low-GPC allele matured 1.95 days later than related lines (p-value < 0.0001). This difference was evident in both the dryland and irrigated fields; however, the difference was largest in the irrigated plots where the low-GPC lines matured 2.54 days later (p-value < 0.0001) (Figure 2.3). The low-GPC lines matured only 1.375 days later in the dryland environments (p-value = 0.0073).

In 2016 there was high variability between the different families for grain yield. There was a decrease in yield associated with to the low-GPC allele in the Amsterdam and Lewis backgrounds with a decrease of 351.24 kg/ha (p-value = 0.0585) and 1033.5
kg/ha (p-value < 0.0001) respectively. However, there was an increase in yield for the low-GPC allele in the Hockett and Karl backgrounds with an increase of 354.8 kg/ha (p-value = 0.0367) and 380.7 kg/ha (p-value = 0.0126) respectively (Figure 2.4). Note that the Karl background was only evaluated in the normal nitrogen treatment in 2016.

Nitrogen’s impact on yield was only present in the irrigated treatment where increased nitrogen caused an increase in yield of 803.7 kg/ha (p-value = 0.0014). During the 2017 season, there was no evidence of a change in yield for the low-GPC allele apart from the lines in the Hockett background in which there was a mean increase in yield of 765.8 kg/ha (p-value < 0.0001) and the Lewis background where there was a decrease in yield of 418 kg/ha (p-value = 0.0683) (Figure 2.4). The impact of nitrogen on yield was only seen in the dryland fields where yield increased by 767.5 kg/ha (p-value = 0.0145). There was no interaction between QTL and nitrogen.

In 2016 there was no consistent effect caused by the QTL on harvest index but we did see effects in individual families. The Hockett background lines with the low-GPC allele harvest index increased by 0.019 (p-value = 0.0029). The Karl background lines with the low-GPC allele harvest index, in the normal nitrogen treatment only, increased by 0.024 (p-value = 0.0029) (Figure 2.5). The Lewis background lines with the low – GPC allele harvest index, across both nitrogen treatments, decreased by 0.0458 (p-value = 0.0001). There was no impact of the increased nitrogen treatment in the 2016 analysis. In 2017 there was no evidence of the QTL influencing harvest index in any of the tested germplasm. The increased nitrogen treatment was linked to an increase in harvest index of 0.0137 when compared to the normal nitrogen treatment (p-value = 0.0051).
In the 2016 season, there was a QTL effect on the percentage of plump kernels only in the lines with the Hockett background. In the Hockett lines, those with the low-GPC allele had an increase in plumps of 9.03% (p-value = 0.008) (Figure 2.6). There was no effect of nitrogen on kernel plumpness in the 2016 data. In 2017, across the families in our trial, there was no consistent impact on plumps for the low-GPC allele. However, in the Hockett background there was a 12.47% increase in plump kernels (p-value < 0.0001), while the Karl background showed a 5.75% decrease for the low-GPC allele (p-value = 0.0075) (Figure 2.6). The increased nitrogen treatment in 2017 showed a decrease in plumpness in all families by a mean of 2.35% (p-value = 0.0667). The increased nitrogen effect was strongest in the Karl background, in which the high-nitrogen treatment had plumpness decreased by 6.3% across dryland and irrigated treatments (p-value = 0.0054).

In 2016, we observed no consistent effect in test weight associated with the QTL. However, we did see an increase in test weight of 2.14 kg/hL in the Hockett background low-GPC lines (p-value < 0.001) and a decrease of 2.82 kg/hL in the Amsterdam background low-GPC lines (p-value < 0.0001), and a decrease of 3.58 kg/hL in the Lewis background low-GPC lines (p-value < 0.0001) (Figure 2.7). We observed no change in the Karl background. We also observed no impact of the nitrogen treatment in the 2016 season on test weight. In 2017 we observed no consistent effect in test weight associated with the QTL. However, we did again see an increase in test weight of 2.84 kg/hL in the Hockett background low-GPC lines (p-value < 0.0001) and a decrease of 2.13 kg/hL in
the Amsterdam background low-GPC lines (p-value < 0.0001) (Figure 2.7). We observed no impact of the nitrogen treatment on test weight in 2017.

In 2016, there was no evidence of a difference in plant height between the low-GPC and high-GPC alleles (Figure 2.8). The high nitrogen treatment was associated with an increase in height of 2.43 cm with the Karl family excluded, which was only planted in the normal nitrogen treatment due to seed shortage (p-value = 0.0162). In 2017 there was no difference in average plant height between the alleles but we did see that plant height was significantly impacted by the nitrogen treatment. Plants receiving the high nitrogen treatment were 3.04 cm taller than plants with normal nitrogen applied (p-value = 0.0097).

We also saw a large increase in plant height in the irrigated treatments. In 2016 the irrigated treatment was an average 17.9 cm taller than the dryland treatment (p-value < 0.0001) and in 2017, the irrigated plots averaged 10.9 cm taller than the dryland treatments (p-value < 0.001).

In 2016, there was slight evidence of an increase in tiller numbers in the Karl type background in which the low-GPC variety (Karl) had an increase of 16.07 tillers/meter (p-value = 0.12) compared to its high-GPC NIL (Figure 2.9). The high nitrogen treatment also increased tiller numbers by 15.74 tillers/meter across all lines (p-value = 0.0039). In 2017 we observed evidence of an increase in tiller numbers in the Karl background only. In this background the low-GPC allele had 25.55 tillers/meter more (p-value = 0.0051). Nitrogen also impacted tiller numbers; the high nitrogen treatment had an increase of 15.64 tillers/meter across all varieties (p-value = 0.009).
Malt Quality Parameters

Storage proteins are partially degraded during the malting process. One important question was whether differences in grain protein caused by the investigated QTL (Figure 2.2) are also measurable in malt protein concentration or if, possibly depending on the genetic background, these differences disappear. In 2017 (no data for 2016, due to the methods used), there was less protein on average of 7.73 mg/g in the low-GPC lines versus the high-GPC lines across all family backgrounds (p-value < 0.0001) (Figure 2.10). The family with the greatest decrease was the Hockett background with a decrease of 11.843 mg/g (p-value < 0.0001), while the Lewis background decreased 7.0 mg/g (p-value < 0.001), and the Amsterdam type decreased 4.375 mg/g (p-value = 0.0078). We found no evidence of an effect of soil nitrogen levels on malt protein.

A fraction of the malt protein remains in the wort as soluble protein. In 2016, the effect of the QTL on soluble wort protein was inconsistent. The Amsterdam background decreased by an average 2.4 mg/g for the low-GPC allele across both nitrogen levels (p-value 0.0427) and there was no evidence of a difference in the Hockett and Lewis backgrounds (Figure 2.11). The high nitrogen treatment had an increase in soluble wort protein levels by increasing the levels 1.35 mg/g (p-value = 0.053) when compared to the normal nitrogen treatment. In 2017, the same inconsistent effect was present across families. The Amsterdam background was still the only background with evidence of a QTL effect as it decreased by an average 4.08 mg/g (p-value 0.0496) for the low-GPC allele across both nitrogen levels (Figure 2.11) and there was no evidence of a difference
in the Hockett and Lewis backgrounds. There was no evidence of a difference in soluble wort protein between the nitrogen treatments.

In 2016, there was no evidence of a difference in average free amino nitrogen (FAN) levels caused by the low-GPC allele except for the Amsterdam family in which we observed that the lines with the low-GPC allele had a decrease of 28.35 ppm (p-value = 0.0122) (Figure 2.12). The high nitrogen treatment also caused a consistent increase of 13.2 ppm (p-value = 0.0697).

In 2017, there was a decrease in FAN levels in the low-GPC varieties across all treatment with the largest decrease of 46.25 ppm in the Amsterdam low-GPC lines (p-value = 0.007) (Figure 2.12). We observed no impact of nitrogen in the 2017 season.

In 2016, across all family backgrounds and treatment combinations, the low-GPC allele showed a decrease in diastatic power (DP) of 53.12 °ASBC (p-value < 0.0001) (Figure 2.13). There is no evidence of an interaction with family, nitrogen or irrigation treatment. The increased nitrogen treatment was linked to an increase in DP of 32.88 °ASBC (p-value = 0.0036). In 2017, across all family backgrounds and treatment combinations the low-GPC allele is linked to a decrease in DP of 42.005 °ASBC (p-value < 0.0001) (Figure 2.13). There is no evidence of an interaction with family, nitrogen or irrigation treatment. The increased nitrogen treatment tended to cause a small increase in DP across all treatment combinations but this increase was not statistically significant.

We found evidence of a small increase (with the low-GPC allele) in α-amylase of 16.39 DU in the Amsterdam background only in 2016 (p-value < 0.0001) (Figure 2.14). We also found evidence of a small decrease in the Hockett background in which the low-
GPC lines have an average of 4.46 DU less α-amylase than related lines with the alternate allele (p-value = 0.0215). Increased nitrogen gave a mean α-amylase increase of 5.797 DU (p-value < 0.0001). In 2017 we found weak evidence of increased α-amylase in the Amsterdam background low-GPC lines of 16.56 DU when compared to their high-GPC sister line (p-value = 0.0749) (Figure 2.14). However, we found no evidence of an effect caused by the nitrogen treatment.

Malt kernel color was only analyzed in the 2016 season. Across all treatment combinations, the low-GPC lines were 2.06 units lighter than the high-GPC lines (on a scale of 1 to 100) (p-value = 0.0071). The increased nitrogen treatment caused no change in color (Figure 2.15).

No significant effects of the analyzed QTL or nitrogen fertilization (in both years) were found for malt extract, β-glucans, and turbidity (see appendix, Figures 1-3).
Figure 2.2. Grain protein concentration by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.3. Maturity date in Julian days by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.4. Grain yield by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.5. Plant harvest index by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.6. Grain plumpness by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.7. Grain test weight by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.8. Plant height by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.9. Plant tillers per meter of row by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.10. Malt protein concentration by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.11. Soluble wort protein by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.12. Free amino nitrogen by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.13. Diastatic Power by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.14. α-amylase activity by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Figure 2.15. Malt kernel color by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Discussion

Quality requirements on malting barley producers are demanding and prices can change substantially due to small changes in grain quality. A critical factor for acceptance as malt is protein < 13%. Protein can be too high due to lack of water or high temperatures during grain fill, or too much nitrogen application. Therefore, it is critical to understand the relationship between variety (genetic makeup) and management. A novel low-grain protein allele has been used to improve barley varieties for Montana. The results of this study indicate that the low-GPC allele improves quality stability for at least some malt traits under variable nitrogen fertilization and water availability.

To quantify and understand these interactions, we tested lines differing in the allelic state of the chromosome 6H grain protein QTL for important agronomic and malt quality characteristics under dryland and irrigated conditions and at both recommended and high (~1.5 times the recommended level) nitrogen fertilization levels. Our results indicate that the tested QTL had a consistent impact, across irrigated and dryland environments as well as normal and high nitrogen levels, on maturity date, GPC, malt protein, and diastatic power.

GPC was consistently lowered (Figure 2.2), which is advantageous, as protein levels are often too high, particularly under dryland conditions. The low malt protein was highly correlated to the low grain protein, Previous research has shown that the QTL is linked to decreased hordein storage protein (Dailey et al., 1988; Jukanti et al., 2008). Decreased GPC across all treatment conditions is highly beneficial to malt barley farmers as they will be less likely for their grain to be rejected for malt.
Maturity date was delayed for the low-GPC lines in this study (Figure 2.3), which has been previously associated with the GPC locus (Jukanti et al., 2008). A gene within this locus, *HvNAM-1*, codes for a transcription factor. An allele of *HvNAM-1* delays senescence, allowing more time for grain fill. More starch can be deposited through the longer grain fill, decreasing percent grain protein and increasing yields. An orthologue in wheat (*Gpc-B1*) has also been shown to influence senescence timing (Assaf Distelfeld et al., 2008; Uauy, Brevis, et al., 2006).

One problem identified by our research may be the decrease in diastatic power (Figure 2.13) that was observed in the low-GPC lines; however, this decrease was small and all lines still fell within the acceptable range specified by the American Malting Barley Association which recommends that DP be above 120 °ASBC for adjunct two-row and between 110-150 °ASBC for all malt two-row.

We also see changes in grain plumpness (Figure 2.6) that are associated with the QTL; however, increased in the low-GPC lines are seen in the Hockett background while a decrease was seen in the Karl background. Yield (Figure 2.4) also showed mixed results with increases associated with the low-GPC allele in the Hockett and Karl families and small decreases observed in the Lewis and Amsterdam families.

Nitrogen fertilizer levels also played an important role in determining the quality of barley to be used for malt in our data. While yield is improved with a high nitrogen treatment (Figure 2.4), GPC also increased which could potentially cause grain rejection. We also saw a decrease in plump kernels in the 2017 season, and an increase in soluble wort protein in the 2016 season. Free amino nitrogen increased and DP increased with
high nitrogen levels as well, both of which have specific acceptance ranges for maltsters. With these interactions, use of the low-protein allele in new varieties could allow for increased nitrogen application and the improved yield that is associated, while still maintaining acceptable protein levels. The potential to maintain low grain protein even in presence of higher N application in such germplasm has previously been mentioned (Weston et al., 1993). It has also long been noted that GPC and diastatic power are positively correlated (Foster, Peterson, & Banasik, 1967; Igartua, Hayes, Thomas, Meyer, & Mather, 2002; Peterson & Foster, 1974; Rasmusson & Glass, 1967; Streeter & Pfeifer, 1966; Weston et al., 1993), which our data set corroborated as well (compare Figures 2.2 and 2.13).

The consequences of the difference in fertilizer rate used in these experiments were small. The additional yield may be tempting but careful calculation should be made based on farm history and cultivar knowledge before increasing nitrogen for yield alone.

It has also been pointed out that lines containing the low-GPC allele were not being utilized due to low DP or unacceptably dark seed color (Goblirsch, Horsley, & Schwarz, 1996; Weston et al., 1993). Our results confirm that DP is slightly decreased in the low-GPC lines (Figure 2.13), however, at all locations, nitrogen, and irrigation regimes in our study, DP exceeded acceptable standards for AMBA quality guidelines. We also evaluated the lines for seed color and did find evidence of a difference between the alleles. However, this we found the low-GPC lines to be lighter in color but the difference was negligible in our experiment. This evaluation was performed only for one field season so we recommend that programs utilizing this low-GPC allele continue to
evaluate this trait as it could be dependent on background, location, or treatment conditions.

**Conclusion**

This research shows the implications of soil nitrogen levels on malt quality and how the analyzed chromosome 6H low-GPC allele can help maintain acceptable malt quality. While the studied QTL is not a complete solution to high grain protein content, the low-protein allele does substantially decrease GPC and malt protein levels which will enable farmers to grow malt barley with a decreased risk of rejection due to unacceptably high GPC. Importantly, no negative effects of the low-protein allele were found on a series of malt quality characteristics across two different years of experimentation, using various genetic backgrounds.
CHAPTER THREE

BARLEY PERFORMANCE UNDER RAINFED AND IRRIGATED CONDITIONS: INFLUENCE OF A CHROMOSOME 6H LOCUS CONTROLLING EXPRESSION OF A GLYCINE-RICH RNA-BINDING PROTEIN

Introduction

Drought tolerance is a highly sought-after trait in the rainfed production regions of the American West. Interest in this trait has been heightened due to climate change, and due to the westward movement of barley production in the US caused by the increasing pressure to plant corn and soybean on higher producing Midwestern land. One solution to an increasing risk of drought would be to select cultivars with enhanced drought tolerance.

A glycine-rich RNA-binding protein, acting as an RNA chaperone, has been identified in barley (Hordeum vulgare) (Jukanti et al., 2008). This protein, called Hordeum vulgare Glycine-Rich RNA-Binding Protein 1 (HvGR-RBP1) has been shown to be a functional homologue of Arabidopsis thaliana Glycine-Rich RNA-Binding protein 7 (AtGRP7) and is suspected of providing improved drought tolerance in barley (Tripet et al., 2014).

Research in Arabidopsis has shown that AtGRP7 plays an important role in regulating floral transition, and that its expression is upregulated during whole-plant senescence (Gepstein et al., 2003; Streitner et al., 2008). Rice transgenic lines expressing
the AtGRP7 and similar AtGRP2 proteins have improved grain yield and recovery under drought stress conditions. *AtGRP7* is expressed abundantly in stomatal guard cells, and is directly involved in stomatal regulation (Kim et al., 2008; Yang et al., 2014).

In addition to controlling the differential expression of *HvGR-RBP1*, the QTL also contains a gene that controls grain protein concentration. Previous work from this laboratory has demonstrated that lines containing the Karl low-GPC allele are later-senescing when compared to their NILs, with slower degradation of leaf proteins (including Rubisco) and of chlorophylls, and decreased leaf amino acid levels (Heidlebaugh et al., 2008). Karl is also slower to begin storage protein accumulation in developing kernels and ultimately has lower GPC in mature kernels (Heidlebaugh et al., 2008; Jukanti & Fischer, 2008; Jukanti et al., 2008). In addition, previous studies also indicate that the QTL promotes flowering time and earlier senescence (A. Distelfeld, Avni, & Fischer, 2014; Lacerenza et al., 2010).

González, Martín, and Ayerbe (González, Martín, & Ayerbe, 1999) suggest that osmotic adjustment capacity (OA) and leaf stomatal conductance are important traits in selecting for drought tolerance and that variation exists in cultivated barley varieties for their ability to regulate stomatal conductance (1999). Stomatal conductance improves drought tolerance by actively managing stomatal aperture during a drought (Teare, Kanematsu, Powers, & Jacobs, 1973).

The current research was conducted over two years of field experiments to determine the impact of HVgpc.6H, with differential expression of *HvGR-RBP1*, on grain yield, total biomass, and stomatal conductance in two sets of near-isogenic lines under
normal growing conditions with supplemental irrigation and under rainfed growing conditions. Barley grain yield and total biomass were used as indicators because they provide a metric of season-long plant productivity. In addition to these measurements, we also collected measurements of stomatal conductance and thermal images to assess canopy temperature depression throughout the field season. These data were collected to test the hypothesis that lines containing the Lewis-type allele with increased expression of *HvGR-RBP1* expression have improved agronomic performance and different stomatal regulation during the season. In addition, our experiments determined if thermal canopy depression can be used as a higher-throughput alternative to direct stomatal conductance measurements.

**Materials and Methods**

**Plant Material**

This study utilized two pairs of near-isogenic barley varieties/lines named: ‘Lewis’/‘21_7, and ‘Karl’/’10_11 To study the effect of the high expression *HvGR-RBP1* allele on grain yield biomass and stomatal conductance. These near-isogenic lines (NILs), in the Lewis and Karl backgrounds, were created by backcrossing the high-GPC / high *HvGR-RBP1* expression locus from the Lewis background into the Karl background creating the line 10_11, and backcrossing the Karl low-GPC / low *HvGR-RBP1* expression locus into the Lewis background creating the line 21_7 (Jukanti et al., 2008).

Two additional families from the ‘Hockett’ and ‘Amsterdam’ backgrounds were also used due to variation at the QTL of interest. The variety Hockett contained the high-
GPC allele and the related lines MT124673, MT124128, MT090182, and MT090190 contained the low-GPC allele. The variety Amsterdam contained the high-GPC allele and the related lines MT124027, and MT124071 contained the low-GPC allele.

The varieties and lines Amsterdam, Hockett, 10_11, and Lewis, all contained the chromosome 6H high-protein QTL and have potentially high-expression of the HvGR-RBP1 (verified for Lewis and 10_11; Jukanti et al., 2008; Lacerenza et al., 2010). These lines will henceforth be referred to as the high-GPC lines. The varieties/lines MT090182, MT090190, MT124027, MT124071, MT124673, MT124128, Karl, and 21_7 were selected as lines containing the low-GPC allele and suspected low-expression of HvGR-RBP1 (verified for Karl and 21_7; Jukanti et al., 2008; Lacerenza et al., 2010). These lines will henceforth be referred to as the low-GPC lines. The pedigree of lines included is shown in Figure 3.1.
Experimental Design

The experiment was planted at the Arthur Post Research Farm in Bozeman, MT, United States (45°40’40.78 N, 111°09’07.14 W) during the summers of 2016 and 2017. At the Post Farm field experiments, irrigated and dryland plots were grown 200 m from each other in 2016 and 20 m in the 2017 season. Both years included a nitrogen fertilizer treatment split-plot randomized complete block layout.

In each season, field soil samples were analyzed for total nitrogen content by AGVISE Laboratories (Benson, MN) on a field level in 2016 and a replication level in 2017. Nitrogen fertilizer, in the form of urea (46-0-0), was top dressed after planting but
before a rainfall or irrigation event to ensure that nitrogen was not lost to the atmosphere, to achieve the desired soil nitrogen treatment.

The nitrogen treatment consisted of two levels, a normal nitrogen level, and a high treatment which was recommended rate plus 56 kg/ha. The normal nitrogen application was calculated using the Montana Barley Production Guide calculation for malting barley nitrogen application which was \((61.77 \text{ grams } N \times \text{Expected Yield in kg})/\text{hectare}\).

At the 2016 Post Farm experiment, the estimated rate was 112 kg/ha for the normal treatment and 168 kg/ha for the high treatment. The 2017 Post Farm experiment used a normal rate of 97.5 kg/ha and a high rate of 168 kg/ha.

In both field experiments, irrigated and dryland plots were grown in the same field in a split plot randomized complete blocks design. The varieties were planted in 3-row plots (1.2 x 3.65 m) in the 2016 field season and in 4-row plots (1.2 x 5.48 m) in 2017. Soil water potential was tracked at the 2017 Post Farm location using 16 Watermark granular matrix sensors arranged with 8 sensors split between two depths, 25 and 50 cm, in each field (Model 200SS, The Irrometer Company, Inc., Riverside, CA) (Figure 3.2). Soil water potential was then logged hourly on a 900M data logger by the same manufacturer. Post Farm plots collected 119 mm of rainfall from May to July during the 2016 season and during the same time in 2017 the plots received 124.5 mm. All plots had adequate pre-season rainfall to reach soil field capacity.

A total of approximately 150 mm of irrigation was added to the irrigated plots over 3 events throughout the season to ensure plots did not go below the wilting point.
These three irrigations are visible for the 2017 season in the plot below which shows soil water potential increasing at three points on July 12th, July 22nd, and July 5th. Irrigation was suspended in the first week of July to begin dry down for harvest. The 2016 Post Farm fields were harvested on 08/30/2016. The 2017 Post Farm fields were harvested on 09/07/2017.

Figure 3.2. Soil Water Potential of the 2017 field plots separated by rainfed and irrigated plots. Each environment was measured at both 25 cm and 50 cm below the soil surface.

Stomatal Conductance Measurement

As conductance of water vapor through leaf stomata is closely related to the conductance of carbon dioxide which is, in turn, an estimator of photosynthetic production, plants were measured for stomatal conductance using a leaf porometer.
(Model SC-1, Decagon Devices, Pullman, WA). Stomatal conductance measurements are reported in units of mmol of H₂O per square meter per second (mmol/m²s⁻¹).

Conductance was measured on the uppermost fully developed leaf in only normal nitrogen level plots in the 2016 season and on both the normal nitrogen and high nitrogen plots in the 2017 season. All assays were performed between early- and mid-afternoon on sunny days, to minimize variation which could be caused by changing light conditions. A minimum of five leaves were measured in each plot on each day of measurement.

Canopy Temperature Depression Measurement

Thermal images of barley plots were taken with a thermal camera (Model E40, FLIR Systems, Wilsonville, OR, USA). Images were taken from 0.5 m north of each plot with the camera facing south at approximately 2 p.m. throughout the summer. Pixel temperature values were extracted from each image using “Flir Tools” software provided by the camera manufacturer. Values were then sorted by temperature to identify warm soil temperature outliers, which were excluded. Temperatures recorded are those of the plot’s green biomass.

Statistical Analysis

Statistical analysis was conducted to determine the effects of the chromosome 6H QTL on important agronomic characteristics in both the irrigated and dryland environments using mixed models for each quality trait. Analysis was done using R version 3.5.4 (R Development Core Team, 2017), with the “lme4” (Bates, Sarkar, Bates,
& Matrix, 2007), and “lsmeans” packages (Lenth, 2016). Plots were produced using the GGplot2 package and annotated with the cowplot package (Hadley, 2015; Wilke, 2016).

The different field sites were analyzed individually using a randomized complete block with three replications per location in the 2016 season of both dryland and irrigated environments. The 2017 season used a split plot randomized complete block design with nitrogen as the whole plot and three replications in both dryland and irrigated environments.

Results

Stomatal Conductance

During the 2016 and 2017 field seasons were analyzed separately. We found that the two pairs of NILs reacted differently over the season and within each irrigation treatment. We observed an opposite effect of the QTL between the Karl and Lewis type background for stomatal conductance in 2016. The high-GPC allele line 10_11 from the Karl background had a season average stomatal conductance increase of 29 mmol/m²·s⁻¹ compared to the low-GPC allele NIL (p-value = 0.066) in the dryland and no measurable difference in the irrigated plots (Figure 3.3 A, B). In the Lewis background, we observed the Lewis variety carrying the high-GPC allele to have a season average stomatal conductance decrease of -35.8 mmol/m²·s⁻¹ (p-value = 0.071) in the dryland and -27.728 mmol/m²·s⁻¹ (p-value = 0.012) in the irrigated environments compared to its low-GPC allele NIL 21_7 (Figure 3.3 C, D).
The trend for the QTL effect matched that of 2016 for the 2017 stomatal conductance results. The high-GPC allele line 10_11 from the Karl background had a stomatal conductance increase of 34.69 mmol/m²s⁻¹ (p-value = 0.0038) in the dryland and 18.11 mmol/m²s⁻¹ (p-value = 0.124) in the irrigated environments over the low-GPC lines (Figure 3.4 A, B). We observed the high-GPC line, Lewis, had a season average stomatal conductance decrease of -50.83 mmol/m²s⁻¹ (p-value < 0.0001) in the dryland and -79.06 mmol/m²s⁻¹ (p-value < 0.0001) in the irrigated environments when compared to its low-GPC NIL (Figure 3.4 C, D).

**Canopy Temperature Depression**

In the 2017 season, canopy temperature depression (CTD) was measured on all plots including the ones for which stomatal conductance was also determined (Figures 3.5 and 3.6). The apparent trend of the CTD (Figure 3.5 slightly lower temperatures in 10_11 (vs Karl) and Lewis (vs 21_7)) are not significant. We found no evidence of a difference in CTD between the two QTL types across all irrigation and nitrogen treatments, and no significant family background interactions. We also had no evidence of a difference in means between the high and low-GPC alleles of the Hockett and Amsterdam families.

We also contrasted the alleles during our approximate mean grain fill period (7/13/17 to 8/5/17) to exclude terminal senescence and found no evidence of a difference in means or any interaction with family background, nitrogen, or irrigation.

We found no evidence of a difference in CTD between the high and normal nitrogen treatments in either the dryland or irrigated treatments when looking at all
collected data but when sub-setting only the approximate mean grain fill period we saw that the high nitrogen treatment had a mean 0.71 °C cooler in the irrigated treatment (p-value < 0.001) and a mean 0.63 °C cooler in the dryland treatment (p-value = 0.0026) than the normal nitrogen treatment in their respective irrigation environments.

We also observed that the irrigated treatment was an average of 1.8 °C cooler in the normal nitrogen treatment (p-value < 0.001) and 1.9 °C cooler in the high nitrogen treatment (p-value < 0.0001) when compared to the dryland environments during the dates we collected data throughout the season. We found no evidence of an interaction with family background and temperature depression across irrigation and nitrogen treatments.

While these results may not fully support the use of CTD as a replacement for stomatal conductance assays, we noted that CTD was highly correlated with maturity date, plant height, and yield which could be potentially useful (Figure 3.7). We were also able to see difference between family background which does indicate a genetic component that is visible with CTD measurements.

Grain Yield

In 2016 there was high variability between the different families for grain yield. There was a decrease in yield associated with the low-GPC allele in the Amsterdam and Lewis backgrounds with a decrease of 351.24 kg/ha (p-value = 0.0585) and 1033.5 kg/ha (p-value < 0.0001) respectively. However, there was an increase in yield for the low-GPC allele in the Hockett and Karl backgrounds with an increase of 354.8 kg/ha (p-value = 0.0367) and 380.7 kg/ha (p-value = 0.0126) respectively (Figure 2.4). Note that
the Karl background was only evaluated in the normal nitrogen treatment in 2016. Nitrogen’s impact on yield was only present in the irrigated treatment where increased nitrogen caused an increase in yield of 803.7 kg/ha (p-value = 0.0014). During the 2017 season, there was no evidence of a change in yield for the low-GPC allele apart from the lines in the Hockett background in which there was a mean increase in yield of 765.8 kg/ha (p-value < 0.0001) and the Lewis background where there was a decrease in yield of 418 kg/ha (p-value = 0.0683) (Figure 2.4). The impact of nitrogen on yield was only seen in the dryland fields where yield increased by 767.5 kg/ha (p-value = 0.0145). There was no interaction between QTL and nitrogen.

We did, however, notice a correlation between yield and stomatal conductance (Figure 3.8).

**Maturity Date**

Maturity date of the lines carrying the low-GPC allele averaged over family background 2.0 days later when compared to their NILs (p-value < 0.0001). However, this is most likely caused by the *HvNAM-1* gene, as an orthologue in wheat (*Gpc-B1*) has been previously shown to influence senescence timing (Assaf Distelfeld et al., 2008; Uauy, Brevis, et al., 2006). There was no observed interaction with the different family backgrounds and maturity date.
Figure 3.3. 2016 Post Farm field study barley (*Hordeum vulgare*) stomatal conductance values by treatment combination. Each point includes the mean of at least five conductance measurements from each of three replicated plots, with the standard error indicated by bars. Dryland environment conductance of the Karl type varieties (A). Irrigated environment conductance of the Karl type varieties (B). Dryland environment conductance of the Lewis type varieties (C). Irrigated environment conductance of the Karl type varieties (D).
2017 Stomatal Conductance by Irrigation Treatment

A  Dryland Conductance

B  Irrigated Conductance

C  Dryland Conductance

D  Irrigated Conductance

Figure 3.4. 2017 Post Farm field study barley (*Hordeum vulgare*) stomatal conductance values by treatment combination. Each point includes the mean of at least five conductance measurements from each of three replicated plots, with the standard error indicated by bars. Dryland environment conductance of the Karl type varieties (A). Irrigated environment conductance of the Karl type varieties (B). Dryland environment conductance of the Lewis type varieties (C). Irrigated environment conductance of the Karl type varieties (D).
Figure 3.5. 2017 Post Farm field study barley (*Hordeum vulgare*) canopy temperature depression from ambient field temperature of plots given the recommended nitrogen. Each point represents the average pixel temperature from each of three replications with bars indicating standard error. Dryland environment temperature depression of the Karl type varieties (A). Irrigated environment temperature depression of the Karl type varieties (B). Dryland environment temperature depression of the Lewis type varieties (C). Irrigated environment temperature depression of the Karl type varieties (D).
Figure 3.6. 2017 Post Farm field study barley (*Hordeum vulgare*) canopy temperature depression from ambient field temperature of plots given the recommended nitrogen. Each point represents the average pixel temperature from each of three replications with bars indicating standard error. Dryland environment temperature depression of the Karl type varieties (A). Irrigated environment temperature depression of the Karl type varieties (B). Dryland environment temperature depression of the Lewis type varieties (C). Irrigated environment temperature depression of the Karl type varieties (D).
Figure 3.7. 2017 Post Farm field study scatter plot correlation matrix of variables correlating with season average canopy temperature across both nitrogen and irrigation treatments.
Discussion

The regulation of stomata in response to both endogenous and environmental signals is highly complex and, for both basic and applied reasons, has long been a very actively studied subject (Taiz, Zeiger, Moller, & Murphy, 2015). While stomata generally open during the day under high light intensities, they control water loss by (partially) closing when plants grow under water-limiting conditions, especially after midday. For this reason, stomatal conductance in this study was assayed between early to mid-
afternoon, maximizing the likelihood of observing differences in stomatal regulation between lines with low vs. high \textit{HvGR-RBP1} expression.

We used a leaf porometer to measure the difference in stomatal conductance of the uppermost fully developed leaf at multiple dates throughout the growing season under both dryland and irrigated conditions for NILs that differed in allelic state at the GPC QTL. All assays were performed between early to mid-afternoon on clear, sunny days to minimize variation caused by changing light conditions.

We hypothesized that the introduction of the allele associated with high \textit{HvGR-RBP1} expression might impact stomatal regulation during drought stress (Kim et al., 2008). In the two backgrounds we tested, the results were inconsistent. The Karl background had increased stomatal conductance with the high \textit{HvGR-RBP1} expression QTL and the Lewis background had decreased stomatal conductance with the high \textit{HvGR-RBP1} expression QTL. These results were consistent in both years of the experiment. From these data, it does appear that the QTL is impacting stomatal behavior; however, its effects may be dependent on the genetic background tested.

Observed differences in stomatal conductance were not associated with significant differences in grain yield. We saw that the lines with increased expression of the \textit{HvGR-RBP1} also senesced earlier across the experiments. This result was also visible in previous studies. This earlier senescence, which is most likely caused by the \textit{HvNAM-1} gene which has been shown by the latest barley genome sequence to be approximately 5.4 cM away from \textit{HvGR-RBP1} (Mascher et al., 2017), which could be confounding yield or drought tolerance effects of the \textit{HvGR-RBP1} gene.
The canopy temperature depression (CTD) measurements did not consistently match the conductance measurements (see Figures 2 and 3). This may have been due to the high variability of the measurements, or because stomatal conductance was always measured on the uppermost leaves, while CTD provided an average across the canopy including ears (with awns and glumes) at later dates. It may become necessary to compare different imaging distances and angles, and to improve data extraction methods to enhance the value of CTD in future studies.

The CTD measurements did provide insight into the mechanisms of grain yield in genotypes used in our study. Average canopy temperature was highly negatively correlated with maturity date, plant height, and grain yield. Grain yield has been previously been shown to correlate with CTD in wheat (Amani, Fischer, & Reynolds, 1996). It has also been demonstrated that CTD can help understand the physiological basis of improved grain yield in wheat (Karimizadeh & Mohammadi, 2011). The biological significance of this result needs further analysis, but it supports the idea that canopy temperature depression may be useful in selecting for grain yield (Figure 3.7).

Conclusion

We found no consistent evidence of a difference in stomatal conductance or canopy temperature depression linked to *HvGR-RBI* expression levels. These results, however, are limited to the conditions of our 2016 and 2017 seasons.

Results are based on two pairs of near-isogenic lines for the conductance data and four families for the 2017 thermal canopy temperature depression data. Both the stomatal
conductance and thermal canopy temperature depression measurements would benefit from additional field seasons with increased replication.

We were able to show that canopy temperature depression may be a usable phenotype to help select higher yielding barley cultivars.

Another approach to this problem would be using a recombinant-inbred line population to analyze that effect of the QTL as there would be approximately 5.5% of the population with recombination at this QTL.
REFERENCES CITED


APPENDICES
APPENDIX A

ADDITIONAL FIGURES FOR CHAPTER TWO
Appendix Figure 2.16. Total malt extract by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Appendix Figure 2.17. β-glucan by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
Appendix Figure 2.18. Turbidity by treatment combination and family background. Each bar represents the mean of three replications of the lines representing the two QTL types of the given family. The 2016 and 2017 seasons were analyzed separately. The error bars represent standard errors.
APPENDIX B

ADDITIONAL FIGURES FOR CHAPTER THREE
Figure 3.9. 2017 Post Farm field study scatter plot of yield (kg/ha) plotted by the season long average stomatal conductance of the Karl and Lewis background varieties.