GENETICS AND MAPPING OF QUANTITATIVE TRAIT LOCI OF FEED QUALITY-RELATED TRAITS IN BARLEY (*HORDEUM VULGARE L.*)

by

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ABSTRACT

The agricultural sector continues to outpace other industries in Montana. Cattle and calf production are 50% of Montana's agricultural cash receipts. Based on its moderate protein content and lower price, barley is an alternative to corn in finishing cattle diets. Most of Montana barley is produced for animal feed. The objective of this project is to encourage calf producers to retain more feeder calves in Montana by developing improved value feed barley for both the barley grower and cattle producers.

Barley has been criticized for cattle feed because of its rapid digestion in the rumen, which can result in many digestive disorders such as bloat and lactic acidosis in cattle fed on high barley diets. A potential approach to limit this problem associated with feeding barley is to select barley cultivars that have a slower rate of digestion in the rumen. Selection of barley grain for low dry matter digestibility (DMD), low acid detergent fiber (ADF) content and high starch content should allow progress in breeding for improved feed quality.

The aim of the current thesis is to use DNA marker technology coupled with different barley mapping and validation populations and DMD assays to identify the chromosomal location of genes modifying grain composition and digestibility. The obtained QTLs can be used to transfer the low DMD trait from exotic germplasm resources to high yielding cultivars. The largest QTL impacting DMD that we detected in this project resides on barley chromosome 2H very close to *Vrs1*. That gene also impacts plant and seed development. The strong correlation among DMD, particle size and yield and the overlapping QTLs which are controlling the variation in these traits around *Vrs1*, may be due to pleiotropic effects or closely linked QTLs. If this is a pleiotropic effect, gene transfer into 2-rowed backgrounds will be impossible. If, however, linkage is the cause of the association between *Vrs1* and DMD-QTL, then larger mapping populations should permit us to better map the gene and identify useful recombinants.

CHAPTER 1

LITERATURE REVIEW

Introduction

History and Taxonomy

Barley is taxonomically classified within the family Poaceae, tribe Triticaeae and genus *Hordeum*. *Hordeum* is comprised of 30 species, nearly three fourths perennial. Most *Hordeum* species are diploids (2n=14) while the remaining are tetraploid (2n=28) and hexaploid (2n=42) (Bothmer, 1992). Archeological findings show that agriculture started in the Neolithic Near East with barley along with emmer and einkorn wheats as the first cultivated crops. These findings suggested that barley, in the form that is known today, existed and was used from 16000 B.C. in the Nile River valley in Egypt (Wendorf *et al.*, 1979). The center of origin of cultivated barley has been reported to be the Fertile Crescent of the Middle East (Zohary and Hopf, 1988). Harlan (1968) reported evidence of early barley cultivation in Ethiopia, Tibet, Afghanistan and India but considerably later than that in the Middle East. All cultivated barley varieties have non-brittle heads, which allow the spikes to stay intact after ripening. This trait is in contrast with wild barley in which heads are always brittle. Non-brittleness is controlled by a mutation in either one of two tightly linked genes (Bt_1, Bt_2) on chromosome 3H (Takahashi, 1955; Nilan, 1964).

Cultivated barley shows close similarity with a group of wild and weedy barley genotypes, which are traditionally grouped as *Hordeum spontaneum* C. Koch. or *Hordeum vulgare* L. subsp *spontaneum*. *Hordeum spontaneum* is

annual, brittle, two-rowed, diploid and predominantly self-pollinated. It is the only wild barley form cross compatible and fully interfertile with cultivated barleys. The hybrid shows normal chromosome pairing and segregation in meiosis (Zohary and Hopf, 1988). *Spontaneum* heads are separated into individual arrow-like triplets at maturity, structures that, together with their retained awns, act like arrows as they plant themselves. These are highly specialized devices, which ensure the survival of the plant under non-cultivated conditions. These devices allow the wild form of barley to spread to other places. Under cultivation, the non-brittle mutants were selected by man for reaping, threshing and sowing (Zohary and Hopf, 1988). Zohary's findings (1971) showed that most archeological remains dating from 7000 to 6000 B.C. constitute two-rowed forms, while six-rowed forms do not become common until after 6000 B.C.

Cultivated barley is adapted to and produced over a wider range of environmental conditions than other cereals. It can grow at latitude of 70°N in Norway, in the desert on the fringe of the Sahara desert in Algeria and below the equator in Ecuador and Kenya. In addition, it was observed at elevations up to 4200 m on the Altiplano and slopes of the Andes in Bolivia (Nilan and Ullrich, 1993), and at 330 m below sea level near the Dead Sea (Harlan, 1968). It is relatively cold tolerant and is considered the most drought, alkali and salt tolerant among the small grains (Smith, 1995). The relatively early maturity and low water use of barley are the major factors for adaptation to

drought and temperature extremes. Barley is not well adapted to acid and wet soil conditions (Poehlman, 1985).

Types of barley

Due to its wide adaptation, barley has a diversity of agronomic, morphologic and industrial properties. The spike in barley consists of three spikelets attached at each node of the rachis. In two-rowed barley, the central spikelet (floret) of the triplet is fertile, the lateral florets have reduced sexual parts but usually have the lemma and palea. In six-rowed barley, all three spikelets are fertile (Ried and Wiebe, 1968). Kernel shape varies between two-rowed and six-rowed types. All kernels of two-rowed barley are symmetrical with size varying by location within the spike. In six-rowed types the kernels produced from the central spikelets are symmetrical, and the remaining kernels produced in the lateral spikelets have a slightly twisted appearance. Several investigations have studied the fertility of the lateral florets. The intermediate type, in which the awnless lateral florets exhibit fertility greater than that found in the two-rowed and less than that occurring in the six-rowed type, was obtained by crossing two-rowed and six-rowed barley (Harlen and Hayes, 1920). Row type is controlled by a single recessive gene *(v) vrs1* (six-row) on chromosome 2H, while lateral floret fertility is controlled by a single recessive gene (*i)* on chromosome 4H (Nilan, 1964).

Barley may be awned or awnless, an awn being a slender, apically tapering extension of the glumes and the lemma (Ried and Wiebe, 1968). Awns may be rough or smooth. The rough type usually has barbs or hairs that

angle toward the apex. Awns can be replaced by a trifurcate (three-forked) appendage called a hood, and such cultivars are called hooded barley. It is believed that awns photosynthesize as well as transpire. The photosynthetic tissues of awns greatly add to the area of the head (Briggs, 1978). Awn type is controlled by five genes on different chromosomes: *lk* (awnless) on chromosome 2H, lk_2 (short awn) on chromosome 1H, *k* (hooded awn) on chromosome 4H, lk_5 (short awn) on chromosome 5H and *r* (smooth awn) on chromosome 7H.

The barley kernel is a single seeded fruit called a caryopsis. The caryopsis is enclosed between the lemma and palea. The caryopsis consists of pericarp, integuments, aleurone layer, endosperm and embryo (Ried, 1985). The pericarp is a protective covering over the entire kernel. Integuments are outer cell layers enveloping the nucleus of the ovule and differentiating into the seed coat. The endosperm is formed in the embryo sac by cell division after fertilization to provide nutritive tissue, such as starch and proteins, to the developing embryo. The aleurone layer contains protein bodies and enzymes involved with endosperm digestion (Briggs, 1978). In most barley cultivars, the lemma and palea adhere to the kernel, a trait desirable for malting (Burger and LaBerge, 1985). Cultivars in which the grain threshes free of these appendages are called hull-less or naked. Hull-less barley is most frequently found in areas of the world where barley is used for human food. In countries like the United States where the majority of barley is used for livestock feed or malting, hulled varieties predominate.

The aleurone is a layer of cells, usually two to four cells thick, lying just inside the hull, pericarp, testa, and nuclear tissues, and is part of the endosperm (Ried and Wiebe, 1968). Unlike the remainder of the endosperm, these are living cells filled with dense cytoplasm that contain a wide array of organelles: rough endoplasmic reticulum, mitochondria, proplastids and aleurone grains. Grain color in barley is determined in the aleurone layer as colorless, white, yellow, blue or various shades of these. For example, when the anthocyanin pigment is present in the aleurone layer of the caryopsis a blue color is formed.

Barley production

Although barley can survive and produce grain under a wide array of environmental conditions, the best growth and grain production conditions are on well drained, fertile loam soils under relatively cool temperatures (15-30°C) and moderate annual rainfall (50-100 cm) (Nilan and Ullrich, 1993). Northern and central European countries produced 66% of the world's barley in 2001 on 20% of the world's harvested barley area (FAO statistics, 2001). In contrast, subtropical dry climates of North Africa produced 1.6% of the world's barley on approximately 6% of the world's barley acreage. In that year, the world average harvested area of barley was 53.9 million hectares with a yield of 139.6 million tons Barley acreage constitutes approximately 8% of all the harvested area of cereal crops.

Barley is the fourth ranking cereal crop after corn, wheat and sorghum in the United States (Nilan and Ullrich, 1993). The five top ranking barley-

producing states are North Dakota (1.73 million tons), Idaho (1.09 million tons), Montana (0.82 million tons), Washington (0.46 million tons) and Colorado (0.19 million tons) (NASS statistics, 2001). These states produced about 75% of the total 5.43 million tons produced in the United States in 2001 on approximately 76% of the total barley harvested area.

Montana's climate is distinctly continental with extremes in both temperature and precipitation. Fall and winter precipitation across the area is low (8.1- 10.1 cm). April through August, precipitation ranges from 15 to more than 22 cm (Black, 1983). The main crop in Montana is spring wheat (3.75 million hectares) followed by winter wheat (1.45 million hectares) then spring barley (0.384 million hectares) (Montana Agricultural Statistics, 2003). Total barley seeded acreage accounted about 8% of total Montana planted area. Barley is produced under irrigated (224,000 hectares), re-cropped (384,000 hectares) and summer fallow (342,000 hectares) cropping conditions (Montana Agri. Statistics, 2003). Barley sales accounted for about 5.8% of the Montana commodity receipts equivalent to 98.6 million dollars (Montana Agri. Statistics, 2003). Variability of production and income is a major characteristic of dryland barley farming. Drought within and across years is a major problem. Under dryland conditions over a 10-year period (1991-2000), annual yield averaged 2.22 tonsha $^{-1}$, while the average yield of irrigated barley was 4.4 tons ha^{-1}. Nearly 57% of the acres seeded to barley, in both dry land and irrigated farms, were malting varieties and the rest were feed (Montana Agri. Statistics, 2003). Harrington, a malting variety, is the top variety planted on

46% of the Montana's barley acreage followed by Haybet (10.3%), a hay barley, and Baronesse (4.2%), a feed barley (Montana Agri. Statistics, 2003).

Barley cultivated in the United States is classified into four general groups that originated from different areas around the world. The first, Manchuria group, are six-rowed, awned, spring-type varieties, typified by Morex. This group is believed to have originated in Manchuria. They tend to shatter extensively in dry climates. This group is grown mainly in the upper Mississippi Valley and is predominantly used for malting. The second group is the Coast group. These varieties trace to North African ancestry and are grown in California, Arizona and the Inter-Mountain Region. They are six-rowed, awned, and include the variety Steptoe. They mature relatively early and are not prone to shatter. They have a spring growth habit but may be fall or winter seeded in California and Arizona where winters are mild. The third group is Tennessee Winter**.** Varieties of this group trace to the Balkan-Caucasus Region or Korea. They are six-rowed, awned and have winter habit. This group includes varieties such as Kamiak. These varieties are grown in the southeastern quarter of the United States. The fourth group, Two-rowed barleys, as illustrated by Harrington, includes types tracing to Europe and Turkey. The Turkish type is adapted to areas with marginal rainfall. Varieties in this group are grown principally in the Pacific and Inter-Mountain Regions and to some extent in the Northern Great Plains. Varieties are mainly spring type though two-rowed winter varieties are known. Some varieties are used mainly for malting, others for feed (Forster, 1987).

Uses of Barley

Barley is grown mainly for livestock feed, human food and malting. Half or more of the barley grown in the United States is used for livestock feed because it is a short season, early maturing crop grown in both irrigated and dryland areas (Smith, 1995). The entire kernel is used in feed generally after grinding or steam rolling. Malt sprouts from malting as well as brewers' grain byproducts are also valuable livestock feeds. Although barley's energy is not easily utilized by animals, it does have higher protein content than other feed cereals, especially corn, which reduces the need for a protein supplement in the feedstuff (Kellems and Church, 2002). The amount of barley sold for use as feed depends on the relative price of corn and other feed grains.

Around 40% of the barley crop is used for malting in the United States (Nilan and Ullrich, 1993). For malting, barley is steeped in water under controlled conditions allowing it to germinate or sprout and is referred to as green malt. The starch is hydrolyzed by alpha and beta amylases into maltose and dextrins, and proteins are broken down by enzyme action. Malt is primarily an intermediate product and requires further processing. Nearly 80% of malted barley is used for beer production, 14% for distilled alcohol products and 6% for malt syrup, malted milk and breakfast foods (Dickson et al., 1968). The brewing industry uses both six-rowed and two-rowed barleys. Two-rowed varieties are higher in test weight and kernel plumpness and six-rowed barley has superior enzyme characteristics that are crucial in beverage production. Brewers evaluate malt based on total protein, soluble protein extract,

final/coarse difference, diastatic power and alpha amylase (Burger and LaBerge, 1985).

Barley consumption as a human food is overshadowed by its use in livestock feed and malting with approximately 2% of the United States barley production used for food products (Smith, 1995). Most barley used for food is used as either pearl barley or barley flour. Pearling is a polishing process that removes the outer husk and part of the bran layer of the kernel. Barley flour, a byproduct of pearling, is used for baby foods and breakfast cereals, or mixed with wheat flour in baking. Barley flat bread and porridge is widely consumed in North Africa and part of Asia. In general, barley is used as a food source in the regions where other cereals do not grow well due to the environmental limits such as latitude, low rainfall, soil salinity, high elevation and short growing season conditions (Nilan and Ullrich, 1993). Hull-less barleys are preferred for food applications, where a minimal amount of cleaning is required prior to processing. Barley is a good source of dietary fiber, includes soluble and insoluble fiber fractions and contains inhibitors of cholesterol biosynthesis (Qureshi et al., 199; Ranhotra et al , 1991).

Barley is also grown as a hay crop. Based on nutritive parameters, forage should have optimum dry matter concentration for proper fermentation after ensiling, high digestibility to maximize intake, efficiency of conversion by animals and high protein concentration to reduce requirements of supplemental protein. Young plants have about 85% moisture and 14-16% protein (Briggs, 1978). If the whole plant is used, it must be cut when the yield

is sufficient, the nutritive value is suitable and before maturation of awns. Awns can affect the palatability of barley and cause irritation inside the cow's mouth. Therefore, the hooded or awnless barley is preferred for haymaking. Barley hay should be dried rapidly and handled gently to minimize head loss. When barley is used as hay, low nitrate accumulation potential would be advantageous. Nitrate accumulation in the barley can result in nitrate toxicity at levels of 1,130-2,260 ppm No_3-N or 5,000-10,000 ppm NO_3 on a dry basis for cattle (Cash et al., 2002). Sowing a mixture of barley and legumes can enhance the feed value. Winter barley also may be grazed until the stems begin to elongate.

Properties of feed constitutes

Morrison (1956) defined a nutrient as any feed constituent, or group of feed constituents of the same general chemical composition, that aids in the support of animal life. The functions of these nutrients are to serve as structural materials for building and maintaining the body, as sources of energy for animal activities, and to regulate and control animal body functions (Perry et al., 1999). The basic nutrients that are found in varying quantities in animal feed include water, carbohydrate, fat, protein, vitamins and minerals (Matsushima, 1979).

The animal obtains its water from three sources: drinking water, water present in its food and metabolic water formed within the body as a result of oxidation. Water functions in the body as a solvent in many enzymatic reactions, in the transport of other nutrients and in maintenance of normal

body temperature (Perry et al., 1999). Water can also be a resource for minerals; cattle receive from 20-40% of NaCl, 7% -28% of Ca, 6-9% of Mg and 20-40% of their sulfur requirements from water (Kellems and Church, 2002). Beef cattle water consumption ranges from 8.4-12.5 l per 100 kg bodyweight depending on many factors such as animal age, air and water temperature, protein and salt content of the ration, physiological conditions of animal and water quality (Matsushima, 1979).

In general, cereal grains contain 8-14% crude protein, 1-6% fat and 41-72% starch. Barley grain does not differ widely from other grains based on chemical composition. It contains more total protein, higher essential amino acids levels and higher crude fiber than corn and sorghum, but lower starch and lipid content (Kellems and Church, 2002).

Carbohydrates are the primary compounds found in feedgrains. In ruminants, carbohydrates are classified into two groups, nitrogenous free extract (NFE) and fibrous carbohydrates. NFE is degraded in the rumen into monosaccharides, disaccharides and starch (Cecava, 1995). Starch consists of amylose and amylopectin. In the small intestine, starch and disaccharides are broken down into 6-carbon sugars by amylases (Perry et al., 1999). Due to its composition, NFE is considered the most important component in cattle feedstuff because it represents the most important energy source in finishing diets. Fibrous carbohydrates, crude fiber, or cell wall carbohydrates include less degraded fractions as cellulose, hemicellulose, pectin and lignin (Perry et al., 1999). Cereal grains are the major source of starch in finishing diets.

Most feedstuff proteins are degraded into peptides and amino acids by ruminal microorganisms. The dietary amino acids are divided into essential and nonessential amino acids. Ruminant animals can survive on diets that do not contain protein but contain a nitrogen source because of the microbial organisms in the gastrointestinal tract, which can synthesize about 50% of both essential and nonessential amino acids (Kellems and Church, 2002b; Bowman and Sowell, 2002). Protein and amino acids play a prominent role in animal physiology and nutrition. That role is more important for young beef cattle that have higher protein requirements. Optimization of protein supply to growing or lactating ruminants improves efficiency of protein utilization and feed intake (Cecava, 1995).

Fats or lipids are a group of substances that are characterized by their insolubility in water and solubility in organic solvents (Cecava, 1995). Fats are classified as untrue fats such as cholesterol and wax, and true fats. True fats are important sources of energy and can supply the animal with 2.25 times more energy than carbohydrates by weight (Matsushima, 1979). As well as being an energy source, fats are important thermal insulators (McDonald et al, 1995). In the rumen, fat is hydrolyzed to glycerol and constituent fatty acids (Kellems and Church, 2002b). In beef cattle nutrition, common feedstuffs contain low levels of fat ranging from none to 2% in hays, 4-5% in grains, 7-10% in distillery by-products, 13% in rice bran and up 98% in oils and tallow. According to feeding standards, a diet based on a combination of hay, corn and oil meal contains less than 4% fat (Cecava, 1995).

Ruminant Nutrition and Metabolism

Many of the organic components of feed are in the form of large insoluble molecules, which have to be broken down into simpler compounds before they can pass through the mucous membrane of the gastrointestinal tract (GI), and into the blood and lymph. That degradation process is termed digestion, while the passage of the digested nutrients into the blood or lumph is termed absorption (McDonald et al., 1995). Animals are classified according to their digestive physiology into nonruminant and ruminant animals. Nonruminant animals such as pigs, rabbits, horses, poultry, cats and dogs, have a single compartment stomach and do not ruminate. Ruminants move rumen-reticulum contents back to the mouth for further particle size reduction. Nonruminants, except horses, make poor utilization of high fiber feedstuffs (Gray, 1992). Ruminants such as cattle, sheep and goats have a multi-compartment stomach consisting of the rumen, reticulum, omasum and abomasum (Perry et al., 1999). Ruminants are able to make effective utilization of high fiber feed by the microbial fermentation processes in the rumen. Unlike nonruminants, ruminants do not produce salivary amylase, so there is no starch digestion in the mouth. Absorption is the movement of the end products of digestion from the digestive tract into the blood or lymph system and is accomplished by diffusion through the semi permeable membranes that line much of the digestive tract (Perry et al., 1999).

Digestion and Absorption

Carbohydrates Nonfiber carbohydrates are mainly degraded in the rumen. That digestion is dependent on enzymatic activities of a range of microorganisms, which include Bacteroides ruminicola, B. amylophilus and Streptococcus bovis (Kotarski et al., 1992; Russell and Rychlik, 2001). The factors that influence the rate and extent of starch digestion in the rumen include source of starch, diet composition, grain processing, amount of feed consumed per unit time, rumen microbes species and dietary additives (Huntington, 1997).

Grains that have hard testa or husk, such as corn and sorghum, are less affected by rumen microbial activities so they have a slower rate of starch degradation, while grains that have a thin testa such as wheat, barley and oat, are rapidly degraded (Herrera-Saldana et al., 1990). Grain processing such as cracking or rolling increases the endosperm surface area exposed to rumen microbes, which allow these microbes to degrade the starch more rapidly compared with the whole grain. The main end products of microbial nonfibrous carbohydrate digestion in the rumen are methane and volatile fatty acids (VFA) such as propionic, butyric and acetic acid. With rapid starch fermentation (degradation), the VFA and lactic acids may accumulate in the rumen and increase the risk of acidosis (Kotarski et al., 1992) caused by the overgrowth of starch-fermenting, lactate-producing bacteria (Owens et al., 1998). Another disorder caused by rapid starch fermentation is bloat. Bloat normally occurs when froth is formed in the rumen and inhibits the eructation

process. The gas accumulates rapidly, compressing the lungs and causing asphyxiation (Kellems and Church, 2002c).

Whereas little starch or other α-linked glucose polymers reach the small intestine on high forage diets (Heald, 1952), considerable amounts of starch escape ruminal digestion on high grain diets (Sutton, 1985; Ørskov, 1986; Huntington, 1997). The basis of starch digestion in the small intestine is enzymatic hydrolysis (Huntington, 1994). Pancreatic amylase acts on starch to produce maltose. Intestinal maltase secreted by the intestinal wall converts maltose to glucose (Perry et al., 1999). Starch can also be digested in the large intestine by pancreatic and intestinal amylases (Ørskov, 1986).

Fibrous carbohydrates are usually retained in the rumen for periods of time, as long as 10 days, to allow for microbial digestion (Kellems and Church, 2002c). A range of cellulolytic and non-cellulolytic microorganisms break down the cellulose into cellobiose and glucose (Cheng et al., 1991; Flint and Forsberg, 1995). The remaining cellulose is digested in the large intestine by microbial fermentation (Perry et al., 1999).

Carbohydrates are absorbed as glucose in the small intestine into the bloodstream. It is believed carbohydrates combine with phosphorus for absorption across the villi of the small intestine (Cecava, 1995).

Carbohydrates constitute about 80% of barley grain (MacGregor and Fincher, 1993). They are divided into starch, sugars, and non-starch polysaccharides such as ß-glucan, cellulose and arabinoxylans.

Starch is the main compound of barley carbohydrates (NRC, 1996). Starch content represents up to 65% of kernel dry weight (MacGregor and Fincher, 1993). Starch content is affected by environmental conditions (Kong et al., 1995), grain type and hull type (Kong et al., 1995; Li et al., 2001). Most barley starches contain two major components: amylopectin (74-78%) in which chains of α-(1$\rightarrow$4) D-glucopyranose units are branched through α-(1$\rightarrow$6) linkages, and amylose (22-26%) that contain straight chains of D-glucopyranose units linked α-(1$\rightarrow$4) (Newman and Newman, 1992). The waxy gene (*wx*) located on chromosome 1H (Nilan, 1964), produces starch that is 96-100% amylopectin versus a 3:1 ratio of amylopectin and amylose in normal barley (Xue *et al.*, 1997). Waxy barley contains less starch and greater amounts of free sugars such as sucrose, maltose and hexose than normal barley (Xue et al., 1997). A mutant discovered in the barley variety Glacier (Glacier Ac38) has increased amylose level (44%) (Merritt, 1967).

The composition and properties of the non-starch, cell wall polysaccharides are of importance in relation to the nutritional quality of barley grain. Non-starch polysaccharides are comprised of two major components (1$\rightarrow$3) and (1$\rightarrow$4)β-D-glucans (β-glucans) and arabino- (1$\rightarrow$4)-β-D-xylans (arabinoxylans), with smaller amounts of cellulose (MacGregor and Fincher, 1993). The cell wall of the starchy endosperm of barley grain contains a high proportion of β-glucans (75%) and low proportion of arabinoxylans (20%) and cellulose (about 2%) (Shewry and Morell, 2001). While the aleurone cell walls

contain approximately 71% arabinoxylan and 26% β-glucan (Bacic and Stone, 1981).

β-glucans consist of linear chains of β-glucosyl residues polymerized through both (1→3) and (1→4) linkages (MacGregor and Fincher, 1993). Variation in endosperm β-glucan content is controlled by both genetic and environmental factors (Fastnaught et al., 1996). β-glucan content increases during grain development (Fastnaught et al., 1996). In the brewing process, a high β-glucan content in malting barley may lead to a reduced rate of wort filtration. This is due to high viscosity imparted by the β-glucan that adversely affects the recovery of malt extract and may lead to haze formation in beer (Böhm and Kulicke, 1999; Zhang et al, 2002).

High β-glucan content barley has been implicated in problems encountered in animal feeding, particularly in nonruminant animals such as poultry and pigs (Newman and Newman, 1992). Barley β-glucan has been associated with the formation of gels in aqueous media that are believed to be responsible for the excretion of sticky droppings, which reduce the water holding capacity of the litter on which broilers are reared. This can cause hock problems and damage to the breasts of birds, thus reducing quality and market value of meat products (McNab and Smithard, 1992).

Arabinoxylans contain a backbone structure of D-xylopyranose residues linked by β-(1→4)- glycosidic bonds with units such as L-arabinofuranose attached as branches by β-(1→2)- or β-(1→3)-linkages (Viëtor et al., 1991). Arabinoxylans alone or synergistically with β-glucans may be involved in

influencing the rate of wort separation (Han, 2000). Arabinoxylans, as β-glucans, also make an important contribution to the viscosity of gut contents in chickens and will thereby adversely affect feed digestibility and growth rate (MacGregor and Fincher, 1993).

More than 96% of the total grain cellulose is present in the hull (MacLeod and Napier, 1959). Cellulose is (1→4)-β-glucans containing up to several thousand β-gluco-pyranosyl residues linked through (1→4)-β-linkages (MacGregor and Fincher, 1993). Cellulose is indigestible in nonruminant animals, contributing only a small amount of available energy through the production of volatile fatty acids in the large intestine (Newman and Newman, 1992). In ruminants, cellulose contributes considerably more energy and carbon skeletons through fermentation in the rumen (Newman and Newman, 1992).

Proteins Protein digestion is more complex than that of carbohydrates. In the rumen, some proteins are broken down to peptides and amino acids by ruminal microbes such as Peptostreptococcus anaerobius, Clostridium aminophilum and C. sticklandii (Russell and Rychlik, 2001). While the majority of dietary protein is degraded to ammonia, a portion is recycled into the rumen as urea in saliva (McDonald, 1954). The extent of urea recycled to the rumen, both in saliva and by transfer across the rumen wall, is markedly influenced by the nature of the diet (Obara et al., 1991). Ammonia is incorporated into amino acids by ruminal microbes, and results in forming microbial proteins. The extent of protein digestion in the rumen is affected primarily by rate of protein

degradation and rate of passage of digesta from the rumen (Cecava, 1995b). The rate of protein digestion is influenced by the protein's physical and chemical properties, particle size of ingested materials and speed of digestion in the rumen (Kellems and Church, 2002b). The amount of protein in the intestine is the sum of microbial protein, intermediate protein breakdown product (IPBP) and dietary protein that escaped rumen degradation. These proteins are degraded by trypsin and certain other proteases of the pancreatic juice to produce amino acids and other IPBP. The remaining portion of IPBP is digested by intestinal peptidases to amino acids (Perry et al., 1999).

The nutritional significance of protein degradation was confirmed by Chalmers *et al.* (1954), who showed that casein, which is rapidly degraded in the rumen, is utilized more efficiently when administered post-ruminally. Many attempts have been made to shift the site of protein digestion from the rumen to the small intestine by chemical and physical treatments such as heat denaturation (Chalmers et al., 1954) and expander treatment (PrestlØkken, 1999).

Proteins are absorbed as ammonia across the wall of the rumen into the bloodstream. The absorbed ammonia is transported to the liver where it is synthesized into urea. Urea may be transferred to the kidneys for excretion in the urine, pass into saliva and then return back into the rumen, or pass into the bloodstream and back into the gut (Cecava, 1995b). The main form of absorbed proteins is amino acids with absorption occurring in the small intestine. The fate of these amino acids may be twofold. First is the

resynthesis of tissue protein and other nitrogen-containing constituents such as enzymes, hormones and milk. The second fate is deamination in the kidneys and liver (Cecava, 1995b).

Proteins account for 8-15% of the dry weight of the mature barley grain (Shewry, 1993). Protein content is affected by environmental and genetic factors (Kong et al, 1995). Barley contains more total protein and higher levels of lysine, tryptophan, methionine and cystine than corn (Kellems and Church, 2002). Osborne (1924) classified the proteins according to their solubility in different solvents into four groups. Barley proteins are prolamins (hordeins), which are extracted by aqueous alcohol, albumins that are soluble in water, globulins which are soluble in salt solutions and glutelins which are soluble in strong acid or alkali (Shewry, 1993). Hordeins can be classified into four groups: B hordeins (polymeric S-rich prolamins) account for 70-80% of the prolamins fraction, C hordeins (monomer S-poor prolamins) account for 10-20%, D hordeins (polymeric high molecular weight prolamins) account for 5% and γ-hordeins (S-rich prolamins) account for 5% (Shewry and Morell, 2001). The major effect of high grain nitrogen is an increase in the proportion of hordein (Kirkman et al., 1982). Increases in hordeins reduce the lysine fraction in the total protein (Newman and Newman, 1992).

Fats In the rumen, fat is hydrolyzed to free unsaturated long chain fatty acids by rumen bacteria (Garton, 1977). Long chain fatty acids are not absorbed directly from the rumen. When they reach the small intestine they are mainly saturated and unesterified (McDonald et al., 1995). In the small

intestine, bile plays a role in forming fat micelles, which contact microvilli of the intestinal mucosa (Cecava, 1995). Certain compounds of bile secreted by the liver react with fat to form soap and glycerol. Pancreatic lipase secreted by pancreas acts on the fat to form fatty acids, glycerol and monoglycerides (Perry et al., 1999).

Fats, in the form of free fatty acids, are absorbed in the upper small intestine and travel via lymphatic circulation in the form of chylomicrons, which consist of triglycerides and small amounts of phospholipids, free fatty acids, cholesterol and cholesterol esters (Cecava, 1995).

Barley generally contains about 2-3% fat (Welsh, 1978) with some varieties containing up to 6% (Andersson *et al* , 1999). Lipid and total fatty acid contents are affected by barley genotype and environment (DeMan, 1985). In general lipid content does not vary much with grain protein content or edaphic variation (Morrison, 1993). In contrast, lipid content in the high lysine barley mutant Ris∅1508 is 3.6% compared with about 1.8% in the parental variety Bomi (Shewry *et al.*, 1979). The main classes of barley lipids are nonpolar lipids (67-78%), phospholipids (14-21%) and glycolipids (8-13%) (Morrison,1993). Approximately 8% of barley oil is non-saponifiable compounds including carotenoids, tocopherols and isoprenoid products (Newman and Newman, 1992). Carotenoids and tocopherols are of interest in clinical nutrition because of their antioxidative properties (Anderson and Theron, 1990). Dietary D-α tocotrienol was identified as an inhibitor of cholesterol biosynthesis in livers of experimental animals because of its ability

to suppress 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Qureshi et al., 1986). Hypercholesterolmic chicks were fed on barley oil (558 ppm -α tocotrienol), maize oil (23 ppm -α tocotrienol) and margarine for 10 days. The serum cholesterol levels of chicks which consumed barley oil were 172 ± 10 $mg \cdot dl^{-1}$compared with 227 ± 15 $mg \cdot dl^{-1}$ and 262 ± 50 $mg \cdot dl^{-1}$ for those fed maize and margarine respectively (Wang et al, 1990).

Minerals and Vitamins The ash content of barley accounts 2-3%, with the major components being P, K and Ca and lesser amounts of Cl, Mg, S and Na (Newman and Newman, 1992).

About 60-80% of the total phosphate in grains is present as phytate, *myo*-inositol 1,2,3,4,5,6-hexabisphosphate (Raboy and Gerbasi, 1996). Most phytate is localized in the aleurone (90%) with only 10% in the embryo (Hatzack *et al.*, 2000). Because of the high density of negatively charged phosphate groups in phytate, it decreases the bioavailability of other mineral elements such as Ca, Zn, Fe, K, and Mg for both humans and non-ruminants (Hatzack et al., 2000). Phytase is added to animal diets to break down phytate and increase the availability of organic phosphorus (Bedford, 2000). However, ruminants have the ability to produce phytase and hydrolyze phytate in the rumen (Nelson et al., 1976). Some feed processing methods such as heat treatment can reduce the phytate degradation in the rumen and phytate-P availability (Park *et al.*, 2000). The approaches to produce low phytate grain include overexpression of phytases in transgenic cereals and mutational

breeding of low-phytate mutants (Larson et al., 1998; Rasmussen and Hatzack, 1998; Hatzack et al., 2000; Raboy, 2001).

Barley grain contains no vitamins A, B_{12}, C or D, although the carotenoids and sterols that are present may act as precursors for A and D respectively (Briggs, 1978). Barley grain also has a low concentration of thiamine, pyrodoxine, riboflavin and panthothenic acid (Kellems and Church, 2002).

Chemical and physical characterization of barley feed quality

The feed quality of barley is influenced by many factors including chemical and physical characteristics. The complexity of these factors has contributed to inconsistent data on feed quality of barley.

Physical factors

Most physical characters of barley grain such as plumpness, 1000 kernel weight and bushel weight are important for variety improvement but their influence on feed quality is not clear (Bhatty, 1993). Because feed barley is utilized as processed products rather than whole grain, the importance of these physical factors may be lost during barley processing (Bhatty, 1993).

The hull constitutes 10-13% of the dry weight of barley (Bhatty et al., 1975). Because the major components of the hull are cellulose, hemicellulose, lignin and pectin (Bhatty, 1993), hull-less varieties have reduced fiber and increased starch contents (Yang et al., 1997), which is reflected in increased digestibility and energy value for the nonruminant animals (Beames et al., 1996). Bowman et al. (2001) concluded that hull-less world collection barleys

had greater starch and lower acid detergent fiber (ADF) than the hulled barleys. The absence of hull increased the ruminal dry matter digestibility by 5.5% in a comparison between 16 hulled and six hull-less varieties (Lehman et al., 1995). A side effect of feeding ruminants hull-less barley is increased digestion rate that accumulates ruminal acid (Lehman et al., 1995). Increasing ruminal acid will decrease the ruminal pH and cause an increased incidence of liver abscesses, bloat, acidosis and laminitis (Hunt, 1996; Zinn et al., 1996; Yang et al., 1997).

Based on many studies, two-rowed barley contains more starch and less fiber than six-rowed barley (Kong et al., 1995). This may be the main reason for higher animal performance with two-rowed barley than six-rowed barley (Boss and Bowman, 1996; Ovenell-Roy et al., 1998). Also, two-rowed barley has a higher ruminal digestibility than six-rowed barley (Lehman et al., 1995; Bowman et al., 2001).

Hardness or kernel texture has been described as the most important single characteristic that affects the functionality of common wheat (Shewry and Morell, 2001). It affects a range of characters including milling, end-use properties and wheat classification. Grain texture reflects the ease with which the endosperm can be separated into fragments and particles. Kernel hardness has received little attention in barley breeding programs. This character is important for grain quality of malting varieties, where the soft-textured varieties generally malt better than the hard-textured types (Allison et al., 1976; Brennan et al, 1996). For feed varieties, Beecher et al. (2002) found

negative correlation between barley kernel hardness, as measured by single kernel characterization system (SKCS), and dry matter digestibility (DMD). They concluded that development of hard barley (high SKCS) might benefit feed quality. This suggestion is supported by an association between high feed quality, large particle size and low DMD (Bowman et al., 2001). Surber et al. (2000) indicated a relationship between particle size and feeding quality of barley.

In ruminants where barley starch degradation occurs mainly by microorganisms, large particle size provides a small surface area for the microorganism colonization. Thus the starch digestion rate is reduced and subsequently effects animal performance (Ewing et al., 1986; Bowman et al., 2001).

In barley endosperm, starch consists of a mixture of large lenticular granules 15-25 µm in diameter, type A, and smaller irregularly shaped granules < 10 µm in diameter, type B (MacGregor and Fincher, 1993). Although type A comprises about 10% of starch granules, it represents about 90% of total starch weight (Borém et al., 1999). The ratio between type A and B is associated with the ratio between amylopectin and amylose, respectively. For example, in high amylose barley starch, type B granules are larger and type A granules are smaller than the corresponding granule types in normal barley starch (MacGregor and Fincher, 1993).

Only α-amylase and glucoamylase enzymes have the ability to hydrolyze significant amounts of both starch granule types (Slack and Wainwright, 1980;

Takahashi et al., 1985). Type B granules are hydrolyzed faster than type A by α-amylase and glucoamylase, potentially due to differences in the surface area in contact with the enzymes (Morrison et al., 1986; McHale, 1986). Two recent studies report that there is no association between malting quality and starch granule traits, such as surface area of A granules, surface area of B granules, volume of A granules, volume of B granules, proportion of A to total starch by volume and ratio of number B/A granules (Oliveira et al., 1994; Borèm et al, 1999).

Chemical factors

Recent research identified that chemical factors including starch content, acid detergent fiber (ADF) and dry matter digestibility (DMD) are important barley feed quality traits (Surber et al, 2000).

Crude fiber value reflects the indigestible portion of cell wall such as lignin and fibrous carbohydrates of the feed (Perry et al., 1999). Both ADF and neutral detergent fiber (NDF) procedures are used to estimate crude fiber. NDF, which is the residue after extraction with boiling neutral solutions such as sodium lauryl sulfate and EDTA, consists mainly of lignin, cellulose and hemicellulose. ADF, the residue after refluxing with 0.5 M sulfuric acid and CTAB, is the crude lignin and cellulose fractions (McDonald et al., 1995).

NDF and ADF are affected by environment and cultivar (Kong et al., 1995). Feed efficiency in beef cattle fed barley-based diets declined as the ADF content of barley increased (Engstrom et al., 1992). Two-rowed barleys contain less NDF and ADF than six-rowed; therefore, the nutritional value of

two-rowed barley is higher than six-rowed (Kong et al., 1995). Hull-less barley also has lower NDF and ADF than hulled barley (Kong et al., 1995; Zinn et al., 1996).

In order to correctly measure the supply of absorbed feedstuff, the digestibility of this feedstuff must be assessed. There are several methods for measuring the dry matter degradation of barley in the rumen. In an *in vivo* trial, feedstuff is given to the animal in known amounts and the output of feces measured (Matsushima, 1979). This method has been used widely in barley feed experiments (Bradshaw et al., 1996; Boss and Bowman, 1996; Noon et al., 1998). Although accurate for digestibility, *in vivo* analysis is extremely expensive and not realistic to evaluate a large number of samples (Khorasani et al., 2000). It also requires a large amount of barley grain per sample.

The second method, *in vitro*, determines digestibility by reproducing the reactions that take place in the animal digestive tract using enzymatic procedures, hydrochloric acid and rumen fluid in a laboratory setting (McDonald et al., 1995). While it can be rapid and inexpensive, this method does not closely simulate physiological conditions of ruminants (McNiven et al., 2002). Digestibility is generally lower than that determined using *in vivo* techniques and corrective equations are required to relate one measure to another (McDonald et al., 1995).

The *in situ* or *in sacco* method involves the placement of non-digestible bags containing barley grain, as whole or cracked grain, into the rumen of a cannulated cow for a certain time interval. Then the amount of dry matter that

has disappeared from the bag is measured (Herrera-Saldana et al., 1990; Khorasani et al., 2000). This method provides digestion analysis within the rumen itself and reduces the need for ruminal simulation. The measurement of rumen degradation of feedstuff incubated in a nylon bag can be affected by a variety of factors such as bag and sample size, bag material, pore size, sample processing, animal diet, feeding level and frequency, bag insertion and removal procedures, location of the bag in the rumen, rinsing procedure, microbial correction and incubation time (Vanzant et al., 1998).

Bowman et al. (1996) tested the *in situ* procedure with various durations and grain processing procedures and developed a standardized procedure for evaluating barley DMD. In that procedure, barley grains were cracked through a Buehler mill to simulate dry rolling processing, placed in 10 x 20 cm nylon bags and incubated in the rumen of a cannulated cow for three hours.

Genotypic effects on *in vitro* DMD (Kemalyan et al., 1989), *in situ* DMD (Khorasani *et al.*, 2000) and *in vivo* DMD (Boss and Bowman, 1996) have led to an increased emphasis on barley selection based on DMD. Bowman et al. (2001) reported that *in situ* DMD was affected by genotype, spike type and grain type. Their results indicated that selection for low DMD with other grain quality parameters should allow progress in improving barley feed quality.

Animal performance reflects both physical and chemical characteristics of barley and the interaction between them. It is often measured by average daily gain, carcass grade at slaughter time, and feed efficiency.

The meat from barley fed calves tends to be more highly marbled and higher in color than meat from corn fed calves (Beauchemin et al., 1997). Carcass grade may also be variety dependent with steers fed Harrington exhibiting higher carcass quality than those fed Medallion or Gunhilde (Boss and Bowman, 1996).

Average daily gain (ADG) varies among barley varieties and is related to digestible starch intake (Boss and Bowman, 1996). ADG of steers fed Boyer was 12% greater than those fed Steptoe (Preston and Herlugson, 1980). They reported differences in energy content of the grains. Medallion had higher net energy for maintenance (NE_m) than either Harrington or Gunhilde and higher net energy for gain (NE_g) than Gunhilde (Boss and Bowman, 1996).

Animal performance can be predicted through a lab analysis of characters including starch content, ADF and DMD (Surber et al., 2000). Negative significant correlations were found between ruminal DMD and ADG ($r = -0.36$), NE_m ($r = -0.37$), NE_g ($r = -0.59$) and gain/feed ($r = -0.37$) of barley (Surber et al., 2000). These negative correlations between DMD and animal performance characters indicate that cattle fed barley with a slower rate of digestion performed better in the feedlot and had higher energy resulting in greater gain/feed that those fed high DMD barley.

While starch intake was significantly correlated with ADG ($r = 0.53$), starch content was negatively correlated with ADF ($r = -0.45$). ADF is the most significant variable influencing energy value (Engstrom et al., 1992; Fairbairn et al., 1999), and low lignin and cellulose will reduce the amount of ADF

content (Hunt, 1996) resulting in increased ADG. Starch content was negatively correlated with ADF (r = -0.48) and positively correlated with NE_g (r = 0.37), NE_m (r = 0.34) and gain/feed (r = 0.33) (Surber *et al.*, 2000), which indicates high starch content will decrease non-digestible components that result in increased gain/feed.

Particle size after rolling was positively correlated with gain/feed (r = 0.35) but negatively correlated to both *in vivo* DMD (r = -0.68) and dry matter intake (DMI) (r = -0.31) (Surber et al., 2000). Based on this relationship, large particle size will decrease DMD resulting in increased ADG.

Measurements of *in situ* DMD, ADF, starch content and particle size after dry rolling of barley grain contribute to our ability to predict performance of cattle fed high barley diets. The combination of lab analysis could be used to estimate barley NE_g, NE_m, ADG and gain/feed (Surber et al, 2000).

Genetic improvement of barley for cattle feed

Improvement of barley varieties has involved many different characters. In the USA, increased yield, greater disease resistance and improvement of quality parameters have been the most important goals (Anderson and Reinbergs, 1985). Foster (1987) reported that feed or nonmalting varieties have been developed with the major emphasis being high yield for producers with limited consideration for feed quality. The neglect of feed quality is related to difficulties in screening breeding material for quality traits, lack of well-defined quality parameters and lack of a premium in the marketplace for improved feed cultivar (Foster, 1987). Steptoe was one of the best-yielding

varieties when grown in the Pacific Northwest and Intermountain regions (Muir and Nilan, 1973), but the lack of feed tests resulted in failure to identify its poor feed quality before release as a feed variety (Foster, 1987; Surber et al., 1998). Recently, the feedlot parameter can be predicted through lab tests such as starch content, DMD, ADF and particle size. Bowman et al. (2001) concluded that selection for low DMD, low ADF, large particle size after dry rolling and high starch content will help the barley breeder improve feed quality by broadening the genetic variation of selected parents. Following these criteria, Valier was recently released by Montana State University Experiment Station and is the first barley variety to have documented feed quality prior to its release (Blake et al., 2002).

The improvement of barley has been accomplished by utilizing different resources of genetic variability. *Hordeum* germplasm consists of primary, secondary and tertiary gene pools (Bothmer et al., 1992). The primary gene pool of cultivated barley is comprised of breeding lines, released varieties, landraces still available in some parts of the world and the wild barley progenitor, *H. spontaneum*. There is no biological limitation for gene transfer between these forms, although the presence of undesired genes can create difficulties in their use as breeding materials (Bothmer et al., 1992). The secondary gene pool comprised of *H. bulbosum* is widely used breeding material, the hybrid between H. *vulgare* and *H. bulbosum* has high meiotic pairing and the two species are considered to share the common basic genome (Subrahmanyam and vonBothmer, 1987). The tertiary gene pool

includes all other *Hordeum* species. Crossing between the tertiary gene pool plants and cultivated barley mostly produce unfertile plants.

In barley, as in many other crop species, there is limited genetic variation in economical traits due to domestication (Martin et al., 1991; Hayes et al., 1997). The two distinct barley germplasm pools are two-rowed and six-rowed barley (Powell et al., 1990; Ortiz et al., 2002). Using coordinate analysis, Martin et al. (1991) indicated that two-rowed and six-rowed gene pools are well-differentiated. They concluded that both 2-rowed and 6-rowed malting barley cultivars have a narrow genetic base.

Even though feed barley varieties have variation in feed quality parameters such as DMD, variation is far more limited that that found in the barley world collection (Surber et al., 1998; Khorasani et al., 2000; Bowman et al., 2001). Using parents high in DMD, a breeding program will produce individuals that have high DMD values. To broaden the variation of DMD and other quality parameters, Bowman et al. (2001) surveyed 1480 accessions of the USDA world barley core collection, which includes varieties, breeding material and landraces from all over the world. They found that DMD values of these accessions varied from 8.2% to 62.1%, while a diverse array of cultivars varied from 42% to 56%. After screening the entire collection of 1480 lines, the study concentrated on 73 selected accessions, 36 with high and 37 with low DMD to evaluate trait stability and correlations between DMD and starch content, ADF, and particle size after dry rolling. While significant correlations were observed among these characters, individual lines showed unique

combinations of these traits. Six lines, including PI370970, were selected for further experimentation based on low DMD, high starch content, large particle size and low ADF content.

Genetic control of quality parameters

In general, agronomic and grain quality traits are affected by many factors such as genotype, environment and genotype x environment (G x E) interaction. Heritability provides an estimate of the potential effectiveness of selection for a trait within a population, and depends on the relative importance of environment and genetic factors in expression of the trait. Genetic factors include both total genetic and additive genetic variances (Fehr, 1987). Inheritance can follow either quantitative or qualitative patterns. Qualitative traits such as aleurone color, awn type, rachilla hair length, hull color, dwarfness, growth habit and resistance to some diseases are controlled by one or two genes. Quantitative traits are controlled by allelic variation at multiple genes (Foster, 1987). Every population is unique, and population structure determines the complexity of genetic variation underlying a trait. Some traits traditionally considered to be classic quantitative characters include diastatic power, grain yield, lodging and plant height. The heritabilities of these characters varied from high 82% (diastatic power) to medium 66% (lodging score and plant height) and low 44 % (grain yield) across an array of populations (Hockett and Nilan, 1985). Prior to this project, little effort had been made to assess genetic variation for barley feed quality parameters such as DMD, starch content, particle size and ADF.

Several genes such as *amoI* on chromosome 2H and *stb* on chromosome 5H are known to have an impact on starch content (Hockett and Nilan, 1985). While starch content is affected by environment and variety (Kong et al., 1995) G x E interaction effect has been reported to be low on this trait (Molina-Cano et al., 1997). The broad sense heritability of starch content is 33% based on five varieties at six locations (Molina-Cano et al, 1997) .

ADF is affected by environment and genotype (Kong et al, 1995; Bowman et al., 2001). A significant line x location interaction was detected for ADF (Frègeau-Ried et al., 2001), while Molina-Cano et al. (1997) concluded that there is no G x E interaction for crude fiber. Selection for low crude fiber can start in early generations because of its high heritability (80%) (Molina-Cano et al., 1997). Frègeau-Ried et al (2001) reported that additive x additive epistasis was detected for NDF and ADF, and selection intensity for these traits should not be too severe early in the selection program.

Less is known about the genetic basis of grain hardness in barley than in wheat (Beecher et al., 2001). Variation in wheat grain hardness is controlled by allelic variation in one major gene, *Ha*, linked to three structural genes *puroindoline a* (*pinA*), *puroindoline b* (*pinB*) and *Gsp-1a* on chromosome 5D (Baker, 1977; Giroux and Morris, 1998). Hordoindolines are the homologs of puroindoline genes in barley (Gautier et al., 2000) on chromosome 5H (Beecher et al., 2001). Three *hinA* and two *hinB* sequence types were found, which indicates substantial allelic variation at this locus (Beecher *et al.*, 2001). A QTL mapped to the *HinA/HinB/Gsp* region on the short arm of chromosome

5H explained 22% of the SKCS hardness variation in the Steptoe x Morex population (Beecher et al., 2002).

Identification of QTL for traits influencing barley feed quality

Lande and Thompson (1990) proposed marker-assisted selection (MAS) as a method that allows complex traits to be screened in the early generations of breeding programs. Barr et al. (2001) identified nine key steps in the identification of molecular marker(s) linked to traits for use in MAS including identifying parents differing in trait(s) of interest, developing a population of plants segregating for these trait(s), screening the population for the trait(s), constructing a linkage map of the population to locate a QTL associated with the trait(s) of interest, identifying molecular marker(s) that co-segregate with the trait(s) of interest, marker validation of these marker(s) in different background, producing clear and simple protocols for assaying the marker(s), modifying breeding strategy to optimize use of MAS relative to alternative selection techniques and implement into breeding programs. A quantitative trait locus (QTL) is the location of gene(s) that affect a trait that is measured on a quantitative scale (Tanksley, 1993). QTL identification analysis consists of four components: a segregating population, segregating markers, phenotypic values for the individuals from measurement of trait(s) of interest and association of the phenotypic data for the trait with genotypic data using an appropriate statistical approach.

Segregating markers

A segregating marker provides information regarding the allelic status (maternal homozygous, paternal homozygous or heterozygous) of each of the individuals in a population. These inheritance patterns can be monitored and scored. These markers include morphological, biochemical and molecular markers. An ideal marker set would have the following characteristics: a high degree of informativeness per marker (multiple alleles at high frequency in target populations), co-dominant inheritance, frequent distribution throughout the genome, easy to access and assay, high reproducibility and easy exchange of data between laboratories (Weising et al., 1995). Although there is no certain type of marker that fulfills all of these criteria, using a combination of different markers can provide an adequate marker set.

Over the past 50 years, many genetic maps have been constructed in barley using morphological markers (Smith, 1951; Nilan, 1964; David and Wiebe, 1968; Hockett and Nilan, 1985; von Wettstein-Knowles, 1992; Franckowiak, 1997). These markers are distributed along the seven chromosomes such as *msg1* (genetic male sterile), *f7* (chlorina seedling) on 1H, *yst4* (yellow streak), *e* (elongated outer glume), *Vrs1* (two/six rowed spike) *f-sh* (chlorine seedling) on 2H, *al* (albino lemma), *als* (absent lower laterals) on 3H, *gl* (glossy leaf), *yh* (yellow head) on 4H, *r* (rough awn), *s* (short rachila hair) on 5H, *o* (orange lemma) on 6H and *wx* (waxy endosperm), *n (nud)* (naked caryopsis), *lk2* (short awn) on 7H (Franckowiak, 1997). Although morphological markers are easy to score, some are difficult to use for mapping

studies because they are often recessive in nature, may exhibit epistatic effects, pleiotrophy, incomplete penetrance and expression may be affected by environments or may be apparent at only one stage of a plant's development.

Biochemical markers which include isozyme and storage proteins are based on separation of enzymes or proteins followed by specific staining of distinct protein subclasses (Weising et al., 1995). In isozyme analysis, the starch or polyacramide gel is stained for a particular enzyme by adding a substrate and dye under the appropriate reaction conditions, resulting in a band in the position where the enzyme has migrated according to their polypeptide size and charge. Isozymes have been used in building linkage maps of barley (Hockett and Nilan, 1985; von Wettstein-Knowles, 1992). Barley isozymes such as *Pre1*, *Pre2* (Peroxidases), *Est1*, *Est2* (Esterases) and *Pgd2* (6-Phosphogluconate dehydrogenase) are located on 2H, 3H and 1H respectively (Shin et al., 1990).

Hordeins are the main storage proteins of barley seed. Their biochemical characteristics and migration in SDS-PAGE allow the differentiation of four types (Shewry and Morell, 2001). *Hor2* and *Hor1* loci on chromosome 1H encode B-hordeins and C-hordeins respectively, and D hordeins are encoded by *Hor3* on the same chromosome (Blake et al., 1983). Biochemical markers have advantages and disadvantages. Advantages include their relatively low cost (Burow and Blake, 1997) and co-dominant nature, which allows distinguishing heterozygotes from homozygotes. The disadvantages of using

these biochemical markers, especially isozymes, in linkage maps are the limited number of identified markers unevenly distributed on the genetic map (Nielsen and Scandalios, 1974), assay methods are time and labor consuming and environment can effect protein or enzymes expression.

Because environments have no effect on DNA level or structure, DNA-based molecular markers are more widely used than other markers types. A wide variety of techniques can be used to detect DNA variations. Molecular markers can be classified into 3 categories: hybridization-based DNA markers such as RFLP; PCR-based DNA markers such as RAPD, SCAR, STS, SSR and AFLP, and DNA chip-based microarray such as SNP.

Restriction fragment length polymorphism (RFLP) relies on use of restriction enzymes to digest DNA into specific fragments. Restriction enzymes recognize specific base pair combinations and cleave the DNA strand at these sites. Mutations such as deletions, insertions and translocations may cause changes at these restriction sites (Botstein et al., 1980). These fragments are separated by electrophoresis, blotted to membrane by Southern blotting and hybridized by labeled DNA that recognizes specific sequences. Polymorphisms are detected as length differences. RFLP is a co-dominant, highly reproducible marker and has no phenotypic effect. RFLPs are particularly useful as anchor markers in comparative linkage maps of different plant species (Ahn and Tanksley, 1993; Hernández et al., 2001). However, RFLP analysis is labor intensive, time consuming uses radioactive probes and requires large amounts of pure DNA.

RFLPs were the first molecular markers to be used in the human genome (Botstein et al., 1980) and then were adopted for plant genome mapping (Weber and Helentjaris, 1989). Barley genetic maps have been constructed using RFLP markers (Shin et al., 1990; Kleinhofs et al., 1993). The NABGMP constructed a comprehensive barley genome map using the Steptoe x Morex population, which covers 1250 cm with 1325 RFLP loci (Kleinhofs and Graner, 2001).

The random amplified polymorphic DNA (RAPD) technique was discovered by two independent groups (Williams et al., 1990; Welsh and McClelland, 1990). RAPD markers were generated by using a single, short (10mer) arbitrary sequence oligonucleotide to detect complementary sites across relatively short distances within the genome. The presence of these complementary sites in a genotype will result in the primer amplifying a DNA fragment, while the absence of the complementary site will result is absence of this DNA fragment. RAPD markers are typically dominant, and the inability to distinguish between heterozygous and homozygous is a main drawback. RAPD analysis does not require pre-sequence information (Weising et al., 1995), and is a relatively quick and simple process. However, it exhibits a lack of reproducibility between laboratories (Weeden et al., 1992). RAPDs have been used in many applications including construction of linkage maps in many plant species including barley (Costa et al., 2001; Hernández et al., 2001; Tacconi et al, 2001).

The amplified fragment length polymorphism (AFLP) technique was described by Vos et al. (1995). It combines the restriction site recognition element of RFLP analysis with the exponential amplification aspects of PCR-based markers. Using several different restriction enzymes and primers provides a high degree of flexibility, which enables efficient scanning of the genome for polymorphism. Using methylation-sensitive restriction enzymes, such as *Hpa*II and *Pst*I, increases the distribution of AFLP markers throughout the genome relative to using non methylation-sensitive restriction enzymes such as *EcoR*I and *Mse*I (Young et al., 1999). Using a combination of *Pst*I/*Mse*I primers was more efficient in detecting polymorphism than *Eco*RI/*Mse*I primers (Castiglioni et al., 1999). *Pst*I/*Mse*I AFLP markers were generally found to be randomly distributed across the chromosome, while *Eco*RI/*Mse*I AFLP markers were clustered on centromeric regions of the chromosomes of maize (Castiglioni et al., 1999) and soybean (Young et al., 1999). In barley, Kanazin and Blake (2000) found that more than 50% of the detected *Msp*I sites were adjacent to centromeres, while around 20% of the *Hpa*II sites mapped near centromeres. Some studies support that there are large amounts of repetitive sequences distributed in centromeric regions, and functional genes seem to concentrate in non-centromeric regions (Carles et al., 1995; Barakat et al., 1997). No prior sequence knowledge is required for AFLP markers, making this technique very suitable for analysis of germplasm, biodiversity and genetic relationship studies (Ellis et al., 1997). Even though these markers are dominant, they can be scored co-dominantly (Waugh et al., 1997; Castiglioni et al., 1999), making this technique more suitable for genetic

mapping studies relative to arbitrarily primed PCR techniques. High marker densities can be obtained with modest efforts (Qi et al., 1998). The reproducibility and reliability of AFLPs allow construction of high-resolution maps in barley (Becker et al., 1995) and soybean (Keim et al., 1997), as well as identification of closely linked genetic markers for a specific phenotype such as potato R1 locus (Meksem et al., 1995) and the *Mla* locus in barley (Schwarz et al., 1999).

Sequence tagged site (STS) markers were identified by Olson et al. (1989). STS analysis consists of PCR amplification of a region in the genome using a pair of primer (18-22 bp) sets, which direct amplification of a sequence from a specific locus. STS primers are designed based on cloning and sequencing mapped RFLP products (Tragoonrung et al., 1992; Larson et al., 1996), AFLP products (Shan et al., 1999) and RAPD products (Naik et al., 1998). STS derived from RAPD products can be referred as SCAR (Paran and Michelmore, 1993). STS polymorphisms are insertion/deletion polymorphisms that can be read directly from agarose gels and point mutation polymorphisms that can be identified by digestion with restriction enzymes after polyacrylamide gel electrophoresis (Tragoonrung et al., 1992). STS is easy to detect with moderate-resolution analytical techniques; unlike the dominant nature of RAPD and AFLP, sequence-tagged-sites provide co-dominant markers. Disadvantages of STS development include the effort and time required to sequence large numbers of RFLP markers, although this has been mitigated by the recent development of huge expressed sequence tag

databases for barley and several other crops. Using primer sets developed from sequences of RFLP probes, Blake et al. (1996) amplified a series of 135 barley-specific markers using a set of 115 STS primers. STS markers have been used in constructing barley linkage maps (Shin et al., 1990; Kleinhofs et al., 1993; Larson et al., 1996; Sayed-Tabatabaei, 1999; Mano et al, 1999).

Simple sequence repeats (SSR) or microsatellites are short tandem repeats of di- tri- and tetra-nucleotides. The first report of microsatellites in plants was made by Condit and Hubbel (1991). Ma et al. (1996) found that $(AT)_n$ dinucleotides are the most abundant SSR type in plants. SSR discovery starts with the construction of a small-insert genomic library, then screening with a number of microsatellite probes. Following this methodology, 568 barley SSRs were discovered (Ramsay et al., 2000). The main drawbacks of SSR are high development cost, extensive DNA cloning and sequencing. Advantages of SSR are co-dominant nature, ease in genotyping and use as anchor markers within a species. SSR have proven to be multiallelic; the number of alleles varied between 5-15/microsatellite (Struss and Plieske, 1998). This high allele status ranked them as a high polymorphism information content (PIC) marker that increases the informativeness of SSRs. SSRs have been used in many types of barley genetic analyses, including linkage mapping (Liu et al., 1996; Ramsay et al., 2000; Pillen et al., 2000), genetic diversity (Struss and Plieske, 1998; Macaulay et al., 2001) and varietals discrimination (Russell et al., 1997).

Single nucleotide polymorphism (SNP) occurs as point mutation, which is the substitution of one nucleotide for another. Nucleotide substitution is a common type of mutation and can be classified as a transition or transversion mutation. Kowk and Gu (1999) defined the development of SNP markers as a six step process as follows: acquisition of DNA sequences surrounding the SNP, development of STS markers, identification of the SNP, mapping the SNP to a unique location in the genome, determination of the allelic frequencies of the SNP in the population, and development of a genotyping assay for the SNP. Wang et al. (1998) surveyed 2.3 mega bases of human genomic DNA for SNPs. Out of 3241 candidate SNPs detected, 2227 were located in the genetic map. In plants, construction of a biallelic genetic map in Arabidopsis thaliana was done with 412 SNP markers (Cho et al, 1999). Many approaches to genotype SNPs have been developed such as gel electrophoresis-based genotyping, fluorescent dye-based genotyping and DNA chip-based microarray methods. Gel-based detection of SNPs, which permitted characterization of PCR product by both size and fluorescence, has been developed (See et al., 2000). Based on this method, Kanazin et al. (2001) identified 38 barley loci containing single nucleotide polymorphisms.

Mapping population

Genetic maps of plants are constructed based on a mapping population. The choice of mapping population is dependant on many factors such as the goal of the mapping project, parents chosen for crossing, size of population, cross advancement and generations used for genotypic and phenotypic

analyses (Young, 2001). There are different kinds of populations such as F_2, backcross (BC), recombinant inbred lines (RIL) and doubled haploid (DH).

F_2 populations are developed by selfing F_1 individuals, which are developed from crossing two homozygous parents that show significant differences in some or all traits. Crossing F_1 individuals with one of the parents develops backcross populations. Because an F_2 population is easy to develop and has all possible combination of parental alleles, it has been used as a genetic linkage mapping population in plants (Paterson, 1996).

Barley DH populations are made by generating plants by anther and microspore culture (Kasha and Kao, 1970) or interspecific hybridization between *Hordeum vulgare* and *H. bulbosum* (Kasha and Kao, 1970, Kasha, 1974). The theoretical advantages of DH in self-pollinated plants, such as barley, are the immediate fixation of homozygous genotype in one step, elimination of non-additive genetic variation and time saved in varietal production, while the drawbacks of DH include the variation in varietal responses to *in vitro* culture techniques, possibility of soma- and gameto-clonal variation and the cost-efficiency compared with the conventional procedures (Bjørnstad et al., 1993).

RIL are derived through repeated cycles of selfing one individual per line per generation for five or more generations beyond the F_2 (Burr et al., 1988). Single seed descent is one approach to obtain RILs. The numerous meiosis cycles required to reach homozygosity result in additional opportunities for recombinations different than the parents; these differences in each line

provide a basis for linkage analysis (Young, 2001). This cycling can be time consuming compared with other mapping populations.

The choice of mapping population type depends on marker system used. Each type of population will give a specific segregation ratio at each locus. In F_2, dominant and co-dominant markers segregate 3:1 and 1:2:1, respectively, while the segregation of both marker types is 1:1 in BC, DH and RIL. Using F_2 can maximize the information of co-dominant markers, using DH or RIL can maximize the information obtained by dominant markers. BC and F_2 are not eternal, therefore the source of tissue for DNA or protein is limited. Both DH and RIL populations can produce hundreds of seeds so that unchanging genotypes can be evaluated repeatedly over years and locations in multiple traits (Burr et al., 1988). Also increasing the number of progeny evaluated or the number of replicates allows for the detection of additional regions with relatively smaller effects (Cowen, 1988). Another advantage of DH and RIL is that all information obtained from mapping is cumulative, may be shared between laboratories and allows for extension of the map with additional markers.

Statistical tools for mapping

QTL are identified via statistical procedures that integrate genotypic and phenotypic data and are attributed to regions of the genome at specified levels of statistical probability. Thus, mapping QTL is not as simple as mapping a gene that affects a qualitative trait.

Single marker analysis is the traditional method to detect a QTL in the vicinity of a marker by studying genetic markers individually. That approach was first reported by Sax (1923) in studying linkage between seed color and weight of beans. The difference between phenotypic means provides an estimate of the phenotypic effect of each allele at the QTL. The approach is based on classifying the offspring into one of two classes depending on their genotype at the marker, calculating the mean trait value associated with each class of offspring and comparing the mean trait values for each class to get significant differences. To test whether the inferred phenotypic effect is significant, simple statistical tests, such as t-test, analysis of variance or regression analyses are used (Liu, 1998). A significant value indicates that a QTL is linked to the marker. The further a QTL is from the marker, the less likely it is to be detected statistically due to crossover events between the marker and gene. Single point analysis does not require a complete molecular linkage map. This analysis has some drawbacks such as labor requirement, decreased power to detect a QTL between markers and inability to distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect (Lander and Botstein, 1989). SAS, statistical analysis software, can detect QTL by identifying associations between marker genotype and quantitative trait phenotype by a single marker analysis approach.

QTL simple interval mapping (SIM) is another approach for QTL analysis. A well-known example of SIM is Mapmaker/QTL developed by Lincoln et al.

(1993). The principle behind interval mapping is to test a model for presence of a QTL at many positions between two mapped marker loci. This model uses method of maximum likelihood or regression.

The maximum likelihood approach (Lander and Botstein, 1989) assumes that a QTL is located between two markers. Maximum likelihood involves searching for QTL parameters that give the best approximation for quantitative trait distributions observed for each marker class. Models are evaluated by computing the likelihood of observed distributions with and without fitting a QTL effect. The map position of a QTL is determined as the maximum likelihood from the distribution of likelihood values calculated for each locus. LOD scores are the ratio of likelihood that the effect occurs by linkage to likelihood that the effect occurs by chance. In the presence of more than one QTL per linkage group, the method could either fail to detect any effect at all or could detect a ghost QTL (Martinez and Curnow, 1992).

Interval mapping by regression (Haley and Knott, 1992) was developed primarily as a simplification of the maximum likelihood method. Since the QTL genotypes are unknown, they are replaced by probabilities estimated from the nearest flanking markers. In most cases, regression mapping gives estimates of QTL position and effect that are almost identical to those given by the maximum likelihood method. The approximation deviates only at places where there are large gaps or many missing genotypes. One of the factors that weakens interval mapping is fitting the model for a QTL at only one location. The problems with this approach are that effects of additional QTL will

contribute to sampling variance, linkage of multiple QTL will cause biased estimates and QTL with large effects could mask others of small effect.

The method of composite interval mapping (CIM) was proposed as solution to SIM drawbacks (Jansen and Stam, 1994; Zeng, 1994). CIM will perform QTL analysis in the same way of SIM, except that variance from other QTL is accounted for by including partial regression coefficients from markers (cofactors) in other regions of the genome. CIM can be proposed using the whole marker set as initial co-factors then eliminating them in backward elimination regression procedure (Jansen and Stam, 1994) or in forward selection to identify co-factors (Hackett, 2002). Zeng (1994) proposed the use of marker co-factors to account for the effect of one QTL when searching for another. CIM gives more power and precision than SIM because the effects of other QTL are not present as residual variance. CIM can remove bias caused by QTL linked to the position being tested.

There are many genetic analysis software packages for implementation of CIM such as MQTL, a program for composite interval mapping in multiple environments, which can also perform simple interval mapping. It is restricted to the analysis of data from homozygous progeny (Tinker and Mather, 1995). PLABQTL (Utz and Melchinger, 1996) is a program for both composite interval mapping and simple interval mapping of QTL. PLABQTL like MQTL can analyze data from a trait from more than one environment and identify QTL x environment interactions. Its main purpose is to localize and characterize QTL in mapping populations derived from a biparental cross by selfing or

production of double haploids. QTL Cartographer performs single-marker regression, interval mapping, and composite interval mapping and permits analysis from F_2 or backcross populations. Another feature is the ability to stimulate data sets in QTL Cartographer. It displays map positions of QTL using the GNUPLOT software. A comprehensive list of linkage map and QTL analysis software is available at http://www.stat.wisc.edu/biosci/linkage.html#linkage .

Genotypic information derived from molecular marker data can be used in breeding programs to better estimate the genetic value of individuals submitted to selection. Lande and Thompson (1990) proposed marker-assisted selection (MAS) as a method that allows complex traits to be screened in the early generations of the breeding programs. Barr et al. (2001) classified marker validation of putative QTL in alternative genetic backgrounds as a MAS step. Few studies have been done to validate QTL analysis results, although validation simulations suggest the importance of this analysis to further characterize putative QTL MAS (Melchinger et al., 1998; Utz et al., 2000; Bohn et al., 2001). Spaner et al. (1999) detected a QTL on the “plus” arm of chromosome 5H associated with grain yield, plant height and lodging severity in Harrington x TR306 population. This QTL was validated on a new set of Harrington x TR306 lines that had not been used in the original mapping experiment. They demonstrated that the effect of this QTL may be small and vary among environments. Larson et al. (1996) detected a QTL linked to yield on chromosome 3H in a Steptoe x Morex population that was independently

verified in a Steptoe x Morex backcross population evaluated in different environments. Hayes et al. (1993) identified two QTL that accounted for over 25% of the phenotypic variation in malt extract in a Steptoe x Morex population. Using another sample of lines from the same population, Han et al. (1997) detected QTL with much less variation for these malting characters. This low level of consistency of QTL among the populations maybe due to different sets of QTL segregating in different populations, dominance effects that are not detectable in the population of RIL, epistasis and small population sizes causing inflated estimates of QTL effects and making the information unsuitable for MAS (Melchinger et al., 1998; Utz et al., 2000; Bohn et al., 2001).

In order to detect useful QTL, QTL effects need to be estimated without bias due to sampling and need to explain a sufficient proportion of genotypic variation (Utz et al., 2000). Simulation studies show that statistical sampling has a strong impact on QTL analysis and the overestimated bias in estimated QTL effects can be severe (Utz et al., 2000; Monforte et al., 1999). To overcome this problem, a validation approach has been suggested (Melchinger et al., 1998; Utz et al., 2000). This involves cross validation (CV) to eliminate the bias due to model selection caused by genetic and environmental sampling (Utz et al., 2000), and validation with an independent sample (IV). The independent sample approach uses two independent samples derived from the same parental cross, one being used for QTL

detection and the other for estimation of QTL effects at the respective positions (Lande and Thompson, 1990; Utz et al, 2000).

Research objectives

The objectives of the present research were to identify and validate QTLs associated with feed quality and predict animal feedlot performance using lines derived from mapping and validation populations. These included 150 DH lines from the Steptoe x Morex cross and 140 DH lines from the Harrington x Morex cross. Both populations were developed by the North American Barley Genome Mapping Project (NABGMP). We also assayed 62 Recombinant Inbred Lines derived from a cross between Lewis x Baronesse, 116 recombinant inbred lines derived from a cross of Valier x PI370970 and 179 RILs of Baronesse x PI370970.

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CHAPTER 2

MAPPING THE GENES CONTROLLING VARIATION FOR FEED QUALITY IN THE STEPTOE X MOREX BARLEY POPULATION

Abstract

Barley (*Hordeum vulgare* L.) is a major feed source for livestock in the western regions of North America. Feed quality has been neglected as a selection criterion because of a lack of understanding of the grain characteristics that could be responsive to selection and would improve feedlot performance. A review of feedlot trials involving a wide array of barley cultivars revealed a limited set of grain characteristics that could be used as selection criteria to improve barley feed quality (Surber et al., 2000). Bowman et al. (2001) concluded that selection for low DMD, low ADF, large particle size after dry rolling and high starch content will result in barley varieties less prone to induce bloat and lactic acidosis in cattle. The North American Barley Genome Mapping Project generated an excellent medium density RFLP-based linkage map in a set of doubled haploid lines derived from a cross between Steptoe x Morex (Kleinhofs et al., 1993). We utilized this population to evaluate genetic variation for *in situ* dry matter and starch digestibility, ADF, protein and starch content in order to map QTLs affecting rumnial digestibility of barley. Major QTLs affecting these traits were detected using composite interval mapping.

Introduction

Barley (*Hordeum vulgare* L.) is a major feed source for livestock in the western regions of North America. Improvement of barley varieties has involved many different characters. Foster (1987) reported that feed or nonmalting varieties have been developed with the major emphasis being high yield for producers with limited consideration for feed quality. The neglect of feed quality is related to difficulties in screening breeding material for quality traits, lack of well-defined quality parameters and lack of a premium in the marketplace for improved feed cultivars (Foster, 1987). Steptoe remains one of the highest-yielding varieties when grown in the Pacific Northwest and Intermountain regions (Muir and Nilan, 1973), but a lack of feed trials prior to its release resulted in failure to identify its poor feed quality before release as a feed variety (Foster, 1987; Surber et al , 1998).

There are several methods for measuring the dry matter degradation of barley in the rumen. In *in vivo* trials, feed is given to the animal in known amounts and the output of feces is measured (Matsushima, 1979). This method has been used widely in barley feed experiments (Bradshaw et al., 1996; Boss and Bowman, 1996; Noon et al., 1998). Although accurate for digestibility, *in vivo* analysis is extremely expensive and not realistic as a selection criterion for a large number of samples (Khorasani et al., 2000). It also requires a large amount of barley grain per experimental treatment (at least 5 tons).

The *in situ* method involves the placement of non-digestible bags containing barley grain, as whole, cracked or ground grain, into the rumen of a cannulated cow for a certain time interval. The amount of dry matter that has disappeared from the bag is then measured (Herrera-Saldana et al., 1990; Lehman et al., 1995; Vanzant et al., 1998; Khorasani et al., 2000). This method provides digestion analysis within the rumen itself and reduces the need for ruminal simulation.

Animal performance is often measured by average daily gain (ADG), carcass grade at slaughter time, feed efficiency, diet digestibility and feed intake. Average daily gain (ADG) varies among barley varieties and is related to digestible starch intake (Boss and Bowman, 1996). ADG of steers fed Boyer barley was 12% greater than those fed Steptoe (Preston and Herlugson, 1980). Measurements of *in situ* dry matter digestibility (DMD), acid deteragent fiber (ADF) , starch content and particle size after dry rolling of barley grain contribute to our ability to predict performance of cattle fed high concentration barley diets. The combination of these lab analyses was used to estimate animal performance (Surber et al., 2000). Bowman et al. (2001) concluded that selection for low DMD, low ADF, large particle size after dry rolling and high starch content will result in improved barley feed quality.

Lande and Thompson (1990) proposed marker-assisted selection (MAS) as a method that allows complex traits to be screened in the early generations of breeding programs. Barr et al. (2001) identified nine key steps in the identification of molecular marker(s) that linked to traits to use in MAS. These

steps are identifying parents differing in trait(s) of interest, developing a population of plants segregating for trait(s), screening the population for the trait(s) values, constructing a linkage map of the population to locate QTL(s) associated with the trait(s) of interest, identifying molecular marker(s) that co-segregate with the trait(s) of interest, marker validation of these marker(s) in different backgrounds, producing clear and simple protocols for assaying the marker(s) and modifying the breeding strategy to optimize use of MAS relative to alternative selection techniques.

The North American Barley Genome Mapping Project (NABGMP; Hayes et al., 1993; Kleinhofs et al., 1993) developed a population of 150 doubled haploid lines produced utilizing the pollen of *H. bulbosum* (Kasha et al., 1970) to pollinate F1 florets from a cross of Steptoe x Morex (Chen and Hayes, 1989). Morex is a six-rowed malting barley variety while Steptoe is high yielding variety with poor feed quality. A robust medium-density molecular linkage map was developed using these 150 lines. This population was widely used to locate and study the QTLs of agronomic and malting characteristics (Hayes et al., 1993; Han et al., 1995; Larson et al., 1996).

The objectives of this study were to evaluate genetic variation in the *in situ* DMD and starch digestibility of Steptoe x Morex doubled haploid (DH) population, to map QTLs affecting ruminal digestibility of barley and other feed traits and to estimate the interactions between the detected QTLs.

Materials and Methods

Plant materials

A linkage map was developed using a population of 150 doubled haploid lines from a Steptoe x Morex cross (NABGMP; Hayes et al., 1993; Kleinhofs et al., 1993) was used for the analyses. These lines were grown under rainfed and irrigated conditions at Bozeman, MT during 1992. During the summer of 2000, the lines were planted under irrigated conditions, and the grain samples were used to determine the particle size and dry matter digestibility of the cracked grains. Each of the 150 lines, and the parental lines were planted into an Amsterdam silty loam soil at a seeding rate of approximately 30 seeds/m in single row plots with 3m long rows spaced 0.3m apart. Plots were harvested by a Wintersteiger plot combine and cleaned using a de-awner and blower.

Feed quality estimations

To simulate dry rolling processing before feeding barley, grain samples from each line were individually cracked using a Buehler mill (Buehler-Miag, Braunschweig, Germany). Particle size was determined on cracked samples by dry sieving with two replicates per sample (Fisher et al., 1988). A set of 3350 µm, 2360 µm, 1700 µm, 850 µm and 425 µm sieves along with the bottom collection pan were used to estimate particle size.

Starch, protein and ADF analyses were performed in duplicate on each grain sample. Grain samples from each line were individually ground to pass through 0.5 mm screen using a Udy-Cyclone Mill (Ft. Collins). To calculate the

chemical composition traits on dry basis, dry matter content (DM) was determined by following method AOAC 934.01 (AOAC, 1990). ADF content was determined following the method of Van Soest (Van Soest et al., 1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Crop., Fairport, NY). Starch content was determined by the Amyloglucosidase/α-amylase method (Megazyme, Sidney, Australia). Protein content was determined based on N content (N × 6.25) using Leco FP 528 protein analyzer (Leco Crop., St. Joseph, MI).

In situ DMD and starch digestibility were estimated for the ground grains for 6 hrs and cracked grain for 3 hrs, according to the procedure of Vanzant et al. (1998) using two ruminally cannulated beef cows that consumed low quality grass hay ad libitum and 3.6 kg day-1 barley. Grain samples were ground through a 2-mm screen in a Wiley mill. Duplicate nylon bags (50 μm pore size) for each of the genotypes, containing a 5 g sample, were placed into the rumen of both cows and removed after 3 or 6 hrs (4 bags for each genotype at each time point). Six *in situ* runs were conducted with a total of 48 bags in a cow during each run. After removal from the rumen, bags were washed with cold water until the rinse water was clear, squeezed to remove excess water, and dried at 60° C for 48 h in a forced air oven. Ruminal DMD was calculated according to the following equation: in situ DMD % = 100-{[(dry sample and bag wt. out - bag weight) - (dry blank bag wt. in – dry blank bag wt. out)]/ (sry sample wt.)}*100. Starch analysis was performed on the grain residue in the 6 hrs bags at and *in situ* starch digestibility was calculated.

Simple correlation analysis was carried out for chemical composition traits (ADF content, starch content and protein content) and physical characters (ISDMD, Starch digestibility and particle size) using means over the environments and PROC CORR in SAS (Statistical Analysis System, SAS Institute 1990).

QTL analysis

Analysis of variance was performed on all traits to identify significant genotype and genotype x environment interactions. When genotype effects were significant, QTL analyses were performed using QTL Cartographer 2.0 (Wang et al., 2002). A series of 1000 permutations was run to determine the experiment-wise significant level at P=0.05 of LOD for the trait (Churchill and Doerge, 1994). Composite Interval Mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam, 1994; Zeng 1994) using Model 6 of the Zmapqtl program module. The genome was scanned at 2-cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward-backward method of stepwise regression. The percentage of phenotypic variance explained by a specific QTL value (R^2) was taken as the peak QTL position as determined by QTL cartographer. Total phenotypic variance was calculated using multiple locus models based on type III sums of squares of SAS GLM (Statistical Analysis System, SAS Institute 1990) using the closest marker locus to QTL model identified by CIM and possible epistatic interaction between each marker pairs.

Results and Discussion

Phenotypic variation

Phenotypic variation for feed related traits of the parents and their doubled haploid progeny lines summarized in Table 2-1. For these traits Morex had higher protein content, higher ADF, higher starch content, larger particle size, lower DMD values and lower starch digestibility value for ground grains after 6 hours than Steptoe in both the environments and the combined analysis.

In the present study, even though the variation between parents was narrow for most traits, positive and negative transgressive genotypes over each parent indicted the wide spectrum variation of these traits in the offspring (Table 2-1). Transgressive segregation over parents was observed for protein content, ADF and starch content. In the combined analysis, 25 genotypes were lower than Steptoe for protein content, 95 genotypes were higher than Morex for starch content and 46 genotypes scored as positive transgrants that were lower than Steptoe in ADF (Table 2-1). The same results were indicated for particle size, dry matter and starch digestibility (Table 2-1).

Table 2-1. Means, range and transgressive phenotypes of Steptoe, Morex and their DHs over rainfed, irrigated and combined environments for protein content (CP), ADF, starch content, particle size (PS), DMD after 3h for cracked grains (DMDc), DMD after 6h for ground grains (DMDg) and starch digestibility after 6h for ground grains (Schdig).

Trait	Steptoe	Morex	DHs				
			Positive trans[a]	Negative trans[b]	Min[c]	Max[d]	χ[e]
Starch (%)							
Rainfed	46.4	48.6	76	36	38.3	60.8	49.8
Irrigated	52.7	52.1	102	42	41.0	76.0	55.2
Combined	49.6	52.3	95	37	38.3	66.7	52.5
CP (%)							
Rainfed	13.4	14.5	40	61	12.0	17.5	14.2
Irrigated	11.2	12.6	24	47	10.3	16.6	12.1
Combined	12.3	13.6	25	50	11.4	17.0	13.2
ADF (%)							
Rainfed	3.6	4.6	98	1	3.4	6.8	4.9
Irrigated	5.6	8.7	103	2	2.4	8.7	5.1
Combined	4.6	6.7	46	3	3.3	7.0	5.0
PS (µm)							
Rainfed	1374.7	1439.3	20	78	1074.7	2295.5	1359.4
Irrigated	1267.5	1306.2	16	113	1263.2	1273.8	1312.0
Combined	1321.1	1372.7	31	89	1159.7	1783.5	1329.1
DMDc (%)							
Rainfed	37.8	35.9	22	118	23.2	57.1	42.5
Irrigated	38.0	37.2	84	60	21.2	60.2	36.7
Combined	37.9	36.6	105	40	26.5	56.9	39.6
DMDg (%)							
Rainfed	79.4	78.2	113	28	68.6	82.8	77.3
Irrigated	76.8	74.4	47	63	67.1	85.0	76.1
Combined	78.1	76.3	58	34	69.5	81.5	76.7
Schdig (%)							
Rainfed	96.3	95.5	70	54	88.3	98.2	95.3
Irrigated	98.6	96.4	82	7	90.2	99.3	95.8
Combined	97.5	95.9	66	8	91.2	98.0	95.6

a: Positive transgressive genotypes with low protein content, low ADF than Steptoe and high starch content, larger PS, low DMDc, low DMDg and low schdig than Morex,
b: Negative transgressive genotypes with high protein content, high ADF than Steptoe and low starch content, smaller PS, high DMDc, high DMDg and high schdig than Morex.
c: Minimum value, d: Maximum value, e: mean.

Correlation analysis

Simple correlation analysis was carried out to understand the genetic control of chemical composition traits (starch, ADF and protein content) in

correlation with others feed quality traits (particle size, dry matter and starch digestibility for cracked and ground barley grains).

Table 2-2. Phenotypic correlations among protein content (CP), acid detergent fiber (ADF), starch content (SCH), particle size (PS), DMD after 3h for cracked grains (DMDc), DMD after 6hrs for ground grains (DMDg) and starch digestibility after 6h for ground grains (Schdig) over combined environments of Steptoe x Morex population.

Trait	SCH	CP	PS	DMDc	DMDg	SCHdig
CP	-0.287**					
PS	-0.144*	-0.912				
DMDc	0.102	0.010	-0.551**			
DMDg	-0.059	-0.208**	0.015	0.169		
Schdig	0.058	-0.220**	-0.127	0.204**	0.561**	
ADF	-0.585**	-0.010	0.202**	-0.078	-0.124	0.03

*' ** Significant at P=0.05 and P = 0.01, respectively.

High negative correlations were found between starch content and ADF. Protein content correlated negatively with DMD, particle size and starch content (Table 2-2), In contrast, a positive correlation between protein and ADF was indicated in a group of two-rowed genotypes (Frégeau-Reid et al. 2001). These facts suggested that selection for low ADF should increase starch content and reduce the protein content. Particle size correlated negatively with DMD of cracked grain while the correlation between particle size and both DMD and starch digestibility of ground grains was not significant. That indicated the processing of barley grain for feed product has a significant effect on overall DMD.

QTL analyses

A previously constructed linkage map using 150 genotypes of Steptoe x Morex DH population (Kleinhofs et al., 1993; Hayes et al., 1993) was used to

determine the chromosomal locations of gene(s) controlling variation in chemical composition and digestibility of barley grain (Figure 2-1), Composite interval mapping (CIM) and multiple interval mapping (MIM) were employed to detect QTL and QTL interactions. QTL analyses were carried out using combined data over these environments.

This study indicated that, QTL models explained about 44%, 26% and 61% of the total variation in ADF, starch and protein content, respectively. Four QTLs controlling variation in ADF were detected on chromosomes 2H, 3H, 4H and 6H. Previous studies detected various QTLs controlling ADF variation at 1H, 2H and 4H chromosomes in the Steptoe x Morex population (Ullrich et al., 1996; Han et al., 2003).

In our study, a major ADF-QTL, *Qadf6a*, was located on chromosome 6H and controlled about 9% of the variation. *Qadf2a* was detected on chromosome 2H near CDO474C, explained about 5% of the total variation in ADF. Han et al. (2003) detected a QTL located near CDO474C at chromosome 2H that explained about 14% of the variation in ADF in the same Steptoe x Morex population. The differences in the amount of variation controlled by each of these QTLs could be related to the different QTL detection methods in the two studies or the environments. Previous studies indicated the importance of hull absence on variation for ADF (Kong et al., 1995; Zinn et al., 1996; Bowman et al., 2001). Since both parents and progeny are hulled, it is expected that the variation in Steptoe x Morex population for ADF is narrow (Table 2-1).

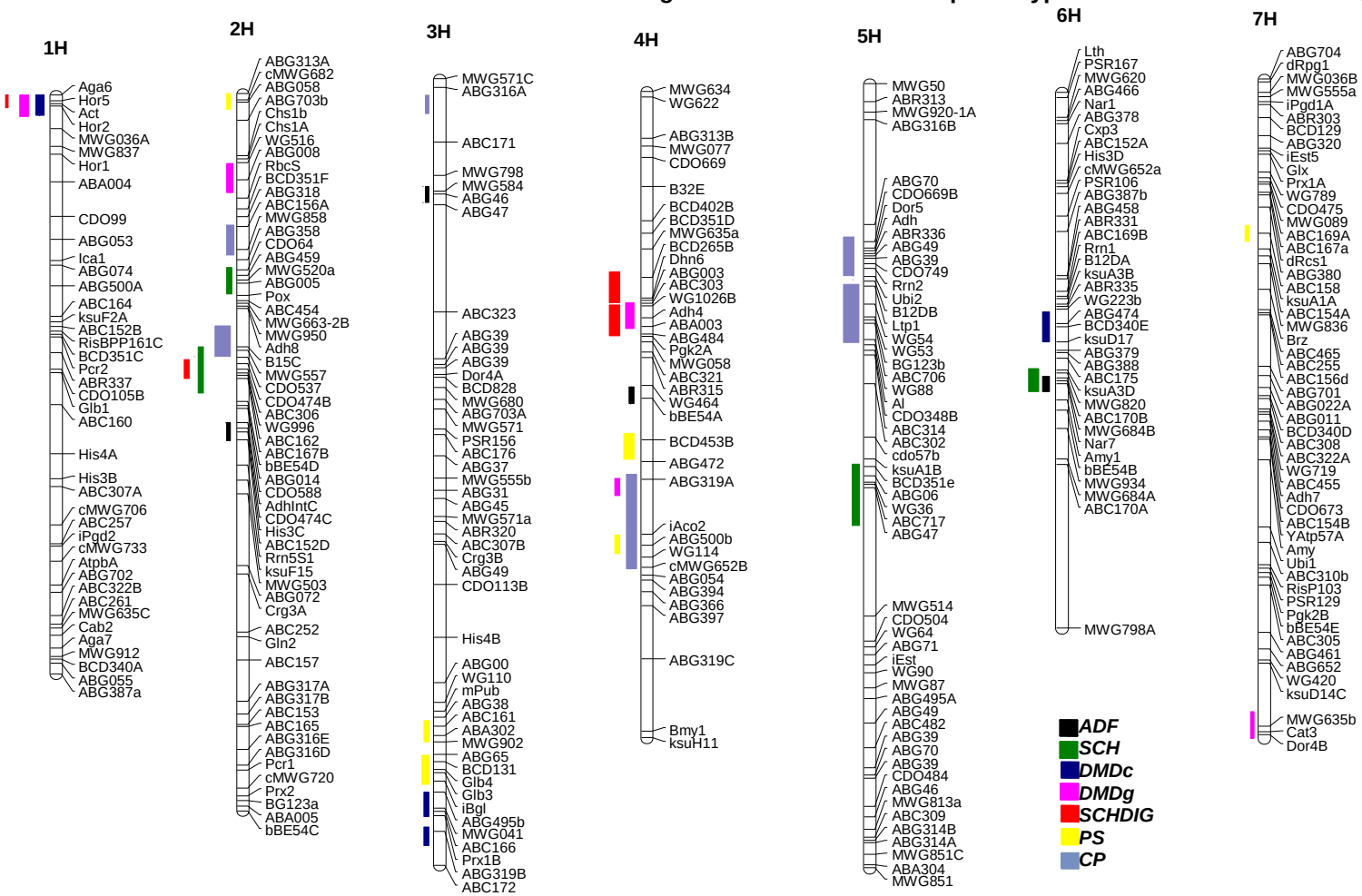
Table 2-3. QTLs for protein content (CP), acid detergent fiber (ADF) and starch content (SCH) identified by composite interval mapping (CIM) of Steptoe x Morex population.

Trait	QTL Name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	allelic efect	R^{2c}	Total R^{2d}
CP (%)								
	Qpro2a	2(2H)	ABC156A	38.61	5.9	0.309	9.2	61.22
	Qpro2b	2(2H)	MWG557	66.81	11.8	-0.457	18.8	
	Qpro3b	3(3H)	ABG316A	6.11	2.7	0.202	4.5	
	Qpro4a	4(4H)	ABG319A	115.01	9.0	-0.358	14.0	
	Qpro5a	7(5H)	Adh6	46.21	7.5	-0.348	13.2	
	Qpro5b	7(5H)	WG541	51.61	12.1	-0.442	20.6	
ADF(%)								
	Qadf2a	2(2H)	CDO474C	88.11	4.90	0.167	4.7	47.77
	Qadf3a	3(3H)	ABG460	30.01	4.20	-0.151	3.8	
	Qadf4a	4(4H)	bBE54A	79.91	2.41	0.826	6.0	
	Qadf6a	6(6H)	Nar7	76.00	3.54	-2.729	8.9	
Starch(%)								
	Qsch2a	2(2H)	WG996	72.81	3.3	1.083	7.0	25.92
	Qsch5a	7(5H)	WG364	107.21	3.2	1.448	9.8	
	Qsch6a	6(6H)	Nar7	76.01	4.4	1.787	12.2	

[b] QTl position in cM.
[b] Peak value of the LOD.
[c] Proportion of phenotypic variance explained by the QTL.
[d] Total phenotypic variances that explained by the model.

QTLs were detected for starch content. Qsch6a had the largest impact and explained about 12% of the total phenotypic variation of starch. Qsch6a and Qadf6a near the Nar7 locus appear to cosegregate, resulting in a negative correlation between starch and ADF. Another 2 QTLs, *Qsch2a* at chromosome 2H and *Qsch5a* at chromosome 5H, were detected but with less effect. No significant QTL x QTL interaction was detected, in contrast with Frégeau-Reid et al. (2001) who detected a Additive x Additive epistasis for starch content.

Figure 2-1. QTL locations for acid detergent fiber (ADF), protein (CP), starch content (SCH), dry matter digestibility for creaked (DMDc) and ground grains (DMDg), starch digestibility (SCHdig) and particle size (PS) for Steptoe x Morex DH population. Bar length indicates the 1-LOD confidence interval and the bar weight indicates the relative phenotypic variation value of each QTL.



Several protein QTLs were detected on the seven barley chromosomes with different populations (Han and Ullrich 1994; Oziel et al., 1996; Mather et al., 1997; Marquez-Cedillo et al., 2000). We detected QTLs associated with protein content on 1H, 2H, 3H and 4H chromosomes (Table 2-3) using CIM. Two major QTLs, *Qpro2b* and *Qpro1b*, accounted for about 39% of the total variation in protein content.

Table 2-4. QTLs for Dry matter digestibility of the cracked grain (DMDc), dry matter digestibility of ground grains (DMDg), starch digestibility of ground grains (Schdig) and particle size (PS) identified by composite interval mapping (CIM) of Steptoe x Morex population.

Trait	QTL Name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	allelic effect	R^{2c}	Total R^{2d}
DMDc (%)								
	Qdmdc1a	5(1H)	Hor5	1.41	4.5	-3.507	12.1	47.43
	Qdmdc2a	2(2H)	ABG005	49.41	2.3	-1.671	7.2	
	Qdmdc3a	3(3H)	ABG495B	190.00	2.7	-1.173	6.6	
	Qdmdc3b	3(3H)	ABG319B	196.21	2.7	-1.177	7.0	
	Qdmdc6a	6(6H)	BCD340E	61.21	2.5	2.036	9.2	
DMDg (%)								
	Qdmdg1a	5(1H)	Hor5	1.41	4.7	-0.677	11.7	35.11
	Qdmdg2a	2(2H)	WG516	22.01	3.4	-0.569	8.5	
	Qdmdg4a	4(4H)	ABA003	58.91	2.7	1.056	10.7	
	Qdmdg4b	4(4H)	ABG319A	103.00	2.3	0.595	6.2	
	Qdmdg7a	1(7H)	Cat3	170.11	4.8	0.489	3.3	
Schdig (%)								
	Qschdig1a	5(1H)	Hor5	1.41	3.2	-0.508	7.9	29.67
	Qschdig2a	2(2H)	ABC306	71.81	5.1	0.311	5.8	
	Qschdig4a	4(4H)	ABG003	54.41	5.1	0.688	12.5	
	Qschdig4b	4(4H)	ABA003	58.91	6.0	0.699	12.7	
PS (µm)								
	Qps2a	2(2H)	ABG358	1.61	4.9	-23.604	5.2	36.16
	Qps3a	3(3H)	ABC161	170.81	2.4	26.432	7.1	
	Qps3b	3(3H)	Glb3	180.81	2.8	29.237	8.3	
	Qps4a	4(4H)	BCD453B	93.71	3.9	43.591	13.4	
	Qps4b	4(4H)	ABG500B	118.31	2.2	-29.943	6.5	
	Qps7a	1(7H)	ABC158	40.21	4.0	21.151	4.5	

[b] QTl position in cM.
[b] Peak value of the LOD.
[c] Proportion of phenotypic variance explained by the QTL.
[d] Total phenotypic variances that explained by the model.

Major QTL explained about 36% of the variation in particle size. The major QTL, *Qps4a* on chromosome 4H, explained about 13% of the variation. Two QTLs were detected on chromosome 3H which explained together about 15% of the variation, Other QTLs were detected on 2H and 7H chromosomes (Table 2-4).

QTLs for DMD of cracked barley were detected on chromosomes 1H, 2H, 3H and 6H. These QTLs explained about 47% of the variation for this trait (Table 2-4). *Qdmdc3a* was located on chromosome 3H, about 10cM from *Qps4a*, a major PS-QTL that could help explain the significant correlation between these two traits (r=0.55).

QTLs for dry matter and starch digestibility of ground barley grains were detected on chromosomes 1H, 2H, 4H and 7H, in aggregate explaining about a third of the total phenotypic variation. *Qschdig4b* and *Qdmdg4a* appeared to cosegregate and explained about 11 % and 13% of the total variation of DMD and starch digestibility. *Qdmdg1a* and *Qdmdg4a* controlled about 12% and 8% of the total variation (Table 2-4). Interestingly, the storage protein complex on the short arm of chromosome 1H was found to affect digestibility of cracked grain also (Table 2-4, Figure 2-1). Variation among hordein haplotypes might contribute to variation in the rate at which endosperm starch granules become available for hydrolysis by rumen microorganisms (McAllister et al., 1993; McAllister et al., 1993 Yanke et al 1993).

In this study, we report the results of QTL mapping of feed quality-determining traits including ADF, starch and protein content using a doubled

haploid population derived from a cross between Steptoe and Morex. This population has sufficient variation in feed-related traits to permit detection of several QTLs for most of the studied traits. A QTL on chromosome 4 had the greatest impact on ground grain digestibility, while a major QTL for particle size and digestibility of cracked grain was located on chromosome 3H. Breeding for improved feedlot performance in barley seems a reasonable objective.

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CHAPTER 3

MAPPING AND VERIFICATION OF QTLS CONTROLLING VARIATION FOR FEED QUALITY IN A TWO-ROWED BARLEY POPULATION

Abstract

Barley (*Hordeum vulgare* L.) is a major feed source for livestock in the western regions of North America. Selection for barley genotypes based on grain characteristics associated with improved feed utilization has received little attention in barley breeding programs. Bowman et al. (2001) concluded that selection for low DMD, low ADF, large particle size after dry rolling and high starch content will result in improved average daily gain in beef cattle.

Since Montana is primarily a 2-rowed barley producing state, we developed a recombinant inbred line population derived from a cross between Lewis, a commonly grown, moderate quality feed barley variety and Baronesse, the highest-yielding 2-rowed variety grown in the Pacific Northwest. Our primary objective was to identify the location of genes contributing to the extraordinary yield potential exhibited by Baronesse. We also hoped to identify QTL contributing to improved feed value and in doing so, develop an improved quality, higher-yielding feed barley variety. A low-density linkage map of this population was constructed (Blake et al., 1998). Variation among lines in both grain yield and feed-related traits was sufficient to detect several QTL that contributed to variation in adaptation as well as variation for ADF, starch content, DMD and starch digestibility. Two chromosome 3 QTL were identified that proved important for agronomic

performance in Montana's dryland environments(Blake et al., 1998) , and these coupled with feedlot performance data resulted in the release of the variety 'Valier', line number 30 from this population (Blake et al., 2002).

In a retrospective analysis, we evaluated this population for potential feedlot-performance related traits. Our objective in this analysis was to see whether marker-assisted selection would have identified lines different from those we advanced to varietal release through large-scale phenotypic evaluation. Through this process, one QTL was discovered that had a large impact on digestibility on chromosome 1H, another QTL on chromosome 7H impacted particle size variation and third on chromosome 6H impacted ADF. These data were included in various selection simulations. As might be expected from a mapping experiment that consisted of a small population, a low-density map and moderate heritability traits, phenotypic analysis proved as productive as would have marker assisted selection. Still, knowing which mapped QTL were likely fixed in 'Valier' provides a basis for further designed crosses and genetic improvement.

Introduction

Barley (*Hordeum vulgare* L.) is a major feed source for livestock in the western regions of North America. Improving the feedlot performance of barley demands knowing both what grain quality characteristics are important, and knowing which of those heritably vary in populations of interest. In other papers we describe which parameters are important determinants of feed quality, and which of those varied among the members of the USDA spring

barley core collection (Bowman et al., 2001; Surber et al., 2000). In this paper we evaluate the progeny of a cross between a high yielding European 2-rowed spring barley variety (Baronesse) and a Montana-adapted, good quality 2-rowed variety (Lewis) to map QTL that might contribute toward development of higher yielding improved quality feed barley varieties adapted to rainfed environments in the Northwest.

In situ DMD, ADF, starch content and particle size after dry rolling of barley grain contribute to variation in the performance of cattle fed high concentration barley diets. These laboratory analyses have been used to predict animal performance (Surber et al., 2000). Bowman et al. (2001) concluded that selection for low DMD, low ADF, large particle size after dry rolling and high starch content would result in barley varieties improved for feedlot performance.

Lewis and Baronesse are both well-adapted two-row barley varieties which are widely grown in Montana and neighboring states. Baronesse is a European feed variety that was planted on over 50 thousand hectares in Montana in 2002 (Montana Agricultural Statistics, 2003), making up 4.2 % of the total state barley acreage. Lewis is a feed and malt variety released by Montana Agricultural Experiment Station and planted to 21 thousand Montana hectares in 2002 (1.8 % of the state total).

The objectives of the current study were to evaluate genetic variation in the *in situ* DMD, ADF, particle size and starch content of a Lewis x Baronesse RIL population, and to map QTLs affecting ruminal digestibility and other feed-

related traits. Selected lines from this population were evaluated in full feedlot trials to test our genetic model.

Materials and Methods

Plant materials

A linkage map was developed using 62 F_5-recombinant inbred lines of Lewis x Baronesse cross (Blake et al., 1998). These lines were grown under rainfed and irrigation conditions at Bozeman, MT during 1995 in two replicates, 1996 in 4 replicates and 1997 in two replicates. Each of the 62 lines, and the parental lines were planted into an Amsterdam silty loam soil at a seeding rate of approximately 30 seeds/m in single row plots with 2m long space 0.3m apart. Grain were harvested using a Wintersteiger plot combine and cleaned using a de-awner and blower.

Feed quality estimations

To simulate dry rolling processing before feeding barley, grain samples from each line were individually cracked using a Buehler mill (Buehler-Miag, Braunschweig, Germany). Particle size was determined on cracked samples by dry sieving with two replicates per sample (Fisher et al., 1988). A set of 3350 µm, 2360 µm, 1700 µm, 850 µm and 425 µm sieves along with the bottom collection pan were used to collect particle size fractions.

Barley grains samples of Lewis x Baronesse population from each genotype at each location were used for compositional analysis. Grain samples from each line were individually ground to pass through a 0.5 mm

screen using a Udy-Cyclone Mill (Ft. Collins). To calculate the chemical composition traits on dry basis, dry matter content (DM) was determined using AOAC method 934.01 (AOAC, 1990). ADF content was determined following Van Soest's method (Van Soest et al., 1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Crop., Fairport, NY). Starch content was determined following the Amyloglucosidase/α-amylase method (Megazyme, Sidney, Australia). Protein content was determined based on N content (N × 6.25) using Leco FP 528 protein analyzer (Leco Crop., St. Joseph, MI).

In situ DMD and starch digestibility were estimated according to the procedure of Vanzant et al. (1998) using two ruminally cannulated beef cows that consumed low quality grass hay *ad libitum* and 3.6 kg day-1. Grain samples were ground through a 2-mm screen in a Wiley mill. Duplicate nylon bags (50 μm pore size) containing a 5 g sample from each genotype were placed into the rumen of both cows and removed after 3 hrs (4 bags total for each genotype at each time point). After removal from the rumen, bags were washed with cold water until the rinse water was clear, squeezed to remove excess water, and dried at 60° C for 48 h in a forced air oven. Ruminal DMD was calculated according to the following equation: *in situ* DMD % = 100-{[(dry sample and bag wt. out - bag weight) - (blank bag wt. in – blank bag wt. out)]/ (sample wt. in* DM)}*100. Starch analysis was performed on the grain residue in the bags at 6 and 24 h, and in situ starch digestibility was calculated.

Feedlot performance trails and predication studies

Eight genotypes were selected from the mapping population for a large-scale animal performance study. These selected genotypes and the two parents were grown under dryland conditions near Havre, Montana in the spring and summer of 1999. These genotypes were divided into 2 locations. LB13, LB30 and parent parental controls were fed to animals at the feedlot facility at the Montana State University experimental feedlot near Bozeman, MT, while LB2, LB5, LB6, LB32, LB48 LB57 and parents were fed to animals at the animal facility at Northern Agricultural Research Center feedlot near Havre, MT. At each location 80 steers were used in the study. The steers were allocated into 16 pens (each pen with 5 animals). At the Havre location the pens were organized in a completely randomized design (CRD) with 2 pens for each barley genotype. In the Bozeman trial the animals were organized in a randomized complete block design (RCBD) with 4 replications.

Grain compositional data including ADF, starch percentage, DMD and particle size of these eight genotypes were used to estimate the predicted values of net energy available for maintenance (NE_m), net energy available for gain (NE_g), feed efficiency ratio (FE) and average daily gain (ADG) using the predication equations of Bowman et al. (2001). These predictions were then compared to our observed values gained from the large feedlot experiments.

The study was divided into 2 periods. During the first period (70 days) steers were fed 85% barley diets based on a blend of Baronesse and Lewis at both locations. During the second period (70 days) the steers were fed 85%

barley diets based on one of the genotypes or the parents. Diets were balanced to contain 13% protein. A 28-day adaptation period was used to adjust steers to their individual diets. Steers were allowed *ad libitum* access to water and were offered feed once daily. Steers were weighed every 28 days throughout the trial. Diet and fecal samples were composited by pen and analyzed for dry matter (AOAC, 1997), acid insoluble ash (Van Keulen and Young, 1997) and starch (AOAC, 1997).

Steers were slaughtered when 70% were visually estimated to grade choice. Hot carcass weight was recorded at slaughter and all other carcass traits were taken after a 24 h chill. Marbling score, the intermingling or dispersion of fat within the lean, was estimated using a 1-10 scale (Boggs and Merkel, 1990). Yield grade is based on a 1-5 scale, and is dependent on the yield of boneless and closely trimmed retail cults, while the quality grade is the estimation of tenderness and flavor of the meat. These parameters were assigned by a USDA grader.

Statistical analysis

Simple correlation analysis was carried out for grain composition traits including ADF content, starch content, ISDMD, Starch digestibility and particle size using means over environments utilizing PROC CORR in SAS (statistical Analysis System, SAS institute 1990).

Two models were proposed to estimate the additive effects of significant loci modifying ADF, *in situ* DMD, particle size and starch content. The ‘simple’ model utilized the one locus/trait with the greatest R^2 (the ‘biggest QTL’ for

each trait). We then estimated the additive effects of each of these major QTLs for each trait as follows: $p_{yi} = X_a + A_y$, where, X_y is the mean of trait y, A_y is the additive effect of the significant QTL and $_i$ is the genotype. These QTL included one marked by GGCTGB4 on chromosome 1(7H) for Particle size (PS), GGCATL3 locus on chromosome 5(1H) for DMD and starch digestibility (SCHdig) and a QTL marked by HPAP2B5 on chromosome 6(6H). The second model, the multiloci/trait model, estimated the additive effects of all the detected QTLs that affected each trait as follows: $p_{yi}=X_a+ A_{y1}+A_{y2}+\ldots.+A_{yn}$, where X_a the mean, A_{y1} is the additive effect of the first locus affect trait y, $_n$ is the number of QTLs affecting the trait y and $_i$ is the genotype. These phenotypic (*p*) values for each trait and each selected genotype were used to predict FE and ADG. Simple correlation analyses were conducted among the measured feedlot parameters and these predicted quality parameters.

QTL analysis

QTL analyses were performed using QTL Cartographer 2.0 (Wang et al., 2002) for individual environments and combined over environments. A series of 1000 permutations was run to determine the experiment-wise significant level at P=0.05 of LOD for the trait (Churchill and Doerge, 1994). Composite Interval Mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam, 1994; Zeng 1994) using Model 6 of the Zmapqtl program module. The genome was scanned at 2-cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward-backward method of stepwise regression. The percentage of

phenotypic variance explained by a specific QTL value (R^2) was taken as the peak QTL position as determined by QTL cartographer.

Results and Discussion

Phenotypic variation

Phenotypic values for feed related traits of the parents and the selected lines from the Lewis x Baronesse population are summarized in Table 3-1. For these traits Lewis had higher ADF, higher starch content, larger particle size, lower DMD and starch digestibility values than Baronesse in both the environments and the combined analysis.

Correlation analysis

Simple correlation analysis was carried out to demonstrate relationships among compositional traits and other feed quality traits in the Lewis x Baronesse population. Starch percentage was negatively correlated with ADF, and larger particles resulted in a reduced rate of total dry matter digestion and starch digestion. Starch digestibility and DMD apparently measure the same fundamental trait. (Table 3-2).

Table 3-1: Means, range and transgressive phenotypes of Lewis, Baronesse and their RILs over rainfed, irrigated and combined environments for protein content, acid detergent fiber (ADF), starch content, particle size (PS), dry matter digestibility (DMD) and starch digestibility for cracked grains (SCHdig) of Lewis x Baronesse population.

Trait	Lewis	Baronesse	RILs				
			Positive trans[a]	Negative trans[b]	Min[c]	Max[d]	χ^e
Starch (%)							
Rainfed	54.82	51.53	19	4	50.21	56.58	54.05
Irrigated	54.39	52.11	49	4	48.66	57.94	55.43
Combined	54.60	51.82	37	1	50.01	56.74	54.75
ADF (%)							
Rainfed	5.51	4.51	4	26	4.22	6.73	5.49
Irrigated	-	-	-	-	-	-	-
Combined	-	-	-	-	-	-	-
PS (µm)							
Rainfed	1082.13	1068.48	44	6	1055.50	1192.83	1093.75
Irrigated	1072.73	1045.83	11	10	1019.13	1134.00	1062.26
Combined	1077.60	1057.15	25	5	1053.05	1138.48	1078.15
DMD (%)							
Rainfed	48.18	53.49	24	11	42.59	58.13	49.77
Irrigated	52.77	55.35	21	16	44.67	63.53	53.34
Combined	50.48	54.42	19	7	46.03	58.66	51.62
SCHdig (%)							
Rainfed	56.77	63.21	18	16	52.10	71.21	60.23
Irrigated	60.63	68.48	9	17	53.93	78.48	65.59
Combined	58.70	65.84	7	14	54.60	72.34	62.98

a: Positive transgressive genotypes with lower ADF than Baronesse and higher starch content, larger PS, lower DMD and lower schdig than Lewis.
b: Negative transgressive genotypes with higher ADF than Lewis and lower starch content, smaller PS, higher DMD and higher schdig than Baronesse.
c: Minimum value, d: Maximum value, e: mean.

Table 3-2. Phenotypic correlations among acid detergent fiber (ADF), starch content (SCH), particle size (PS), dry matter digestibility (DMD) and starch digestibility for cracked grains (SCHdig) over combined environments of Lewis x Baronesse population.

Trait	SCH	PS	DMD	SCHdig
PS	-0.073			
DMD	-0.024	-0.539**		
SCHdig	0.102	-0.440**	0.923**	
ADF§	-0.227*	0.137	-0.080	-0.195

*' ** Significant at P=0.05 and P= 0.01, respectively.
§ Correlation between ADF and other traits based on 1997 data only.

QTL analyses

A low-density linkage map was constructed using the 62 F_5-deriveded genotypes of the Lewis x Baronesse RIL population (Blake et al., 1998). This was used to determine the chromosomal locations of gene(s) contributing to variation in agronomic performance, digestibility and grain composition (Figure 3-1), Composite interval mapping (CIM) was employed to detect QTLs. QTL analyses were carried out using data combining environments over years.

Five QTLs controlling variation in ADF were detected on chromosomes 2H, 6H and 7H (Table 3-3). Previous studies detected various QTLs that controlling the ADF variation at 1H, 2H and 4H chromosomes in the Steptoe x Morex population (Ullrich et al., 1996; Han et al., 2003; Abdel-Haleem et al., submitted). In the present study, one of the major ADF-QTLs, *Qadf-2a*, was located on chromosome 2H near ABG602. This QTL controlled about 27% of the variation in ADF. Han et al. (2003) detected a QTL located near ABG019 on chromosome 2H that explained about 28.4% of the variation in ADF of Steptoe x Morex population. According to the barley consensus map, ABG019 and ABG602 are about 5 cM apart on barley chromosome 2H (Grain genes, 2004). This suggests that allelic variation at the same gene may bear responsibility in both populations. *Qadf-2a* was flanked by ABG602 and stscac. Stscac was found to be linked to a grain yield QTL in the same population (Blake et al., 1998).

While multiple QTL were detected for each measured trait, the most interesting included one on barley chromosome 2H, near stscac and ABG602,

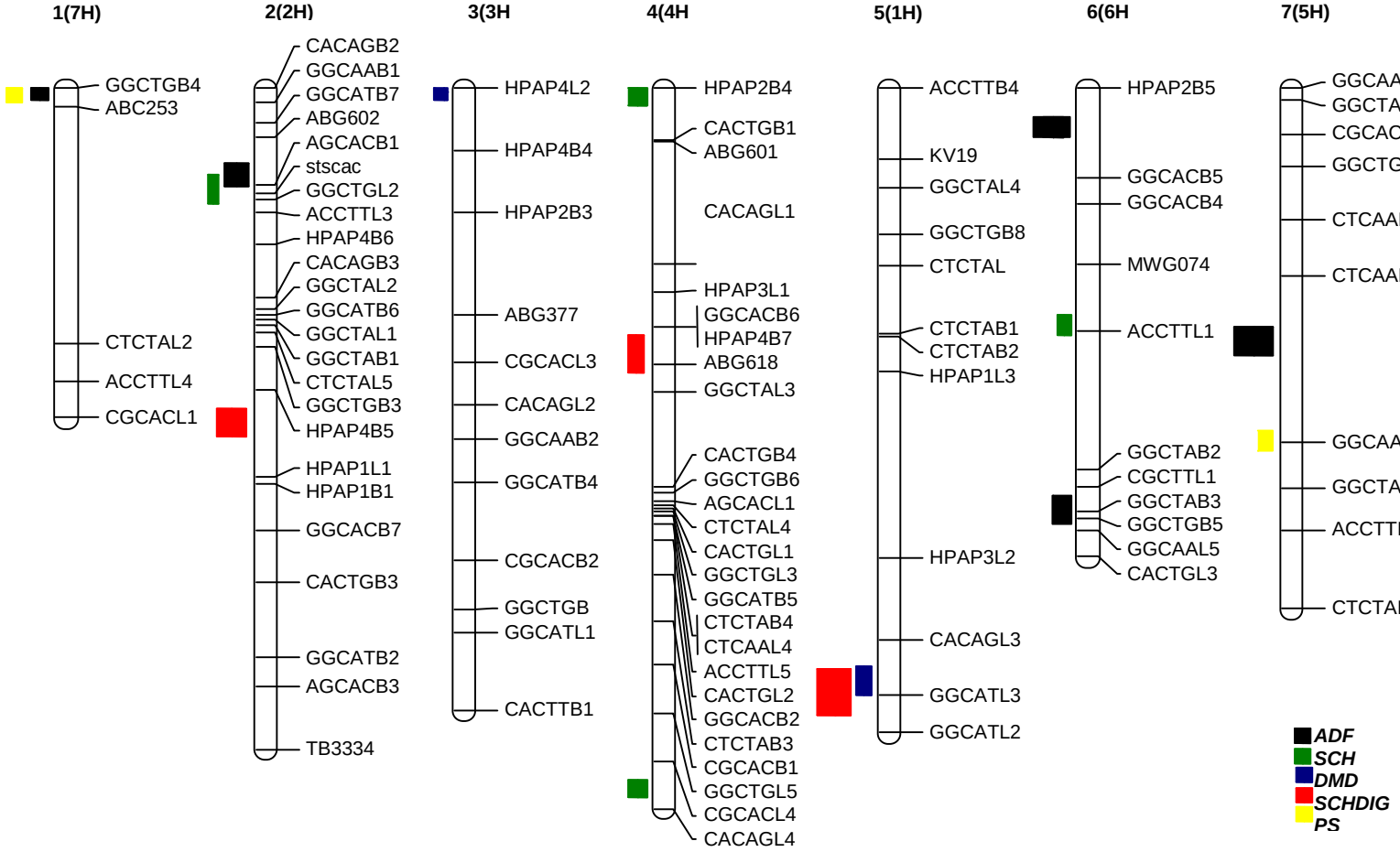
one on chromosome 5(1H) near GGCATL3, one on chromosome 1(7H) near GGCTGB4, and one on chromosome 6(6H) hear HPAP2B5 (Table 3-4). The gene on chromosome 2(2H) was interesting because it had a profound impact on both yield (Blake et al., 1998) and starch content and ADF, and the lines advanced to large-scale trial were fixed for the 'Baronesse' allele at this locus due to its potent agronomic value.

Table 3-3: QTLs for acid detergent fiber (ADF), starch content (SCH), dry matter digestibility (DMD), starch digestibility(SCHdig) and paricle size (PS) using composite interval mapping (CIM) for the Lewis x Baronesse population over combined environments.

Trait	QTL Name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	allelic effect	Positive[c] allele	Rp[2d]	R[2e]
SCH (%)									
	Qsch-2b	2(2H)	stscac	36.47	2.5	0.558	B	10.26	37.9
	Qsch-4b	4(4H)	HPAP2B4	2.01	3.3	0.549	B	16.95	
	Qsch-4c	4(4H)	CACAGL4	241.71	2.1	0.662	L	18.87	
	Qsch-6a	6(6H)	ACCTTL1	83.58	2.9	0.597	L	13.60	
ADF (%)									
	Qadf-1a	1(7H)	GGCTGB4	0.01	3.4	0.289	L	16.79	48.9
	Qadf-2a	2(2H)	ABG602	28.78	3.2	0.468	L	26.45	
	Qadf-6a	6(6H)	HPAP2B5	14.01	2.5	0.420	B	34.94	
	Qadf-6b	6(6H)	GGCTAB3	145.52	4.1	0.342	B	18.72	
	Qadf-7a	7(5H)	CTCAAL2	88.87	3.1	0.403	L	37.40	
DMD (%)									
	Qdmd-3a	3(3H)	HPAP4L2	2.01	2.5	1.526	L	14.38	23.1
	Qdmd-5a	5(1H)	GGCATL3	206.03	3.0	1.210	L	17.40	
SCHdig (%)									
	Qschdig-2a	2(2H)	HPAP4B5	113.70	3.0	2.217	B	28.72	34.8
	Qschdig-4a	4(4H)	ABG618	94.82	3.1	1.763	B	15.01	
	Qschdig-5a	5(1H)	GGCATL3	206.03	4.8	2.032	L	30.00	
PS (µm)									
	Qps-1a	1(7H)	GGCTGB4	0.01	3.0	7.062	L	15.85	40.1
	Qps-7a	7(5H)	GGCAAL4	121.75	3.0	6.860	L	14.05	

[b] QTL position in cM.
[b] Peak value of the LOD.
[c]: Positive allele for high SCH, large PS, low DMD and low Schdig of Baronesse (B) or Lewis(L)
[d] Proportion of phenotypic variance explained by the QTL.
[e] Total phenotypic variances that explained by the model.

Figure 3-1. QTL locations for acid detergent fiber (ADF), starch content (SCH), dry matter digestibility for cracked grains (DMD), starch digestibility (SCHdig) and particle size (PS) for the Lewis x Baronesse population. Bar length indicates the 1-LOD confidence interval and the bar thickness indicates the relative phenotypic variation value for each QTL.



If three genes each have two alleles, we expect eight possible genotypes. Five of these eight recombinant genotypes were available within the set of lines fixed for the 'Baronesse' allele at stscac. In total, we evaluated eight selected lines which represented these five available genotypes to see how well their performance matched genotypic expectations (Table 3-4).

Table 3-4: Means of feed quality characteristics for selected recombinant inbred lines carrying Lewis allele (L) or Baronesse allele (B) at locus GGCTGB4 on chromosome 1(7H) for particle size (PS), at GGCATL3 locus on chromosome 5(1H) for dry matter digestibility (DMD) and starch digestibility (SCHdig) and locus HPAP2B5 on chromosome 6(6H) for ADF over combined environments.

	Allele			Feed quality				
	7H	1H	6H	SCH (%)	DMD (%)	SCHdig (%)	PS (µm)	ADF(%)§
LB2	L	B	B	54.66	48.00	57.35	1076.30	5.09
LB5	B	B	L	55.87	50.76	60.73	1059.20	4.90
LB6	L	B	B	55.90	53.63	64.39	1073.10	4.92
LB13	L	B	L	55.83	50.04	60.64	1075.50	4.95
LB30	B	B	L	54.59	45.99	55.34	1069.80	4.74
LB32	B	B	L	54.12	46.43	53.64	1075.30	6.23
LB48	L	L	B	55.30	44.40	55.07	1155.90	6.27
LB57	B	L	L	55.41	54.10	65.65	1071.00	5.23
Lewis				54.60	50.48	58.7	1077.60	5.51
Baronesse				51.82	55.42	65.84	1057.15	4.51

§ ADF estimation based on 1997 data only.

Simple correlation analysis of starch content, particle size, in situ DMD and ADF percentage for these 8 lines was strongly (and significantly) positive for Feed Efficiency (FE), but not significant for ADG. This suggests that compositional analyses of barley prior to purchase could provide feedlot operators with economic value (Table 3-5).

Table 3-5: Predicted (based on grain composition) and adjusted (based on genotype) estimates of average daily gain (ADG) and feed efficiency (FE) parameters using our 8 recombinant inbred lines from Lewis x Baronesse population.

Genotype	Predicted		Adjusted (model 1)		Adjusted (model 2)	
	ADG (kg/d)	FE (kg/100kg)	ADG (kg/d)	FE (kg/100kg)	ADG (kg/d)	FE (kg/100kg)
LB13	1.42^{abc}	15.62^{ab}	1.44	15.73	1.39	15.73
LB30	1.56^{a}	16.68^{ab}	1.42	15.73	1.37	15.91
LB2	1.52^{abc}	16.25^{ab}	1.47	15.63	1.44	15.68
LB5	1.47^{abc}	16.36^{ab}	1.42	15.73	1.38	15.45
LB6	1.46^{bc}	15.91^{b}	1.47	15.63	1.42	15.10
LB32	1.43^{ba}	15.42^{ab}	1.42	15.73	1.35	15.17
LB48	1.50^{a}	16.58^{a}	1.43	15.34	1.49	14.16
LB57	1.48^{c}	16.37^{b}	1.39	15.44	1.51	15.01

§ Means with different letters are significantly different at P>0.001

Table 3-6: Feedlot performance (observed) and carcass characteristics of steers finishing diets based on 8 recombinant inbred lines form Lewis x Baronesse population and parents at 2 animal facilities at Bozeman and Havre.

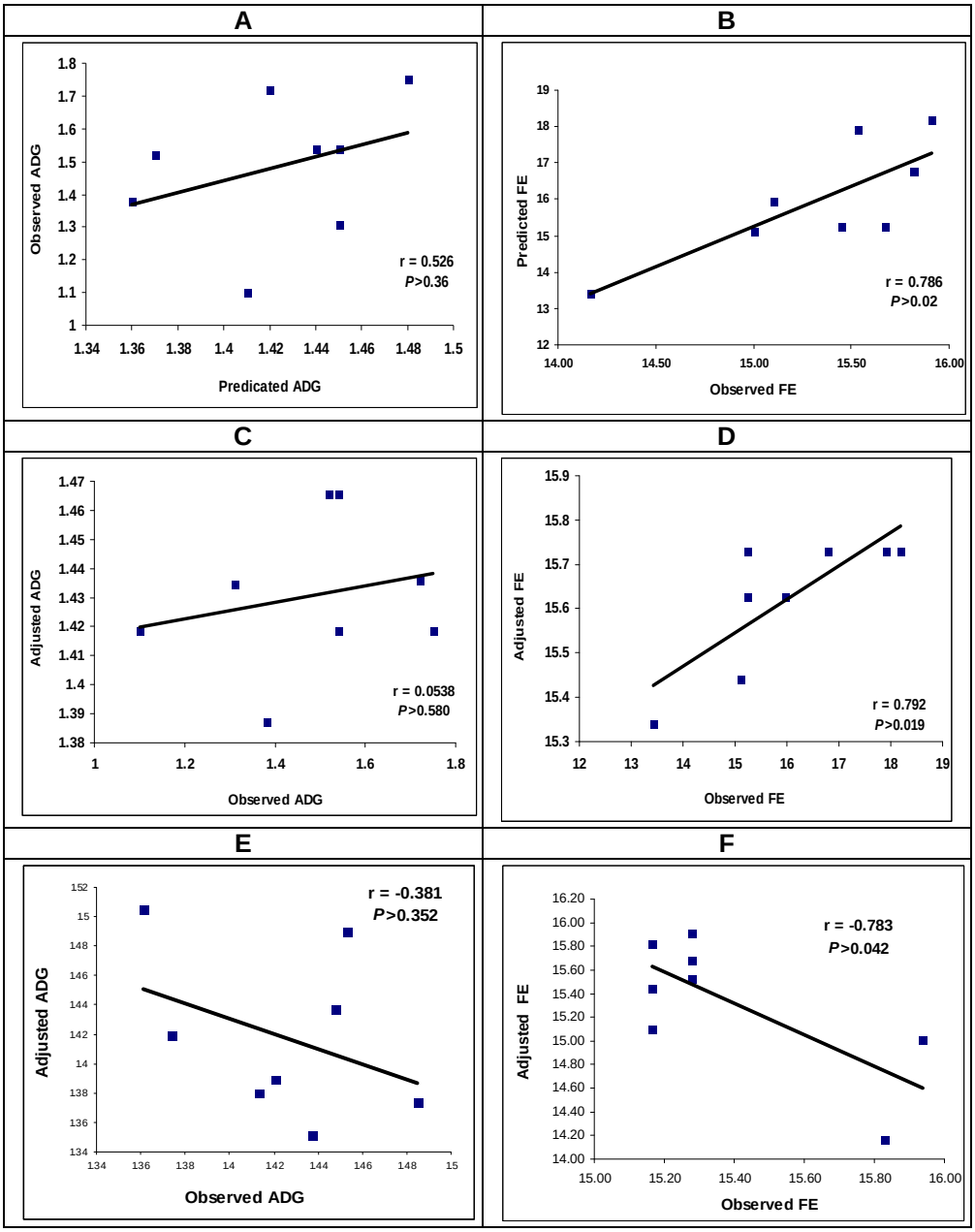
Genotype	ADG (kg/d)	DMI (kg/d)	FE (kg/100kg)	Marbling score	Quality grade	yield grade
Bozeman trial						
LB13	1.72	9.64	17.9^{a}	3.7	11.2	2.3
LB30	1.75	9.59	18.2^{b}	3.7	11.3	2.5
Lewis	1.46	9.18	17.0	3.7	11.3	2.3
Baronesse	1.47	9.18	17.5	3.7	11.2	2.2
P§	***0.07***	***0.55***	***0.05***	***0.99***	***0.90***	***0.28***
Havre trial						
LB2	1.54^{c}	10.11^{d}	15.23	4.7^{a}	12.2^{b}	2.4
LB5	1.10^{a}	8.67^{a}	15.23	5.5^{b}	13.3^{c}	3.3
LB6	1.52^{c}	9.52^{cb}	15.96	4.3^{a}	12.0^{ab}	2.6
LB32	1.54^{c}	9.17^{ab}	16.79	4.6^{a}	12.3^{b}	2.8
LB48	1.31^{b}	9.76^{cd}	13.42	4.4^{a}	12.1^{a}	2.7
LB57	1.38^{bc}	9.13^{ab}	15.11	4.4^{a}	12.1^{ab}	2.7
Lewis	1.40	8.94	15.66	4.7	12.3	2.8
Baronesse	1.41	9.03	15.61	4.5	12.2	2.7
P§	***0.006***	***0.001***	***0.47***	***0.05***	***0.03***	***0.39***

§ Means with different letters are significant according to the significant level of each trait.

In the better-replicated Bozeman trial, significant genotype effects were observed for ADG and FE (P= 0.006 and P=0.0001, respectively), and both recombinant lines provided better feedlot performance than did either parent. At Havre, steers fed LB2, LB32 and LB6 provided higher ADG than steers fed LB5, LB48 and LB57 (Table 3-6). Steers consumed more dry matter from the selected recombinants than the parents, resulting in similar feed efficiencies for all lines. Interestingly, in the cold, dry Havre environment steers produced higher quality scores than did those at Bozeman, and our poorest-performing barley line in terms of ADG produced the highest quality carcasses.

The two genetic models (Simple or Complete) failed to predict barley performance better than did prediction utilizing compositional analysis. Indeed, the Complete Model failed to predict grain performance (Table 3-5). Surprisingly, utilizing the ‘Three Biggest’ QTL provided about as much insight into grain feedlot value for feed efficiency as did grain composition. This suggests that our low density map may have failed to mark critical genes, or that the predictive value of our adjacent markers is inadequate.

Figure 3-2: Correlation analysis between observed and predicted average daily gain (A) and feed efficiency (B) based on 8 recombinant inbred lines from Lewis x Baronesse population. Observed values were obtained by direct feedlot measurement and Predicted values were those based on grain composition data, utilizing the prediction equations of Bowman et al. (2001). Panels C through F present correlations between feedlot performance and predictions based on genotype utilizing either the Simple model (C and D) or the Complete Model (E and F).



The objective of this exercise was to determine whether QTL analysis would assist or detract from varietal development in a real-world barley variety development project to improve feed quality. Baronesse, while agronomically excellent, was found to be relatively high in ADF, low in starch, and fermented relatively rapidly in the rumen. Lewis proved better at starch deposition, and its grain fermented more slowly. Among the 62 recombinant lines we evaluated, two provided feed quality better than either parent, and one of these was agronomically desirable (line 30, released as the variety ‘Valier’).

This QTL analysis, utilizing a small mapping population and a relatively sparse map, is likely to represent the level of resource available to many plant breeders during a varietal development program. Our assessment of this experiment suggests that selection based on phenotype for traits that are real ‘quantitative traits’ (i.e. that involve allelic variation for several genes with similar, relatively minor effects) is at least as effective as is marker-assisted selection. To paraphrase a mentor ‘If you’re going to select for yield, you’d better be measuring yield’ (Pers. Comm., Ted Bingham). In this case, selecting for feedlot performance, or even the primary grain compositional characteristics contributing to variation for feedlot performance is as or more reliable than is utilizing markers from a sparse mapping dataset.

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CHAPTER 4

THE GENETICS OF VARIATION IN FEED QUALITY IN A WIDE 2- BY 6-ROWED BARLEY RIL POPULATION

Abstract

Annually, about 70% of the North American barley crop is fed to livestock. Barley grain has been criticized as a cattle feed because of its rapid digestion in the rumen, which may result in digestive disorders such as bloat and lactic acidosis in cattle. Substantial heritable variation for characteristics contributing to variation in barley feedlot performance was reported in a survey of the USDA barley core collection (Bowman et al., 2001). In situ dry matter digestibility, particle size after dry rolling, kernel hardness, starch and fiber content, all have been found to play a role in feedlot performance of barley as a feed grain (Surber et al., 2000). Reducing the rate of in rumen barley dry matter digestibility is predicted to result in significantly improved animal health and average daily gain, if starch content is high and ADF is low (Surber et al., 2000; Bowman et al, 2000).

In this paper we report the results of a QTL mapping project based on a recombinant inbred line population derived from a cross between a commonly grown feed barley cultivar (Valier) with moderate dry matter digestibility (DMD), and a line from the core world collection exhibiting far slower DMD. One gene or a linked cluster of genes on barley chromosome 2H tightly linked to the morphology-modifying *Vrs1* was found to dramatically impact DMD. Since *Vrs1* is known to impact several characters that could impact DMD,

experiments were conducted to attempt to test whether this variation is the result of pleiotropy or linkage.

Introduction

Barley is an important component of the rainfed small grains production system that dominates Northern North America. Barley grown in harsh environments tends be higher in grain protein concentration than desired by maltsters. High protein, low test weight barley is commonly utilized in cattle feeding rations, and can be used in high concentrate beef cattle diets with excellent results (McDonnell et al., 2003).

The cow-calf production system that dominates the livestock industry in Montana is based on producing feeder calves that are sold at weaning and transported to out-of-state feedlots where they are fed to slaughter weight. In 2003, Montana produced approximately 1,451,000 beef cows. Of those, approximately 70,000 cattle were finished in feedlots in the state, while about 1.1 million head were sold as calves and transported to feedlots in the Midwest and Southern Plains.

Approximately 70% of the Montana barley crop is used for cattle feed, about 700,000 mt. (Montana Ag Stats, 2003). Improving the quality of barley as a feed grain could improve the economics of Western beef cattle production and permit the development of a more robust regional cattle feedlot industry.

Barley has been criticized for cattle feed because of its rapid digestion in the rumen, which results in digestive disorders including bloat and lactic

acidosis in cattle (Kellems and Church, 2002). A potential approach to limit this problem associated with feeding barley is to select barley cultivars that have a slower rate of digestion in the rumen.

Several grain characteristics impact barley feed quality. Surber et al. (2000) found that net energy content, feed efficiency and average daily gain of cattle fed barley could be effectively predicted from measures of dry matter digestibility (DMD), acid-detergent fiber (ADF) percent, particle size after dry rolling and starch percent. Selection of barley grain for slow DMD, low ADF content, large particle size after dry rolling and high starch content should permit selection for improved feed quality. The first feedlot-tested improved feed quality barley variety utilizing these parameters as selection criteria, ‘Valier’ (PI610264), was released to producers in 2001 (Blake et al, 2002).

Limited genetic variation is available for feed quality related grain characters in modern barley varieties adapted to Western North America. A survey of the spring barley core collection revealed far greater variation for these traits (Bowman et al., 2001). Several lines were identified that had normal or high starch content, low fiber content and extraordinarily low DMD. One of these, PI370970, a six-rowed hulless landrace line from Switzerland, was crossed to ‘Valier’, a 2-rowed covered barley variety, and a population of recombinant inbred lines was derived. These were subject to two years’ trials in rainfed and irrigated conditions at the A.H. Post Research Farm near Bozeman, Montana. Agronomic traits and *in situ* DMD of grain samples from

the RILs were determined and the chromosomal location of genes modifying these effects determined.

We report that the largest QTL impacting *in situ* DMD resides on barley chromosome 2H very close to *Vrs1*, a gene that impacts plant and seed development in many ways, including determining whether the head is 2-rowed or 6-rowed. Previous work suggested that grain from 6-rowed varieties degraded slightly but significantly slower than grain from 2-rowed varieties (Bowman et al., 2001). The dramatic effect of this QTL was twice that of the effect previously observed. *Vrs1* resides in a moderate to low recombination region of chromosome 2H (Kunzel et al., 2000), so the physical distance between *Vrs1* and other genes in this region that might impact DMD could be large, even though recombinants might be very infrequent.

The most straightforward way segregation at *Vrs1* might impact DMD is through the variation in kernel size conditioned by the 6-row head type. Kernels on 6-rowed heads vary much more dramatically in terms of size and shape than do those on 2-rowed heads. Variation in kernel size could result in inefficient milling of kernels through the Buehler mill, resulting in a broader spectrum of particle sizes. Large particles tend to degrade in the rumen at slower rates than do smaller particles. To test this hypothesis, we size-fractionated cracked samples from the entire population and reran the samples through our DMD analysis and QTL analysis.

We also tested the impact of the *Vrs1* gene using validating populations derived from a two-rowed x six-rowed cross using RIL of the Baronesse x

PI370970 population and a previously developed mapping population, the Harrington x Morex population. Dr. Patrick Hayes and Ann Corey provided sufficient grain from this previously mapped population to enable us to perform QTL analysis for DMD.

Material and Methods

Plant materials

A mapping population of 116 F_5-recombinant inbred lines was derived by single seed descent from a cross between Valier (PI610264) and the Swiss landrace line PI370970. Valier (PI610264) is two-rowed, hulled spring barley cultivar, developed by the Montana Agricultural Experiment Station and released for commercial production (Blake et al., 2002). PI370970 is a six-rowed hulless barley landrace line from Switzerland selected from the USDA world collection of barley's spring core collection based on its low DMD value, large particle size, high starch content and low ADF content (Bowman et al. 2001).

Two additional barley populations were used to validate the detected QTL. One hundred seventy nine F_6-recombinant inbred lines were produced via single seed descent of a cross between Baronesse and PI370970. Baronesse is two-rowed feed barley variety with high DMD. One hundred forty doubled-haploid lines were produced utilizing the pollen of H. bulbosum (Kasha et al 1970) to pollinate F_1 florets from a cross of Harrington x Morex (Chen and Hayes, 1989). Morex is a six-rowed malting barley variety while Harrington is

two-rowed malting variety, and both are high in DMD compared with PI370970.

Field experiments

One hundred sixteen F_4–derived Valier x PI370970 recombinant inbred lines, Valier, PI370970 and check varieties were planted at the A.H. Post Research Farm near Bozeman, MT during the 2002 cropping season. During the 2003 cropping season, $F_{5:7}$ entries of Valier x PI370970 the parents and the check varieties were grown with and without irrigation. Entries, in both experiments, were arranged in a randomized complete block design with two field replications at the A.H. Post Research Farm. Entries were planted into an Amsterdam silty loam soil at a seeding rate of approximately 30 seeds/m in 2m long single row plots spaced 0.3m apart. Plots were harvested by a Wintersteiger plot combine and cleaned using a de-awner and blower.

Heading date, plant height and grain yield were measured in each experiment. Heading date was estimated as the time at which 50% of the spikes burst through the leaf sheath. Plant height was measured in cm at the physiological maturity from the soil surface to the tip of the spike for each plot, excluding the awns. Yield was measured as the weight of the grains per plot and converted to kg ha^{-1}. Plots were harvested by a Wintersteiger plot combine and cleaning using a de-awner and blower.

The Baronesse x PI370970 population was planted at the A.H. Post Research Farm in summer 2003 with one replicate. Entries were planted at a

seeding rate approximately 30 seeds/m in single row plots 2m long space 0.3m apart. The entries were irrigated as needed.

The Harrington x Morex population was grown at Klamath Falls, Oregon in the summer of 1994, and the seed generously provided by Dr. Pat Hayes. Plot size and management followed local practices.

DMD and particle size estimation

To simulate dry rolling, grain samples from each line of each population were cracked using a Buehler mill (Buehler-Miag, Braunschweig, Germany). Particle size was determined on cracked samples by dry sieving with two replicates per sample (Fisher et al., 1988). In situ DMD estimation was done according to the procedure of Vanzant et al. (1998) using two ruminally cannulated beef cows that consumed low quality grass hay ad libitum and 3.6 kg day_{-1} of barley. Four 5g samples for each entry were placed in 10 x 20-cm, 50-µm pore size polyester bags (Ankom Technology, Fairport, NY). Thirty polyester bags consisting of twenty-eight polyester bags containing experimental samples, one blank bag and one bag containing Harrington as a check variety were placed in the rumen of each of the two ruminally cannulated cows at the same time and incubated for 3 h. After removal from the rumen, the bags were manually rinsed under cold water until the wash water ran clear. The bags were dried at 60°C for 48 h and then weighed. Dry-matter content of the cracked barley samples was estimated by measuring the DM content of ≈120 samples and calculating the mean value. Ruminal DMD was calculated according to the following equation: in situ DMD % = 100-(((dry

sample and bag wt. out - bag weight) - (blank bag wt. in – blank bag wt. out))/ (sample wt. in* DM)))*100

To eliminate the seed size effect, one replicate of the irrigated trial was utilized as a source of grain to determine whether variation in particle size was a large contributor to the *Vrs1* linked DMD QTL effect. Samples were fractionated by dry-sieving (Fisher et al., 1988) using 5 sieve sizes. The fraction that remained atop the intermediate sieves that retained particles greater than 850 µm and 1700 µm was supplied to the cannulated calves for DMD estimation as described above.

Morphological and Proteins markers

Two morphological markers were scored, N/n (hulled vs hulless caryopsis) and *vrs1*/*Vrs1* (six- vs two-rowed spike). The nomenclature of the morphological markers followed the recommendations of Francowiak (1997). Hordein polymorphisms were assayed using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from the mature ground single seed according to Blake et al. (1982). The B, C and D hordein protein banding patterns were derived from the variation at Hor-2, Hor-1 and Hor-3 loci on chromosome 1H.

DNA marker analysis

Three week old seedling leaf tissue from a single F_5 plant represented each RIL for DNA marker analysis and linkage map construction. DNA isolation was performed according to the procedures of Dellaporta et al.

(1983). DNA was phenol-chloroform-purified, ethanol-precipitated, re-suspended in Tris-HCl buffer and stored for later use. Microsatellite (SSR) markers (Ramsay et al., 2000; Liu et al., 1996) and STS primers (Blake et al., 1996; sayed-Tabatabaei et al., 1998; Kleinhofs et al., 1993; Kunzel et al., 2000) were screened to identify polymorphism between the parents. Forty five informative STS and SSR markers were selected to screen the whole population (Table 4-1).

The 25-µl PCR reaction mix consisted of 80 ng of forward and reverse primers, 0.5 units of Taq DNA polymerase (Promega, Madison, WI.), 0.2 mM of each dNTPs, 1 X PCR buffer (50 mM KCL, 10 mM Tris-HCl, 1 g L-1 Triton X-1000), 1.5 –2.5 mM MgCl2, 25 ng of template DNA, and distilled H_2O. The PCR amplification consisted of an initial denaturation at 94°C for 3 minutes, followed by 40 cycles of three steps of each: denaturation at 94°C for 30 seconds, annealing for 30 seconds, and elongation at 72°C for 20 seconds. A final elongation step at 72°C for 5 min was performed. The PCR reactions were performed in a 96-well microtiter plates using a Perkin Elmer 9600 Thermal cycler (PE Applied Biosystems, Foster City, Calif.) and a PTC-100 MJ Research (MJ Research, Watertown, Mass.). Amplification products from SSR and STS were separated on 12-18% polyacrylamide or 1% agarose gels in 0.5x TBE (45 mM Tris-borate, 1 mM EDTA {N,N'-1,2-ethanediylbis-[N (carboxymethyl)glycine]} immediately following amplification. Electrophoresis was performed at 200 V for 3 h and visualized by eithdium bromide staining.

Table 4-1: Informative microsatellite (SSR) and sequence tagged site (STS) markers, their chromosomal locations, restriction enzymes, MgCl concentration, annealing temperature (AT°C) and banding sizes of Valier x PI370970 population.

Maker name	Marker	Chrom	Res. enzym	Amplification cond.		Band size	
				MgCl(uM)	AT°C	Valier	PI370970
Bmag211	SSR	1H	-	2.5	52	220, 194, 185	225, 198, 190
Bmag718	SSR	1H	-	1.5	52	190, 170	182, 160
Bmag872	SSR	1H	-	1.5	52	110	130
EBmac501	SSR	1H	-	1.5	52	190	118
Bmag347	SSR	1H	-	1.5	52	118,110	120, 132
Bmag872	SSR	1H	-	1.5	52	125	194
Bmac154	SSR	1H	-	1.5	52	120, 144	115, 122
ABG10	STS	1H	-	2.5	52	872	239
KV19	STS	1H	-	2.5	64	1078	872, 750
Bmag144	SSR	1H,2H	-	1.5	52	220, 300	190, 210
EBmac684	SSR	2H	-	1.5	52	185, 200	175, 197
Hvbkasi	SSR	2H	-	1.5	52	205	195
HVM54	SSR	2H	-	1.5	52	102, 110, 118	95, 115
ABG613	STS	2H	-	2.5	52	132	150
cMWG682	STS	2H	Tsp 509I	2.5	52	271, 210	530
MWG503	STS	2H	MseI	2.5	68	255, 197, 160	255, 450
cMWG699	STS	2H	TaqI	2.5	62	281, 235	281, 260
HVM60	SSR	3H	-	1.5	52	115	105
Bmac0067	SSR	3H	-	1.5	52	190	180
Bmag013	SSR	3H	-	1.5	52	155, 194	120, 151
ABG483	STS	3H	-	2.5	52	1050, 230, 194	1050, 155
ABC396	STS	3H	MspI	2.5	52	220	200
Bmac0030	SSR	4H	-	1.5	52	182, 170	170, 160
Bmag353	SSR	4H	-	1.5	52	118,130	110,100
BMag310	SSR	4H	-	1.5	52	135, 175	175, 194
HVM67	SSR	4H	-	1.5	52	100	90
ABG54	STS	4H	-	2.5	52	165	174
PinA	STS	5H	DdeI	1.5	52	600	190, 320
GMS0027	SSR	5H	-	1.5	52	125, 190	160, 182
Bmag323	SSR	5H	-	1.5	52	110, 180, 190	101, 170, 185
Bmac113	SSR	5H	-	1.5	52	194, 230	184, 210
Bmag222	SSR	5H	-	1.5	52	182	175
TB21-22	STS	5H	-	2.5	52	120, 210	153, 210
MWG514	STS	5H	-	2.5	52	1050	730
HVD007	SSR	5H	-	1.5	52	194, 175	194
EBmac602	SSR	6H	-	2.5	52	320,210	190,260
Bmag770	SSR	6H	-	1.5	52	170, 194	170, 194, 230,250
Bmag500	SSR	6H	-	1.5	52	145, 230	105
Bmag914	SSR	7H	-	1.5	52	175, 194	185, 200
Bmag900	SSR	7H	-	1.5	52	110	118
Bmag135	SSR	7H	-	1.5	52	210, 240	194
ABG380	STS	7H	-	2.5	52	310, 420, 510	420
ABC305	STS	7H	Tsp 509I	2.5	52	118, 150	118, 140,
ABC255	STS	7H	MspI	2.5	52	320, 270	600
EBmac0679	SSR	4H	-	1.5	52	130, 140	120,132

STS loci that required digestion were sequenced using the ABI 377 automated DNA sequencer in a 4% denaturing polyacrylamide gel containing 8 M urea and 1x TBE at 1200 V for 6h. Restriction-site allelic variants at STS

loci were digested with 2 units of the appropriate restriction enzyme, as shown in Table 4-1, before characterizing them on a 10% polyacrylamide gel. Markers that were polymorphic between the parents were screened on the entire population.

Amplification Fragment Length Polymorphism (AFLP) analysis was done as described by Vos (1995) with some modifications. A 15-µl digestion reaction mix including 100 ng of genomic DNA was digested with 3 units of restriction enzymes *EcoRI* and *HpaII* for 2 hrs at 37°C, and then the reaction was terminated at 65°C for 10 min. The digested mixture was ligated with ligation mixture that contained *HpaII* and *EcoRI* adapters in 1x ligation buffer (New England Biolabs, Boston, MA) and 1 unit of T4 ligase (New England Biolabs, Boston, MA) in a total volume of 20µl at 37°C for 3 hrs. The pre-amplification reaction contained 1 µl of ligation product in 30 µl of 0.1 mM each dNTP, 1x buffer (Promega) 25 nM $MgCl_2$ and 0.5 units *Taq* and 30 ng of *HpaII* and *EcoRI* linker primers (H0 and E0 primers) without selective bases (Table 4-2).

Selective amplification was carried out using primers having 3 selective nucleotides to reduce the complexity of the products. Combinations of 4 EcoRI+3 primers and 8 HpaII+3 primers were used (Table 4-2). The selective amplification reaction mix included 1 µl of pre-amplification product in 20 µl of 0.1 mM dNTP, 1x buffer (Promega), 25 nM $MgCl_2$, 0.5 units *Taq*, 5 ng of *EcoRI* fluorescently tagged primer and 30 ng of *HpaII* primer, thermocycler conditions were described by Vos (1995).

Table 4-2: AFLP adapters and primers used in construction of Valier x PI370970 linkage map.

Primer/adapter		sequence
EcoRI adapter		5'CTCGTAGACTGCGTACC3'
		3'CATCTGACGCATGGTTAA5'
E0 (universal primer)		5'GACTGCGTACCAATTCA3'
EcoRI+3 primers	E1	E0+ACT
	E2	E0+ATC
	E3	E0+AGC
	E4	E0+ACC
HpaII adapter		5'GACGATGAGTCCTGAG3'
		3'TACTCAGGACTCGC5'
H0 (universal primer)		5'GATGAGTCCTGAGCGGC3'
HpaII+ primers	H1	H0+CGA
	H2	H0+CAT
	H3	H0+CTC
	H4	H0+CGG
	H5	H0+CGC
	H6	H0+CAG
	H7	H0+CGT
	H8	H0+CAA

PCR reactions were made in PTC-100 MJ Research (MJ Research, Watertown, Mass.). AFLP products were resolved and detected using 4% denaturing polyacrylamide gels containing 8 M urea and 1x TBE at 250 V for 3 h on an ABI 377 automated DNA sequencer. Internal molecular weight standards (Genescan-500 ROX, Applied Biosystems) were loaded with each sample, permitting automated molecular weight estimations to be made for all AFLP fragments between 50 and 500 bases long. AFLP fragments were scored using Genographer (Benham et al., 1999). Each polymorphic AFLP was identified by the code referring to the combination of *EcoRI*+3 and *HpaII*+3 primers followed by the estimated molecular marker size of the DNA fragment

Map construction and segregation analysis

Segregation and linkage analyses were done using Joinmap 3.0 (Van Ooijen and Voorrips, 2001). For each marker, a X^2 test was used to identify the markers showing segregation distortion, with the expectation of equally frequent Valier: PI370970 alleles. A minimum LOD score of 3.0 was used to initially assign markers to groups and Kosambi's mapping function was used to calculate the map distances. Markers were scored either A (homozygous for the Valier allele), B (homozygous for the PI370970 allele) or H (heterozygous).

Statistical analysis

Analysis of variance was performed for each environment separately in a complete block design and then combined over environments (Steel and Torrie 1980). In the combined analysis the environments, blocks and genotypes were considered as random effects (statistical Analysis System, SAS institute 1990). Variance components were estimated using the restricted maximum likelihood (REML) method of SAS PROC MIXED.

Heritability on plot mean and family mean bases were estimated as $h^2=(\sigma^2_G/(\sigma^2_G + \sigma^2_{GEN} + \sigma^2_e)$ and $h^2= \sigma^2_G /(\sigma^2_G + (\sigma^2_{GEN}/e) + (\sigma^2_e/e\ r))$ respectively (Nyquist, 1991; Holland et al., 2003) where σ^2_G genetic variance among the entries, σ^2_{GEN} variance of genetic x environment interaction, σ^2_e variance of experimental error, e number of environments and r number of replications. Because the data were unbalanced, e and r were computed as harmonic mean of environments and harmonic mean of total replications across all environments, respectively (Holland et al, 2003).

QTL analysis

Analysis of variance was performed on all traits and environments to identify significant genotype and genotype x environment interactions. When genotype effects were significant, QTL analyses were performed using QTL Cartographer 2.0 (Wang et al., 2002). A series of 1000 permutations was run to determine the experiment-wise significant level at P=0.05 of LOD for the trait (Churchill and Doerge, 1994). Composite Interval Mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam, 1994; Zeng 1994) using Model 6 of the Zmapqtl program module. The genome was scanned at 2-cM intervals and the window size was set at 10 cM. Cofactors were chosen using forward-backward stepwise regression. The percentage of phenotypic variance explained by a specific QTL value (R^2) was taken as the peak QTL position as determined by QTL cartographer. The total R^2 including the significant QTLs in the model was calculated using MIM method of QTL cartographer. Multiple interval mapping (MIM) was applied to test the QTL effects including additive and QTL x QTL interactions by simultaneous analysis of the QTLs in multiple regression models. MIM performed with the significant QTLs that selected by CIM model.

The major DMD QTL detected in the Valier x PI370970 population was validated in the Baronesse x PI370970 population using head type (*Vrs1*) as the categorizing variable. Single marker analysis was performed using Proc GLM (statistical Analysis System, SAS institute 1990).

Results

Map construction

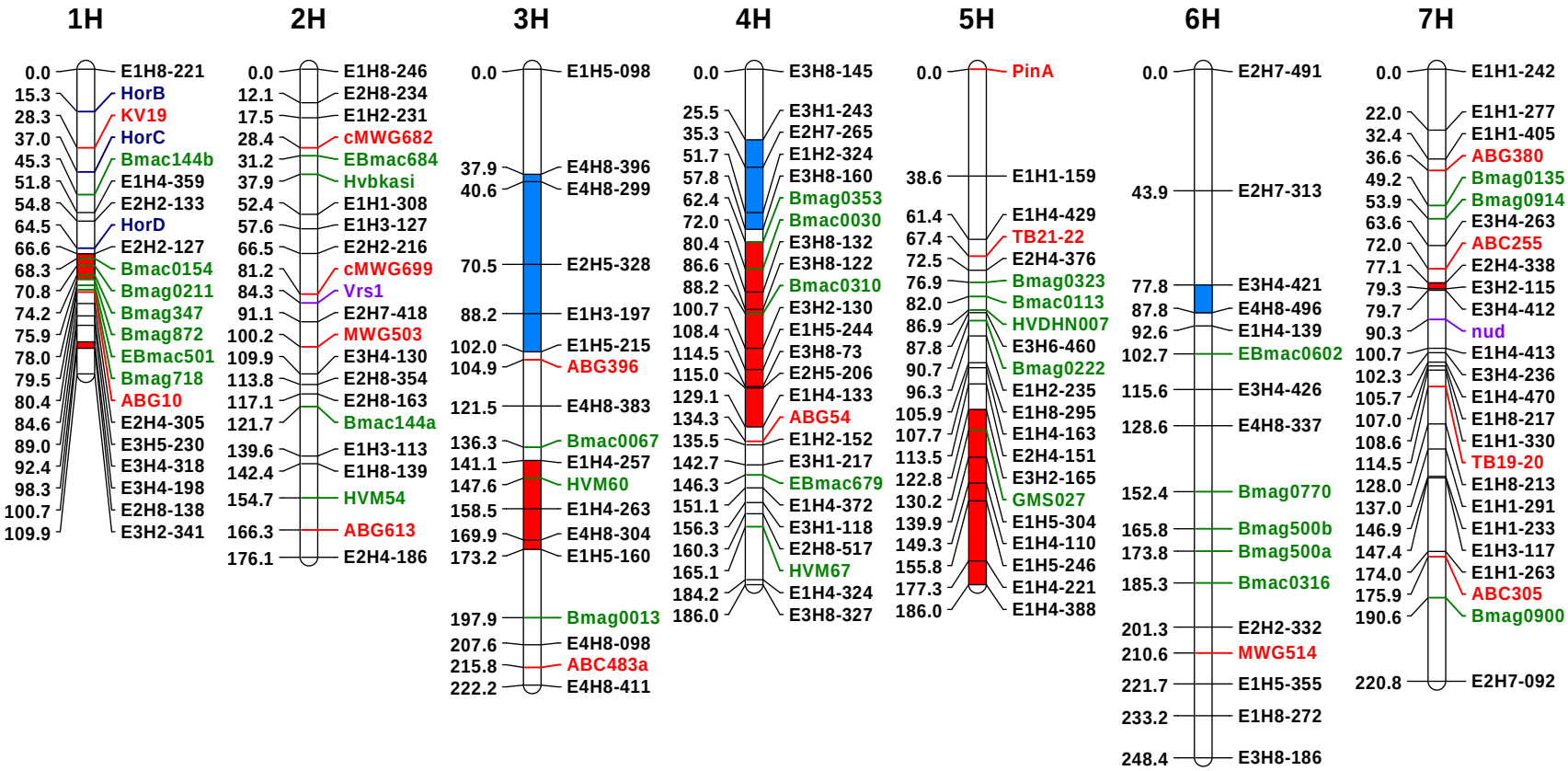
Thirty two SSR, 15 STS, 3 hordein proteins and 2 morphological markers were selected and utilized as anchor markers spanning the barley genome (Table 4-3). Anchor markers were mapped to their expected previously published positions with minor modifications (Kleinhofs et al., 1993; Kunzel et al., 2000; Liu et al, 1996; Ramsay et al, 2000).

Eighteen primer pairs provided a total of 120 polymorphic AFLP markers which were scored and included in the linkage map (Figure 4-1). The number of polymorphic markers per primer combination ranged from 1 using E3H6 to 14 markers using E1H4 with average 6.77 useful markers per primer combination. Of these one hundred twenty potential markers, 98 fit well within the anchored linkage map.

Table 4-3: Number of informative markers, chromosome length and marker density of Valier x PI370970 linkage map.

Chrom	length cM	Markers						Density cM/marker
		AFLP	SSR	STS	Protein	Morph	Total	
1H	109.9	10	7	2	3	0	22	4.99
2H	176.1	13	4	4	0	1	22	8.00
3H	222.2	13	3	2	0	0	18	12.34
4H	186.0	19	5	1	0	0	25	7.44
5H	186.0	14	5	2	0	0	21	10.94
6H	248.4	11	5	1	0	0	17	14.61
7H	220.8	18	3	3	0	1	25	8.49
Total	1349.4	98	32	15	3	2	150	8.99

Figure 4-1: Linkage map of Valier x PI370970 population including 32 SSR (green), 14 STS (red), 3 horedins (blue), 2 morphological (purple), and 98 AFLP (black) markers. Colored bar parts indicated the distortion cluster toward Valier (red) or PI370970 (blue).



Seven linkage groups corresponding to the seven barley chromosomes were separated at LOD 5.0- 8.0, the map covered 1349.4 cM with average separation of 8.99 cM (Figure 4-1). Chromosome 7H had the greatest number of markers numbers (25) while chromosome 6H had the fewest (17).Segregation distortion was observed for about 36% of the mapped markers. The most distorted markers were AFLPs (Figure 4-1).

Phenotypic variation

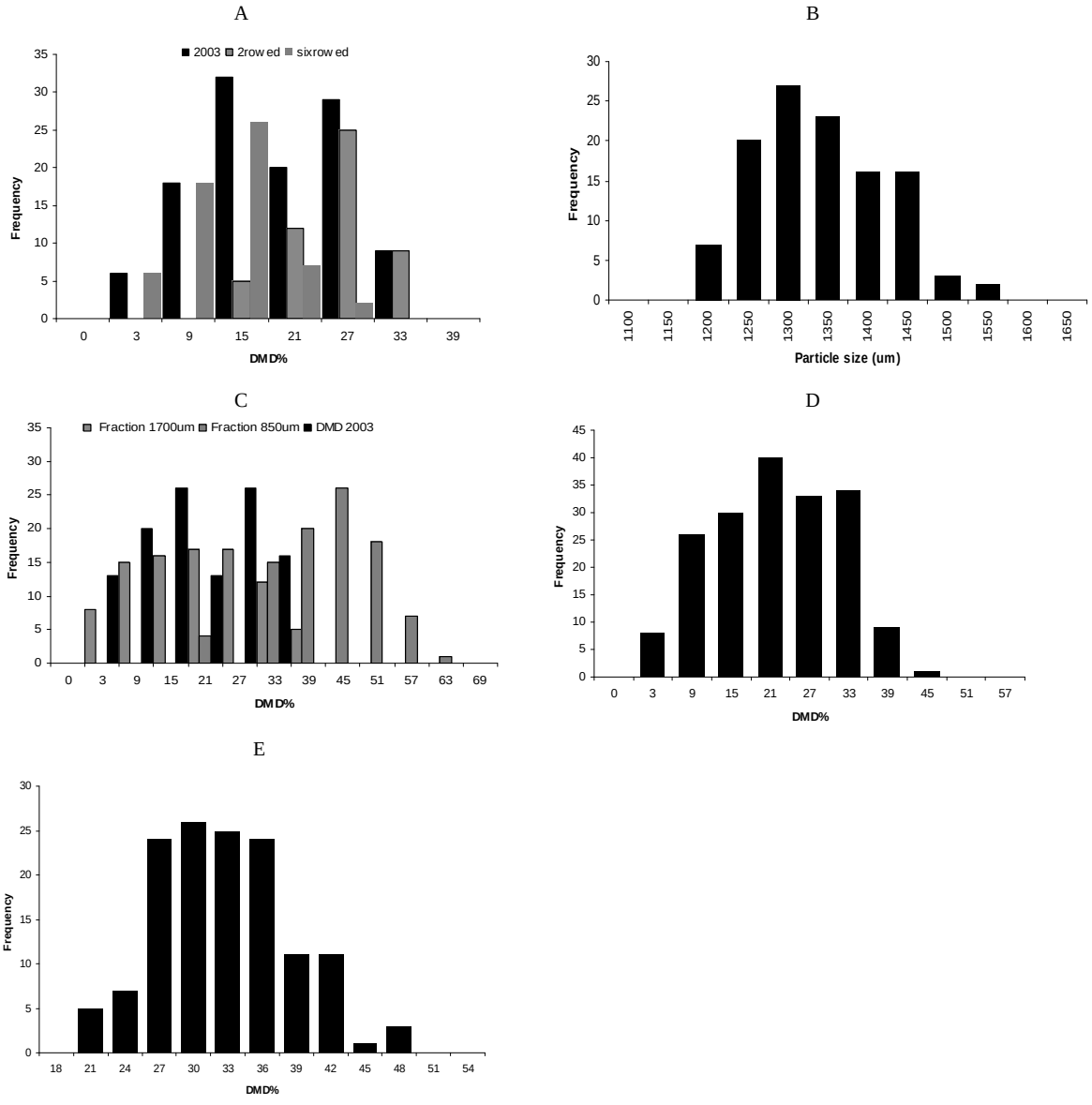
DMD and particle size distributions of the mapping population parents and progeny are summarized in Table 4-4, Figure 4-2E and Figure 4-2C. For both traits Valier had higher DMD and smaller particle size than PI370970 in both environments and the combined analysis. There was little evidence of either positive or negative transgressive segregation for either character (Table 4-4).

Table 4-4: Means, range, transgressive phenotypes of Valier, PI370970 and their RILs over rainfed, irrigated and combined environments for dry matter digestibility (DMD) and particle size (PS).

Trait	Valier	PI370970	RILs				
			Positive trans[a].	Negative trans[b].	Min[c]	Max[d]	χ[e]
DMD (%)							
Rainfed	35.27	13.78	16	5	4.95	40.82	22.87
Irrigated	31.56	5.24	8	24	3.18	37.59	21.32
Combined	33.42	9.51	9	9	7.19	38.84	22.02
1700µm diameter	33.13	6.51	4	18	4.61	49.15	22.94
850µm diameter	49.60	34.54	8	30	23.44	65.28	46.46
PS (µm)							
Rainfed	1267.27	1471.89	9	14	1180.25	1715.26	1359.47
Irrigated	1247.00	1462.08	21	10	1182.18	1632.92	1365.47
Combined	1257.13	1466.99	17	12	1201.27	1576.21	1362.51

[a]: Positive transgressive genotypes with lower DMD and bigger particle size than PI370970,
[b]: Negative transgressive genotypes with higher DMD and smaller particle size than Valier,
[c]: Minimum value, [d] Maximum value, [e]: population mean.

Figure 4-2: Phenotypic frequency distribution of (A) dry matter digestibility (DMD) in whole population of Valier x PI370970 (black bars), six-rowed subpopulation (light bars) and two-rowed subpopulation (white bars), (B) Particle size of Valier x PI370790, (C) DMD of Valier x PI370970 for whole particle fractions (black bars), 1700 µm fraction (white bars) and 850 µm fraction (light bars), (D) DMD of Baronesse x PI370970 population and (E) Harrington x Morex population.



Analysis of variance for DMD and particle size traits within each environment and the ANOVA combined over environments showed significant genotype effects for DMD and particle size (P< 0.001) (Table 4-5). In DMD, the combined analysis indicated significant environmental and G x E effects, but the G x E interaction was much smaller than the genetic variance (Table 4-5). There was no G x E interaction for particle size (Table 4-5). When we sorted the data by head type, two distinct DMD categories were revealed. Six rowed RILs had low DMD and were similar to PI370970, while two-rowed lines were similar to Valier (Figure 4-2B). Similar findings were observed in the Baronesse x PI370970 population (Table 4-12).

Table 4-5: Mean squares for genotypes (MS_G), environments (MS_E), genotype X environment (MS_{GE}) and error (MS_{ER}), genetic (σ^2_G) and genotype X environment (σ^2_{GE}) variance components and heritability for DMD and particle size at rainfed, irrigated and combined environments for Valier x PI370970 population.

Trait	MS_G	MS_E	MS_{GE}	MS_{ER}	σ^2_G	σ^2_{GE}	h^{2a}	h^{2b}
DMD (%)								
Rainfed	98.09**	nd	nd	23.48	37.87	nd	61.80	76.10
Irrigated	199.95**	nd	nd	14.91	92.63	nd	86.10	92.50
Combined	252.49**	290.94**	43.25**	19.14	53.11	12.33	62.82	82.80
Particle Size (µm)								
Rainfed	14175**	nd	nd	9001	2742	nd	23.75	37.85
Irrigated	22220**	nd	nd	5611	8388	nd	60.01	74.76
Combined	26968**	2154^{ns}	9315^{ns}	7275	4535	1032	35.55	65.82

a: heritability on plot-basis, b: heritability on family mean-basis,
**: P<0.001, ns: not significant, nd: not determined.

Selected particle size fractions retained on the 1700 µm and 850 µm screens were evaluated for DMD (Figure 4-2C). The >1700 µm fraction accounted for 60% of the total ground grain, while the 850µm to 1700µm

fraction represented about 20% of the ground product. The larger fraction degraded more slowly than did the smaller particle size fraction.

The validation populations, Baronesse x PI370970 and Harrington x Morex, have distinctly different DMD means and distributions (Table 4-12, Figure 4-2D and 4-2E). The mean DMD for the Baronesse x PI370970 population was 24.7%, similar to the Valier x PI370970 mean of 21%. The mean DMD of the Harrington x Morex population was 34.2%.

Heritability was calculated for each environment and combined data based on plot-basis and family mean-basis for DMD and particle size traits. The heritability estimations for all environments were reasonably high for DMD and intermediate for particle size (Table 4-5). In general, lower heritability estimates were obtained using plots rather than family means. Heritability on plot-basis ranged from 61.80 to 86.10% for DMD and 37.85% to 65.82% for particle size, while the heritability on family mean-basis ranged from 76.1 to 92.5% for DMD and 23.75 to 60.01 for particle size (Table 4-5). These results suggest that genetic variation in this population is large, and that few genes are responsible for the bulk of the variation in both DMD and particle size.

Correlation analysis

DMD was positively correlated with plant height and yield and negatively with particle size. Particle size was negatively correlated with plant height and yield. Yield was negatively correlated with heading date and positively correlated with plant height (Table 4-6).

Table 4-6: Phenotypic correlation coefficients for heading date (HD), plant height (PH), yield (YD), dry matter digestibility (DMD) and particle size (PS) of Valier x PI370970 population.

Trait	HD	PH	YD	DMD
PH	-0.001			
YD	-0.431**	0.493**		
DMD	0.055	0.382**	0.604**	
PS	0.043	-0.379**	-0.559**	-0.769**

*' ** Significant at *P*=0.05 and *P*= 0.01, respectively.

QTL analyses

To determine the chromosomal locations of gene(s) impacting DMD, particle size and other traits, a 150 marker linkage map was constructed using the 116 members of the Valier x PI370970 RIL population. Composite interval mapping (CIM) and multiple interval mapping (MIM) methods were employed to detect QTL. QTL analyses were carried out using data from Bozeman rainfed and irrigated experiments, and the combined data from these environments.

Plant height, heading date and grain yield QTL

QTL were detected for heading date, plant height and yield. There were 2 major QTLs for heading date (*Qhd2a* and *Qhd7a*) which that explained about 25% and 40% of the total phenotypic variation, respectively. The *Vrs1* gene had a large impact on plant height, while five other QTL contributed less and less consistently to variation for height. About half the phenotypic variation in grain yield could be accounted for the action of two genes, one of which appeared coincident with *Vrs1* on chromosome 2H and one on chromosome 7H. The results of analysis by composite interval mapping (CIM) and multiple

interval mapping (MIM) were broadly similar, with MIM providing better evidence of epistatic interactions (Table 4-7, 4-8).

Table 4-7: QTLs for heading date (HD), plant height (PH) and yield (YD) of Valier x PI370970 population identified by composite interval mapping (CIM) over rainfed, irrigated and combined environments.

Trait	QTL name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	R^{2c}	Total R^{2d}
HD (days)							
Rainfed	*Qhd2a*	2H	Hvbkasi	37.21	12.84	27.04	78.81
	Qhd3a	3H	E4H8-396	32.01	4.20	13.21	
	Qhd7a	7H	ABG380	36.61	17.41	38.56	
Irrigated	*Qhd2a*	2H	Hvbkasi	37.21	14.34	28.63	76.68
	Qhd3a	3H	E4H8-396	32.01	2.84	4.33	
	Qhd7a	7H	ABG380	36.61	19.52	46.72	
Combined	*Qhd2a*	2H	Hvbkasi	37.21	13.72	27.37	77.76
	Qhd3a	3H	E4H8-396	34.01	3.77	08.70	
	Qhd7a	7H	ABG380	36.61	19.52	46.72	
PH (cm)							
Rainfed	*Qph2a*	2H	EBmac684	31.21	5.50	14.40	27.38
	Qph5a	5H	TB21-22	69.41	4.30	12.98	
Irrigated	*Qph2a*	2H	EBmac684	31.21	7.29	20.01	38.77
	Qph4a	4H	Bmag0030	62.41	4.11	11.53	
	Qph7b	7H	E1H3-117	147.41	2.89	7.23	
Combined	*Qph2a*	2H	EBmac684	31.21	8.25	21.41	66.82
	Qph2b	2H	Vrs1	78.51	2.58	7.53	
	Qph4b	4H	Bmag0353	61.81	2.92	9.53	
	Qph5a	5H	TB21-22	65.41	4.23	19.17	
	Qph5b	5H	Bmag0323	76.51	3.77	9.18	
YD (ton/ha)							
Rainfed	*Qyd2c*	2H	E2H8-234	12.11	2.30	5.03	33.27
	Qyd2b	2H	Vrs1	86.31	4.68	13.17	
	Qyd7a	7H	ABG380	36.41	5.68	15.07	
Irrigated	*Qyd2b*	2H	Vrs1	86.31	2.72	7.49	39.10
	Qyd3a	3H	HVM60	151.61	2.35	8.95	
	Qyd4c	4H	E2H7-265	37.31	2.62	9.49	
	Qyd7a	7H	ABG380	36.41	4.50	13.17	
Combined	*Qyd2b*	2H	Vrs1	86.31	6.31	16.68	33.85
	Qyd7a	7H	ABG380	36.41	6.85	17.17	

[a] :QTl position in cM, [b] :Peak value of the LOD,[c] :Proportion of phenotypic variation explained by the QTL, [d] :total phenotypic variation that explained by the model.

Table 4-8: Estimated QTL effects and epistatic interaction detected for heading date (HD), plant height (PH) and yield (YD) of Valier x PI370970 population identified by multiple interval mapping (MIM).

Trait	QTL name	Nearest marker	QTL[a] position	Genetic effects Additive	Genetic effects Epistatic	$R^{2\,b}$	Total R^{2c}
HD (days)							
Rainfed	*Qhd2a* (2H)	Hvbkasi	37.21	2.539	-	22.5	71.30
	Qhd3a (3H)	E4H8-396	32.01	1.698	-	9.0	
	Qhd7a (7H)	ABG380	36.71	-3.162	-	35.7	
	Qhd2a X *Qhd7a*			-	0.922	4.8	
	Qhd3a X *Qhd7a*			-	0.896	-0.7	
Irrigated	*Qhd2a* (2H)	Hvbkasi	36.21	2.351	-	27.0	80.60
	Qhd3a (3H)	E4H8-396	28.00	1.405	-	7.9	
	Qhd3b (3H)	Bmag0013	198.00	0.725	-	4.0	
	Qhd7a (7H)	ABG380	37.60	-2.784	-	40.3	
	Qhd2a X *Qhd7a*			-	-0.592	1.4	
Combined	*Qhd2a* (2H)	Hvbkasi	37.21	2.474	-	26.5	62.80
	Qhd3a (3H)	E4H8-396	31.00	1.593	-	9.4	
	Qhd7a (7H)	ABG380	37.00	-3.227	-	45.1	
	Qhd2a X *Qhd7a*			-	-0.503	1.8	
PH (cm)							
Rainfed	*Qph2a* (2H)	Hvbkasi	31.31	2.105	-	16.9	34.30
	Qph5a (5H)	TB21-22	69.40	-1.608	-	9.3	
	*Qph7*a (7H)	ABG380	63.70	-1.096	-	4.7	
	Qph2a X *Qph5a*			-	-0.850	3.4	
Irrigated	*Qph2a* (2H)	Hvbkasi	31.30	2.899	-	15.4	54.50
	*Qph4*a (4H)	Bmag0353	62.51	2.644	-	11.4	
	Qph5a (5H)	E1H1-156	38.70	-2.408	-	5.1	
	Qph7a (7H)	E1H3-117	147.50	-2.425	-	5.7	
Combined	*Qph2a* (2H)	Hvbkasi	31.30	2.651	-	21.7	66.10
	Qph2b (2H)	cMWG699	78.51	1.668	-	10.3	
	Qph4a (4H)	Bmag0353	61.81	1.704	-	11.6	
	Qph5a (5H)	TB21-22	65.41	-2.572	-	17.8	
	Qph4a X *Qph5a*			1.530	-	4.7	
YD (ton/ha)							
Rainefd	*Qyd2c* (2H)	E2H8-234	12.20	-141.17	-	03.9	53.00
	Qyd2b (2H)	cMWG699	78.50	288.29	-	21.4	
	Qyd7a (7H)	ABG380	36.70	303.2	-	20.7	
	Qyd2a X *Qyd7a*			-	171.89	07.0	
Irrigated	*Qyd2b* (2H)	cMWG699	71.50	276.85	-	13.7	57.80
	Qyd3b (3H)	HVM60	151.60	272.44	-	13.1	
	Qyd4b (4H)	E2H7-265	39.30	281.55	-	13.5	
	Qyd7a (7H)	ABG380	36.40	318.81	-	17.5	
Combined	*Qyd2b* (2H)	cMWG699	73.50	218.60	-	14.5	55.40
	Qyd3b (3H)	HVM60	149.60	166.98	-	7.9	
	Qyd7a (7H)	ABG380	37.60	316.93	-	21.7	
	Qyd2b X *Qyd7*a				132.76	3.4	

[a] QTl position in cM, [b] :Proportion of phenotypic variation explained by the QTL,c :total phenotypic variation that explained by the model.

DMD-QTL

Composite interval mapping and multiple interval mapping identified two loci on chromosome 2H and one on chromosome 7H that accounted for between 60% and 70% of the total phenotypic variation for dry matter digestibility. The gene(s) near *Vrs1* produced the largest effect, accounting for between 30% and 46% of the total phenotypic variation (Table 4-9, Fig. 4-3).

Table 4-9: QTLs for dry matter digestibility (DMD) and particle size (PS) of Valier x PI370970 population identified by composite interval mapping (CIM) over rainfed, irrigated and combined environments.

Trait	QTL Name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	R^{2c}	Total R^{2d}
DMD (%)							
Rainfed	*Qdmd2a*	2H	EBmac684	30.41	7.27	20.27	64.44
	Qdmd2b	2H	Vrs1	83.21	13.34	30.92	
	Qdmd6a	6H	E1H5-355	227.71	3.46	13.25	
Irrigated	*Qdmd2a*	2H	EBmac684	30.41	4.78	08.04	64.56
	Qdmd2b	2H	Vrs1	84.31	25.75	46.81	
	Qdmd7a	7H	ABG380	38.61	6.10	9.71	
Combined	*Qdmd2a*	2H	EBmac684	30.41	8.31	15.40	66.80
	Qdmd2b	2H	Vrs1	84.31	24.63	46.82	
	Qdmd7a	7H	ABG380	38.61	2.85	04.58	
PS (µm)							
Rainfed	*Qps2a*	2H	EBmac684	30.41	4.15	19.2	19.20
Irrigated	*Qps2a*	2H	E1H8-246	0.01	3.41	6.74	53.50
	Qps2a	2H	EBmac684	30.41	3.98	11.37	
	Qps2b	2H	Vrs1	84.31	11.27	24.95	
	Qps7a	7H	ABG380	38.61	4.19	10.44	
Combined	*Qps2a*	2H	EBmac684	30.41	9.38	29.57	50.35
	Qps2b	2H	Vrs1	84.31	7.54	16.68	
	Qps7a	7H	ABG380	34.41	2.37	7.85	

[a]:QTL position in cM,[b] :Peak value of the LOD, [c] :Proportion of phenotypic variation explained by the QTL. [d]: Total phenotypic variations explained by model.

Particle size-QTL

QTLs were detected in the different environments for particle size trait. *Qps2a* was observed in the rainfed and irrigated environments and the

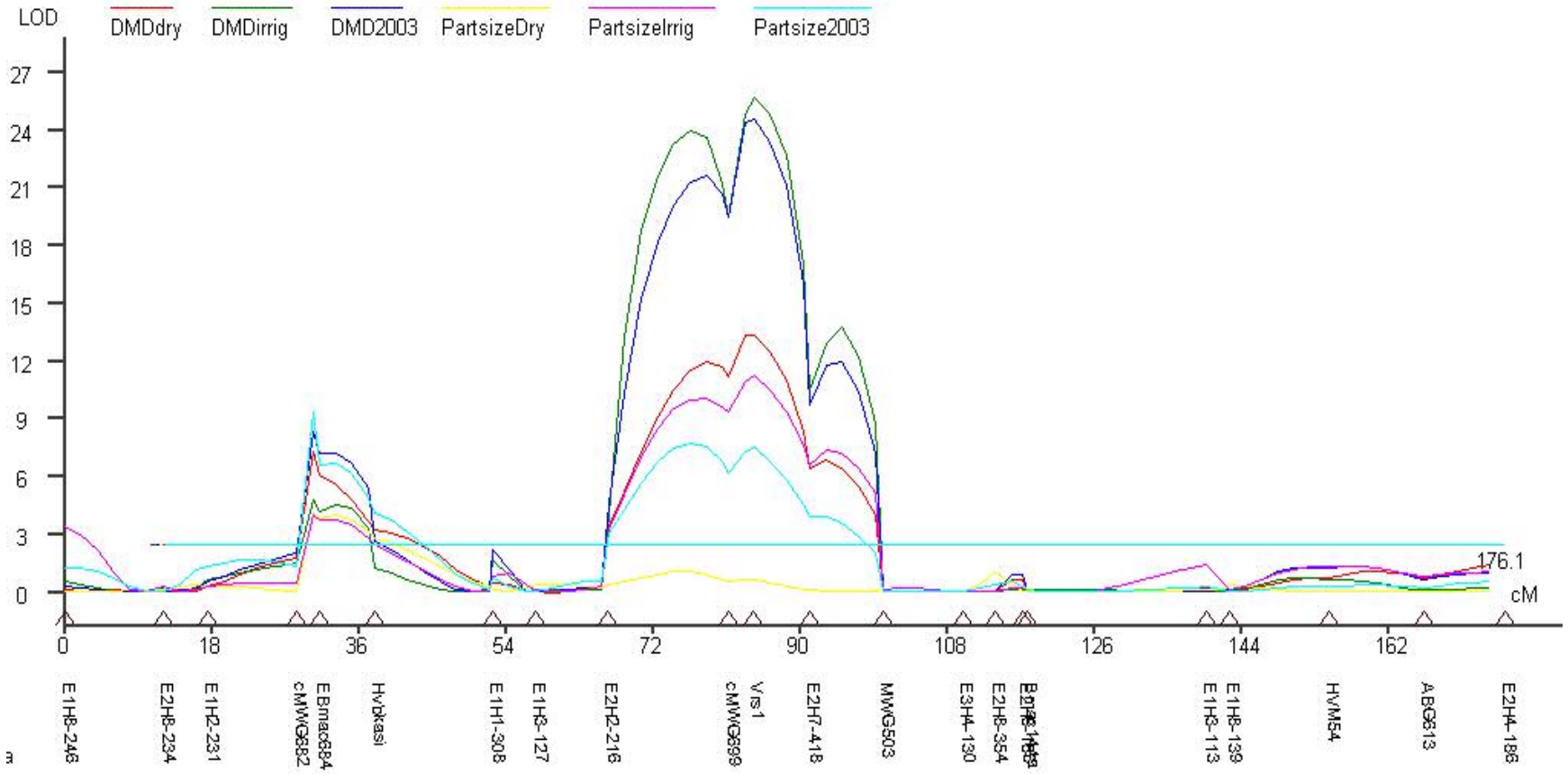
combined analysis. Another QTL, *Qps2b* at chromosome 2H, was detected in irrigated environment and combined analysis only (Table 4-9, Figure 4-3). The CIM model explained about 70 % of the variation in particle size in this population. The major QTL, *Qps2b* at or near *Vrs1*, explained as much as 50% of the variation for particle size. The major QTLs that were detected by CIM were also detected by MIM model (Table 4-10). No significant QTL x QTL interactions were detected by MIM.

Table 4-10: Estimated QTL effects and epistatic interaction detected for dry matter digestibility (DMD) and particle size (PS) by multiple interval mapping (MIM) for Valier x PI370970 RILs population over rainfed, irrigated and combined environments.

Trait	QTL name	Nearest marker	QTL[a] position	Genetic effects Additive	Epistatic	R^{2b}	Total R^{2c}
DMD (%)							
Rainfed	*Qdmd2a* (2H)	Hvbkasi	30.41	3.775	-	25.4	73.97
	Qdmd2b (2H)	Vrs1	84.40	4.152	-	34.6	
	Qdmd6a (6H)	E1H5-355	226.70	2.008	-	14.0	
Irrigated	*Qdmd2a* (2H)	EBmac684	30.41	2.260	-	8.2	84.55
	Qdmd2b (2H)	Vrs1	84.31	7.506	-	62.0	
	Qdmd7a (7H)	ABG380	38.61	2.817	-	11.1	
	Qdmd2a X *Qdmd2b*			-	-1.147	1.2	
	Qdmd2a X *Qdmd7a*			-	-1.117	2.1	
Combined	*Qdmd2a* (2H)	EBmac684	30.41	2.749	-	15.4	78.73
	Qdmd2b (2H)	Vrs1	84.41	5.637	-	53.1	
	Qdmd7a (7H)	ABG380	39.60	1.675	-	06.6	
	Qdmd2a X *Qdmd2b*			-	-1.099	02.4	
	Qdmd2a X *Qdmd7a*			-	0.799	01.1	
	Qdmd2b X *Qdmd7a*			-	0.740	01.3	
PS (µm)							
Rained	*Qps2a* (2H)	EBmac684	30.41	-36.666	-	21.8	77.00
	Qps2b (2H)	Vrs1	78.50	-87.152	-	53.5	
	Qps2a X *Qps2b*			-	-0.85	01.7	
Irrigated	*Qps2a* (2H)	EBmac684	30.41	-43.448	-	19.2	58.77
	Qps2b (2H)	Vrs1	84.40	-49.587	-	26.0	
	Qps7a (7H)	ABG380	35.40	-34.981	-	13.6	
Combined	*Qps2a* (2H)	EBmac684	31.30	2.651	-	24.5	71.90
	Qps2b (2H)	Vrs1	78.51	1.668	-	35.6	
	Qps7a (7H)	ABG380	61.81	1.704	-	11.9	

[a] QTl position in cM, [b] :Proportion of phenotypic variation explained by the QTL,[c] :total phenotypic variation that explained by the model.

Figure 4-3: Chromosome scan of the dry matter digestibility (DMD) and particle size QTLs on the barley 2H chromosome of Valier x PI370970 population over rainfed (dry), irrigated (Irrig) and combined (2003) environments.



Effect of kernel size variation on DMD

Dry rolling processing is performed to barley grain to crack it before the final feed is mixed. The Valier x PI370970 population is the result of a cross between two and six rowed parents, so its segregants differ in the consistency of seed diameter and size (6-rows are more variable than are 2-rows). To reduce the effect of seed size variation on cracking efficiency, selected fractions of cracked barley samples from the Bozeman irrigated location were chosen to reestimate DMD and to reassess QTL location (Table 4-11).

Table 4-11: QTLs for dry matter digestibility (DMD) for selected sieved fractions identified by composite interval mapping (CIM) over irrigated environment, for whole fractions, 1700-µm fraction and 850-µm fraction of Valier x PI370970 population.

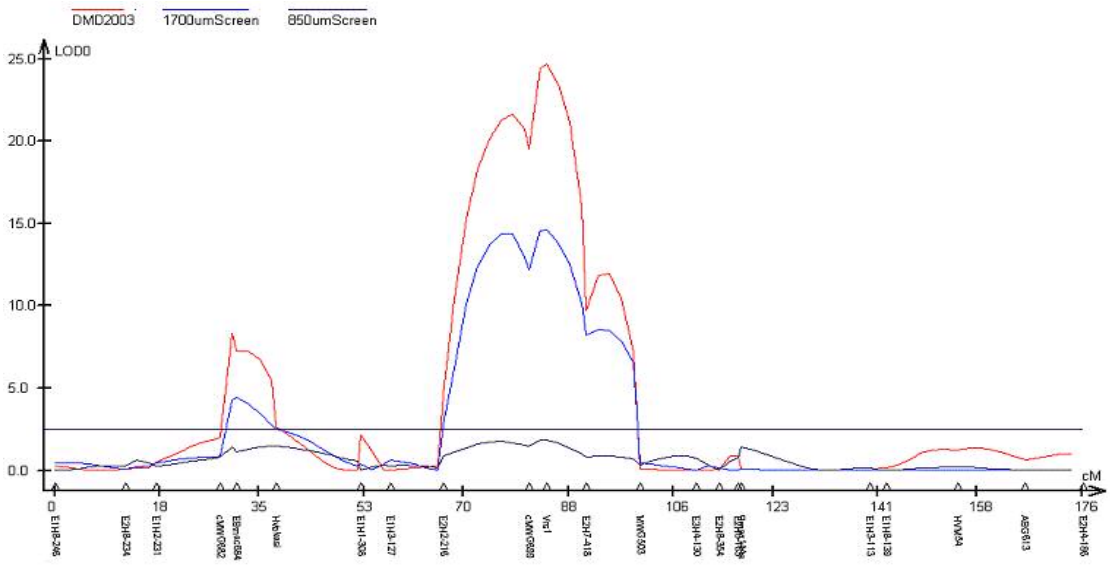
	QTL name	Chrom	Nearest marker	QTL[a] position	LOD[b] score	R^{2c}	Total[d]
Irrigated	*Qdmd2a*	2H	EBmac684	30.41	4.78	08.04	64.56
	Qdmd2b	2H	Vrs1	84.31	25.75	46.81	
	Qdmd7a	7H	ABG380	38.61	6.10	9.71	
1700µm fraction	*Qdmd2a*	2H	EBmac684	31.21	4.14	10.94	38.94
	Qdmd2b	2H	Vrs1	84.31	9.31	28.00	
850 µm fraction	*Qdmd1a*	1H	E12H2-133	54.81	2.53	8.44	28.62
	Qdmd2b	2H	Vrs1	84.31	2.15	7.10	
	Qdmd6a	6H	Bmag500a	173.81	3.43	13.08	

[a]:QTL position in cM,[b] :Peak value of the LOD, [c] :Proportion of phenotypic variation explained by the QTL, [d]: Total phenotypic variations explained by model.

Two QTLs were detected for DMD using the >1700µm fraction and three QTLs were observed with the 850 µm to 1700µm fraction. The gene near *Vrs1* locus significantly affected the DMD in the >1700 µm fraction while it was only marginally significant (LOD 2.15) in >850 µm fraction (Table 4-11, Figure 4-4). *Vrs1* appeared to control about 28% and 7.1% of the phenotypic variation in

the two fractions, respectively. The same gene near *Vrs1* controlled about 46% of the variation in unfractionated grain from the irrigated location (Table 4-11).

Figure 4-4: chromosome scans of dry matter digestibility (DMD) of combined environment (2003) and selected fractions 1700 µm and 850 µm QTLs on chromosome 2H of Valier x PI370970 population.



Validation of low DMD QTL

Two barley populations were used to validate low DMD QTL that been detected in the mapping population. F_6-recombinant inbred lines of Baronesse x PI370970 population and the doubled haploid Harrington x Morex population were used. Both populations derived from 2-rowed x 6-rowed cross. It assumed that different population types and different genetic backgrounds will provide more opportunity to validate DMD QTL. Single marker analysis indicated that Vrs1 had a significant impact on DMD in the Baronesse x PI 370970 population (Table 4-12). The magnitude of the effect was dramatically

different, however. *Vrs1* controlled about 45.25% of the total variation in this population.

Table 4-12: Mean values of dry matter digestibility (DMD) for population, parental and genotypes classes, and percent of variation explained by *Vrs1* locus for Baronesse x PI370970 validation population.

Population	DMD%	SE[a]	Pr>F	R^{2b}
Baronesse x PI37097	24.71	7.035	<0.0001	45.25
Baronesse	39.55	-	-	-
PI370970	9.71	-	-	-
two-rowed (Vrs1)	30.32	6.795	-	-
six-rowed (vrs1)	17.50	7.333	-	-

[a] :standard deviation, [b] :calculated by R^2 x 100.

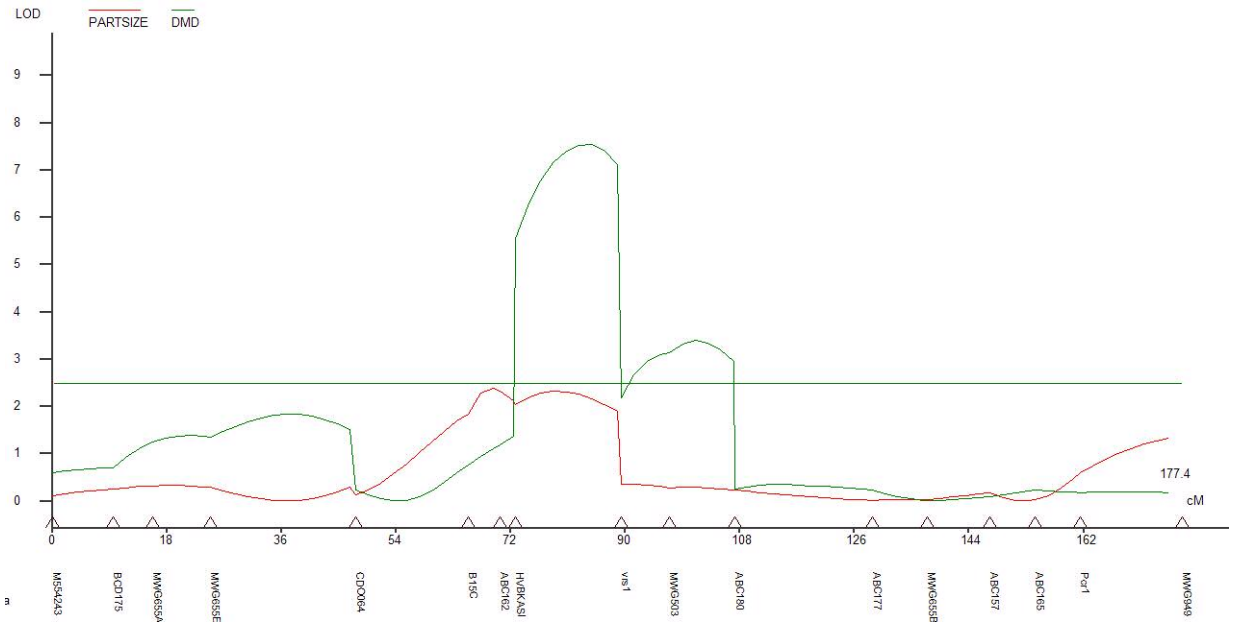
Utilizing the previously published linkage map with the Harrington x Morex population, CIM revealed three QTLs on 2H and 6H that explained about 39.5% of the DMD variation (Table 4-13, Figure 4-5). The main QTL was flanked with Hvbkasi-*Vrs1* and accounted about 22.2% of the variation, while the second QTL that was flanked by markers MWG503- ABC180 account for about 8.9% of the variation. QTLs controlling particle size were detected at 5H and 7H, and only explained about 17.9% of the total variation in particle size in this population, but no QTL have been indicated on 2H (Table 4-13).

Table 4-13: QTLs for dry matter digestibility (DMD) and particle size (PS) of Harrington x Morex population identified by composite interval mapping (CIM).

Trait	Chrom	Marker interval[a]		QTL[b] position	LOD[c] score	Additive effect	R^{2d}	Total R^{2e}
		Left	right					
DMD (%)	2H	Hvbkasi	*Vrs1*	84.71	7.55	2.731	22.21	39.59
		MWG503	ABC180	101.01	3.39	1.945	08.94	
	6H	ABC302A	mSrh	20.21	2.52	-1.697	08.44	
PS (µm)	1H	MWG2031	ABC310B	81.61	2.56	14.631	8.77	17.9
	5H	cMWG706	ABG702B	92.01	3.56	-14.981	9.13	

[a] :flanking markers, the bold marker is the nearest marker,[b] :QTL position in cM,[c] :Peak value of the LOD, [d]:Proportion of phenotypic variation explained by QTL, [e] : total phenotypic variation explained by the model.

Figure 4-5: Chromosome scans of dry matter digestibility (DMD) and particle size QTLs on chromosome 2H of Harrington x Morex population.



Discussion

Map construction

Anchor markers enabled the development of a practically useful map largely based on AFLPs in the Valier x PI370970 RIL population (Figure 4-1). The SSR markers were mapped to their expected positions with the exception of EBmac684 and Bmac144. EBmac684 previously mapped to the short arm of chromosome 5H (Ramsay et al., 2000), while in the present study, it was mapped to the short arm of chromosome 2H (Hori et al., 2003). This marker linked to Hvbkasi, a position in agreement with its location within the Oregon Wolfe Barley population (http://barleyworld.org/). Bmac144 produced products that were mapped to positions on chromosomes 1H, 2H, 4H, 5H and 6H in the

Lina x H.spontaneum Canada Park population (Ramsay et al., 2000) and at 1H, 2H, 3H and 5H in OWB population (Costa et al., 2001). We were able to identify polymorphisms that mapped to chromosomes 1H and 2H.

The methylation-sensitive restriction enzyme *HpaII* was used in combination with *EcoRI* to avoid AFLP markers clustering in recombination-deficient, heavily methylated regions of the genome (Qi et al., 1998 Powell et al., 1997; Costa et al., 2001). Use of *HpaII* provided a low level of polymorphism but a relatively uniform distribution of markers along the seven linkage groups. About 13% of the total map length consisted of regions showing segregation distortion in the direction of the 'Valier'-derived alleles, while about 8% of the total map was distorted in the direction of PI370970 alleles (Figure 4-1).

QTL analysis

The Valier x PI370970 mapping population provides excellent diversity for DMD, particle size, heading date, plant height and grain yield. This phenotypic variation reflects the large genetic differences between the two inbred parents, one of which is a modern 2-rowed feed barley and the other a land race six-rowed line selected for low DMD. Both CIM and MIM detected the same major QTLs that control the variation in the studied traits (Table 4-7 - 4-11).

Correlation analysis indicated that grain yield and plant height were correlated with DMD and particle size. Quantitative trait loci for heading date, plant height, and grain yield were mapped to identify potential pleiotropic effects among the studied traits. Major QTLs controlling the variation of

heading date have been detected on each of the barley's seven chromosomes (Laurie et al. 1994 and 1995). The genes with largest impact are *Ppd-H1* and *Ppd-H2* genes on the short arm of chromosome 2H and long arm of chromosome 1H, respectively. In the present study, 2 major QTLs controlled the variation for flowering time (heading date). *Qhd2a* is located on the short arm of chromosome 2H at the expected location of *Ppd-H1* and *Qhd7a* on chromosome 7H at the expected site of the *eps7S* gene. If these assertions are correct, Valier contributed the 'earliness' allele for *Ppd-H1*, while PI370970 contributed the earliness allele at *eps7S*. This interaction resulted in early and late flowering transgressive segregants comparing with the parents, similar to that reported in the Steptoe x Morex population (Kleinhofs et al., 1993; Hayes et al., 1993). These findings agree with those of Laurie et al. (1994, 1995) as we found that *Ppd-H1* was associated with plant height, while *eps7S* was associated with both plant height and yield.

Vrs1 and ABG380 were the markers nearest the grain yield QTL on chromosomes 2H and 7H, respectively. QTLs located at or near *Vrs1* (Marquez-Cedillo et al., 2001; Kjaer and Jensen, 1996; Powell et al., 1990) have long been implicated in grain yield variation. ABG380 was also previously reported to be linked to genes associated with yield variation (Zhu et al., 1999). In our study *Vrs1* and ABG380 controlled about 16% and 17% of the grain yield phenotypic variation, respectively. Positive epistatic effects were detected between these loci.

For DMD, *Qdmd2b* is located at or near *Vrs1* in single and combined environment analyses (Table 4-6 and 4-7, Figure 4-3). DMD QTL had additive effects and showed no significant epistatic interactions. *Qdmd2a* and *Qdmd7a* appear to be coincident with *Qhd2a* and *Qhd7a* and are likely pleiotropic effects of variation in flowering date.

Particle size variation was controlled by two major QTLs that located on chromosome 2H and one on 7H. Qps2b is found near the *Vrs1* gene and overlaps with DMD. As expected, there was a highly significant negative correlation between DMD and particle size. *Qps2a* and *Qps7a* are located near or at the two main flowering date QTL, and are likely pleiotropic effects.

Vrs1 is well-known to control variation for kernel morphology and uniformity (Marques-Cedillo et al., 2001). We attempted two lines of experimentation to assess whether the variation we observed associated with *Vrs1* is the result of pleiotropy or linkage. If segregation at *Vrs1* is responsible for variation in DMD, than we expect to see similar magnitude variation in any population from a 2-row x 6-row cross. When we assayed the Harrington x Morex population, *Vrs1* appeared to be significantly associated with variation for DMD, but the additive effect of variation at *Vrs1* was less than half that observed in the Valier x PI370970 population (Tables 4-10, 4-13). Variation for DMD could have been due to thin grain passing through our milling system relatively unground. We utilized sieved fractions of the milled product to obtain a more uniform ground product for DMD estimation. In this case, the ground products should show no QTL at *Vrs1* if the QTL was a milling artifact. In this

case, one of the sieved fractions showed a significant QTL while the other did not (Table 4-11). Plainly, the question of pleiotropy or linkage remains unresolved.

In another attempt to test the impact of the *Vrs1* gene DMD, we assayed 2-row x 6-row RIL population derived from Baronesse x PI370970. This population showed a *Vrs1* impact on DMD similar in magnitude to that of our mapping population. Single marker analysis for Baronesse x PI370970 indicted segregation at the same locus that controlled low DMD in the Valier x PI370970 population (Table 4-12). Thusfar, no obviously convincing recombinants between the DMD QTL and *Vrs1* have been identified in either population.

The overlapping QTLs which are controlling the variation in DMD, particle size and yield around *Vrs1*, may be due to pleiotropic effects or closely linked QTLs. Allard (1988) reported that *Vrs1* affect the developmental process with strong influences on many quantitative traits including heading date, plant height and spike length. Using DH and SSD populations Powell et al. (1990) indicated the linkage between *Vrs1* and number of agronomic characters. Marquez-Cedillo et al. (2001) reported difficulty in distinguishing between the linkage and pleiotropy for yield, yield components and malting characters, and proposed that the low resolution of the Harrington x Morex DH linkage map could be in part responsible. The efficiency of using low DMD QTL for marker assisted selection (MAS) to reduce the rate of barley grain digestion will depend on the answer to the question whether DMD-QTL is tightly linked to

Vrs1 or is a pleiotropic effect. Two-rowed varieties are preferred in the arid Northern plains. If this is a pleiotropic effect, gene transfer into 2-rowed backgrounds will be impossible. If, however, linkage is the cause of the association between *Vrs1* and *Qdmd2b*, then larger mapping populations should permit us to better map the gene and identify useful recombinants. Experiments with much larger populations are now underway.

In this study, we report the results of a QTL mapping project based on a recombinant inbred line population derived from a cross between a commonly grown barley cultivar (Valier) with moderate Dry Matter Digestibility (DMD), and a line from the core collection of the USDA National Small Grains Collection exhibiting far slower DMD. One gene or a linked cluster of genes on barley chromosome 2H tightly linked to the morphology-modifying *Vrs1* was found to dramatically impact DMD and particle size. This QTL was validated on other populations with different backgrounds. The magnitude of the effect conferred by the ‘low DMD’ allele contributed by PI370970 is more than twice that provided by the *Vrs1* allele contributed by Morex. If pleiotropy is responsible for this effect, then the PI370970 *Vrs1* allele has a more extreme impact on grain characteristics than the more widely utilized allele found in North American 6-rowed varieties.

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CHAPTER 5

QUANTITATIVE TRAIT LOCI OF ACID DETERGENT FIBER AND STARCH CONTENT IN A HULLED X HULL-LESS BARLEY POPULATION

Abstract

Cultivated barley, *Hordeum vulgare*, is the major feed grain for livestock in the Northern Plain and Northwestern United states due to the short day growing season that limits the planting and production of corn. Substantial heritable variation for characteristics contributing to variation in barley feedlot performance was reported in a survey of the USDA barley core collection (Bowman et al., 2001). Starch and fiber content in addition to in rumen dry matter digestibility and particle size after dry rolling, have all been found to play a role in feedlot performance of barley as a feed grain.

Here we report QTL mapping studies based on a recombinant inbred line population derived from a cross between a commonly grown barley cultivar (Valier) and a line from the core collection of the USDA National Small Grains Collection, PI370970. In total, 15 main effect QTLs affecting ADF, starch and protein content were identified using CIM and MIM methods. The *N* gene that controls adherent (*N-)* vs. non-adherent (*nn*) lemma and palea on chromosome 7H was found to affect these traits. This pleiotropic effect resulted in significant correlations among ADF, starch and protein content The model predicting net energy available for maintenance from grain characteristics suggests that several of the lines derived from this population could provide up to 20% greater energy than corn.

Introduction

Cultivated barley, *Hordeum vulgare*, is the fourth ranking cereal cop after corn, wheat and sorghum in the United States. Half or more of the barley grown in the United States is used for livestock feed with the remaining used for human food and malting. Barely is the major feed source for livestock in the Northern Plain and Northwestern United states due to the short growing season that limits the planting and production of corn. Barley grain does not differ widely form other grains based on chemical composition. It contains more total protein, higher essential amino acids levels and higher crude fiber than corn and sorghum, but lower starch and lipid content (Kellems and Church, 2002).

The hull constitutes 10-13% of the dry weight of barley (Bhatty et al., 1975). Because the major components of the hull are cellulose, hemicellulose, lignin and pectin (Bhatty, 1993), hull-less varieties have less fiber and more starch (Yang et al., 1997), which is reflected in increased digestibility and energy value for nonruminant animals (Beames et al., 1996). Bowman et al. (2001) concluded that hull-less world collection barleys had greater starch and lower acid detergent fiber (ADF) than hulled barleys, a finding that confirmed earlier results (Kong et al., 1995; Zinn et al., 1996). NDF and ADF are affected by environment and cultivar (Kong et al., 1995), and two-rowed varieties tend to be lower in ADF than do 6-rowed varieties (Kong et al, 1995) .

Surber et al. (2000) was able to predict the net energy content, feed efficiency and average daily gain of cattle fed barley using dry matter

digestibility (DMD), acid-detergent fiber (ADF), starch particle size and starch content. Using these selection criteria should permit development of improved feed quality barley varieties. The first feedlot-tested improved barley variety, 'Valier' (PI610264) was released to producers in 2001 utilizing these criteria for varietal selection (Blake et al, 2002).

Our prior work demonstrated that far more variation for feed quality-related traits could be found in exotic accessions of barley than in the commonly utililzed North American germplasm pool. A survey of the spring barley core collection revealed enormous variation for DMD, ADF and starch content (Bowman et al, 2001). Several lines were identified that had normal or high starch content, low fiber content (ADF) and low DMD. One of these, PI370970, a six-rowed hulless landrace line from Switzerland, was crossed to Valier, a 2-rowed covered barley variety, and a population of recombinant inbred lines was derived.

The objective of this study was to map the location of genes contributing to variation for ADF, starch and protein content and predicate bovine performance of animals fed the lines from this recombinant inbred line population.

Material and Methods

Plant materials

A mapping population of 116 F_5-recombinant inbred lines was derived by single seed descent (SSD) from a cross between Valier (PI610264) and the

Swiss landrace line PI370970. This population was used to detect the ADF, starch and protein QTLs. Valier (PI610264) is two-rowed, hulled spring barley cultivar, developed by the Montana Agricultural Experiment Station and released for commercial production (Blake et al., 2002). PI370970 is a six-rowed hull-less barley landrace line from Switzerland selected from the USDA world collection of barley's spring core collection based on its high starch content, low ADF content and low DMD (Bowman et al 2001).

One hundred seventy nine F_6-recombinant inbred lines were produced via SSD of a cross between Baronesse and PI370970. This population was used to validate the detected QTL(s) for ADF. Baronesse is a two-rowed feed barley variety with high ADF.

Field experiments

One hundred sixteen F_4–derived Valier x PI370970 recombinant inbred lines, Valier, PI370970 and check varieties were planted at the A.H. Post Research Farm near Bozeman, MT during the 2002 cropping season. During the 2003 cropping season, F_{5-7} entries of Valier x PI370970, the parents and check varieties were grown with and without irrigation. Entries at the both experiments were arranged in a randomized complete block design with two replications at the A.H. Post Research Farm. Entries were planted into an Amsterdam silty loam soil at a seeding rate of approximately 30 seeds/m in single row plots 2m long space 0.3m apart. Plots were harvested by a Wintersteiger plot combine and cleaned using a de-awner and blower.

The Baronesse x PI370970 population was planted at the A.H. Post Research Farm in summer 2003 with one replicate. Entries were planted at a seeding rate approximately 30 seeds/m in 2m long single row plots spaced 0.3m apart. The entries were irrigated as needed. Plots were harvested by a Wintersteiger plot combine and cleaned using a de-awner and blower.

Chemical composition determination

Duplicate grain samples from each line were individually ground to pass through a 0.5 mm screen using a Udy-Cyclone Mill (Ft. Collins). To calculate the chemical composition traits on dry basis, dry matter content (DM) was estimated (AOAC, 1999). ADF content was determined following the Van Soest et al. method (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Crop., Fairport, NY). Starch content was determined using the Amyloglucosidase/α-amylase method (McCleary et al., 1994). Protein content was determined based on N content (N × 6.25) using a Leco FP 528 protein analyzer (Leco Crop., St. Joseph, MI). The Baronesse x PI370970 population was used to validate the major ADF-QTL by analyzing ADF content using samples for each genotype in duplicate analysis and each line was categorized for hull retention.

Chemical composition form present study and DMD and particle size (Chapter 4) were used to identify net energy available for maintenance (NE_m) using the following equation, NE_m=1.87833 - 0.00794 (ISDMD) + 0.00025097 (starch content).

Statistical analysis

Analysis of variance was performed for each environment separately in complete block design, and then combined over environments (Steel and Torrie 1980). In the combined analysis the environments, blocks and genotypes were considered as random effects (statistical Analysis System, SAS institute 1990). Variance components were estimated using restricted maximum likelihood (REML) method of SAS PROC MIXED.

Heritability on plot mean and family mean bases were estimated as $h^2=(\sigma^2_G/(\sigma^2_G + \sigma^2_{GEN} + \sigma^2_e)$ and $h^2= \sigma^2_G /(\sigma^2_G + (\sigma^2_{GEN}/e) + (\sigma^2_e/e\ r))$ respectively (Nyquist, 1991; Holland et al., 2003) where σ^2_G genetic variance among the entries, σ^2_{GEN} variance of genetic x environment interaction, σ^2_e variance of experimental error, e number of environments and r number of replications. Because the data were unbalanced, e and r were computed as harmonic mean of environments and harmonic mean of total replications across all environments, respectively (Holland et al, 2003).

Simple correlation analysis was carried out for chemical composition traits (ADF content, starch content and protein content) using means over the environments and PROC CORR in SAS (statistical Analysis System, SAS institute 1990). Physical traits (DMD and particle size) and agronomic traits (yield, heading date and plant height) data (Chapter 4) were used to correlate with the chemical composition traits.

QTL analysis

Analysis of variance was performed on all traits and environments to identify significant genotype and genotype x environment interactions. When genotype effects were significant, QTL analyses were performed using QTL Cartographer 2.0 (Wang et al., 2002). A series of 1000 permutations was run to determine the experiment-wise significant level at P=0.05 of LOD for the trait (Churchill and Doerge, 1994). Composite Interval Mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam, 1994; Zeng 1994) using Model 6 of the Zmapqtl program module. The genome was scanned at 2-cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward-backward method of the stepwise regression. The percentage of phenotypic variance explained by specific QTL value (R^2) was taken for the peak QTL position as determined by QTL cartographer. The total R^2 including the significant QTLs in the model was calculated using MIM method of QTL cartographer (Kao et al., 1999). Multiple interval mapping (MIM) was applied to test the QTL effects including additive and QTL x QTL interactions by simultaneous analysis of the QTLs in multiple regression models. MIM performed with the significant QTLs that selected by CIM model.

Results and Discussion

Phenotypic variation

Protein, ADF and starch contents of the parents and their recombinant inbred lines for Valier x PI370970 are summarized in Table 5-1. PI370970 had higher protein and starch content and lower ADF than Valier in both environments and the combined analysis. The RIL population was highly variable and transgressive segregation over parents was observed for these traits (Table 5-1). Since the hull constitutes for about 10% of the barley grain dry weight, it is expected that hull-less varieties should have less fiber content and more starch than hulled varieties (Kong et al., 1995; Zinn et al., 1996; Yang et al., 1997; Bowman et al., 2001), and this expectation was met (Table 5-2). Small effects of kernel morphology variation, conditioned by the two-rowed or six-rowed habit of the progeny could be discerned for starch and protein content (Table 5-3). Each of the traits showed high heritability (Table 5-4).

Combination of lab analyses such as *in situ* DMD, ADF, starch content and particle size after dry rolling could be used to estimate net energy available for maintenance from barley grains (NE_m) (Surber et al., 2000). The NE_m of corn, as high moisture grains, is large in NE_m (2.33 Mcal/kg) comparing with barley grains (2.06 Mcal/kg) (NRC, 1995). The model predicting NE_m from grain characteristics suggests that several of the lines derived from this population could provide up to 20% greater energy than corn (Table 5-1).

Table 5-1: Means, ranges and transgressive phenotypes of Valier, PI370970 and their RILs over combined environments for protein content, acid detergent fiber (ADF) and starch content.

Trait	Valier	PI370970	RILs				
			Positive trans[a]	Negative trans[b]	Min[c]	Max[d]	χ^e
Protein (%)							
Rainfed	16.76	20.05	22	9	15.42	22.01	18.93
Irrigated	15.35	18.37	15	4	14.69	20.49	17.20
Combined	16.06	19.21	21	6	15.35	20.92	18.12
ADF (%)							
Rainfed	5.31	2.20	32	17	1.20	7.70	3.63
Irrigated	5.70	2.90	32	25	1.22	9.79	4.13
Combined	5.50	2.55	41	22	1.35	8.65	3.89
Starch (%)							
Rainfed	50.67	53.15	18	26	40.43	57.96	49.85
Irrigated	49.06	53.87	16	45	40.31	57.87	49.93
Combined	49.87	54.33	29	29	42.14	56.44	49.90
NEm							
Combined	2.377	2.521	2	14	2.153	2.569	2.327

a: Positive transgressive genotypes with low protein content, low ADF, high starch content and high NEm than PI370970,
b: Negative transgressive genotypes with high protein content, high ADF, low starch content and low NEm than Valier,
c: Minimum value, d Maximum value:, e: mean.

Table 5-2: Means, ranges and standard deviations of the hulled and hull-less subpopulations of Valier x PI370970 population over combined environments for protein content, acid detergent fiber (ADF), starch content and net energy available for maintenance (NE_m).

Trait	Min[a]	Max[b]	χ^c	σ^d
Protein (%)				
Hulled	15.348	20.810	17.785**	1.271
Hull-less	15.393	20.713	18.525	1.409
ADF (%)				
Hulled	1.687	8.651	4.988**	1.261
Hull-less	1.338	6.899	2.443	1.095
Starch (%)				
Hulled	42.139	53.580	48.248**	2.794
Hull-less	47.603	56.438	52.020	2/368
NEm (Mcal/kg)				
Hulled	2.153	2.419	2.280**	0.057
Hull-less	2.223	2.569	2.398	0.074

a: Minimum value, b: Maximum value, c: mean, d: Standard deviation, *, ** significant of pairwise comparison between hulled and hull-less subpopulation at P<0.05 and P<0.001resoectively, ns: non significant.

Table 5-3: Means, ranges and standard deviations of the six-rowed and two-rowed subpopulations of Valier x PI370970 over combined environments for protein content, acid detergent fiber (ADF), starch content and net energy available for maintenance (NE_m).

Trait	Min[a]	Max[b]	χ[c]	σ[d]
Protein (%)				
Six-rowed	15.350	20.430	17.895*	1.312
Two-rowed	16.320	20.920	18.362	1.126
ADF (%)				
Six-rowed	1.500	8.650	3.985 ns	1.986
Two-rowed	1.340	7.150	3.757	1.446
Starch (%)				
Six-rowed	42.140	56.300	49.346*	3.461
Two-rowed	44.910	56.440	50.586	2.743
NEm (Mcal/kg)				
Six-rowed	2.230	2.570	2.362**	0.081
Two-rowed	2.150	2.460	2.295	0.075

a: Minimum value, b: Maximum value, c: mean, d: Standard deviation, *, ** significant of pairwise comparison between six-rowed and two-rowed subpopulation at P<0.05 and P<0.001resoectively, ns: non significant.

Table 5-4:.Mean squares for genotypes (MS_G), environments (MS_E), genotype X environment (MS_{GE}) and error (MS_{ER}), genetic (σ^2_G) and genotype X environment (σ^2_{GE}) variance components and heritabilities for Protein, acid detergent fiber (ADF) and starch content for rainfed, irrigated and combined environments.

Trait	MS_G	MS_E	MS_{GE}	MS_{ER}	σ^2_G	σ^2_{GE}	h^{2a}	h^{2b}
Protein (%)								
Rainfed	3.73**	nd	nd	0.56	1.75	nd	75.85	85.01
Irrigated	2.97**	nd	nd	0.52	1.29	nd	71.70	82.82
Combined	5.18**	255.45**	1.29**	0.54	1.06	0.43	52.29	73.13
ADF (%)								
Rainfed	5.22**	nd	nd	0.32	2.48	nd	88.56	93.86
Irrigated	7.75**	nd	nd	1.06	3.37	nd	75.93	86.21
Combined	11.98**	26.87**	0.81 ns	0.69	2.85	0.06	79.02	93.20
Starch %)								
Rainfed	22.49**	nd	nd	13.03	4.47	nd	75.93	86.21
Irrigated	29.95**	nd	nd	7.30	11.39	nd	60.99	75.61
Combined	40.75**	0.36ns	11.47ns	10.13	7.39	0.52	40.42	71.70

a: heritability on plot-basis, b: heritability on family mean-basis
**: significant at P<0.001, ns: not significant, nd: not determined.

Correlation analysis

Simple correlation analysis was carried out to understand the genetic control of starch, ADF and protein in correlation with others field and feed quality traits (Abdel-Haleem et al., chapter 1). A high negative correction was found between starch content and ADF (-0.773). Protein content correlated negatively with ADF and starch content (Table 5-5). ADF correlated significantly and negatively with particle size. Starch content positively correlated with DMD and negatively with particle size.

Table 5-5: Phenotypic correlation among heading date (HD), plant height (PH), yield (YD), protein content (CP), acid detergent fiber (ADF), starch content (SCH),dry matter digestibility (DMD) and particle size (PS) of Valier x PI370970 population.

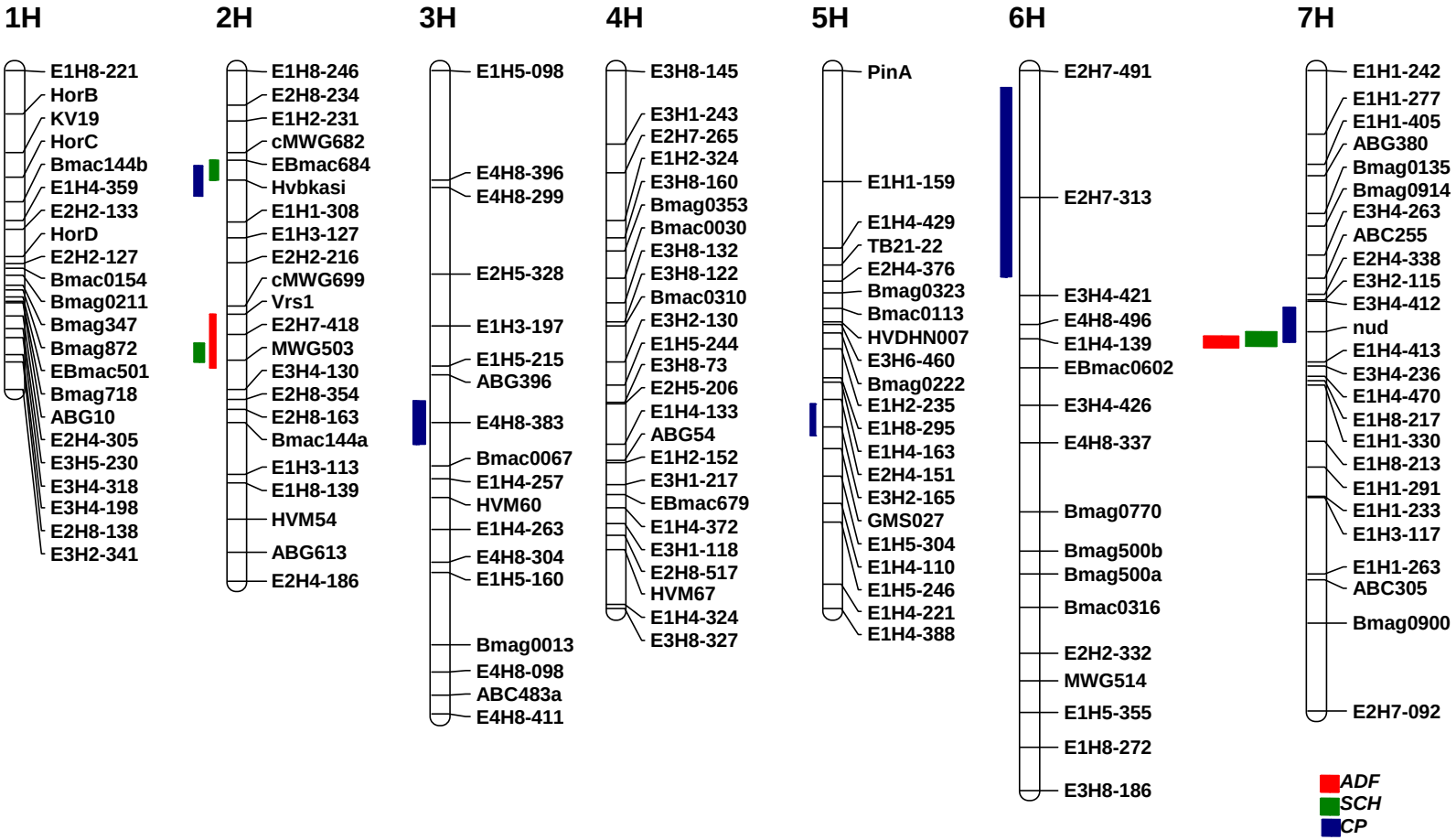
Trait	HD	PH	YD	CP	ADF	SCH	PS	DMD
ADF	0.058	0.037	0.106	-0.499**				
SCH	-0.088	0.169	0.188	0.247**	-0.773**			
PS	-	-	-	-0.120	0.219**	-0.382**		
DMD	-	-	-	-0.109	0.109	0.319**		
NEm	-	-	-	0.172*	-0.644*	0.700**	0.222**	-0.453**

*' ** Significant at P=0.05 and P= 0.01, respectively.

QTL Analysis

To determine the chromosomal locations of gene(s) controlling variation in ADF, starch and protein content, a 150 marker linkage map was constructed using the 116 members of the Valier x PI370970 RIL population (Figure 5-1).

Figure 5-1. QTL locations for acid detergent fiber (ADF), protein (CP), starch content (SCH) for Valier x PI370970 population. Bar length indicates the 1-LOD confidence interval and the bar weight indicates the relative phenotypic variation value of each QTL.



Composite interval mapping (CIM) and multiple interval mapping (MIM) methods were employed to detect QTL and QTLs interactions. QTL analyses were carried out using data from Bozeman rainfed and irrigated experiments, and the combined data of these environments.

The *N* gene was found on the long arm of 7H chromosome in agreement with several prior papers (Fedak et al., 1972; Heun 1992; Becker and Heun 1995; Franckowiak 1997; Qi et al., 1998; Costa et al., 2001; Kikuchi et al., 2003). *Qadf7a, Qcp7a and Qstarch7a* all appear to cosegregate with *N*, a finding we expected. About 15% of the total phenotypic variation for protein content, about 40% of the variation for adf content and about 45% of the variation for starch content was explained by segregation at this locus (Table 5-6). Interesting QTL modifying grain protein content were observed on chromosomes 3H and 6H. A gene on chromosome 2H, likely the photoperiod response gene *Ppd-H1*, had a significant impact on grain starch content.

The results of analysis by composite interval mapping (CIM) and multiple interval mapping (MIM) were broadly similar (Table 5-6, 5-7). No significant QTL x QTL interaction was detected by MIM in contrast with Frégeau-Reid et al. (2001) who detected a Additive x Additive epistasis for starch content. Using Steptoe x Morex population 2 QTLs for starch content were identified (Ullrich,et al., 1996).

Table 5-6: QTLs for protein content (CP), acid detergent fiber (ADF) and starch content identified by composite interval mapping (CIM) for Valier x PI370970 population.

Trait	QTL Name	Chrom marker	Nearest position	QTL[a] score	LOD[b]	R^{2c}	Total R^{2d}
CP (%)							
Rainfed	*Qcp3a*	3H	E4H8-383	123.51	2.51	12.32	64.11
	Qcp5a	5H	E1H5-246	153.31	2.39	15.33	
	Qcp6a	6H	E2H7-313	57.91	2.98	22.35	
	Qcp7a	7H	Nud	87.71	3.62	14.11	
Irrigated	*Qcp2a*	2H	Hvbkasi	37.91	4.03	12.90	44.61
	Qcp3a	3H	E4H8-383	125.51	3.02	12.72	
	Qcp5b	5H	E3H2-165	122.81	2.47	7.50	
	Qcp7b	7H	ABG380	40.61	2.09	10.97	
Combined	*Qcp2a*	2H	Hvbkasi	37.91	3.64	10.32	60.90
	Qcp3a	3H	E4H8-383	123.51	4.49	15.08	
	Qcp5b	5H	E3H2-165	122.81	2.83	7.73	
	Qcp6a	6H	E2H7-313	55.91	2.23	13.24	
	Qcp7a	7H	Nud	87.71	3.94	14.53	
ADF (%)							
Ranfied	*Qadf2a*	2H	MWG503	95.11	2.24	3.84	61.12
	Qadf6a	6H	Bmag500b	165.81	2.63	3.57	
	Qadf7a	7H	Nud	92.31	24.42	53.71	
Irrigated	*Qadf2a*	2H	MWG503	93.11	3.60	6.50	46.73
	Qadf6a	6H	Bmag500b	165.81	2.70	4.23	
	Qadf7a	7H	Nud	92.31	16.06	36.00	
Combined	*Qadf2a*	2H	MWG503	95.11	3.03	5.02	41.83
	Qadf7a	7H	Nud	92.31	19.10	39.81	
Starch (%)							
Rainfed	*Qsch7a*	7H	Nud	92.31	12.74	40.52	49.26
	Qsch7c	7H	E1H1-263	147.41	2.35	5.13	
Irrigated	*Qsch2a*	2H	Hvbkasi	33.21	3.66	7.43	68.17
	Qsch2b	2H	MWG503	97.11	7.44	19.96	
	Qsch7b	7H	ABG380	36.61	2.61	5.35	
	Qsch7a	7H	Nud	92.31	14.51	35.43	
Combined	*Qsch2a*	2H	Hvbkasi	33.21	5.24	10.01	68.90
	Qsch2b	2H	MWG503	97.11	5.35	12.23	
	Qsch7a	7H	Nud	92.31	19.23	46.66	

[b] QTl position in cM., [b] Peak value of the LOD.
c Proportion of phenotypic variance explained by the QTL.
d Total phenotypic variances that explained by the model.

Table 5-7: Estimated QTL effects and epistatic interaction detected for protein (CP), acid detergent fiber (ADF) and starch content by multiple interval mapping (MIM) for Valier x PI370970 population over rainfed, irrigated and combined environments.

Trait	QTL Name[a]	Nearest Marker	QTL Position	Genetic effects Additive	Epistatic	R^2
CP (%)						
Rainfed	*Qcp3a (3H)*	E4H8-383	125.50	0.603	-	15.0
	Qcp5a (5H)	E1H5-246	153.31	-0.449	-	9.7
	Qcp6a (6H)	E2H7-313	59.90	0.548	-	14.9
	Qcp7a (7H)	Nud	86.70	-0.266	-	5.8
	Qcp6a X *Qcp7a*			-	-0.532	15.8
Irrigated	*Qcp2a* (2H)	Hvbkasi	38.90	0.471	-	10.7
	Qcp3a (3H)	E4H8-383	125.51	0.464	-	12.3
	Qcp5b (5H)	E3H2-165	122.90	0.374	-	7.5
	Qcp7b (7H)	ABG380	41.60	-0.427	-	12.5
Combined	*Qcp2a* (2H)	Hvbkasi	38.00	0.385	-	7.1
	Qcp3a (3H)	E4H8-383	123.51	0.494	-	13.6
	Qcp5b (5H)	E2H3-165	122.90	0.370	-	6.5
	Qcp6a (6H)	E2H7-313	55.90	0.430	-	10.6
	Qcp7a (7H)	Nud	87.70	-0.463	-	14.7
ADF (%)						
Rainfed	*Qadf2a* (2H)	MWG503	96.10	-0.302	-	2.9
	Qadf6a (6H)	Bmag500b	165.81	0.265	-	4.5
	Qadf7a (7H)	Nud	92.30	1.324	-	66.5
	Qadf2a X Qadf7a			-	-0.267	4.5
Irrigated	*Qadf2a* (2H)	MWG503	94.10	-0.514	-	5.6
	Qadf7a (7H)	Nud	92.30	1.585	-	63.2
	Qadf2a X Qadf7a			-	-0.300	4.2
Combined	*Qadf2a* (2H)	MWG503	97.10	-0.432	-	5.1
	Qadf7a (7H)	Nud	92.30	1.472	-	69.5
	Qadf2a X Qadf7a			-	-0.260	3.90
Starch (%)						
Rained	*Qsch2a* (2H)	Hvbkasi	36.20	0.816	-	5.9
	Qsch7a (7H)	Nud	93.30	-2.288	-	44.2
Irrigated	*Qsch2a* (2H)	Hvbkasi	35.20	1.315	-	11.8
	Qsch2b (2H)	MWG503	97.10	2.003	-	23.0
	Qsch7a (7H)	ABG380	38.60	0.626	-	4.9
	Qsch7b (7H)	Nud	93.30	-2.626	-	39.4
Combined	*Qsch2a* (2H)	Hvbkasi	35.20	1.032	-	11.3
	Qsch2b (2H)	MWG503	97.10	1.098	-	12.4
	Qsch7a (7H)	ABG380	38.60	0.558	-	4.5
	Qsch7b (7H)	Nud	93.30	-2.266	-	47.6

[a] QTl position in cM, [b] :Proportion of phenotypic variation explained by the QTL,[c] :total phenotypic variation that explained by the model.

Validation of ADF-QTL

We utilized a parallel population derived from a cross between Baronesse and PI370970 to verify the importance and magnitude of the impact of the *N* gene on starch, protein and ADF content. Single marker analysis indicated that the *N* gene controls about 60% of ADF variation in the validation population (Table 5-8). The data from mapping and validation populations suggested that crossing hulled x hull-less parents expressed wide variation in ADF (Table 5-1, 5-8).

Table 5-8: Mean values for acid detergent fiber (ADF) for parental and genotypes classes, and percent of variations explained by N locus for Baronesse x PI370970 validation population.

Population	ADF%	SE[a]	Pr>F	R^{2b}
Baronesse x PI37097	3.80	1.196	<0.0001	64.77
Baronesse (hulled)	5.32	-	-	-
PI370970 (hulless)	2.01	-	-	-
Hulled subpopulation (n)	5.10	1.466	-	-
Hulless subpopulation (N)	1.81	0.574	-	-

[a] standard deviation.
[b] calculated by R2 x 100.

In this study, we report the results of a QTL mapping of feed quality traits such as ADF, starch and protein content using a recombinant inbred line population derived from a cross between a commonly grown barley cultivar (Valier), two-rowed, hulled, high ADF and low starch content, and a line from the core collection of the USDA National Small Grains Collection, PI370970, two-rowed, hull-less, low ADF and high starch content. One gene or a linked cluster of genes on barley chromosome 7H tightly linked to the grain morphology-modifying *N* gene was found to affect ADF, protein and starch content. This QTL was validated using an additional population. While two-rowed hull-less genotypes had lower ADF and higher starch content than two-

rowed hulled genotypes, the two classes were broadly similar in grain yield. The correlation between ADF and starch content is negative and their QTLs, Qstch7a and Qadf7a, overlap at the *N* gene.

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CHAPTER 6

CONCLUSION

The Montana economy is dependant on five major industries, agriculture, mining, gas and oil production, wood and paper products and tourism. A comparison among these industries shows that the agricultural sector continues to outpace other industries (Montana Agriculture Statistics, 2003).

Cattle and calf production are 50.1% of all the agricultural cash receipts from marketings during 2002, which earned about 966 million dollars. The cow-calf production system that dominates the livestock industry in the state is based on producing feeder calves to be sold at weaning and leave the state. In fiscal year 2003, Montana produced approximately 1,451,000 beef cows. Of those, approximately 70,000 cattle were finished in feedlots in the state, while about 1.1 million were sold as calves and transported to feedlots in the Midwest and Southern Plains.

The total number of bushels of barley produced in Montana was 38,000,000, which put Montana is ranking at 3rd out of all the 50 states. Most of Montana barley is produced for animal feed. One objective of the Feed Barley for Rangland Cattle project is to modify the current cow-calf production system to retain the feeder calves in Montana by feeding Montana barley to produce a value-added product for both the barley grower and cattle producers.

Barley has been criticized for cattle feed because of its rapid digestion in the rumen, which results in digestive disorders such as bloat and lactic

acidosis in cattle fed on high barley diets (Kellems and Church, 2002). A potential approach to limit this problem associated with feeding barley is to select barley cultivars that have slower rate of digestion in the rumen. The feed quality of barley is influenced by many factors including chemical and physical characteristics. The complexity of these factors has contributed to inconsistent data on feed quality of barley. Animal performance is often measured by average daily gain, carcass grade at slaughter, feed efficiency, diet digestibility and animal intake. Recent research determined that chemical factors including starch content, acid detergent fiber (ADF) and in situ dry matter digestibility (ISDMD) are important barley feed quality traits. Surber et al. (2000) predicted animal performance using the parameters of ISDMD, ADF, particle size after rolling and starch content. Selection of barley grain for low DMD, large particle size after rolling, low ADF content and high starch content should allow progress in breeding for improved feed quality.

Lande and Thompson (1990) proposed marker-assisted selection (MAS) as a method that allows complex traits to be screened in the early generations of breeding programs. Barr et al. (2001) identified nine key steps in the identification of molecular marker(s) linked to traits for use in MAS. These including identifying parents differing in trait(s) of interest, developing a population of plants segregating for these trait(s), screening the population for the trait(s), constructing a linkage map of the population to locate a QTL associated with the trait(s) of interest, identifying molecular marker(s) that co-segregate with the trait(s) of interest, validating these marker(s) in different

backgrounds, producing clear and simple protocols for assaying the marker(s) and modifying breeding strategy to optimize use of MAS within the breeding program.

The general objectives of the present research were to identify and validate QTLs associated with feed quality and predict animal feedlot performance using lines derived from mapping and validation populations. These included 150 DH lines from the Steptoe x Morex cross and 140 DH lines from the Harrington x Morex cross. Both populations were developed by the North American Barley Genome Mapping Project (NABGMP). We also assayed 62 Recombinant Inbred Lines derived from a cross between Lewis x Baronesse. Both Lewis and Bronesse are well-adapted 2-row barley varieties that are grown in Montana and surrounding states. This cross represents a 'real world' test of the technology within a practical crop improvement context. Baronesse is a European two-row feed variety with high ISDMD and ADF, that was planted on over 50,400 hectares (4.2 % of the total state barley acreage) in Montana. Lewis is a two-row feed and malt variety released by Montana Agricultural Experiment Station planted to 21,000 hectares (1.8 %) (Montana Agricultural Statistics, 2003). We also evaluated 116 recombinant inbred lines derived from a cross of Valier x PI370970. Valier (PI610264) is a two-rowed, hulled spring barley cultivar, developed by the Montana Agricultural Experiment Station and released for commercial production, while PI370970 is a six-rowed hulless barley landrace line from Switzerland selected from the USDA world collection of barley's spring core collection based on its low

ISDMD value, large particle size, high starch content and low ADF content. Finally, we utilized 197 RILs of Baronesse x PI370970 cross as a validation population.

In the first chapter, Steptoe x Morex doubled haploid population, was used to evaluate genetic variation in the in situ dry matter and starch digestibilities, ADF, protein and starch content, and map QTLs affecting rumnial digestibility of barley and other feed traits and estimated the interactions between the detected QTLs. Positive and negative transgressive genotypes over each parent were indicated. This indicates the wide spectrum variation of these traits in the offspring. Significant correlations were detected among many of these traits. Major QTLs affecting these traits were detected using composite interval mapping. QTLs controlling dry matter digestibility and starch digestibility of the ground grain were detected on chromosome 4 (4H), near STS marker ABA003, while particle size and dry matter digestibility of the cracked grains were affected by QTLs/gene(s) located on chromosomes 3 (3H), 4 (4H) and 6 (6H). These results indicated ABA003 as a diagnostic marker associated with the variation of digestibility of ground grains in rumen in this population. A major QTL for ISDMD of cracked grains was located on chromosome 3(3H), about 10cM from a major particle size-QTL. Since the two traits are correlated (r=0.551), the distance between the QTL peaks may imply measurement error. This may be a pleiotropic response, a gene affecting both particle size and ISDMD. A marker for the gene controlling the B hordeins on chromosome 5(1H) was associated with variation in dry matter digestibility and

starch digestibility. Variation among hordein haplotypes might contribute to variation in the rate at which endosperm starch granules become available for hydrolysis by rumen microorganisms. We believe that this observation, associating B-hordein haplotype variation for starch digestibility, to be novel.

QTLs for ADF and starch content overlapped on chromosome 7(5H) at Nar7. The tight negative correlation between starch and ADF and the different allelic effect on these QTLs suggested that Nar7 locus if validated in another population, could work as anchor marker in MAS programs for selection genotypes with low ADF and high starch content.

Since Montana is primarily a 2-rowed barley producing state, we developed a recombinant inbred lines population derived from Lewis x Baronesse cross to find significant transgressive segregation for feedlot performance. Linkage map of this population was constructed. The variation in feed-related traits was sufficient to detect several QTL that controlling the variation for ADF, starch content, ISDMD and starch digestibilities using composite interval mapping. As found in Steptoe x Morex population, ADF and starch content were correlated and overlapped in the same chromosomal region, at chromosome 2H. For dry matter digestibility and starch digestibility several QTLs were detected. A QTL for starch digestibility was detected on chromosome 4(4H) and flanked with ABG618, this marker was mapped about 20 cM distant from ABA003. The QTL centered on this locus controlled variation in starch digestibility of the Steptoe x Morex population.

Another QTL that had impact on digestibility was detected on chromosome 5(1H). One of the major goals of QTL mapping is to select markers that linked to genes contributing to variation in the trait of interest. QTL consistency across different environments and background is important component for MAS. This QTL, on chromosome 5(1H), was detected in different tested environments so we used it as selection criteria to select eight genotypes that have Lewis or Baronesse alleles at that locus, varied dramatically for dry matter digestibility and have the appropriate high-yield genes. Feedlot studies were conducted to correlate the lab and feedlot data, as expected low ISDMD and large particle size genotypes are required for good animal performance. Feedlot performance studies suggested genotypes that are superior to the parents in animal performances characteristics and could be used to improve the feeding quality of barley.

In barley, as in many other crop species, there is limited genetic variation in economic traits due to domestication and intensive selection for these traits. Martin el al. (1991) concluded that most feed barley cultivars have been developed from existing cultivars that derive from a very limited germplasm pool. The genetic variation for these feed-related traits in U.S. adapted barley lines is limited, and that was obviously noticed in the narrow variations among the parents of the previous 2 populations (Steptoe, Morex, Lewis and Baronesse). Unadapted germplasm has long been recognized as a source for trait variation not found in adapted lines. Incorporation of the traits of interest with the restriction of genetic drag from the source germplasm has been

improved through the development of QTL mapping and MAS for such traits. In our attempt to broad the genetic variation of barley for DMD, 1500 accessions of the USDA world core collection were screened to select low DMD parents (Bowman el al., 2001). Several lines were identified that had normal or high starch content, low ADF and extraordinarily low ISDMD. One of these, PI370970, was crossed to ‘Valier and a population of recombinant inbred lines was derived.

We found that the largest QTL impacting ISDMD resides on barley chromosome 2(2H) very close to *Vrs1*, a gene that impacts plant and seed development in many ways, including determining whether the head is 2-rowed or 6-rowed. Previous work suggested that grain from 6-rowed varieties degraded slightly, but significantly slower than grain from 2-rowed varieties (Bowman et al., 2001). The dramatic effect of this QTL was twice that of the effect previously observed. *Vrs1* resides in a moderate to low recombination region of chromosome 2H (Kunzel et al., 2000), so the physical distance between *Vrs1* and other genes in this region that might impact DMD could be large, even though recombinants might be very infrequent.

The strong correlation among ISDMD, particle size and yield and the overlapping QTLs which are controlling the variation in these traits around *Vrs1*, may be due to pleiotropic effects or closely linked QTLs. Allard (1988) reported that *Vrs1* affects the developmental process with strong influences on many quantitative traits including heading date, plant height and spike length. Marquez-Cedillo et al. (2001) reported difficulty in distinguishing between

linkage and pleiotropy for yield, yield components and malting characters, and proposed that the low resolution of the linkage Harrington x Morex DH map could be in part responsible.

If segregation at *Vrs1* is responsible for variation in ISDMD, then we expect to see similar magnitude variation in any population from a 2-row x 6-row cross. When we assayed the Harrington x Morex population, *Vrs1* appeared to be significantly associated with variation for ISDMD, but the additive effect of variation at *Vrs1* was less than half that observed in the Valier x PI370970 population. In another attempt to test the impact of the *Vrs1* gene ISDMD, we assayed another 2-row x 6-row RIL population derived from Baronesse x PI370970. This population showed that *Vrs1* impact on ISDMD similar in magnitude to that of our mapping population. Thusfar, no obviously convincing recombinants between the DMD QTL and *Vrs1* have been identified in either population.

The most straightforward way segregation at *Vrs1* might impact ISDMD is through the variation in kernel size conditioned by the 6-row head type. Kernels on 6-rowed heads vary much more dramatically in terms of size and shape than do those on 2-rowed heads. Variation for ISDMD could have been due to thin grain passing through our milling system relatively unground. We utilized sieved fractions of the milled product to obtain a more uniform ground product for ISDMD estimation. In this case, the ground products should show no QTL at *Vrs1* if the QTL was a milling artifact. In this case, one of the sieved

fractions showed a significant QTL while the other did not. Plainly, the question of pleiotropy or linkage remains unresolved.

The efficiency of using low DMD QTL for marker assisted selection (MAS) to reduce the rate of barley grain digestion will depend on the answer to the question whether DMD-QTL is tightly linked to *Vrs1* or is a pleiotropic effect. Two-rowed varieties are preferred in the arid Northern plains. If this is a pleiotropic effect, gene transfer into 2-rowed backgrounds will be impossible. If, however, linkage is the cause of the association between *Vrs1* and DMD-QTL, then larger mapping populations should permit us to better map the gene and identify useful recombinants. Experiments with much larger populations are now underway.

In a continuing study, we report the results of a QTL mapping of feed quality traits such as ADF, starch and protein content using the Valier x PI370970 population. One gene or a linked cluster of genes on barley chromosome 7H tightly linked to the grain morphology-modifying *N* gene was found to affect ADF, protein and starch content. This QTL was validated using the Baronesse x PI370970 population. While two-rowed hull-less genotypes had lower ADF and higher starch content than two-rowed hulled genotypes, the two classes were broadly similar in grain yield. The correlation between ADF and starch content is negative and their QTLs, overlap at the *N* gene, confirming the potential value of hulless feedgrains.

As the current understanding of the variation of animal performance is limited and *in vivo* evaluation of barley genotypes is very expensive. There are

two ways to estimate animal feedlot performance for barley improvement. First, we may evaluate breeding lines by using calf performance directly. If we have a population of 500 lines and we are using 20 calves/ barley line, we would require 10,000 acres of land to grow the barley and 10,000 calves to test it. Second, we may select the promising lines for this population for the feedlot performance trials based on predication of performance of cattle fed on high barley diets utilizing measurements of DMD, starch content, particle size and ADF. Our study suggests that net energy available for maintenance (NE_m) and average daily gain are significantly influenced by barley genotypes. The NE_m of corn, as high moisture grains, is high (2.33 Mcal/kg) comparing with barley standards (2.06 Mcal/kg). Our present model of NE_m and average daily gain suggests that lines derived from the Valier x PI370970 population could provide greater feedlot performance than corn.