A Study of the Biochemical, Physical and Genetic Factors Influencing Malt Extract in Barley (*Hordeum vulgare* L.)

by

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Declaration

I hereby declare that this work does not contain any material previously submitted for another degree or diploma in any university or tertiary institution. To the best of my knowledge and belief, it does not contain any material previously published or written, except where due reference is made in the text.

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Helen Marie Collins

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List Of Abbreviations

%db	Percentage calculated on a dry basis
α	alpha
β	beta
°C	Degrees centigrade
μ	microns
μm/min/g	DP, α-amylase and β-amylase units: micromoles of maltose equivalents released per minute per gram dry weight
ABB	Australian Barley Board
ASBC	American Society of Brewing Chemists
UA	Adelaide University
BLUPS	Best linear unbiased predictors
db	Calculated on a dry basis
DP	Diastatic Power
DSC	Differential Scanning Calorimeter
EBC	European Brewing Convention
EBC HWE	Hot water extract measured using the EBC method
FAN	Free amino nitrogen
g	grams
GOPOD	Glucose determining reagent (glucose oxidase/peroxidase reagent)
HPAEC-PAD	High performance anion exchange chromatography with pulsed amperometric detector
HWE	Hot water extract
IOB	Institute of Brewing
IOB HWE	hot water extract measured using the IOB method
J/g	joules per gram
KI	Kolbach Index
LOD	LOD score, log of odds
M	Molar
MAS	Marker assisted selection
mg/L	Milligrams per litre
mJ	milli Joules
mL	millilitres
mm	millimetres
mW	milli watts
NIR	Near Infrared Spectroscopy
pI	isoelectric point
RIL	Recombinant inbred lines
RVA	Rapid Visco Analyzer
RVU	Rapid Visco Analyzer units
SD	Standard deviation
SE	Standard error
SG	Specific gravity
SKCS	Single Kernel Classification System
SP/TP	Soluble Protein to Total Protein Ratio
U/kg	Units per kilogram of malt
VIDA	Victoria Institute of Dryland Agriculture
NBMMP	National Barley Molecular Marker Program

Abstract

Beer is produced from malted barley (barley germinated under controlled conditions), water, hops and yeast via a number of complex steps. The second of these involves the extraction of the soluble material from the malt with warm water in a process called mashing. The soluble material that is extracted from the malt during the mashing process is referred to as malt extract (HWE), and is used as a guide for both domestic and overseas customers in determining the quality of the malt or barley they are purchasing. HWE is variety dependent and is rated as the most important parameter for the release of new malting barley varieties in Australia (MBIBTC, 2001). This study investigated the biochemical and genetic factors influencing high levels of malt extract in a number of barley varieties.

To investigate the biochemical factors influencing high levels of malt extract, twelve Australian and international barley varieties were grown at four sites in Southern Australia. Based on their levels of HWE, these varieties fell into three groups, high malt extract varieties, medium malt extract varieties and feed varieties. Using these varieties, various barley, malt, wort and spent grain traits were investigated. Statistical analysis showed that malt extract is related to thirteen key parameters involving either an individual trait or a group of traits. These included grain size, grain hydration during malting, barley husk content, malt β-glucan levels, limit dextrinase activity, activity of other starch degrading enzymes, size of the large starch granules and RVA peak viscosity, protein modification (in the wort), cell wall modification (in the wort), wort monosaccharide levels, the percent of the original malt remaining in the spent grain and the components of the spent grain. This analysis has been used to identify the key breeding targets that can be used to select for new varieties with improved levels of HWE.

HWE is a complex genetic trait, controlled by up to 24 QTL (regions of the genome). In this study, the key QTL associated with HWE under Australian environmental

conditions were identified. Six regions of the barley genome were found to be associated with malt extract in three barley mapping populations. Five regions were found to be associated with HWE in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations on chromosomes 1H, 2H, 3H, 4H and 5H, and a single region was found on chromosome 2H in the Galleon/ Haruna Nijo mapping population.

These six regions of the barley genome and an additional two regions, found to be associated with HWE in the Chebec/ Harrington population (Collins et al., 2003), were assessed further to identify other traits that have also been found to be associated with these regions. By doing this it is possible to gain a greater understanding of the underlying physical or biochemical bases of elevated HWE. The region on chromosome 3H was found to be associated with genes conferring differences in plant height, which caused a cascade of differences in plant types that influenced the overall level of HWE. These regions on chromosomes 1HS, 2HS, 2HL and 5HL were responsible for causing differences in the levels of modification of the grain during germination, which impacted the HWE. These differences in modification levels were due to differences in the speed of germination caused by either, a hormonally based difference, caused by a gene or genes in the region, or a physical property of the grain, such as husk content.

Markers from four of these regions, on chromosomes 1HS, 2HS, 2HL and 5HL, and two regions found in the Chebec/ Harrington mapping population on chromosomes 1HL, 5HL, were validated using a number of breeding populations with a high HWE variety as a parent. The allele from the high HWE parent was found to be associated with a significant increase in HWE in four regions, two regions on chromosome 2H and two regions on chromosome 5H. The use of these markers to select for improved HWE will to lead to increased levels of HWE. By mapping, validating and assessing the influence of these regions of the barley genome on HWE, this study has successfully shown that marker-assisted selection (MAS) will be a valuable aid for the selection of high HWE varieties. An understanding of how the use of these regions, to select for high HWE, will influence the grain quality, as a whole, will enable breeders to release varieties tailored to the needs of the target environments and markets.

Chapter 1

Introduction

The barley industry in Australia is a multi-million dollar business. Between 1998 and 2002 Australia, on average, produced 5.9 million tonnes of barley annually (GRDC Grain Yearbook, 2003), approximately 40% of which was classified as malting grade and used to produce beer. Each year Australia exports barley for malting to China and other Asian countries, as well as South America. Malted barley is exported to Japan, Philippines, Thailand, Korea and other Asian and South America countries. Whilst each of these countries have individual specifications for the quality of the barley they purchase, they generally require clean, bright grain with high levels of malt extract and appropriate levels of hydrolytic enzymes.

Beer is produced by a complex series of steps, using four main ingredients, barley, hops, water and yeast. Before the barley can be used it is modified in a process called malting. This involves germinating barley under controlled conditions before halting the growth by kilning. This finished malt is then ground and the soluble material is extracted with hot water in a process called mashing. Finally yeast is added and fermentation converts the simple sugars extracted from the malt into ethanol. Additionally, brewers may include other ingredients, known as adjuncts, such as wheat, maize, rice or sugar. These not only add different flavours and aromas to the beer but also act as an additional (and cheaper) source of carbohydrates.

Malt extract refers to all of the soluble material, including carbohydrates, protein and cell wall material that are extracted from the malt during the mashing process. These soluble materials are either used for yeast nutrition during fermentation or form part of the final beer product. Therefore it is important to achieve not only a high level of malt extract, but also a good quality malt extract with the right balance of sugars for fermentation, nitrogen compounds for yeast nutrition and soluble proteins for foam stability. However the factors contributing to a good malt extract are poorly understood and large variations occur between barley varieties.

Hot water extract (HWE) is a measure of the soluble materials extracted from malt in a laboratory small-scale mash (Briggs *et al.*, 1981). This gives an indication of how a malt will perform in a full scale brewing situation. It is rated as the most important parameter for the release of new malting barley varieties in Australia (MBIBTC, 2001), and is used as a guide for both domestic and overseas customers in determining the quality of the malt or barley they are purchasing.

The Australian malting variety Schooner was released in 1983 and quickly became the dominant malting variety grown in Southern Australia (Jefferies and Wheeler, 1991). However Schooner's malting quality was outclassed some years ago by varieties such as Harrington and Alexis from Canada and Europe, causing loss of market share in the lucrative Japanese market (Powell, 1997). The release of good malting varieties such as Franklin and Arapiles in the early 1990's improved the quality of malt exported from Australia but unfortunately these varieties are only suitable for growing in high rainfall areas. Whilst a number of new varieties have been released, such as Sloop and Picola, that may finally have an impact on the amount of Schooner grown, 20 years after its release, Schooner is still a dominant variety in Southern Australia. In 2001, the Southeastern regions of Australia, encompassing South Australia and Victoria planted Schooner on 30% and 50% respectively of the total area on which barley was grown (ABB Grain Ltd Survey). If Australia is to regain market share in Japan and increase share in other expanding Asian markets such as China it is imperative that varieties with HWE levels up to 3% higher than Schooner be released within the next few years. For this reason it is important to gain a greater understanding of the factors contributing to malt extract, which will assist Australian barley breeders in selecting for varieties with high malt extract that are adapted to Australian growing conditions.

The aim of this study is to improve our knowledge of malt extract by investigating both the biochemical and genetic bases of HWE. To date, most of the research investigating the biochemistry of malt extract has been based upon correlation studies of carbohydrate and protein profiles. The problem with wort carbohydrate and protein profiles is that they do not always relate back to the original barley. While brewers may have an understanding of what chemical composition they want in the wort, it is

often difficult to relate this to the barley or malt and in particular to differences between barley varieties. Before breeders can be expected to efficiently select for the complex mixture of sugars, proteins and soluble carbohydrates, a thorough understanding of how these compounds relate back to the original barley is needed. The relationship between the many compounds within the barley grain, including the hormones, hydrolytic enzymes, proteins and carbohydrates is complex and intricate. How these react and change during the malting and mashing processes is at best fragmented, with many gaps still to be filled.

An understanding of the genetic basis of HWE will enable breeders to implement marker-assisted selection (MAS) as an aide to traditional breeding techniques to select for varieties with improved levels of malt extract. This method has the potential to dramatically decrease the time taken to screen new breeding lines for particular traits. However before breeders can implement MAS, markers need to be found and evaluated for their potential usefulness. It is also important to gain an understanding of how these markers will not only impact upon the trait of interest, but also other traits associated with the particular region of the genome.

Twelve Australian, European, Canadian and Japanese barley varieties were selected to investigate the biochemical basis of HWE. These were chosen for either their good malting quality or their good agronomic adaptation to Southern Australian conditions and were grown at a number of sites to examine the influences of site and variety on malt extract. Samples were malted and mashed, and the composition of barley, malt, wort and spent grain was investigated.

To investigate the genetic factors influencing HWE, three barley mapping populations, established in Australia in conjunction with the National Barley Molecular Marker Program (NBMMP), were studied. These populations were made from crosses between a high malt quality international parent and a good agronomic Australian parent. One of the aims of establishing these populations was to determine the genetic basis for high malt quality traits in backgrounds that are agronomically suitable to Australian growing conditions. Using these populations it was possible to search for regions of the barley genome that were associated with high levels of HWE.

Once regions of the barley genome were established that were associated with improved levels of HWE, the impact of selecting for these regions in genetic backgrounds other than the mapping population used to identify them has been investigated. The aim of this part of the study was to assess the usefulness of these regions to barley breeders when using MAS. Additionally other malting quality and physical traits were investigated to see whether they were associated with these regions to gain an understanding of how the selection of these markers would influence the trait of interest but also other traits.

Chapter 2

Literature Review

Malt extract is a measure of the soluble material that is extracted from the malted barley grain during the mashing process. It contains the soluble products of the degradation of the carbohydrates and proteins from the malt which themselves originate from the grain. To fully understand malt extract it is not only necessary to understand the processes involved during mashing but also the processes involved during malting, deriving back to the components of barley and malt. To gain an understanding of the differences among barley varieties in malt extract levels it is important to take a holistic approach and investigate the whole process from the original barley grain to the final wort product. The following chapter contains a discussion of the biochemical and molecular bases currently known to influence malt extract and includes a description of the composition of the grain and malt.

2.1 Barley Cytology and Genetics

Barley belongs to the family of grasses known as Gramineae, the tribe Triticeae and the genus *Hordeum*. Generally, cultivated barley belongs to the species *vulgare* (Nilan and Ullrich, 1993). It is diploid with seven pairs of chromosomes (Nilan and Ullrich, 1993). These are designated as chromosomes 1H-7H as recommended by the 7th International Barley Genetics Symposium held at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada in 1996 (Barley Genetic Newsletter, 1997).

2.2 Grain Structure and Composition

A barley grain is composed of a number of regions, including the husk, pericarp and testa, the embryo, the aleurone layer and the starchy endosperm (Figure 2.2). The chemical composition of the barley grain primarily consists of starch, protein and cell

wall materials, such as beta glucan and arabinoxylan, and low levels of lipids, soluble sugars and minerals (Table 2.2.1).

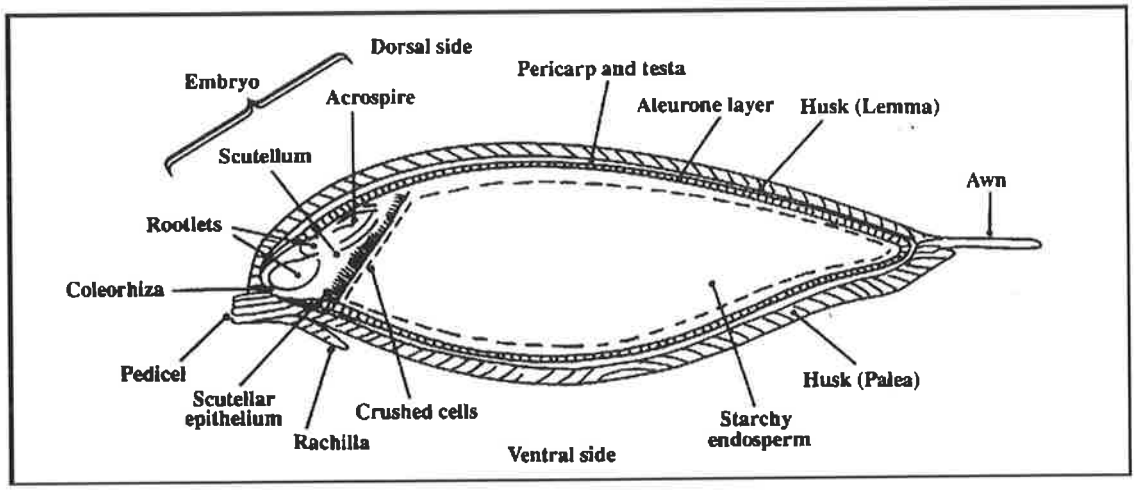


Figure 2.2.1: A schematic representation of a barley grain showing important tissue (Stuart, 1997)

Table 2.2.1: The approximate chemical composition of a barley grain. (Briggs *et al.*, 1981; MacGregor and Fincher, 1993)

Starch	63-65%
Protein	10-12%
Cell wall materials	13-16.5%
Lipids	2-3%
Soluble sugars	1-2%
Minerals	2%
Other substances	5-6%

2.2.1 The Husk, the Pericarp and the Testa

The husk is a protective layer covering virtually the whole seed. It has two sections, the lemma on the dorsal side of the grain, and the palea on the ventral or the furrowed side of the grain. In hulled barley, the lemma and the palea are fused to the pericarp, whilst in hulless barley they are not attached to the grain and are removed during threshing. The husk makes up about 10% of the dry weight of the grain (Palmer and

Bathgate, 1976) and contains cellulose, lignin, pentosans, mannan, uronic acids, silica and hemicellulose. Cellulose is the major constituent of the husk, accounting for 30% of the husk dry weight. Most of the cellulose and lignin of the barley grain is contained within the husk. The husk contains no starch and virtually no sugars (Briggs *et al.*, 1981).

The pericarp lies just below the husk. In developing grains, the pericarp contains chlorophyll and photosynthesizes, causing the grains to be green during development. As the grain matures the cells in the pericarp die and the grains become golden in color (Briggs *et al.*, 1981).

The testa is the seed coat, and is usually fused to the pericarp. It covers the whole grain except a small region at the embryo end where it is penetrated by the micropyle. The cells within the testa are coated with wax, preventing water, oxygen and microbes from entering the grain and soluble compounds, such as amino acid and sugars from exiting the grain. Water can only enter the grain at the base, probably via the micropyle (Briggs *et al.*, 1981).

2.2.2 The Embryo

The embryo contains the sections of the seed required to start germination of the new plant. It accounts for 2-5% of the dry weight of the barley grain and consists of two major sections, the scutellum and the embryonic axis (Briggs *et al.*, 1981). The embryonic axis contains the rootlet and leaf initials. The rootlet initials are covered by the root sheath or coleorhiza. They grow towards the micropyle. The leaf initials are surrounded by the coleoptile. This protects the shoot until it emerges above soil.

In between the endosperm and the embryonic axis is the embryonic tissue known as the scutellum. The layer of cells between the endosperm and the scutellum is known as the scutellar epithelium. Soluble sugars pass from the endosperm to the embryonic axis via the scutellar epithelium and the scutellum. The scutellum, along with the aleurone layer, is thought to be responsible for the production of hydrolytic enzymes (Palmer and Bathgate 1976). The embryo is made up of 34% protein, 14 to 17%

lipids, 20 to 25% soluble sugars, 16% cell walls and 5 to 10% inorganic ash (Briggs *et al.*, 1981).

2.2.3 The Aleurone Layer

The aleurone layer lies between the testa and the starchy endosperm. It is about three cells thick and, together with the scutellum, is responsible for the production of hydrolytic enzymes (Palmer and Bathgate, 1976). The aleurone layer is composed of 42% cell wall material, 7% gums, 20% lipids, 20% proteins, soluble sugars, minerals, anthocyanins and phytic acid (Briggs *et al.*, 1981).

2.2.4 The Starchy Endosperm

The starchy endosperm makes up 75% of the dry weight of the grain (Briggs *et al.*, 1981). It is composed of 84% starch, 9% protein and 7% cell wall material (Briggs *et al.*, 1981). When the grain is mature, the cells in the starchy endosperm are dead and do not contain either nuclei or cytoplasm. The thin walled cells contain starch embedded in a protein matrix. The endosperm is the main source of carbohydrates extracted from barley for use during the malting and brewing processes. Following is a discussion of the three main constituents of the endosperm.

2.2.4.1 The Starch Granules

Barley starch is composed of amylopectin, amylose, protein, lipids and inorganic ions (MacLeod and Wallwork, 1992). The quality and composition of the starch is dependent on both genetic and environmental factors (MacLeod and Wallwork, 1992). Barley starch normally contains 20 to 30% amylose and 70 to 80% amylopectin (Merritt, 1967; Morrison *et al.*, 1984), however both high amylose and low amylose (waxy) genotypes exist. These low and high amylose varieties are generally used for human food or animal feed and are not used in malting and brewing. The ratio of amylose to amylopectin is genetically determined (MacLeod and Wallwork, 1992; Dunn *et al.*, 1996).

Amylopectin consists of D-glucose α-(1,4) linked chains, interconnected with α-(1,6) bonds. This results in small molecules with highly branched complex structures (MacGregor and Fincher, 1993). Amylose consists of α-(1,4) linked D-glucose in long chains, forming a helix in solution. During mashing, amylose is degraded completely to simple sugars by amylolytic enzymes (MacGregor and Fincher, 1993).

Starch in the endosperm of barley is accumulated in starch granules, in two distinct sizes (Mäkelä *et al.*, 1982). Large granules are approximately 25μm in diameter and lenticular in shape. Small starch granules are approximately 2μm in diameter and irregular in shape (Briggs *et al.*, 1981; Van Den Berg and Gheeraert, 1991). Whilst large starch granules make up approximately 90% of the volume of the endosperm they usually only contribute about 10% of the total number of starch granules (Briggs *et al.*, 1981; Mäkelä *et al.*, 1982). However, Goering *et al.* (1973) showed that the number of large starch granules can vary from 2.6% to 15.4% of the total number of starch granules and small starch granules can be from 6.2% to 30.6% of the total starch weight.

There are large differences in the reported values for amylose concentration of small and large starch granules (MacGregor and Fincher, 1993). In general it appears that small granules contain the same amount or slightly less amylose than large granules (Georing and Dehaas, 1974; MacGregor and Ballance, 1980a; Stark and Yin, 1986; MacGregor and Fincher, 1993).

Bathgate and Palmer (Bathgate and Palmer, 1972) measured the protein concentration of small and large starch granules. They found that small granules contain 98% starch and 1.5% protein, the remaining 0.5% being composed of lipids and phosphorus material. Of the 1.5% protein, the majority is on the surface of the granule. Large starch granules only contain about 0.2% protein, 0.1% of which is on the surface.

2.2.4.2 Protein

In general, the protein in the barley grain consists of 30% hordeins, 30% glutelins, 10% globulins and 30% albumins and free amino acids (Brandt, 1976). Much of the protein within the endosperm is bound to the large and small starch granules and this

protein has been shown to be mainly hordeins (Slack *et al.*, 1979). This protein acts as a barrier stopping the starch granules from being attacked by α-amylase (Slack *et al.*, 1979).

2.2.4.3 Cell Wall Material

The cell walls within the endosperm of a barley grain consist of approximately 75% (1→3)(1→4)-β-glucan, 20% arabinoxylan and small amounts of cellulose and glucomannan (Fincher, 1975). In contrast, the cell walls of the aleurone layer contain 71% arabinoxylan and 26% (1→3)(1→4)-β-glucan, while the cell walls of the husk contain high levels of cellulose, silica and lignin with only small levels of (1→3)(1→4)-β-glucan (MacGregor and Fincher, 1993).

(1→3)(1→4)-β-Glucan consists of polymerized chains of glucosyl residues linked at the (1→3) position and the (1→4) position. These chains vary in size, molecular structure and solubility, and form viscous solutions (MacGregor and Fincher, 1993). Overall, the (1→3)(1→4)-β-Glucans contribute between 4 and 7 % of the total weight of the grain (MacGregor and Fincher, 1993) and this is influenced by both genotype and environment (Stuart *et al.*, 1988).

Arabinoxylans consist of mainly arabinose and xylose and are therefore often referred to as pentosans. Barley arabinoxylans usually consist of a backbone of (1→4)-β-xylopyranosyl residues linked together with a single α-l-arabinofuranosyl side chain (MacGregor and Fincher, 1993). These chains vary in size, molecular structure and solubility. Arabinoxylans make up 4-8% of the final grain weight (Han and Schwarz, 1996), the majority of which is in the outer layers. Only 25% of the total arabinoxylans in the grain are in the endosperm (Han and Schwarz, 1996). As with the β-glucans, the arabinoxylans form viscous solutions.

2.3 Malting

Before barley can be used in brewing it must be modified in a process called malting. This involves germinating barley under controlled conditions to produce hydrolytic

enzymes, or in some cases release the hydrolytic enzymes already present from bound forms. At the same time, the cell walls and proteins in the endosperm are partially or totally broken down to expose the starch granules. This process is referred to as modification. The maltster will aim to optimize the level of modification, as too little will hinder the brewing process and too much will cause the loss of valuable starch.

There are three main steps in the malting process- steeping, germination and kilning. Barley is generally stored at 10 to 15% moisture but needs to be at a much higher moisture content to germinate. During steeping, barley is soaked in water to raise the moisture content to between 42 and 46%. This causes the grain to undergo hormonal changes, which trigger the commencement of the germination process. Steeping takes approximately 24 hours (Bamforth and Barclay, 1993).

The second step in malting is germination. During this stage hydrolytic enzymes are produced in both the scutellum and the aleurone layer, and pass into the endosperm. These enzymes attack and breakdown the protein, cell wall material and starch in the endosperm to produce energy for the developing plant. Seedling growth is allowed to proceed until the grain has reached the required level of modification. This is dependant on the level of protein and cell wall modification required by the brewer. In the Australian malting industry, seedlings are usually allowed to grow for 3 to 4 days (Bamforth and Barclay, 1993).

The final step in the malting process is kilning. This involves stopping the germination process by raising the temperature of the grain. Over a 24-hour period the temperature is slowly raised to about 80°C and the moisture content of the grain is reduced to approximately 3 to 4%. Whilst the primary aim of kilning is to stop the germination process, it also produces flavours, colors and aroma compounds that are important for the final product. However, important, unstable enzymes may also be degraded (Bamforth and Barclay, 1993).

2.3.1 Starch Modification

During malting, starch granules undergo a number of changes. These include the partial or complete removal of protective proteins and cell wall material to allow

amylolytic enzymes to attack. Bathgate and Palmer (Bathgate and Palmer, 1973) digested large and small starch granules from both barley and malt with exogeneous α-amylase. They showed that large starch granules in mashes made from malt are hydrolyzed much faster by α-amylase than mashes made from barley. Small starch granules from barley are highly resistant to α-amylase attack during mashing, while small granules from malted barley are more susceptible during mashing. This indicates that the starch granules have undergone changes during the malting process that allow enzymes to attack during the mashing process.

Small starch granules are attacked by enzymes preferentially to large granules during malting. This is probably due to the larger surface area associated with small granules (Bertoft and Kulp, 1986; MacGregor and Morgan, 1986). By the end of the malting process the malt contains about 50% fewer small starch granules than the original barley (Bathgate and Palmer, 1973) while only limited degradation occurs to the large starch granules (MacLeod and Wallwork, 1992).

At least four starch degrading (amylolytic) enzymes are produced within the grain during malting, all of which play a part in the breakdown of starch granules during malting and mashing. These are α-amylase, β-amylase, limit dextrinase and α-glucosidase (Bamforth and Barclay, 1993). The combined activity of these four enzymes is known as diastatic power (DP) and is often measured on malt samples in place of the individual enzymes.

2.3.1.1 α-Amylase

α-Amylase is the main enzyme responsible for the degradation of intact starch granules. It is an endoenzyme that hydrolyses α(1,4) bonds (MacGregor and Fincher, 1993). Since only trace amounts are found in ungerminated barley, the majority of α-amylase is synthesized during malting (MacGregor, 1980). After malting, 7% of the total α-amylase is located in the embryo and 93% in the endosperm. Of the endosperm component, 86.5% is produced in the aleurone layer and 6.5% in the embryo (Briggs, 1964; MacGregor and Marchylo, 1986). MacGregor (1980) showed that degradation of the starch granules appears to occur initially from the scutellum. It

has therefore been suggested that α-amylase originating from the aleurone layer only plays a minor part in the degradation of starch during malting, but plays a large part during mashing (MacGregor, 1980).

α-Amylase in malted barley exists in two isoforms (isoenzymes) that can be separated by isoelectric point (pI) (MacGregor, 1978; MacGregor and Ballance, 1980b). α-Amylase I has a low pI and α-amylase II has a high pI. A third α-amylase isoenzyme was thought to exist but this was shown to be a complex of α-amylase II and a small molecular weight protein (Weselake *et al.*, 1985; MacGregor and Marchylo, 1986). α-Amylase I is more efficient than α-amylase II at degrading starch granules (MacGregor and Morgan, 1986), but both types degrade small starch granules much faster than large starch granules (Bertoft and Kulp, 1986). Large starch granules are highly resistant to attack by α-amylase II but much less resistant to α-amylase I (MacGregor, 1980).

The optimal temperature for α-amylase activity is about 65°C, however after mashing at 75°C for 60 minutes it has been shown that 70% of the activity still remains in the mash (Inkerman *et al.*, 1997). The main products formed by α-amylase degradation of starch granules are small linear dextrins and highly branched dextrins (MacGregor and Morgan, 1986). The highly branched dextrins are the product of the degradation of amylopectin (Merritt, 1967).

2.3.1.2 β-Amylase

β-Amylase is an exoenzyme that removes maltose residues from the non-reducing ends of starch molecules. It is not capable of degrading intact starch granules but instead helps clean up the products from α-amylase attack (Maeda *et al.*, 1978). β-Amylase is accumulated in the starchy endosperm during grain development and in mature barley exists in both a bound and a free form (Sopanen and Laurière, 1989). During germination the bound form is activated and released by proteolytic enzymes (Guerin *et al.*, 1992).

Maltose formation during mashing is due almost entirely to the action of β-amylase, while α-amylase and limit dextrinase only play minor roles (Piendl, 1973; MacGregor and Fincher, 1993). Optimum maltose formation occurs from 60°C to 65°C, while optimum activity for β-amylase is at 63°C (Piendl, 1973).

β-Amylase is not very heat-stable during mashing. β-Amylase activity reduces slowly at a mashing temperature of 63°C, however if the temperature is raised above 63°C the activity level reduces quickly (Stenholm *et al.*, 1996). Consequently, mashes at 63°C produce higher levels of extract and fermentable sugars than mashes at 65°C (Stenholm *et al.*, 1996), which is the normal temperature of an infusion mash. Mashes at 70°C only produced 80% of the fermentable sugars yielded at 63°C (Stenholm *et al.*, 1996).

2.3.1.3 Limit Dextrinase

Limit dextrinase is a debranching enzyme that hydrolyses α-(1,6) glucosidic linkages in amylopectin, and branched α-dextrins (Longstaff and Bryce, 1993). Levels of limit dextrinase are low in ungerminated barley but increase during malting (Manners and Yellowlees, 1973). High levels of limit dextrinase are important to convert branched dextrins to oligosaccharides during mashing (Macri *et al.*, 1993).

During the initial stages of malting, levels of limit dextrinase are low and the majority is found in a bound form. After the fourth day of germination the amount of free limit dextrinase increases and by the seventh day it has reached 65% of the total limit dextrinase (Kristensen *et al.*, 1993). Kilning at 60°C reduces the levels of the total limit dextrinase but has no effect on the levels of the free form (Longstaff and Bryce, 1991). However, kilning at higher temperatures reduces the levels of free limit dextrinase considerably (Kristensen *et al.*, 1993).

Limit dextrinase is more heat stable during mashing and kilning than β-amylase (Sjoholm *et al.*, 1995; Stenholm *et al.*, 1996). Malt with low limit dextrinase activity produces wort with high levels of branched dextrins (Sjoholm *et al.*, 1995). Since

yeast is only capable of digesting simple sugars, these dextrins are not fermented and will remain in the final product.

2.3.1.4 α-Glucosidase

α-Glucosidase releases glucose from α-glucosides. It converts maltose and small dextrins to glucose (MacGregor and Fincher, 1993). Originally it was thought that α-amylase was the only enzyme capable of degrading intact starch granules (Sun and Henson, 1990) but α-glucosidase is also capable of attacking intact starch granules (Sun and Henson, 1990; Sissons and MacGregor, 1994). It has also been demonstrated that α-amylase and α-glucosidase combined can cause greater degradation of starch granules than would be caused due to the product of the enzymes acting separately (Sun and Henson, 1990). It is not understood at this stage whether the α-glucosidase in this synergism is mopping up the products of the α-amylase or attacking the starch granules directly. Sun and Henson (1990) hypothesized that α-glucosidase may be attacking α(1,6) bonds on the starch granules which inhibit α-amylase attack. The lower activity and amounts of α-glucosidase produced during malting suggest it would only have a secondary role to α-amylase during malting (Sissons and MacGregor, 1994).

2.3.2 Protein Modification

During the malting process proteins present in the grain are partially degraded. Most importantly the proteins adhering to the starch granules are degraded, allowing the starch degrading enzymes access to the starch granules. Approximately 50% of protein is degraded during the malting process (Barrrett and Kirsop, 1971), however this is dependant on the malting conditions. During malting, the level of hordeins tends to decrease and the levels of hydrolytic enzymes and amino acids to increase. Initially, endopeptidases (proteases) cause the formation of peptides that in turn are acted upon by exopeptidases (carboxypeptidases) to form amino acids (Briggs *et al.*, 1981).

2.3.2.1:Endopeptidases (proteases)

Two types of proteases are found in malted barley, the sulphydryl dependent (cysteine) enzymes and the metal activated enzymes (Enari and Sopanen, 1986). The cysteine proteases account for 90% of the total protease activity at pH 5.4, the approximate pH of the endosperm during malting (Enari and Sopanen, 1986). The proteases are synthesized in response to gibberellic acid during malting (Hammerton and Ho, 1986). Proteases are heat labile and are rapidly degraded during mashing and are therefore rate limiting in the protein degradation process (Enri and Sopanen, 1986).

2.3.2.2: Exopeptidases (carboxypeptidases)

The carboxypeptidases degrade the peptides produced by the action of the proteases to form amino acids. High levels of carboxypeptidases are present in barley and these levels increase during malting (Enari and Sopanen, 1986). The carboxypeptidases are heat resistant (Bamforth and Barclay, 1993) and are not thought to be limiting the rate of protein degradation.

2.3.3 Cell Wall Material Modification

The breakdown of the cell walls during malting is one of the most important processes involved in the modification of the grain. The presence of the cell wall material within the endosperm can physically hinder the access of amylolytic enzymes to starch granules. Therefore the partial or complete removal of these during malting is important.

As 75% of the cell walls within the endosperm of the grain are composed of (1→3)(1→4)-β-glucans (Fincher, 1975), the processes involved in the breakdown and removal of these has been studied extensively. The three main types of enzymes responsible for the degradation of the β-glucans within the barley grain are endo-(1→3)-β-glucanase, endo-(1→3)(1→4)-β-glucanase and β-glucan solubilase.

Arabinoxylans only make up about 20% of the cell wall material in the endosperm and therefore have not been investigated as thoroughly as the (1→3)(1→4)-β-

glucans. The breakdown of the arabinoxylans during malting may however also play an important role in the quality of the final product and therefore justifies further investigation. Lee (1996) showed that while up to 90% of β-Glucan is degraded during malting, only about 11-20% of the arabinoxylans are degraded. Three endo-(1→4)-β-xylanases have been purified from malt (Slade *et al.*, 1989) and these are thought to be responsible for the degradation of arabinoxylans. Additionally α-L-arabinofuranosidase and β-xylosidase may also be responsible for the degradation of arabinoxylans (Lee *et al.*, 1998). However not much is known about these arabinoxylan degrading enzymes at this stage.

2.3.3.1: endo-(1→3)-β-glucanase

Endo-(1→3)-β-glucanase (EC 3.2.1.39) hydrolyzes consecutive β-(1→3) linkages in mixed linkage β-glucans (Brunswick *et al.*, 1987). However, as only small amounts of consecutive β-(1→3) linkages are present in endosperm cell walls, their influence is minimal. They have been shown to be stable at 60°C for up to 40 minutes (Ballance and Meredith, 1976).

2.3.3.2: endo-(1→3)(1→4)-β-glucanase

Endo-(1→3)(1→4)-β-glucanase (EC 3.2.1.73) hydrolyzes the 1→4 linkages in mixed linkage cell wall β-glucans when the glucosyl residue is substituted at the C3 position (Brunswick *et al.*, 1987). Two isoenzymes of this enzyme are present in germinated barley. The activity of both isoenzymes is reduced during kilning with little or no activity remaining of isoenzyme I (Loi *et al.*, 1987; Barber *et al.*, 1994). Both enzymes are heat labile and are rapidly degraded at 65°C (Loi *et al.*, 1987; Barber *et al.*, 1994).

2.4. Mashing

Brewing of beer involves two main steps, mashing and fermentation. Before the malt can be mashed it must be milled or ground. The ground malt, called grist, is then mixed with warm water to enable the enzymes produced during malting to act upon the constituents of the malt. At a certain temperature, around 60°C, the starch

gelatinizes, allowing the amylolytic enzymes to degrade the majority of the starch into simple sugars, including maltose. Specific enzymes also degrade some of the proteins and cell wall material. Finally the sweet liquid, called wort is separated from the remaining solid material, called spent grain, using either a mash bed or a mash filter.

There are three main methods of mashing; decoction, infusion and temperature programmed mashing (Briggs *et al.,* 1981). Infusion brewing uses a single temperature system of around 65°C, for anywhere from 30 minutes to several hours. At this temperature the starch will be gelatinized but some of the enzymes will degrade quickly. For this reason infusion brewing is generally used on malts that have been well modified with high levels of enzymes and endosperm breakdown (Briggs *et al.,* 1981).

Decoction brewing involves gradually increasing the temperature of the mash from about 35°C to about 65°C. This is done by periodically removing portions of the mash, boiling it and returning it to the mashing vessel. The boiling process completely degrades the enzymes in that portion but the overall slow increase in temperature of the mash allows the enzymes a greater time to act. For this reason it is suitable for malts that are less well modified (Briggs *et al.,* 1981).

The third type of brewing is called temperature programmed mashing. This involves gradually raising the temperature of the entire mash using a predetermined programme. Temperature programmed mashing allows the enzymes to act for a greater length of time, in a similar way to decoction mashing without the destructive boiling phase and is also suitable for less well modified malts (Briggs *et al.,* 1981).

After filtration, the wort is boiled with hops to release the flavours and aromas of the hops and sterilize the wort. This arrests all enzyme activity. The wort is cooled and moved to a fermentation vessel where yeast is added (Briggs *et al.,* 1981).

Fermentation is the process by which the yeast converts simple sugars, produced during mashing, into ethanol and carbon dioxide. The wort must contain not only

high levels of simple sugars, but also adequate levels of amino acids and oxygen for yeast reproduction and nutrition. Compounds in the wort not utilized by the yeast will pass through the brewing process and add flavours and aromas to the final product (Briggs *et al.*, 1981).

The resultant carbohydrate and protein profiles of the wort differ greatly between different malting and mashing regimes, but in general 92% of the solids in wort are carbohydrates (Anderson, 1966) and 5-6% are nitrogenous compounds (Buckee *et al.*, 1976). A typical breakdown of the carbohydrates in wort is:

Dextrins	22%
Maltotetrose	6%
Maltotriose	14%
Maltose	41%
Sucrose	6%
Glucose and Fructose	9%

(Harris *et al.*, 1955).

2.4.1 Starch

The most important process during mashing is the gelatinization of starch granules. The gelatinization of starch is defined as "the collapse of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization" (Atwell *et al.*, 1988). Gelatinization involves the breakdown of the crystalline structure of the starch granule allowing amylolytic enzymes to attack. When starch is mixed with water of increasing temperatures, a number of things happen. Initially the granules slowly expand in volume. At a particular temperature the granules undergo rapid and irreversible swelling, causing the viscosity of the medium to increase. Eventually the molecular order of the starch granule is disrupted and the contents of the starch granule are spilled into the surrounding medium for easy attack by hydrolytic enzymes (MacGregor and Fincher, 1993). The gelatinization temperature of malt starch ranges from about 56-64°C (MacGregor and Fincher, 1993) but this is dependent on both genotype and the environment in which the barley was grown (Tester *et al.*, 1991).

During mashing it is important that a high enough temperature is achieved for the gelatinization of starch. However if the temperature is raised too high, the amylolytic enzymes will be denatured. It has been shown that the activities of α-amylase, β-amylase and limit dextrinase are lower when mashed at 75°C then when mashed at 65°C (Inkerman *et al.*, 1997). Inkerman *et al* (1997) studied the activity of α-amylase, β-amylase and limit dextrinase in wort and found that only α-amylase approached 100% of its total activity in the malt. In contrast β-amylase and limit dextrinase activity only reached 10% and 30% respectively. In fact, the β-amylase activity was no longer measurable after ten minutes at 65°C.

Stenholm *et al.* (1996) studied the effect of mashing temperatures on malt extract formation and fermentable sugar levels. Initially, with the temperature at 48°C, both fermentable sugar and extract levels were low. As the temperature was raised to 63°C, the starch was gelatinized and the levels of extract and fermentable sugars dramatically increased. After a rest at 63°C, the temperature was raised further to 72°C. During this period, the extract and fermentable sugar levels only increased slightly, due to deactivation of β-amylase. This was confirmed by Inkerman *et al* (1997) who showed that the optimal mashing temperature for β-amylase activity was 50°C. However, at this temperature the starch would not be gelatinized (MacGregor, 1980; MacGregor and Ballance, 1980a) and since it cannot act on intact starch granules it could only mop up the products of α-amylase (Maeda *et al.,* 1978). The optimal α-amylase activity was shown to be 60°C and it was only 70% active at 50°C (Inkerman *et al,* 1997).

Large starch granules are hydrolysed preferentially to small granules during mashing (Bathgate and Palmer, 1973). Small starch granules gelatinize at a higher temperature than large starch granules (MacGregor, 1980). Large starch granules gelatinize from 56 to 59°C and would therefore be completely gelatinized during mashing (MacGregor and Ballance, 1980a). Small starch granules gelatinize from 63 to 65°C and are therefore less open to attack during mashing (MacGregor, 1980; MacGregor and Ballance, 1980a). Small granules also have proteins that adhere to their surface

and act as a barrier to attack by enzymes (Macleod and Wallwork, 1992). At the end of mashing, intact small starch granules can be found in the spent grain (Bathgate *et al.*, 1973). These are found in clumps with amorphous proteins and gums (Bathgate *et al.*, 1973).

2.5. Biochemical basis of Malt Extract

Malt extract is a measure of all of the soluble material that can be extracted from the malt during mashing. This material includes the soluble products of the degradation of carbohydrates and proteins that have been formed during the malting and mashing processes.

In the laboratory malt extract is usually estimated by a Hot Water Extract (HWE) procedure. This method of analysis involves the measurement of the level of dissolved solids produced by a laboratory small-scale mash (Briggs *et al.*, 1981). Although this may differ greatly from what would actually occur in the brewhouse, it still gives an indication of how a particular malt or malting variety will perform.

There are three groups that produce recommended methods of analysis of malt. These are:

- American Society of Brewing Chemists (ASBC)- mainly used in North America
- The Institute of Brewing (IOB)- mainly used in Britain
- The European Brewing Convention (EBC)- mainly used in mainland Europe

Asia and Australia use a mixture of all three methods of analysis.

All three of these groups have a method for measuring HWE (ASBC methods of analysis, 1987; EBC anlaytica, 4.5.1, 1987; IoB methods of analysis, 2.3, 1991). The IOB method is based upon the infusion method of brewing (Section 2.4), where water is added to the ground malt at 65°C and held there for an hour. The ASBC and EBC methods are idebtical and based on a temperature programmed mash (Section 2.4) where water is added to the ground malt at 45°C and held there for 30 minutes. The

temperature is raised to 70°C over the next 25 minutes at 1° per minute and is held there for another hour (Figure 2.5.1). Both of these methods have slightly different effects on the biochemistry of the mash, in particular on malt enzymes.

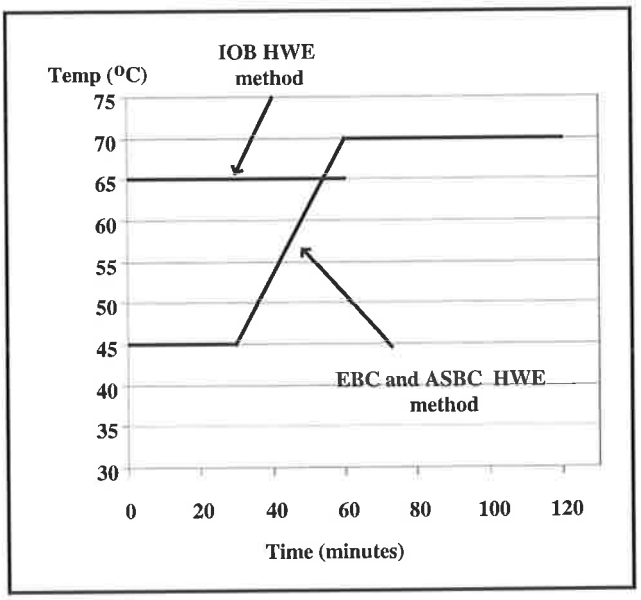


Figure 2.5.1 A schematic representation of the recommended methods of measuring Hot Water Extract showing the different temperatures and time involved in each method.

A number of studies have been carried out comparing malt extract and a number of other traits (Table 2.5.1 and Table 2.5.2). Correlation analysis of components of malt extract can give a greater understanding of malt extract but a causal relationship between malt extract and the variable components must be established. Some significant correlations with malt extract may be due to indirect effects and therefore bias the results obtained. Also differences will occur between correlations studies that investigate "between variety" differences, those based on differences between a few replicates of numerous varieties, and studies that investigate "within variety" differences, those based on numerous replicates of a single or a few varieties. For example the significant negative correlation between malt extract and grain protein is well documented (Anderson *et al.*, 1940; Edmunds *et al.*, 1993; Janes and Skerritt, 1993; Molina-Cano *et al.*, 2000). However this relationship is strongest when established on variation within a single variety. If you were to look at a single

measurement for range of good and poor malting quality varieties the relationship would be much weaker and in some cases no longer significant.

2.5.1 Relationship between malt extract and the physical barley and malt traits

The relationship between malt extract and a number of physical traits is shown in (Table 2.5.1). Strong negative relationships have been established between milling energy and HWE (Alison *et al.*, 1979; Alison, 1986; MacLeod *et al.*, 1993). Milling energy is a measure of the hardness of the barley grain. Varieties with low milling energy have loosely packed endosperm and are referred to as "mealy". Varieties with high milling energy have a tightly packed or "steely" structure. It has been suggested that tightly packed endosperm may slow the movement of enzymes during malting, reducing the modification of the grain (MacLeod and Wallwork, 1992). This was confirmed in a study in which grain hardness was measured using the single kernel classification system (SKCS) (Alexander *et al.*, 1997). One study however has contradicted this (Edmunds *et al.*, 1993) who found no significant relationship between grain hardness and HWE.

2.5.2 Relationship between malt extract and the components of the barley and malt

Malt extract has been compared to the components of the barley and malt in a number of studies (Table 2.5.2). Morgan *et al.* (1983a and 1983b) investigated a number of β-glucan and protein properties of the malt and related these to malt extract. The β-glucan related properties investigated were total β-glucan, soluble β-glucan, falling time, β-glucan solubilase activity and endo-β-glucanase activity. They found that these could account for 76% of the variation in malt extract (Morgan *et al.*, 1983b). They found significant negative correlations between malt extract and the levels of β-glucan soluble in acid and falling time but not the activities of the β-glucan degrading enzymes (Morgan *et al.*, 1983a). In contrast, Stuart *et al.* (1988) found significant ($P<0.01$) positive correlations between malt extract and β-glucanase activity.

The protein related properties investigated by these authors were nitrogen, free amino acids and endopeptidase activity (Morgan *et al.* 1983b). These factors accounted for

32% of the variation in malt extract when measured in the endosperm only but only 6.6% if these traits were measured on the whole grain. Janes and Skerrit (1993) investigated the relationship between the hordein protein and malt extract and found significant negative correlations between groups of the B and D hordeins and malt extract.

Whilst in theory, lowering the husk content of grain sounds like a practical solution to increasing the levels of malt extract, the impact this would have on the hardiness of the grain during development remains to be investigated. Since the husk is also used as a filter to aid in the lautering process the theoretical lower limit of the amount of husk required before the filtering process is adversely affected would need to be investigated.

Relationships established between starch related traits and malt extract can be rather confusing, with many contradictions within the literature (Table 2.5.1). Considering approximately 65% of the barley grain consists of starch (Table 2.2.1) and the products of the degradation of this starch represent the majority of the carbohydrates and sugars within the wort, it should be safe to assume that malt extract would be dependent on the amount of starch contained within the grain. In reality this relationship is vague with some studies finding no relationship with malt extract (Allen *et al.*, 1995, Dunn *et al.*, 1996) and others finding a significant relationship (MacLeod *et al.* 1993, Dunn *et al.*, 1996). This would indicate that even though it is important to have high levels of starch within the grain there must be other factors governing the ability of the starch to yield high malt extract. These factors are likely to include other starch related traits such as granule size and gelatinization temperature. Also, to this authors knowledge there have been no studies comparing the level of malt starch with malt extract.

2.5.3 Relationship between malt extract and the starch properties

Significant correlations have been found between malt extract and the diameter of the large starch granules (Table 2.5.1) (Dunn *et al.*, 1996, Dunn *et al.*, 1997, Allan *et al.*, 1995). In all three of these studies no significant correlations were found between malt extract and small starch granule diameter. This may be due to the preferential

hydrolysis of large starch granules during the mashing process (Bathgate and Palmer, 1973) and the lower gelatinization temperature of the large starch granules (MacGregor, 1980). However, Oliveira *et al.* (1994) found a significant correlation between surface area and volume of the small granules and malt extract but not the surface area and volume of the large granules. They found that malting varieties in general had larger large starch granules than poor malting quality varieties, but overlaps occurred between the two groups. Dunn *et al.*, (1996) found a relationship between the size of the small starch granules and large starch granules indicating that an overall increase in the size of starch granules may increase the levels of malt extract.

Variations in the gelatinization temperature of different malts may cause malts to have differing malt extract potentials. Stenholm *et al.* (1996) studied the effects on malt extract of malts varying in gelatinization temperature, as measured by differential scanning colorimetry (DSC). They compared two samples with malt gelatinization temperatures of 63°C and 67°C. The starch from the sample with the lower gelatinization temperature would have been completely gelatinized at temperatures where the enzymes were most active. For the second sample the levels of β-amylase would have been low by the time the starch gelatinization was complete. The sample with the lower malt gelatinization temperature produced a 6% higher malt extract than the second sample. It must be noted however that the sample with the higher gelatinization temperature also had lower levels of amylolytic enzymes prior to mashing, which may also have influenced the levels of malt extract. The varieties used were not mentioned in the study and it is unclear whether the differences in the malt gelatinization temperatures were varietal based, or due to differences in modification of the same variety. Inadequately modified proteins adhering to the starch granules would not only increase the gelatinization temperature of the starch but also hinder the access of the amylolytic enzymes to the starch granules, reducing the levels of malt extract.

Dunn *et al.* (1997) found no significant varietal or environmental differences in gelatinization temperature of purified barley starch using DSC. Since it is likely that protein and cell wall material adhering to the starch granule would have an influence

on the gelatinization temperature of the starch, differences are more likely to be evident when investigating starch in its native malt environment. It is possible, therefore, that studies looking at the gelatinization temperature of purified starch may give false or misleading information. For example Dunn *et al.* (1997) investigated the pasting properties of a number of varieties using Rapid Visco Analysis (RVA) and found a strong correlation between peak time on purified barley starch and malt extract (r=-0.95). In contrast, when they investigated the barley flour they found no significant correlation with malt extract. This would indicate that proteins and other carbohydrates either masked or changed the starch properties. As the pasting properties were assessed on barley flour, the starch and other material would have undergone changes during the malting process and are therefore likely to have reacted differently to both the purified starch and barley flour.

2.5.4 Relationship between malt extract and the malt enzyme levels

The relationships between amylolytic enzyme levels and malt extract established in the literature are also confusing (Table 2.5.1). Smith and Gill (1986) showed that varieties with higher α-amylase activity had the least amount of starch remaining in the spent grain and the highest malt extract. However they only investigated four varieties grown at one site in one year. Others have found no significant correlations between amylolytic enzyme levels and malt extract levels (Janes and Skerritt, 1993, MacLeod *et al.*, 1993). Arends *et al.*, (1995) found a significant correlation with DP, however it was not a strong relationship (r=-0.29). Surprisingly there was a much stronger relationship with β-amylase and no significant relationship was found with α-amylase. Like protein, the relationship between malt extract and DP identified in the literature is confusing. In general, there is a strong negative relationship between malt extract and DP within a single variety. This is due to the direct influence of protein on each of the two traits. Since enzymes are proteins, their levels increase as protein content increases. Conversely, the more protein there is in the grain the less starch there is, leading to a decrease in the level of malt extract. However the relationship between malt extract and DP can be expected to be different when a number of varieties are investigated. For a number of years breeders have concentrated on selecting malting varieties with high levels of DP and high malt extract. It is likely that there is now an excess of enzymes in high quality malting

varieties and therefore it is likely that enzyme levels no longer have a limiting influence on malt extract. Therefore varieties chosen to investigate the relationship between the levels of DP and malt extract would have a large influence on the significance of the relationship found. For example you would expect to find a different relationship in a study that compares good and poor malting quality varieties and a study that compares just good quality malting varieties.

Many studies have related a number of different malt and barley properties to malt extract. These studies often differ in regard to the relationships found with malt extract. To more fully understand malt extract, an understanding of how the various components of the barley change during the malting and mashing process is needed. For this reason the following study investigates the starch, cell wall material, protein and husk in the barley, malt, wort and spent grain. Other traits, such as grain hardness, starch properties and enzyme levels that have been highlighted in the literature as possibly influencing malt extract have also been investigated.

Table 2.5.1 Correlation studies between Malt Extract and a number of Starch and Cell Wall traits.

	Trait	Correlation[a]	P	Reference	Comment
Starch	Barley Starch Content	-0.09	ns	Allan *et al.*, 1995	
	Barley Starch Content	0.47	ns	Dunn *et al.*, 1996	1993 data
	Barley Starch Content	0.42	P<0.05	Dunn *et al.*, 1996	1994 data
	Barley Starch Content			MacLeod *et al.*, 1993	
	Large starch granule diameter	0.65	P<0.001	Allan *et al.*, 1995	(>4.8μm)
	Proportion of granules	0.69	P<0.001	Allan *et al.*, 1995	Between 4.8 and 18.2
	Proportion of granules	0.59	P<0.01	Allan *et al.*, 1995	>18.2μm
	Proportion of small granules	-0.54	P<0.01	Dunn *et al.*, 1997	
	Mean large starch granule diameter	0.75	P<0.001	Dunn *et al.*, 1997	
	Starch granule surface area	0.43	P<0.05	Oliveira *et al.*, 1994	
	Volume of B (small) granules	0.43	P<0.05	Oliveira *et al.*, 1994	no other correlations
	Mean large starch granule diameter	0.78	P<0.001	Dunn *et al.*, 1996	
	RVA Peak time	-0.95	P<0.001	Dunn *et al.*, 1997	starch
	RVA Peak time		ns	Dunn *et al.*, 1997	barley flour
Cell Wall	Malt β-Glucans	-0.94		Lee, 1997	
	Malt β-Glucans	-0.74	P<0.01	Stuart *et al.*, 1988	
	Malt β-Glucans	-0.62	P<0.01	Ingversen and Jorgensen, 1989	
	Barley β-Glucans	0.46	P<0.001	Allan *et al., 1995*	
	Barley β-Glucans	-0.33	P<0.05	Henry, 1985	
	Barley β-Glucans	0.15	ns	Stuart *et al.*, 1988	
	Barley β-Glucans	-0.53	P<0.05	Henry, 1986	
	Malt Arabinoxylans	-0.74		Lee, 1998	
	Barley Arabinose	-0.42	P<0.05	Henry, 1986	
	Barley Xylose	0.44	P<0.05	Henry, 1986	
	Barley Pentosan	-0.47	P<0.05	Henry, 1986	
	Barley Pentosan and β-Glucan	-0.61	P<0.05	Henry, 1986	

[a]Pearsons correlation (r) unless stated otherwise.

Table 2.5.2 Correlation studies between Malt Extract and a number of Protein, Enzyme, Wort and Physical traits.

	Trait	Correlation[a]	P	Reference	Comment
Protein	Grain Nitrogen	-0.75	P<0.05	Arends *et al.*, 1995	
	Grain Nitrogen	-0.68	P<0.05	Henry, 1985	
	Grain Protein	-0.53	P<0.001	Allan *et al.*, 1995	
	Grain Protein	-0.63	P<0.001	Dunn *et al.*, 1997	
	Grain Protein	-0.87	P<0.001	Dunn *et al.*, 1996	1994
	Grain Protein	-0.80	P<0.001	Dunn *et al.*, 1996	1993
	Grain Protein	-0.45	P<0.001	Janes and Skerritt, 1993	8 variety set
	B hordeins	0.53	P<0.001	Janes and Skerritt, 1993	8 variety set
	D hordeins	0.41	P<0.05	Janes and Skerritt, 1993	8 variety set
	D and B hordeins	-0.52	P<0.001	Janes and Skerritt, 1993	8 variety set
Enzyme	DP	-0.07	ns	Janes and Skerritt, 1993	8 variety set
	DP	-0.29	P<0.05	Arends et al., 1995	
	α-amylase	0.00	ns	Arends *et al.*, 1996	
	ß-amylase	-0.64	P<0.05	Arends *et al.*, 1997	
	limit dextrinase	0.36	P<0.05	Arends *et al.*, 1998	
	Malt ß-Glucanase	0.85	P<0.01	Stuart *et al.*, 1988	
	Malt ß-Glucanase			MacLeod *et al.*, 1993	
Physical	Grain Hardness	r^2=0.80	P<0.001	Alexander *et al.*,1997	SKCS (neg)
	Grain Milling Energy	-0.59		Allison *et al.*, 1979	
	Grain Milling Energy	-0.79		Allison *et al.*, 1979	1 variety removed
	Grain Milling Energy	-0.71		Allison *et al.*, 1986	
	Grain Milling Energy	0.03		Edmunds *et al.*, 1993	
	Grain Size	0.55	P<0.05	Arends *et al.*, 1995	
	Screenings 2.2-2.5mm	-0.33	P<0.05	Henry, 1986	

[a]Pearsons correlation (r) unless stated otherwise.

2.6. Molecular basis of Malt Extract

For more than 100 years, barley breeding programs have been established around the world (Nilan and Ullrich, 1993). These programs have aimed at selecting new varieties with improvements in environmental adaptation, disease resistance and food, feed and malting quality. Screening germplasm for such traits has often been time consuming, expensive and destructive. The screening assays have often required large amounts of seed and consequently many traits have not been measured until several generations after the original cross was made. Most malting quality traits fall into this category, malt extract in particular. In recent years breeding programs have been implementing new techniques to improve the efficiency of the selection process. One of these methods is marker assisted selection (MAS). MAS involves finding a marker located in a region of the genome that has been shown to be linked to a trait of interest and screening the germplasm for the presence or absence of that marker. The main benefit of MAS is that DNA can be extracted from a small sample of leaf material. The method is non destructive to the plant and a number of traits can be screened using a single DNA sample.

Before breeders can use MAS, appropriate markers need to be found. There are six main steps involved in finding useful markers for MAS. These include:

1. *The development of a suitable mapping population.* A barley mapping population is developed by crossing two or more parents to produce F_1 lines. The F_1 are either used to produce doubled haploids or allowed to self through several generations to approach homozygosity.
2. *Establishment of a linkage map for the population.* A linkage map is produced by screening the entire population for the presence or absence of marker loci. There are a number of different marker systems that have been developed that are useful for mapping. These include restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), single sequence repeats or microsatellites (SSR), random amplified polymorphic DNA (RAPD) and a number of other systems. A linkage map is then constructed by calculating the recombination frequencies between individual markers in the population (Jones *et al.*, 1997).

3. *Phenotyping the population for traits of interest.* The entire population is screened for the trait of interest
4. *Establishment of a relationship between marker loci and the phenotypic data.* The genotypic and phenotypic data are statistically compared to establish regions of the genome that are associated with the trait. These are known as quantitative trait loci (QTL).
5. *Validating the marker loci in alternative genetic backgrounds.* Before a marker locus can be used for MAS it is important to establish its usefulness in alternate genetic backgrounds.
6. *Implementing the markers into the breeding program.* If the validation process is successful the marker may now be implemented into the breeding program

2.6.1 QTL found to be associated with Malt Extract

Hayes *et al.* (2001) reported that there are 181 QTL reported in the literature for 29 barley and malt quality phenotypes. These are summarized on the web site http:/www.css.orst.edu/barley/nabgmp/qtlsum.htm. This summary shows a BinMap created by Kleinhofs and Han (2002). Each chromosome has been divided into a number of bins and the QTL have been assigned to these bins. QTL for malt extract have been identified in 8 populations developed by researchers from all over the world. These are schematically shown in Figure 2.6.1, which is based on this BinMap. The populations include:

Steptoe/ Morex: Two "six row" varieties, grown and mapped in USA. Steptoe is a low extract feed quality variety and Morex is a malting quality variety (Hayes *et al.*, 1993; Hayes *et al.*, 1997; Ullrich *et al.*, 1997).

Dictoo/ Morex: Two "six row" varieties. Also grown and mapped in the USA. Dictoo is a winter variety and Morex is a spring variety (Oziel *et al.*, 1996).

Harrington/ TR306: Two "two row" varieties. This population was grown and mapped in Canada. Harrington is a high extract malting variety and TR306 is a feed quality line (Hayes *et al.*, 1997; Mather *et al.*, 1997).

Harrington/ Morex: A cross between two high extract malting quality varieties. These two varieties are the "two row" and "six row" malting quality standards for North America (Marquez-Cedillo *et al.*, 2000).

Calicuchuma –sib/ Bowman: Calicuchima-sib is an ICARDA/CIMMYT "six row" variety; Bowman is a "two row" variety. This population was grown and mapped in the USA (Hayes *et al.*, 1996; Hayes *et al.*, 1997).

Blenheim/ E224/3 and Blenheim/ Kym: These populations were grown in the UK. They are the only populations where the extracts were measured using the IOB method (Thomas *et al.*, 1995; Bezant *et al.*, 1997a; Hayes *et al.*, 1997; Powell *et al.*, 1997).

Chebec/ Harrington: This was grown and mapped in Australia by the National Barley Molecular Marker Program (NBMMP). This is a project involving a number of different laboratories and breeding programs around Australia. Results for this population are as yet unpublished.

Two other populations, developed in Australia, have been used for mapping malting quality traits but not malt extract. These are Galleon/ Haruna Nijo and Clipper/ Sahara (Karakousis *et al.*, 2003a; Karakousis *et al.*, 2003c).

The most extensively studied population is Steptoe/ Morex. Malt extract was first measured in this population in 1991 from four sites and this was repeated in 1992 at a further five sites (Hayes *et al.*, 1993; Hayes *et al.*, 1994; Ullrich *et al.*, 1997). QTL for malt extract were identified on all chromosomes except 3H. However, a number of these were only identified using data from individual sites and consequently have not been included in the summary by Hayes *et al.* (2001). All of the QTL in this summary, from this population have Morex donating the higher malt extract allele (Figure 2.6.1), however Steptoe is responsible for two of the QTL that were identified using data from a single site only (Hayes *et al.*, 1994).

Morex was also used as a parent to develop two other mapping populations, namely Harrington/ Morex and Dictoo/ Morex. Only one region, on the short arm of chromosome 2H, was found to be significantly associated with malt extract in all three of these populations. Morex is responsible for donating the higher malt extract allele in this region, for all three populations. Four other significant regions were also found to be associated with malt extract in the Dictoo/ Morex population, three regions on chromosome 5H and one on chromosome 3H (Oziel *et al.*, 1996).

Two other significant regions were found to be associated with malt extract in the Harrington/ Morex mapping population, both on chromosome 1H (Table 2.6.1) (Marquez-Cedillo *et al.*, 2000). Harrington is responsible for donating the higher allele at these two regions. The region on the long arm of chromosome 1H is flanked by a region found to be associated with malt extract in two other populations with Harrington as a parent (Harrington/ TR306 and Chebec/ Harrington), however in both of these cases Harrington donated the lower allele (Mather *et al.*, 1997; Hayes *et al.*, 2001; Collins *et al.*, 2003).

A region was found to be associated with malt extract on the long arm of chromosome 5H in the two populations Chebec/ Harrington and Harrington/ TR306 (Mather *et al.*, 1997; Hayes *et al.*, 2001; Collins *et al.*, 2003). Harrington donated the higher allele in both populations. Malt extract was also found to be associated with a region on the short arm of chromosome 5H, in the population Harrington/ TR306. This region was not found to be associated with malt extract in any other population.

The other two populations with a common parent are Blenheim/ E224/3 and Blenheim/ Kym (Thomas *et al.*, 1996; Bezant *et al.*, 1997a; Powell *et al.*, 1997). Thomas *et al.* (1996) found 18 regions in the Blenheim/ E224/3 mapping population, that were associated with HWE, and another 22 regions that were associated with HWE when adjusted to a grain nitrogen level of 1.5%. Of these, only four regions were found to be associated with HWE at more than one site. Powell *et al.* (1997) found a further three regions associated with HWE in that population, one of which was in common with the regions Thomas *et al.* (1996) found. Three of these regions could not be assigned to a bin and are not shown in Figure 2.6.1.

Despite Kym generally having lower malt extract than Blenheim, it was responsible for donating the higher allele at five of the eight regions found to be associated with HWE in the Blenheim/ Kym population (Bezant *et al.*, 1997a). Three regions could not be assigned to a bin and are not shown in Figure 2.6.1. Only a single region was found to be significantly associated with HWE in both the Blenheim/ E224/3 and Blenheim/ Kym populations (Thomas *et al.*, 1996; Bezant *et al.*, 1997a; Powell *et al.*, 1997). This region is on the short arm of chromosome 2H and has Blenheim donating the higher HWE allele.

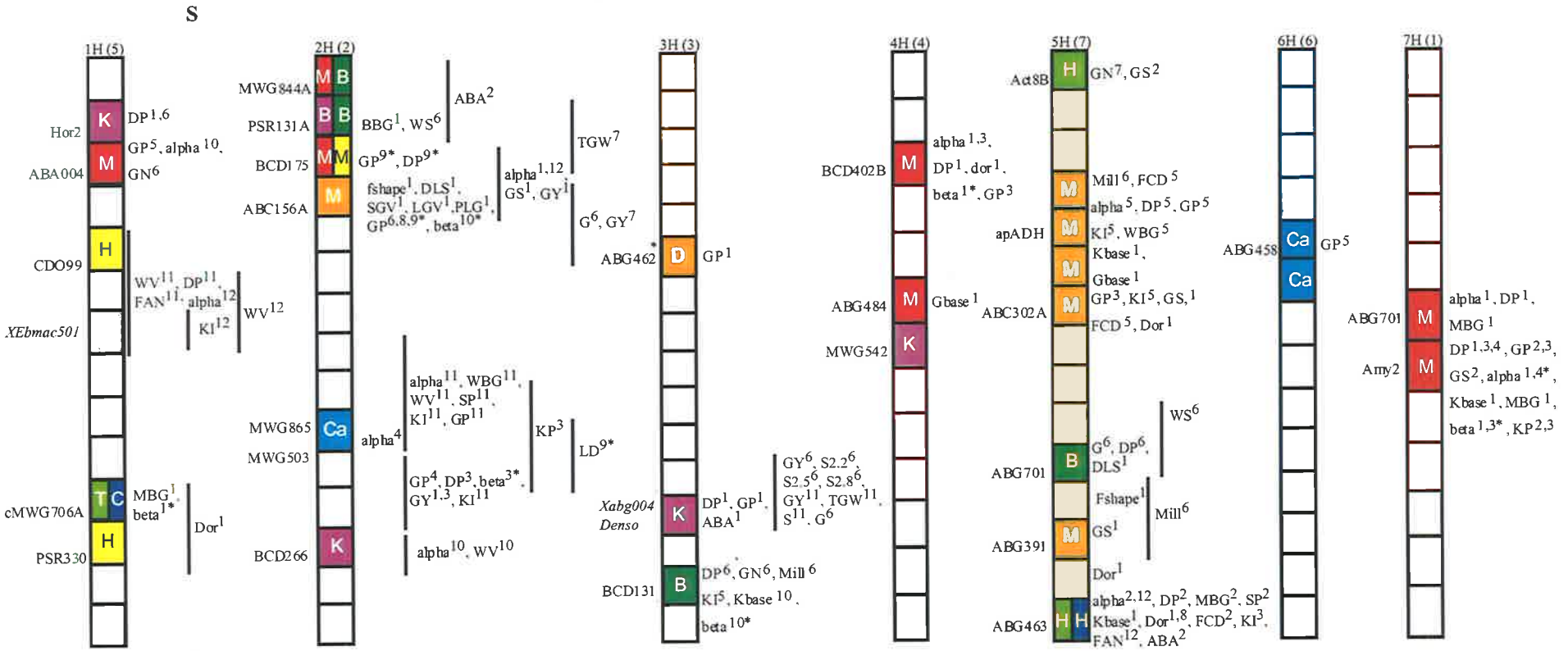
QTL have been found for many other traits associated with malting quality, including many of the traits that were discussed in Section 2.5.4. A single region of the genome is often found to influence a number of different traits. QTL that have been found to be coincident with malt extract QTL are shown schematically in Figure 2.6.1. The majority of regions found to be associated with malt extract have also been found to be associated with other physical or malting quality traits. Whether these regions contain a gene 'cluster', number of closely linked individual genes controlling each trait separately or a single gene that has pleiotropic effects on each trait is currently unknown. However useful information about how a trait of interest is influenced by a region can be gained by investigating other traits that are also associated with that region.

In total more than 24 individual chromosome regions have been found to be associated with malt extract (Figure 2.6.1). The use of molecular markers linked to these regions has the potential to significantly increase the effectiveness of selection of new varieties in barley breeding programs. Before this can occur, it must be established which of these regions are present in material used in Australian breeding programs and whether any other regions are also present. Additionally, the impact of using markers in these regions in breeding populations to select for malt extract needs to be assessed. An understanding of how these regions will not only influence malt extract, but other traits also influenced by the genes in these regions, will ensure no adverse effects occur with the use of MAS.

Figure 2.6.1 A schematic representation of malt extract QTL, based on Hayes *et al.*, (2001), http:/www.css.orst.edu/barley/nabgmp/qtlsum.htm. Each chromosome is separated into a number of regions called Bins as described by Kleinhofs and Han (2002). Coloured squares represent regions found in mapping populations around the world. Markers are listed left of each chromosome. Listed right of the chromosome are other traits found to be associated with each region.

Reference

1. Hayes *et al.,* 1993; Han *et al.,* 1995; Oberthur *et al.,* 1995; Zwickert-Menteur *et al.,* 1996; Han *et al.,* 1997; Larson *et al.,* 1996; Mano and Takeda, 1997; Ullrich *et al.,* 1997; Borem *et al.,* 1999; Zale *et al.,* 2000; Hayes *et al.,* 2001
2. Mano and Takeda, 1997; Mather *et al.,* 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001
3. Marquez-Cedillo *et al.*, 2000; Zale *et al.,* 2000; Hayes *et al.,* 2001
4. Hayes *et al.*, 1996; Hayes *et al.*, 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001
5. Oziel *et al.*, 1996; Zale *et al.,* 2000; Hayes *et al.,* 2001
6. Thomas *et al.*, 1995; Thomas *et al.*, 1996; Powell *et al.*, 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001
7. Bezant *et al.*, 1997a; Bezant *et al.*, 1997b; Zale *et al.,* 2000; Hayes *et al.,* 2001
8. Li *et al.,* 1996; Zale *et al.,* 2000; Barr *et al.*, 2003a; Collins *et al.,* 2003
9. Li *et al.,* 1996; Zale *et al.,* 2000; Karakousis *et al.*, 2003a
10. Zale *et al.,* 2000; Karakousis *et al.*, 2003c
11. Barr *et al.*, 2003b; Panozzo *et al.*, in preparation
12. Moody, unpublished; Panozzo *et al.,* in preparation
13. Asamaya unpublished; Pallotta *et al.,* 2003


S
1H (5)
Hor2
ABA004
CDO99
XEbmac501
cMWG706A
PSR330
DP1,6
GP5, alpha10,
GN6
WV11, DP11,
FAN11, alpha12
KI12
WV12
MBG1,
beta1*
Dor1
2H (2)
MWG844A
PSR131A
BCD175
ABC156A
MWG865
MWG503
BCD266
BBG1, WS6
ABA2
GP9*, DP9*
fshape1, DLS1,
SGV1, LGV1, PLG1,
GP6,8,9*, beta10*
alpha1,12
GS1, GY1
TGW7
G6, GY7
alpha4
alpha11, WBG11,
WV11, SP11,
KI11, GP11
KP3
LD9*
GP4, DP3, beta3*,
GY1,3, KI11
alpha10, WV10
3H (3)
ABG462*
Xabg004
Denso
BCD131
GP1
DP1, GP1,
ABA1
GY6, S2.26,
S2.56, S2.86,
GY11, TGW11,
S11, G6
DP6, GN6, Mill6
KI5, Kbase10,
beta10*
4H (4)
BCD402B
ABG484
MWG542
alpha1,3,
DP1, dor1,
beta1*, GP3
Gbase1
5H (7)
Act8B
apADH
ABG701
ABG391
ABG463
ABC302A
GN7, GS2
Mill6, FCD5
alpha5, DP5, GP5
KI5, WBG5
Kbase1,
Gbase1
GP3, KI5, GS,1
FCD5, Dor1
WS6
G6, DP6,
DLS1
Fshape1
Mill6
GS1
Dor1
alpha2,12, DP2, MBG2, SP2
Kbase1, Dor1,8, FCD2, KI3,
FAN12, ABA2
6H (6)
ABG458
GP5
7H (1)
ABG701
Amy2
alpha1, DP1,
MBG1
DP1,3,4, GP2,3,
GS2, alpha1,4*,
Kbase1, MBG1,
beta1,3*, KP2,3
L
Steptoe (S)/ Morex(M) 1
Harrington (H)/ TR306 (T) 2
Harrington (H) / Morex (M) 3
Calicuchuma (Ca) / Bowman (B) 4
Galleon / Haruna nijo 9
Clipper / Sahara 10
Sloop / Alexis, Sloop-sib / Alexis 11
Dictoo (D) / Morex (M) 5
Blenheim (B) / E224/3 (E) 6
Blenheim (B) / Kym (K) 7
Chebec (C) / Harrington (H) 8
Arapiles /Franklin (F) 12
alpha = alpha amylase
DP = diastaic Power
beta = beta amylase
LD = Limit Dextrinase
Kbase = kilned beta glucanase activity
Gbase = green malt beta glucanase activity
MBG = malt beta glucan
WBG = wort beta glucan
BBG = barley beta glucan
FAN = wort free amino nitrogen
WV = wort viscosity
GP = grain protein
SP = soluble protein
GN = grain nitrogen
KI = kolback index, sol./tot prot
Dor = dormancy
WS = water sensitivity
GS = speed of germination
G = germination
GY = grain yield
S = screenings
Mill = milling energy
FCD = fine course difference
Fshape = mean F shape of small granules
DLS = mean max. diam large granules
SGV = mean strach granule volume
LGV = mean vol large granules
PLG = proportion of large granules
KP = Kernel plumpness
ABA = ABA response during germination
TGW = Thousand grain weight

Chapter 3

Material and Methods

3.1 Micromalting

Screened samples (2.2mm) were malted using a Phoenix Automated Micromalting System® according to the schedule shown in Table 3.1. The program includes a 22hour steep (7 hours wet, 10 hours dry, 5 hours wet) and a 94.5 hour germination at either 15°C or 17°C followed by a 20 hour kiln cycle.

Table 3.1: The Schedule used to malt the samples used in the experiments throughout this work.

Stage	Cycle (hours:minutes)	Set Temperature (° C)	Start Temperature (° C)	End Temperature (° C)
Wash	0:15			
Steep 1	7:00	17/15		
Germination 1	10:00	17/15		
Steep 2	5:00	17/15		
Germination 2	94:30	17/15		
Kiln 1	9:00		30	40
Kiln 2	4:00		40	60
Kiln 3	2:00		60	70
Kiln 4	4:30		70	80
Kiln 5	0:30		25	25

3.2 Hot Water Extract

3.2.1 IOB Hot Water Extract

IOB hot water extract (IOB HWE) was measured using a small-scale version of the IOB method (IOB methods of analysis, 2.3, 1991). This involved accurately weighing 5g of ground malt (0.7mm grind size) and mixing it with 36mls of water at 65°C for 1 hour. A further 5mls of water were added before the samples were filtered (Whatman Number 4) and the specific gravity (SG) was measured using an Anton Paar® DMA57 density meter. The flask was weighed before and after the addition of water to allow calculation of the dilution of the sample. The extract results were calculated as follows:

The dilution of the sample D = amount of water/amount of flour/8

Degrees of gravity G = 1000(SG-1)*D

HWE (% db) = G*2.279/SG * (100/(100-M))

Where: SG is the specific gravity

M is the malt moisture content (%)

3.2.2 EBC Hot Water Extract

EBC hot water extract (EBC HWE) was measured a using small-scale version of the EBC Extract of Malt method (EBC Anlaytica, 4.5.1, 1998). This involved accurately weighing 10g of ground malt (0.2mm grind size) and shaking it with 40mls of water at 45°C for 30 minutes. Next the temperature was raised at 1°C per minute to 70°C. Another 15ml of water was added and the samples were allowed to shake at 70°C for a further 60 minutes. Five mls of water was added, the samples were cooled and another 15mls of water was added. The samples were filtered (Whatman Number 4) before the specific gravity was measured using an Anton Paar® DMA57 density meter. The flask was weighed before and after the water extraction period to calculate the dilution.

The extract content of the wort in °Plato (g of extract in 100g of wort) was calculated using the specific gravity of the wort from the formula:

Extract of wort (°Plato) = (645.656*SG) - 4.66.947 + (208.71*SG^2).

The dilution was calculated from the formula:

Amount of water per 100g of malt = amount of water added *(10/flour weight) *10

Where (10/flour weight) adjusts the calculation to take into account the exact amount of flour added.

From this the extract content of the malt was calculated from the formula:

Extract (%, as is) = extract of wort * (amount of water per 100g of malt + moisture of the malt) / (100-extract of wort)

Extract (% dry basis) = Extract (as is) * 100 / (100 – moisture)

3.3 Physical

3.3.1 Screenings

Screenings were calculated by weighing the proportion of grain that passed through a 2.2mm screen as a percentage of the total weight. This proportion of the sample was discarded and the remaining analysis was preformed on the screened sample.

3.3.2 Malt and Barley 1000 grain weight

Barley and malt 1000-grain weights were measured by the EBC method (EBC Analytica, 3.4, 4.4, 1998). 40g of barley was counted using a Pfeuffer®, Contador automatic seed counter and calculated using the formula:

Weight of 1000 grains (g, dry) = total weight of barley in g * 1000 / number of grains * (100 / (100-moisture))

3.3.3 Milling Energy

Milling Energy was measured using a Comparamill® as described in Allison *et al.*, (1979). The Comparamill® measures the energy required to grind a sample of barley (5g) to flour fine enough to pass through a 0.2mm screen. The results are expressed in Joules (J).

3.3.4 Single Kernel Classification System (SKCS) Weight, Diameter, Length, Moisture and Hardness

Weight, diameter, length, moisture and hardness were measured using The Perten Instruments Single Kernel Classification System (SKCS) as outlined by Panozzo and Ratcliffe (1997). The SKCS takes a sample of 300 seeds and crushes each seed individually. It measures the weight force deformation profile and electrical conductivity of each grain. From this it uses stored algorithms to calculate the diameter, length, moisture content and hardness of each seed.

3.3.5 Grain Hydration

Grain hydration was measured at the end of steep and at 24, 48 and 72 hours into germination, according to Landau *et al* (1995). Forty grains were removed from the micromalter and boiled for 30 seconds. They were then cut in half longitudinally and given a score of 1, 2, 3 or 4 according to the proportion of the endosperm gelatinised. A total score out of 200 was calculated.

3.3.6 Skinnings

The level of skinnings was calculated according to the method outlined in the Australian Barley Board classification manual (ABB, 1998). 100 grains from each sample were visually inspected for husk damage and reported as the percentage of grains damaged. The components of skinning are defined as follows:

Side or back skinnings: "one third or more of the husk is missing from the side or back"

Germ exposed: "husk is removed from the germ end or the germ has been removed"

Chipped: "approximately one third of the grain has been removed at the awn end"

Pearled: "all the husk has been removed and the aluerone layer is exposed"

Split skirts: "the husk is split along the center or side ridges on the back of the grain, at the germ end"

Split backs: "the husk is split along the length of the center ridge on the back of the grain"

Awn skinning: "greater then half of the husk from the awn end towards the center of the garin has been removed"

3.3.7 Moisture

Moisture content was measured by either of two methods. Moisture is expressed as a percentage of the total weight.

3.3.7.1 Near Infrared Spectroscopy (NIR) Moisture Content

NIR moisture content was measured on a Technicon Infraalyzer® 400 Near Infrared (NIR) instrument that was calibrated using the EBC oven moisture method (EBC Analytica, 4.2, 1987) as outlined below, at 104°C for 3 hours. Unless otherwise stated, this method of moisture content assessment was used throughout this study.

3.3.7.2 Oven Moisture Content

Oven moistures, when used, were measured following a small scale version of the EBC oven moisture methods for either malt or barley (EBC Analytica, 3.2 and 4.2, 1998). In both cases 1g of ground flour was weighed into oven moisture tins and dried at either 130°C or 104°C for 1 or 3 hours respectively. The difference in the weight before and after drying allowed calculation of the moisture content.

3.3.8 Germinations, Dormancy, Water sensitivity

Germination tests were based on the IOB germination method (IOB methods of analysis, 1.7, 1991). The 4ml test measures the ability of the grains to germinate and

hence the level of primary dormancy. The 8ml test gives an indication of the secondary dormancy known as water sensitivity.

Two petri dishes were lined with filter paper (Whatman no1) and 100 seeds were placed in each. Four millilitres of water were placed into one of the dishes and 8mls into the other. For the 8ml test the seeds were placed with the ventral (crease) side down. Both dishes were sealed and placed in an incubator at 20°C for 72 hours. At 24 hour intervals the dishes were inspected and the seeds that had germinated were removed and counted. The results were expressed as a percentage of the total.

3.4 Components of Malt and Barley

Malted and unmalted samples were ground on a Udy Mill using a 0.5mm screen prior to analysis for all malt and barley components.

3.4.1 Malt and Barley Protein

Malt and grain nitrogen were measured by the Kjeldahl method. 1g of the milled sample was digested with 20ml of concentrated sulphuric acid and two high selenium catalyst tablets (Univar). Samples were then distilled and titrated using a Kjeltec 1030 Auto Anaylzer. Results were calculated according to the EBC method (EBC Analytica, 3.3.1, 1998) and multiplied by the factor of 6.25 to convert to protein content. The formula used was:

Total nitrogen (%, dry) = titration volume (ml) * 14 / (weight of sample * (100– moisture)

Total protein (%, dry) = Total nitrogen * 6.25

3.4.2 Malt and Barley β-Glucan

Barley and malt β-glucan levels were measured using a Megazyme beta-glucan (mixed linkage) assay kit according to the manufacturers instructions. To remove any glucose present, the malted samples were initially washed in heated aqueous ethanol followed by two washes at room temperature in aqueous ethanol. This step was not needed for barley samples due to the levels of glucose in the grain being negligible.

The method relies on the enzymic breakdown of the β-glucan by Lichenase and β-glucosidase. The amount of residual glucose is then measured spectrophotometrically from the colour development with glucose oxidase/peroxidase reagent (GOPOD). The results were calculated from the equation:

% Beta Glucan = (absorbance of the sample – absorbance of the blank) * F/ Dry weight (mg) * 5.4

Where: F = 100 /Absorbance of a Glucose standard

Dry weight = Weight of sample* (100-Moisture) / 100

3.4.3 Malt and Barley Arabinoxylans and Pentose

Barley and malt total pentose and arabinoxylans were measured using the phloroglucinol reaction (Lee, 1996).

3.4.3.1 Total Pentose

100mg samples of flour were boiled with 5mls of sulphuric acid (0.5M) for 40 minutes. The samples were cooled and centrifuged. 100µl of each was reacted with a mixture of phloroglucinol (20g phloroglucinol in 100ml ethanol) and glacial acetic acid (990ml glacial acetic acid and 18ml hydrochloric acid) in a ratio of 1:18.7, for 25 minutes at 97°C. The samples were then cooled and measured spectrophotometrically at 552 and 510nm. The results were standardised against xylose standards.

3.4.3.2 Arabinoxylan Content

The samples were initially washed in heated aqueous ethanol followed by two washes at room temperature in 50% aqueous ethanol to remove simple sugars. The samples were centrifuged and the supernatant was removed. The above method (Section 3.4.3.1) was then followed. The results were calculated from the following formula:

Arabinoxylan (%db) = (absorbance at 552 – absorbance at 510) * S * D * 0.88 * (1/M) * (1/1000) * 100

Where: S = slope of standard curve

D = dilution factor

1/1000 = conversion factor from μg to mg

M = dry wt of sample in mg

0.88 = adjustment from free pentose to anhydro-pentose

3.4.4 Malt and Barley Husk Content

Malt and barley husk content were measured according to a small-scale version of the EBC method (EBC Analytica, 3.9, 1998). The husk was removed from 5g of sample using a mixture of boiling sodium hypochlorite (12.5% w/v) and sodium hydroxide (125g/L). The change in weight before and after the removal of the husk, calculated on a dry basis, was used to calculate the percentage of husk on the grain. Moisture content after the removal of the husk was measured as described in Section 3.3.7.2.

3.4.5 Malt and Barley Starch Content

Barley and malt starch content were measured using a Megazyme Total Starch assay kit (McCleary *et al.*, 1994) as per the manufacturers instructions. As with the β-glucan method, the malted samples were initially washed in heated aqueous ethanol followed by two washes at room temperature in aqueous ethanol to remove simple sugars present. This step was not needed for barley samples due to the levels of glucose in the grain being negligible.

The method involves the solubilization and enzymic breakdown of starch with thermostable α-amylase. The dextrins were then reduced to glucose with the enzyme amyloglucosidase. As with the β-glucan method, the amount of glucose was measured spectrophotometrically after colour development with GOPOD. The results were calculated from the equation:

Starch % (dry wt basis) = (Absorbance of the sample – Absorbance of the blank)
* F/ weight (mg) * 90* 100 / (100-M)

Where: F = 100 /Abs Glucose

M= Moisture content (%)

3.4.6 Starch Components

Samples were prepared by mixing 0.5mg of barley flour with 1ml of 2% SDS (sodium dodecyl sulphate) and four drops of 1% DTT (dithiothreitrol) for 10 minutes. The samples were centrifuged for 10 minutes and the supernatant was discarded. The samples were then washed in 1ml of water, centrifuged and the supernatant was again discarded. The pellet was resuspended in 150µL of iodine (5%KI/0.5%I) and 150µL of saturated maltose. A drop of the mixed sample was placed on a microscope slide and covered with a small 7mm square coverslip. The sample was analysed on an Axiophot, Zeiss microscope equipped with a digital camera, using the program VideoPro32®. A minimum of 30 fields of view and 2000 starch granules were analysed per sample. Each sample was analysed in duplicate. Measurements were taken for area, width, length, diameter, perimeter and shape.

3.4.7 Malt Flour Gelatinisation Temperature (DSC)

Malt flour gelatinization temperature was measured using a Setaram Differential Scanning Calorimeter (Micro-DSCVIII) (DSC). Approximately 50mg of malt flour was accurately weighed into DSC pans. The weight was made up to 150mg with silver nitrate (0.1mM). The silver nitrate was added to inhibit enzyme activity during the reaction. The pans were sealed and loaded onto the DSC. The temperature was initially held at 1°C for one minute before being raised at 10°C per minute to 100°C. The samples were analysed in duplicate. Onset, end and peak temperatutres were measured in °C, peak height was measured in milli watts (mW), peak area in milli Joules (mJ) and delta H in joules per gram (J/g).

3.4.8 Malt Flour Pasting Properties (RVA)

Malt flour pasting properties were measured using a Rapid Visco Analyzer. 3.5g of malt flour was weighed into a sample cup and mixed with 10.5mls of silver nitrate (0.1mM). As with DSC gelatinization temperatures, the silver nitrate was used to inhibit enzyme activity during the reaction. The sample was placed onto the RVA and stirred with a paddle at 160rpm while being heated. The temperature was initially held at 50°C for one minute, before being raised at 12.5°C per minute to 90°C where it was held for two minutes. Finally the sample was cooled back to 50°C at 12.5°C

per minute. The viscosity was expressed in Rapid Visco Analyzer units (RVU), temperature in °C and time in minutes.

3.5 Enzyme Analysis

Malted samples for all enzyme analysis were ground on a UDY Mill using a 0.5mm screen.

3.5.1 Diastatic Power (DP), α-amylase, β-amylase

DP, α-amylase and β-amylase were measured as described in the SABIP Barley Quality Report (Logue, 1997). The method is based on the assumption that DP is the product of the activities of α-amylase and β-amylase only (Section 2.3.1).

The method involves extracting the enzymes from the flour (250mg) with a sodium chloride (10mls, 10%w/v) solution for an hour at 25°C. A standard starch solution (10mls, 20%w/v) (EBC, 4.12, 1998) was hydrolysed with 50μL of the extract for 10 minutes at 25°C to measure the total activity of the diastatic enzymes. This reaction was stopped by changing the pH of the solution using a sodium hydroxide solution (4ml, 0.5M). A solution of para-hydroxybenzoic acid hydrazide was used to calculate the amount of reducing sugars present in the reduced starch solution using flow injection analysis.

A portion (2mL) of the extract was heated at 65°C for 15 minutes in the presence of calcium ions (20μl, 500mM Calcium Chloride) to denature the β-amylase. The assumption was made that α-amylase was the only diastatic enzyme remaining in solution. The above method was then followed to measure the level of α-amylase remaining in the 100μl of extract. β-amylase was calculated by subtracting the activity for α-amylase from the total activity.

The results for DP, α-amylase and β-amylase were expressed as micromoles of maltose equivalents released per minute per gram dry weight (μm/min/g).

3.5.2 Limit dextrinase

Limit dextrinase activity was measured using a Megazyme limit dextrinase assay kit according to the manufacturers instructions. This method uses a cross linked pullulan polysaccharide that has a dye molecule attached. This compound was subject to attack by limit dextrinase but not other amylolytic enzymes. As the polysaccharide was broken down, dye linked fragments were released into solution. The colour development was measured spectrophotometrically at 590nm. The results were compared to a standard curve supplied by the manufacturer and expressed as units/kg malt (U/kg). "Units" equals one micromole of glucose reducing-sugar equivalents released per minute from pullulan under the defined assay conditions.

3.5.3 β-Glucanase

β-Glucanase activity was measured using a Megazyme Malt beta-glucanase assay kit (azo-barley glucan method) according to the manufacturers instructions. The β-glucanase was extracted from the flour and reacted with an azo-barley glucan substrate. This substrate has a bound dye molecule. As the substrate was attacked by the β-glucanase the dye becomes soluble. The absorbance of the solution was measured spectrophotometrically at 590nm. The β-glucanase activity was directly related to absorbance and calculated from the equation of a calibration curve. Malt β-glucanase activity was measured in Units/kg malt (U/kg). "Units" equals one micromole of glucose reducing-sugar equivalents released per minute under the defined assay conditions.

3.6 Wort Analysis

All wort analysis was carried out on the extract produced from either the EBC or IOB HWE methods (Section 3.2).

3.6.1 Soluble Protein

Soluble protein was measured by either of two methods and expressed as a percentage of the original weight.

3.6.1.1 Kjeldahl method

Soluble nitrogen was measured by the Kjeldahl method. 10mls of wort was digested with 25ml of concentrated sulphuric acid and two high selenium catalyst tablets (Univar) and distilled using a Kjeltec 1030 Auto Anaylzer. The level of protein in the wort was calculated using the factor of 6.25. Calculations for protein levels were taken from either the EBC or IOB methods of analysis as appropriate (EBC Analytica, 2.11, 1998; IOB methods of analysis, 4.9.1, 1991).

EBC Soluble protein (%m/m dry) = (titration*14/volume of wort) * (100*extract of malt)/(10000*extract of the wort(°P))*6.25*(100/(100-moisture))

IOB Soluble protein (%m/m dry) = titration volume*4.910/(specific gravity of wort*(100-moisture))*6.25

3.6.1.2 Spectrophotometric method

The level of soluble protein was measured spectophotometrically according to the ASBC method (ASBC methods of analysis, wort17, 1987). This involves calculating the difference in the absorbances of a sample, diluted in a sodium chloride solution measured at 225 and 215nm. This is compared to a calibration curve developed using the Kjeldahl method (Section 3.6.1.1).

3.6.2 β-Glucan

Wort β-glucan levels were measured using a Megazyme mixed linkage beta-glucan assay kit as described in section 3.4.2. 5mls of wort was initially mixed with 2.5g of ammonium sulphate and left to extract overnight. The sample was centrifuged and the supernatant was removed. The pellet was washed twice with ethanol before being treated as described in Section 3.4.2. The results were calculated from the equation:

% wort β-Glucan = absorbance * F / (Dry weight*5.4)

where F = 100 / absorbance of glucose

3.6.3 Arabinoxylans

Wort arabinoxylan levels were measured by the phloroglucinol method according to Lee (1996) and as described in section 3.4.3. 5mls of wort was initially mixed with 2.5g of ammonium sulphate and left to extract for 2 days. The sample was centrifuged and the supernatant was removed. The pellet was washed twice with ethanol before 5mls of H_2SO_4 was added and the samples were treated as described in Section 3.4.3. The results were calculated from the formula:

Arabinoxylan (%db) = (absorbance at 552 – absorbance at 510) * S * D * 0.88 * (1/5)

Where: S = slope of standard curve

D = dilution factor

0.88 = adjustment from free pentose to anhydro-pentose

3.6.4 Viscosity

Viscosity was measured dynamically using an PAAR AMV200 rolling ball viscometer. This method calculates viscosity by measuring the time it takes for a small ball (stainless steel, diameter: 1.5mm, density: 7.85g/cm^3) to pass through a capillary tube (internal diameter: 1.7mm, angle: 15°) filled with the sample equilibrated to 20°C. Water (high purity) and air were used as standards for calibration purposes. The results were expressed as centipoise (cP).

3.6.5 Free Amino Nitrogens (FAN)

FAN was measured using the standard EBC and IOB ninhydrin method (EBC Analytica, 8.10, 1998; IOB methods of analysis, 8.4, 1991). A diluted solution was reacted with a ninhydrin solution, boiled and measured spectrophotometrically at 570nm. The result was calculated against a glycine standard and expressed as mg/L using the formula:

FAN(mg/L) =(absorbance * 2 * dilution) / absorbance of the glycine standard

3.6.6 Kolbach Index (KI) and Soluble Protein to Total Protein Ratio (SP/TP)

KI and SP/TP represent the ratio between soluble and total protein. KI is a measure of the ratio of soluble to total protein on wort extracted by the EBC hot water extract method. SP/TP is a measure of the ratio when measured on wort extracted by the IOB hot water extracted method. Both methods are expressed as a percentage.

3.6.7 Simple sugar levels

Simple sugar levels were measured by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using a Dionex GP40 gradient pump, a Dionex ED40 electrochemical detector and a Shimadzu SIL-10AD autoinjector. It was fitted with a Dionex CarboPac PA-1 column and PA-1 precolumn. The wort was initially centrifuged to remove all solids and 1μL was diluted to 200μL with high purity water. 10μL of the diluted wort was injected into the column. The wort was eluted using a linear gradient of sodium acetate (16-120mM) in sodium hydroxide (100mM, made from low CO_2, 50% w/w liquid sodium hydroxide) over 34 minutes with a flow rate of 1ml/min. The residual dextrins were removed from the column by increasing the sodium acetate concentration to 400mM over one minute and reducing the concentration back to 16mM over two minutes. The column was allowed to equilibrate at a sodium acetate concentration of 16mM for eight minutes before the next sample was injected. A standard was prepared (6.250nM glucose, 1.950nmols fructose, 1.472 nmols sucrose, 11.735 nmols maltose, 1.991 nmols maltotriose, 1.151 nmols maltotetraose and 0.513 nmols maltohexaose) and measured every 20 samples. The Dionex Peaknet software was used to compare the peak areas of the standards to the peak areas of each sample to determine the concentration of each sugar in nmols/L.

3.7 Spent Grain

The solid material left over after the production of wort is called spent grain. The spent grain samples used in the following analysis were collected from the production of the EBC and IOB HWE (Section 3.2). After the extraction process the samples were filtered through Whatman filter papers (number 4) and the solid material remaining on the filter papers was collected. It was washed with 100ml of water at

70°C and dried at 50°C overnight. The samples were then ground on a Miag mill setting 2.

3.7.1 Spent grain weight

Initially the filter papers were dried at 100°C for three hours before pre weighing. After the spent grain samples were dried a final weight was taken. The moisture content was calculated by drying 0.5g at 130°C for 3 hours. The final spent grain weight was calculated by subtracting the weight of the filter paper from the final weight. The initial and final weights were adjusted to take into account the moisture content. The final weight was reported as a percentage of the original weight.

3.7.2 β-Glucan

β-Glucan content of the spent grain was measured using a Megazyme mixed linkage beta-glucan assay kit as described in Section 3.4.2.

3.7.3 Arabinoxylans and Pentose

Arabinose and pentose content of the spentgrain were measured by the phlorglucinol method (Lee, 1996) as outlined in Section 3.4.3.

3.7.4 Protein

Spent grain nitrogen content was measured by the Dumas combustion method using a Leco FP-2000 system, calibrated against ethylenediaminetetraacetic acid. 0.25g of each sample was analysed. The nitrogen content was multiplied by a factor of 6.25 to convert to protein content and expressed as a percentage of the total weight.

3.7.5 Starch

Spent grain starch analysis was carried out using a Megazyme Total Starch assay kit according to Section 3.4.5. After treating the ground spent grain with 3mL of thermostable α-amylase, the solution was only diluted to 10mL, instead of 100mL to account for the low levels of starch in the spent grain.

3.8 Molecular Analysis

3.8.1 DNA extraction

DNA was extracted from a 15cm piece of leaf material following a mini-prep method adapted from Ragowsky *et al.* (1991) with the following changes. Plant material was crushed placing the plant material in a 10mL tube with ball bearings and shaking for 60 seconds. A single extraction was carried out using 700µL of extraction buffer (0.1M Tris-HCl (pH7.8), 10mM EDTA, 0.1M NaCl, 1% sarkosyl and 2%polyvinyl-polypyrrolidone, insoluble) and 700µL of phenol/chloroform/iso-amylalcohol (25:24:1) for 10 minutes. The phases were separated by centrifugation using a silica gel matrix and the aqueous phase was collected. The DNA was precipitated by the addition of 0.1 volume of sodium acetate (3M, pH4.8) and 1 volume of isopropanol. The pellet was washed with 70% ethanol and dried before resuspension in 50µL of R40 (TE buffer (10mM Tris-HCl, pH8.0, 1mM EDTA) containing 4µg/mL treated RnaseA.

3.8.2 Southern Blot Analysis of DNA

3.8.2.1 Restriction Digestion, Gel Electrophoresis and Transfer of DNA

6µL of DNA was digested with restriction endonucleases in 10X SDB (330mM Tris, pH7.8, 650mM potassium acetate, 100mM magnesium chloride, 40mM spermidine and 50mM dithiothreitrol) for five hours at 37°C. The enzymes used were *EcoRI, EcoRV, HindIII, XbaI, DraI* and *BamHI.* Ficoll dye was added and the digested DNA was separated on a 1% agarose gel overnight. The gel was stained with ethidium bromide (10µg/mL) and photographed. The DNA was transferred to a hybond N^+ nylon membrane using sodium hydroxide (0.4M). The membrane was washed with 2 x SSC (0.3M NaCl, 0.03M Tri-sodium citrate).

3.8.2.2 Oligolabelling and Hybridisation of DNA probes

Plasmid DNA (approximately 50ng), 9mer random primer mix (3μL, 0.1μg/μL) and 2μL λ was boiled for five minutes and quick chilled on ice. 12.5μL of oligolabelling mix (60μM each of dATP, dTTP and dGTP, 150nM Tris-HCl, pH 7.6, 150mM NaCl, 30nM $MgCl_2$ and 300μg/mL BSA), 1μL Klenow (DNA polymerase I) and 3μL [α-^{32}P]dCTP were added and incubated at 37°C for 60 minutes. The radioactively labelled DNA (probe) was separated from unincorporated nucleotides on a G-100 sephadex column.

The membranes were initially soaked in a solution containing 50mL 5 x SSC (0.75M NaCl, 0.075M Tri-sodium citrate), 20mL Denhardt's III (2% BSA, 2% Ficoll 400, 2% polyvinyl-pyrrolidone and 10% SDS) 5mL denatured salmon sperm DNA (5mg/mL) and 25mL water for 30 minutes at 65°C. The membranes were then prehybridised in glass bottles in a solution containing 3mL of 5 x HSB (3M NacL, 100nM Pipes, 25mM Na_2EDTA, pH6.8), 3mL Denhardt's III, 3mL dextran sulphate (25%), 0.5mL denatured salmon sperm DNA (5mg/mL) and 0.5mL water for four hours at 65°C.

0.5mL denatured salmon sperm DNA (5mg/mL) was added to the probe and denatured by boiling for five minutes followed by cooling on ice before being added to the bottle containing the membranes. Hybridisation was allowed to occur at 65°C overnight.

The membranes were washed four times for 20 minutes at 65°C. The washes were:
2 x SSC and 0.1% SDS
1 x SSC and 0.1% SDS
0.5 x SSC and 0.1% SDS
0.2 x SSC and 0.1% SDS.
The membranes were exposed to X-ray film at -70°C for four to eight days depending on the signal strength.

The membranes were stripped by pouring a hot solution of SDS (0.1%) and EDTA (2mM, pH8.0) over the membranes and allowing to them to cool.

3.8.3 Microsattelites

The DNA prepared in section 3.8.1 was diluted with water in the ratio of 1:25 for the PCR reactions. 2.5µL of diluted DNA was mixed with 12.5µL of a solution containing nanopour water (5.775µL), 10 x Reaction buffer (1.25µL, Gibco-BRL, 100mM Tris-HCl, pH8.3, 500mM KCl and 0.1% gelatin), $MgCl_2$ (0.375µL, 50nM), dNTP (4µL, 1.25 µM each of dATP, dCTP, dGTP and dTTP) upper and lower primers (0.25µL each, 0.15mg/mL) and *Taq* DNA polymerase (0.1µL, 5 units/µL, Gibco-BRL). The samples were amplified using one of two touchdown PCR programs as outlined by Karakousis *et al.* (2003b).

The samples were denatured at 95°C for five minutes, cooled on ice and run on an 8% denaturing polyacrylamide gel for four hours. The gel was stained in an ethidium bromide solution (1mg/mL) and photographed using a UV transilluminator.

Chapter 4

Biochemistry of Malt Extract

4.1 Introduction

The brewing process involves a number of complex steps. Initially barley is converted into malt via a controlled germination process. This is followed by the solubilisation and extraction of material from the ground malt with warm water. This process is called mashing and the liquid produced is called wort. After filtration, the soluble material in the wort is either used by the yeast during fermentation to produce alcohol, or remains in the final product. The waste solid material remaining is known as spent grain.

Malt extract refers to the amount of soluble material that can be extracted from the malt during mashing. In the laboratory, malt extract is measured using a small-scale version of the mashing process. The amount of material extracted from the malt is estimated by measuring the specific gravity of the filtered wort produced from the small-scale mash and is referred to as hot water extract (HWE) (Briggs *et al.*, 1981). HWE gives an indication of how a malt will perform in a full scale brewing situation and is used by maltsters and brewers to gauge the quality of the malt or barley they are purchasing. There are two different industry recommended methods for measuring HWE. The first method (IOB HWE) involves mixing coarsely ground malt with water at 65°C for one hour (IOB methods of analysis, 2.3, 1991). The second method (EBC HWE) uses a temperature-programmed mash where water is added to finely ground malt at 45°C and held there for 30 minutes. The temperature is raised to 70°C over the next 25 minutes at 1° per minute and is held there for another hour (EBC anlaytica, 4.5.1, 1998).

HWE is rated as the most important parameter when considering the release of new malting barley varieties in Australia (MBIBTC, 2001), and the release of new varieties with high levels of HWE is an industry priority. However, before barley

breeders can select effectively for the complex mixture of material extracted from the malt, a thorough understanding of how these materials relate back to the original barley is needed. While the reactions and changes during the malting and mashing processes between the many compounds within the barley grain, including the hormones, hydrolytic enzymes, proteins and carbohydrates, have been investigated for many years, our understanding is at best fragmented, with many gaps still to be filled.

To gain a thorough understanding of malt extract it is important to investigate differences between varieties right through the malting and brewing processes. In this chapter, initially, the influences of site and variety on malt extract are examined. This is followed by an investigation of the composition of barley, malt, wort and spent grain. Furthermore, other traits that have been highlighted in the literature as influencing malt extract such as grain hardness and starch properties are also investigated.

The barley varieties assessed in this study were chosen for either their good malting quality or their good agronomic adaptation to Southern Australian conditions. The European, Canadian and Japanese varieties examined have been used extensively as parents in Australian breeding programs due to their high malting quality. A list of these varieties is shown in Table 4.1.1 along with their release dates and countries of origin.

4.2 Materials and Methods

4.2.1 Materials

Twelve and eleven barley varieties were grown at two South Australian sites in 1997 and 1998, respectively. These included malting and feed varieties from Canada, Europe, Japan and Australia, as shown in Table 4.1.1. The sites were Brinkworth and Tuckey in 1997, and Pinery and Maitland in 1998. Grain samples from three replicates of each variety at each site were screened on a 2.2mm sieve before being micromalted.

Table 4.2.1: Details of the varieties used in Chapter 4

Variety	Organisation/ Country of origin	Type	Release date
Alexis	Breun, Germany	Malting	1986
Arapiles	VIDA, Australia	Malting	1993
Barque	UA, Australia	Feed	1998
Chariot	Cambridge, UK	Malting	1991
Chebec	UA, Australia	Feed	1992
Franklin	Tasmania, Australia	Malting	1989
Galleon	UA, Australia	Feed	1981
Harrington	University of Saskatchewan, Saskatoon, Canada	Malting	1981
Haruna Nijo	Sapporo Breweries, Japan	Malting	1981
#Manley	University of Saskatchewan, Saskatoon, Canada	Malting	1983
Schooner	UA, Australia	Malting	1983
Sloop	UA, Australia	Malting	1997

grown in 1997 only

4.2.2 Methods

The results section of this chapter has been divided into three sections:

4.3.1: The influence of site and variety on HWE

In this section I consider the influence of site and variety on both IOB and EBC HWE.

4.3.2: The influence of the components of the malt and barley on HWE

In this section I look at the relationship between a number of malt and barley characteristics and HWE. It has been divided into four sections: Physical barley and malt traits, Components of Barley and Malt, Starch and Cell Wall Degrading Enzymes and Starch Properties.

4.3.3: The influence of the wort and spent grain components on HWE

This section has also been divided into four sections: EBC Wort Components, IOB Wort Components, Spent Grain Components and Comparison of EBC and IOB Wort and Grain Components.

4.2.2.1 Barley, Malt, Wort and Spent grain Analysis

In general, analyses were carried out according to the methods described in Chapter 3. The sections describing each analysis are shown in Table 4.2.2. Preceding malt, wort and spent grain analysis, fifty grams of each sample were malted in a Phoenix Automated Micromalting system at 17°C as described in Section 3.2. Micromalter grain moistures were adjusted to 45% at 24, 48 and 72 hours into germination. Three replications from four sites were analysed for all traits except where indicated otherwise (Table 4.2.2).

4.2.3 Statistics

Pearsons product moment correlations (r) (mean of 3 replicates) between IOB and EBC HWE and other traits were calculated using the computer program Jmp® (version 3.1.6, SAS Institute Inc.). The assumption of normality for each trait was visually assessed and appeared to be met in most cases. Where normality was not met the data was transformed using $\log_{10}$ for further analysis. Scatter plots of each trait against IOB and EBC HWE were inspected to ensure no outliers were biasing the results. Least significant differences were calculated using the computer programs GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust) and Jmp® (version 3.1.6, SAS Institute Inc.).

Generally, when assessing correlation coefficients, the statistical significance of the relationship is quoted. This is based on testing the null hypothesis, which states that there is no relationship within the data set, and therefore the correlation coefficient is statistically equal to zero. The statistical significance is affected by both the sample size and the observed size of r. For example, a simple correlation coefficient of 0.195 calculated from a sample of 100 observations would be significantly different from zero at the 5% level of significance, but in practical terms is not at all indicative of a strong association between the two variables (Snedecor and Cochran 1989 p 187 and p 473). In this study, correlation coefficients have been stated for the individual sites (mean of 3 reps), n=11 and 12. A correlation coefficient of 0.60 is statistically significant at the 5% level for 11 individuals. However, we chose to use the following to classify correlation coefficients as strong, moderate and weak as suggested by

Devore and Peck (1996) as an indication of the strength of the association between the variables:

|r|=0.8 –1 strong relationship

|r|=0.6-0.8 moderate relationship

|r|<0.6 weak relationship.

Thus, not only are all correlations that are termed moderate or strong statistically significant, they are also indicative of a meaningful relationship.

Multiple regression analysis was performed using the following method. To reduce the number of variables, all traits were initially assessed for their relationship with HWE using simple linear regression. Traits that showed a poor relationship with HWE (F pr>0.2) were not included in the analysis. The relationship between each trait was also assessed. Where any two traits were highly correlated, a single trait was chosen for analysis. The remaining traits were compared to HWE using multiple regression analysis (Genstat 5, release 4.1). A single trait was removed at a time from the model until all traits remaining in the model were significant (P<0.05). The residual plots of the final model were inspected to ensure that no outliers were biasing the model.

Best linear unbiased predictors (BLUPS) were calculated on mean IOB and EBC HWE using the statistical model:

HWE = site + site x variety + error

Where *site* is a fixed effect with four levels to compensate for different site means and *site x variety* is a random interaction effect which gives the genetic variance at each site and a correlation matrix (Table 4.3.4 and Table 4.3.8).

From the correlation matrix (Table 4.3.4 and Table 4.3.8) a common effect for each variety can be calculated. The correlation of each site against the common effect is used to calculate a loading for each site (Table 4.3.5 and Table 4.3.9). This loading was used as an indication of the amount of information from each site that was used in the composition of the common effect. For example, if all sites were highly correlated they would have high loadings (around 0.9) and the same information would be obtained from all sites. However, if one of the sites performed differently

and was poorly correlated with the other sites it would have a lower loading in the common effect.

Table 4.2.2: Sections of Chapter 3 describing the analyses undertaken in Chapter 4

Analysis	Section
IOB HWE	3.2.1
EBC HWE	3.2.2
Barley Screenings	3.3.1
[1]Barley Milling Energy	3.3.3
SKCS Weight, Diameter, Moisture and Hardness	3.3.4
Malt and Barley 1000 grain weight (1000GW)	3.3.2
Grain Hydration (GH)	3.3.5
Skinnings	3.3.6
Moisture - Near Infrared Spectroscopy	3.3.7.2
Malt and Barley Protein	3.4.1
Malt and Barley β-Glucan	3.4.2
Malt and Barley Arabinoxylans and Pentose	3.4.3
Malt and Barley Husk Content	3.4.4
Malt and Barley Starch Content	3.4.5
Starch Components	3.4.6
[2]Malt Flour Gelatinisation Temperature (DSC)	3.4.7
[3]Malt Flour Pasting Properties (RVA)	3.4.8
Diastatic Power (DP), α-amylase, β-amylase	3.5.1
Limit dextrinase	3.5.2
β-Glucanase	3.5.3
Wort Soluble Protein - Kjeldahl	3.6.1.1
Wort β-Glucan	3.6.2
Wort Arabinoxylans	3.6.3
Wort Viscosity	3.6.4
Wort Free Amino Nitrogens (FAN)	3.6.5
Kolbach Index (KI) and Soluble Protein to Total Protein Ratio (SP/TP)	3.6.6
[4]Wort simple sugar levels	3.6.7
[5]Spent grain weight	3.7.1
[5]Spent Grain β-Glucan	3.7.2
[5]Spent Grain Arabinoxylans and Pentose	3.7.3
[5]Spent Grain Protein	3.7.4
[5]Spent Grain Starch	3.7.5

[1]Milling Energy was analyzed on samples from Pinery and Maitland only.
[2]Malt Flour Gelatinisation Temperature (DSC) was analyzed on 2 replicates of samples from Tuckey only.
[3]Malt Flour Pasting Properties (RVA) was analyzed on samples from Pinery, Maitland and Tuckey.
[4]Wort simple sugar levels (HPAEC-PAD) were analyzed on samples from Pinery, Maitland and Tuckey.
[5]Spent grain analysis was carried out on samples from Pinery, and Maitland only.

4.3 Results

4.3.1 The influence of site and variety on HWE

4.3.1.1 EBC HWE

Mean EBC HWE for the four sites are shown in Table 4.3.1. Maitland produced significantly higher EBC HWE than all other sites ($P<0.001$) and Tuckey was significantly higher than Pinery and Brinkworth ($P<0.001$). This is likely to be at least partly due to high mean kolbach index (KI) (Table 4.3.2) at Tuckey and Maitland and low KI at Brinkworth and Pinery. Likewise barley protein levels were very high at Brinkworth and low at Tuckey (Table 4.3.2). Higher barley protein tends to lead to lower HWE levels due to the physical relationship between the amount of starch and protein present in the grain.

Brinkworth performed quite differently to the other three sites as indicated by the correlations between the four sites (Table 4.3.3) and the loadings used in the calculation of the common effect (Table 4.3.4). Whereas the other three sites were highly correlated and had high loadings (>0.9), Brinkworth had lower correlations with the other sites and consequently less information was used from this site when calculating the common effect. The genetic variance and heritability were higher at Pinery than the other three sites (Table 4.3.4), indicating the error associated with the environment was lowest at Pinery.

In general the overseas malting varieties (Alexis, Chariot, Harrington, Manley and Haruna Nijo), and the Australian malting variety Franklin, produced the highest EBC HWE at all sites. The mean EBC HWE for Manley is lower than would normally be expected due to being the mean of only two sites, one if which is Brinkworth, the lowest of all sites. This is highlighted by the common effect (Table 4.3.5) where Manley is ranked higher than the varieties Sloop and Schooner. Schooner performed better at Brinkworth than the other three sites, causing it to have a higher, but statistically similar mean EBC HWE to Chebec but a lower common effect.

For the purposes of the following discussion it is useful to place the varieties into three groups based on the common effect (Table 4.3.5). Calculating the common

effect on a data set such as this one gives an indication of how a variety is likely to perform under 'average' conditions. Therefore it is more appropriate to use common effect to place the varieties into groups than mean EBC HWE, as this could be greatly influenced by atypical sites, such as Brinkworth. These groups are:

High: (common effect greater than 0) Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot;

Medium: (common effect between 0 and -1) Sloop, Schooner, Arapiles and Chebec;

Low: (common effect less than -1) Galleon and Barque.

Mean EBC HWE of the three groups is shown graphically in Figure 4.3.1.

Table 4.3.1: Mean EBC HWE (%) at four sites (mean of three reps per site)

Variety	Brinkworth#	Tuckey#	Maitland#	Pinery#	Arithmetic Mean#	St Dev
Alexis	78.2^{ef}	81.9^{bc}	83.3^{c}	80.8^{a}	81.1^{e}	2.1
Arapiles	78.2^{ef}	80.4^{e}	81.1^{f}	79.2^{b}	79.7^{g}	1.3
Barque	74.8^{a}	79.2^{f}	79.6^{a}	76.0^{d}	77.4^{b}	2.4
Chariot	78.5^{e}	81.5^{c}	83.4^{cb}	80.6^{a}	81.0^{ef}	2.1
Chebec	77.6^{f}	81.3^{cd}	82.0^{de}	78.3^{c}	79.8^{g}	2.1
Franklin	80.6^{bc}	81.9^{bc}	82.7^{cd}	81.3^{a}	81.6^{de}	1.0
Galleon	78.4^{e}	79.4^{g}	81.5^{ef}	76.4^{d}	78.9^{a}	2.1
Harrington	80.7^{bc}	83.8^{a}	84.1^{b}	81.2^{a}	82.5^{c}	1.7
Haruna Nijo	81.0^{b}	82.3^{b}	83.3^{c}	81.1^{a}	81.9^{cd}	1.2
Manley	78.5^{e}	81.9^{bc}			80.2^{g}	2.2
Schooner	80.4^{bd}	80.6^{de}	81.2^{f}	78.7^{bc}	80.2^{g}	1.3
Sloop	80.0^{cd}	80.6^{de}	82.2^{de}	78.6^{bc}	80.3^{fg}	1.7
Mean	78.9	81.2	82.2	79.3	80.4	2.2
St Dev	2.0	1.4	1.5	1.9		

#Means within a column followed by a common *superscript* letter are not significantly different (P<0.05).

Table 4.3.2: KI (%), SP/TP (%) and barley protein (%) for each variety at all four sites (mean of three reps) and overall mean.

Variety	Brinkworth			Tuckey			Maitland			Pinery			Mean		
	protein	KI[1]	SP/TP[2]	protein	KI	SP/TP	protein	KI	SP/TP	protein	KI	SP/TP	protein	KI	SP/TP
Alexis	14.5	36.4	32.8	9.1	50.8	43.3	9.7	49.7	43.7	10.6	34.4	29.5	11.0	42.8	37.3
Arapiles	11.7	36.1	31.4	9.1	42.9	36.1	10.8	39.1	32.8	11.0	32.6	28.4	10.7	37.7	32.2
Barque	11.4	33.3	30.3	8.6	48.0	38.3	11.7	37.5	31.7	12.1	28.7	25.3	10.9	36.9	31.3
Chariot	12.7	35.9	30.4	9.4	44.9	37.6	10.1	46.5	40.2	11.3	33.9	30.9	10.9	40.3	38.2
Chebec	12.2	36.9	35.0	8.0	56.3	46.8	10.3	52.0	42.5	11.9	33.8	30.0	10.6	44.8	37.0
Franklin	13.2	36.8	32.7	8.6	51.5	42.9	11.1	45.7	39.0	11.3	37.2	33.5	11.0	42.8	31.9
Galleon	11.6	29.8	25.3	9.2	36.4	29.7	10.5	42.5	35.6	13.0	25.1	20.4	11.1	33.4	34.5
Harrington	12.1	44.1	37.5	8.1	52.9	44.4	10.7	63.6	52.3	10.9	45.5	39.0	10.4	51.5	43.0
Haruna Nijo	12.4	42.3	36.9	11.1	50.2	45.6	13.6	47.8	40.1	13.0	38.6	33.8	12.5	44.7	39.3
Manley	14.0	40.9	36.4	9.3	55.1	44.1							11.6	48.0	34.3
Schooner	11.9	41.1	37.2	9.5	55.3	46.3	12.3	50.5	42.6	12.1	38.8	32.1	11.4	46.4	39.5
Sloop	12.4	45.0	38.9	9.6	50.9	45.2	11.1	53.0	44.4	12.2	37.3	32.8	11.2	46.6	39.9
Mean	12.5	38.2	33.7	9.1	49.6	41.7	11.1	48.0	40.4	11.8	35.1	29.5	11.1	43.0	36.3

[1]based on soluble protein of an EBC HWE, [2] based on soluble protein of an IOB HWE

Table 4.3.3: The relationship (r^2) between four sites for EBC HWE.

	Brinkworth	Tuckey	Maitland	Pinery
Brinkworth	1.000	-	-	-
Tuckey	0.746	1.000	-	-
Maitland	0.746	0.998	1.000	-
Pinery	0.706	0.945	0.945	1.000

Table 4.3.4: Genetic variance, loading in the common effect and heritability (h^2) of EBC HWE from 12 varieties grown at four sites.

Site	Genetic variance	loading	Site mean	h^2
Brinkworth	2.47	0.75	78.8	0.74
Tuckey	1.48	1.00	81.2	0.79
Maitland	1.41	1.00	82.2	0.75
Pinery	3.28	0.95	79.3	0.88

Table 4.3.5: EBC HWE BLUPS (best linear unbiased predictors) and the common effect for four sites

	Brinkworth	Tuckey	Maitland	Pinery	Common Effect
Barque	-3.44	-2.29	-2.24	-3.29	-2.64
Galleon	-0.79	-1.51	-1.47	-2.76	-1.74
Arapiles	-0.68	-0.64	-0.63	-0.32	-0.74
Schooner	0.80	-0.57	-0.56	-0.73	-0.66
Chebec	-1.00	-0.38	-0.37	-0.94	-0.44
Sloop	0.62	-0.38	-0.37	0.74	-0.44
Manley	-0.15	0.46	0.45	0.65	0.53
Chariot	-0.05	0.63	0.63	1.18	0.74
Alexis	-0.18	0.80	0.79	1.38	0.93
Franklin	1.41	0.89	0.86	1.82	1.03
Haruna Nijo	1.67	1.10	1.08	1.69	1.28
Harrington	1.79	1.89	1.83	2.06	2.17

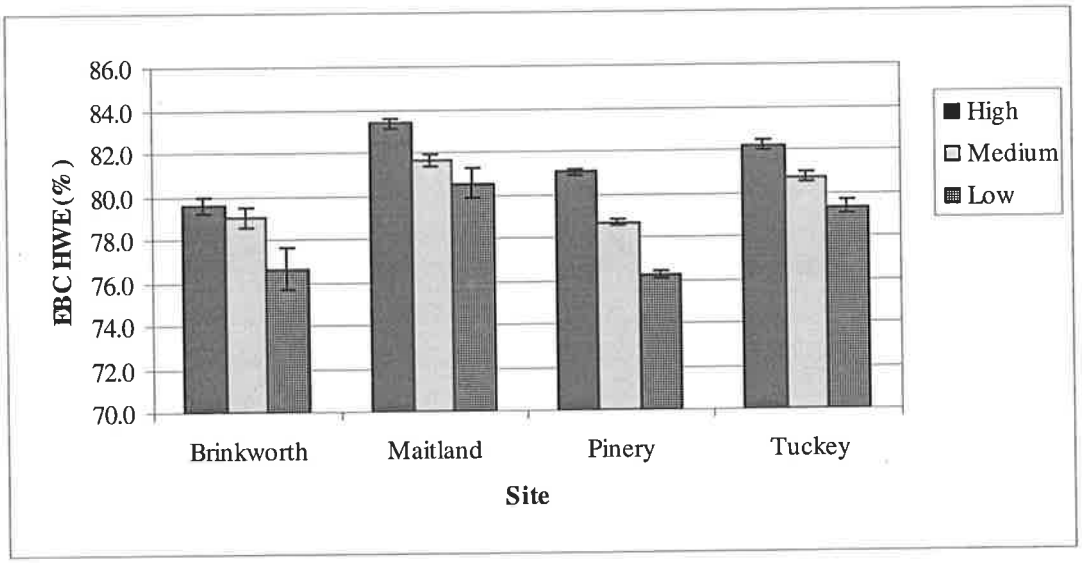


Figure 4.3.1: Mean EBC HWE of three groups based on the common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque.

4.3.1.1 IOB HWE

Mean IOB HWE for the four sites is shown in Table 4.3.6. As for EBC HWE Maitland was significantly higher than all other sites ($P<0.001$) and Tuckey was significantly higher than Pinery and Brinkworth ($P<0.001$). Maitland also had a much lower standard deviation than the other three sites. Again this is likely to be due to the influence of protein and modification on the grain (Table 4.3.2).

Brinkworth showed poor correlations with the other three sites (Table 4.3.7) and therefore had the lowest loading (Table 4.3.8). Tuckey and Pinery produced the strongest correlations and therefore the highest loadings. Maitland produced slightly lower correlations with all sites and therefore produced an intermediate loading of 0.83. Consequently less information was used from Brinkworth and Maitland than Pinery and Tuckey when calculating the common effect. As with EBC HWE, the genetic variance and heritability were higher at Pinery than the other three sites (Table 4.3.8).

Sloop produced the third highest mean IOB HWE, above Franklin and the overseas varieties Alexis, Manley and Chariot. However, this result was influenced by one site, Brinkworth. At all other sites Sloop produced lower IOB HWE than Franklin, Alexis, Manley and Chariot. Consequently, in the calculation of the common effect, Sloop ranked lower than Franklin, Alexis, Manley and Chariot (Table 4.3.9). Chariot performed particularly poorly at Brinkworth. The common effect for Chariot placed it higher in the rankings than would be expected from the over all mean IOB HWE. The rankings for Harrington, Haruna Nijo, Schooner, Chebec, Arapiles, Barque and Galleon remained the same regardless of whether they were ranked on the mean IOB HWE or the common effect.

Using the common effect (Table 4.3.9), the varieties can be divided into three groups:

High: (common effect greater than 0) Harrington, Haruna Nijo, Franklin, Alexis, Sloop, Manley and Chariot;

Medium: (common effect between 0 and -1) Schooner, Arapiles and Chebec;

Low: (common effect less than -1) Galleon and Barque.

Mean IOB HWE of the three groups is shown graphically in Figure 4.3.2. In comparison to the EBC HWE groups Sloop now falls into the second (medium) group.

Table 4.3.6: Mean IOB HWE (%) at four sites (mean of three reps)

	#Brinkworth	#Tuckey	#Maitland	#Pinery	#Mean	Standard deviation
Alexis	76.4^{de}	80.1^{b}	81.7^{a}	78.2^{a}	79.1^{b}	2.2
Arapiles	75.7^{e}	76.7^{d}	78.8^{e}	75.6^{c}	76.7^{d}	1.4
Barque	73.2^{g}	76.4^{d}	77.0^{f}	72.4^{d}	74.7^{e}	2.4
Chariot	74.8^{f}	80.1^{b}	81.9^{a}	78.5^{a}	78.8^{b}	3.0
Chebec	74.8^{f}	79.7^{b}	80.1^{cd}	75.3^{c}	77.5^{cd}	2.7
Franklin	76.7^{cd}	80.1^{b}	80.8^{bc}	78.6^{a}	79.0^{b}	1.7
Galleon	74.0^{g}	73.4^{e}	79.5^{de}	70.3^{e}	74.3^{e}	3.6
Harrington	79.1^{b}	81.5^{a}	82.0^{a}	78.3^{a}	80.2^{a}	1.8
Haruna Nijo	80.5^{a}	80.0^{b}	81.5^{ab}	78.5^{a}	80.1^{a}	1.4
Manley	77.4^{c}	79.8^{b}			78.6^{b}	1.9
Schooner	79.0^{b}	78.6^{c}	79.5^{de}	76.1^{bc}	78.3^{bc}	1.7
Sloop	79.6^{b}	79.3^{bc}	80.7^{bc}	76.9^{b}	79.1^{b}	1.8
Mean	76.7	78.8	80.3	76.2	78.0	2.9
Standard deviation	2.5	2.4	1.7	2.7		

#Means within a column followed by a common *superscript* letter are not significantly different (P<0.05).

Table 4.3.7: The relationship (r^2) between four sites for IOB HWE.

	97Brinkworth	97Tuckey	98Maitland	98Pinery
97Brinkworth	1.00	-	-	-
97Tuckey	0.65	1.00	-	-
98Maitland	0.56	0.81	1.00	-
98Pinery	0.67	0.97	0.83	1.00

Table 4.3.8: Genetic variance, loading in the common effect and heritability (h^2) of IOB HWE from 12 varieties grown at four sites

Site	Genetic variance	loading	Site mean	h^2
Brinkworth	4.85	0.67	76.6	79.9%
Tuckey	4.69	0.98	78.8	84.8%
Maitland	2.05	0.83	80.3	80.2%
Pinery	7.03	1.00	76.2	89.2%

Table 4.3.9: IOB HWE BLUPS (best linear unbiased predictors) and the common effect for four sites

	Brinkworth	Tuckey	Maitland	Pinery	Common effect
Galleon	-2.88	-4.99	-1.44	-5.95	-4.49
Barque	-3.26	-2.87	-2.87	-3.93	-2.97
Arapiles	-0.95	-1.34	-1.27	-1.08	-0.88
Chebec	-1.63	-0.19	-0.30	-0.85	-0.60
Schooner	1.75	-0.18	-0.65	-0.21	-0.16
Sloop	2.35	0.53	0.32	0.63	0.49
Manley	0.67	1.01	0.54	1.20	0.91
Alexis	-0.09	1.38	1.19	1.81	1.36
Chariot	-1.33	1.49	1.36	2.01	1.50
Franklin	0.19	1.54	0.61	2.11	1.56
Haruna Nijo	3.04	1.52	1.07	2.11	1.59
Harrington	2.13	2.11	1.45	2.14	1.67

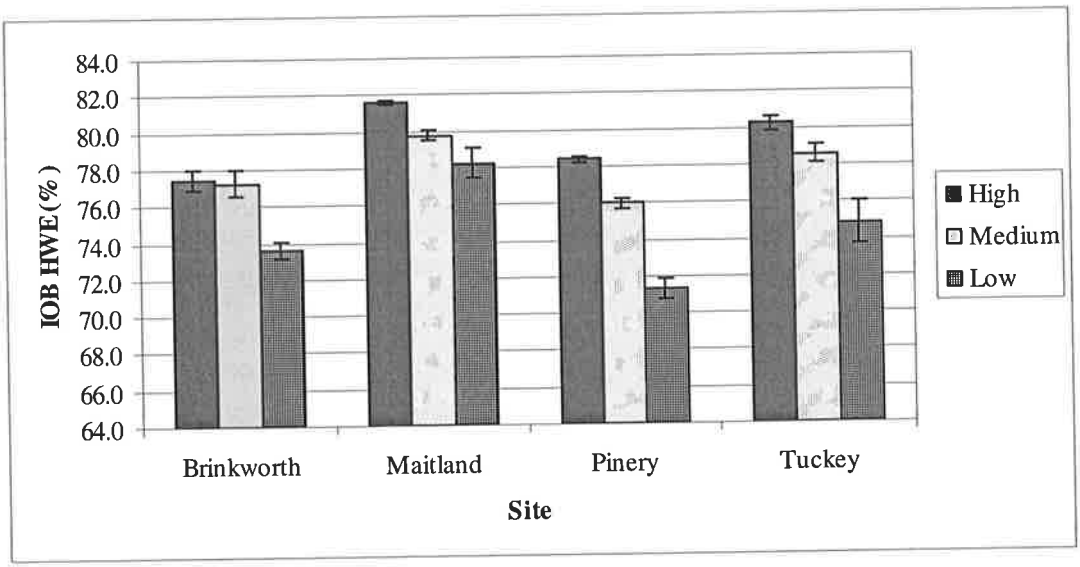


Figure 4.3.2: Mean IOB HWE of three groups based on the common effect (Table 4.3.9) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Sloop, Manley and Chariot; Medium: Schooner, Arapiles and Chebec; Low: Galleon and Barque

4.3.2 The influences of the malt and barley components on HWE

4.3.2.1 Physical barley and malt traits

The means of the physical barley and malt traits are shown in Tables 4.3.10 and 4.3.11. The barley parameters related to grain size included screenings, 1000 grain weight and weight and diameter measured by SKCS. The smallest grained varieties, as indicated by low 1000 grain weight, low SKCS weight, low SKCS diameter and high screenings were Franklin, Harrington, Alexis and Chariot. Manley also produced high levels of screenings. The two Australian feed varieties, Galleon and Barque, produced the highest grain weight but not the lowest screenings. The only size parameter measured on the malted grain was 1000 grain weight. All of the overseas malting varieties and Franklin produced the lowest grain weights, while the two Australian feed varieties, Galleon and Barque produced the highest grain weights.

Franklin produced the hardest grain as well as quite high milling energy, while Haruna Nijo had the softest grain and low milling energy. Franklin, Harrington and Manley had the highest grain hydration scores at all of the measured times. Galleon and Barque had the lowest grain hydration scores at all times.

All correlations between the physical traits and HWE are shown in Table 4.3.12. Additionally each physical trait was divided into the three groups described in section 4.3.1.1 and the mean is shown graphically in Figure 4.3.3. Of all of the size related traits, screening was the most poorly associated trait with HWE, showing only weak correlations with both IOB and EBC HWE at all sites. This is highlighted in Figure 4.3.3 with the medium HWE varieties and low HWE varieties producing similar screening levels. The SKCS diameter of the grain had moderate correlations with EBC HWE but only weak correlations with IOB HWE. SKCS weight, barley 1000-grain weight and malt 1000-grain weight showed strong to moderate relationships with both IOB and EBC HWE at all sites except Brinkworth.

Surprisingly no relationship was found between either milling energy or SKCS hardness and HWE. Figure 4.3.3 shows that the mean of the high HWE varieties for SKCS hardness is significantly higher ($P<0.05$) than the other two groups, whereas the low varieties showed significantly higher milling energy than the other groups.

Table 4.3.10: Barley and malt physical traits, mean of four sites and three replicates

Variety	SKCS Weight (mg)	SKCS Diameter (mm)	SKCS Moisture (%)	SKCS Hardness (HU)	[a]Milling Energy (J)	Screen (% <2.2)	Barley 1000 GW (g)	Malt 1000 GW (g)
Alexis	38.2	2.6	12.3	54.2	471	9.1	34.6	30.0
Arapiles	41.2	2.7	12.1	49.9	481	4.0	37.5	32.1
Barque	46.9	2.8	11.9	36.3	493	3.0	42.8	36.7
Chariot	37.0	2.6	12.2	41.3	419	5.4	33.0	27.2
Chebec	42.9	2.7	12.1	36.0	443	3.4	39.3	32.8
Franklin	36.8	2.5	12.3	67.0	485	11.2	33.2	27.6
Galleon	44.4	2.7	12.0	35.0	472	3.9	40.5	36.1
Harrington	37.4	2.6	12.3	46.2	501	7.8	33.5	26.9
Haruna Nijo	41.9	2.8	12.0	29.5	380	3.4	37.6	30.8
Manley	38.4	2.7	12.1	46.6		10.9	35.4	27.2
Schooner	42.3	2.7	12.1	43.0	469	2.7	38.2	31.2
Sloop	43.1	2.7	12.2	32.8	421	2.8	39.8	31.5
SD	4.2	0.1	0.5	14.1	36	5.7	4.1	5.3
SE	0.4	0.0	0.0	1.2	6	0.5	0.4	0.5

[a]Measured at Pinery and Maitland only,

Table 4.3.11: The physical traits during malting (mean of four sites and three replicates)

Variety	Grain Hydration (out of 200)			
	Steep	24 Hrs	48 Hrs	72 Hrs
Alexis	86	135	142	144
Arapiles	82	133	133	127
Barque	74	123	116	111
Chariot	89	161	151	149
Chebec	92	142	135	128
Franklin	97	161	167	154
Galleon	72	122	126	113
Harrington	104	170	171	174
Haruna Nijo	91	148	147	148
Manley	113	180	166	163
Schooner	89	142	139	133
Sloop	90	150	162	149
SD	23	19	18	22
SE	3	2	2	3

Table 4.3.12: Correlations between EBC HWE, IOB HWE and, barley and malt physical traits measured at four sites

	EBC HWE				IOB HWE			
	Pinery	Maitland	Brinkworth	Tuckey	Pinery	Maitland	Brinkworth	Tuckey
SKCS Weight	***-0.89***	**-0.74**	-0.40	**-0.74**	**-0.75**	**-0.73**	-0.16	**-0.71**
SKCS Diameter	**-0.74**	-0.56	0.03	-0.50	-0.57	-0.54	0.27	-0.42
SKCS Moisture	***0.83***	**0.69**	0.33	0.38	**0.77**	**0.72**	0.46	0.48
SKCS Hardness	**0.64**	0.22	0.05	0.07	0.52	0.20	-0.18	0.01
Milling Energy	-0.28	-0.36			-0.37	-0.44		
Screenings	0.57	0.39	0.03	0.42	0.45	0.35	-0.13	0.41
Barley 1000GW	***-0.90***	**-0.77**	-0.47	**-0.74**	**-0.78**	**-0.76**	-0.28	**-0.65**
Malt 1000GW	***-0.92***	***-0.80***	**-0.60**	**-0.72**	***-0.81***	***-0. 80***	-0.57	***-0.87***
GH Steep	0.41	0.00	0.44	**0.75**	0.45	-0.05	0.40	0.55
GH 24 hours	0.46	0.37	0.43	***0.83***	0.51	0.35	0.41	**0.68**
GH 48 hours	0.45	0.56	0.59	**0.77**	0.50	0.53	0.52	**0.71**
GH 72 hours	***0.87***	0.53	0.53	***0.91***	**0.79**	0.50	0.50	***0.84***

\|r\|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Grain hydration is a measure of the ability of water to filter through the endosperm of the grain during the malting process. A loosely packed, mealy grain will allow the water to spread evenly over the whole of the endosperm, therefore allowing even modification (Landau *et al.*, 1995). Strong to moderate correlations were found between grain hydration at 48 and 72 hours and both IOB and EBC HWE. At both 24 and 48 hours all three groups (Figure 4.3.3) were significantly different ($P<0.05$). However by 72 hours the low varieties were no longer significantly different to the medium extract varieties.

Tuckey, the site with the highest modification levels, was more influenced by grain hydration than the other sites. Brinkworth, an under-modified, high protein site was poorly correlated with all of the physical traits except malt 1000 grain weight. In general EBC HWE showed stronger correlations with the measured physical traits than IOB HWE. The exception to this was moisture content.

Multiple regression analysis was performed at all four sites. At all sites barley 1000GW and SKCS weight were highly correlated and only barley 1000GW was used in the model. A number of traits either showed a very poor relationship with HWE or were highly correlated with other traits and were therefore omitted from the analysis. The traits considered for the analysis for each site are shown in Table 4.3.13 and Table 4.3.14. Either malt 1000GW or barley 1000GW were significant traits in the final models for both IOB HWE and EBC HWE at Pinery, Maitland and Tuckey, indicating that grain weight is an important indicator of HWE potential. The grain hydration score after 48 hours was a significant trait in the models for EBC HWE and IOB HWE at Brinkworth.

Table 4.3.13: The model produced, using multiple regression analysis, to predict EBC HWE from the physical barley and malt traits at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 63.1-0.3479 x malt 1000GW + 2.369 x SKCS moisture	SKCS hardness, SKCS moisture, GH 24hrs, barley 1000GW, malt 1000GW, log screenings	81%
Maitland	EBC HWE = 91.76-0.2690 x malt 1000GW	SKCS diameter, SKCS moisture, GH 48hrs, malt 1000GW, log screenings	34%
Brinkworth	EBC HWE = 26.0+3.88 x SKCS moisture + 0.0420 x GH 48 hours	SKCS diameter, SKCS moisture, GH steep, GH 24hrs, GH 48hrs, GH 72hrs, barley 1000GW, malt 1000GW	30%
Tuckey	EBC HWE = 91.44-0.2642 x barley 1000GW	SKCS diameter, SKCS moisture, barley 1000GW, malt 1000GW, log screenings	43%

Table 4.3.14: The model produced using multiple regression analysis to predict IOB HWE from the physical barley and malt traits at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 92.62-0.4847 x malt 1000 GW	SKCS hardness, SKCS moisture, GH 24hrs, barley 1000GW, malt 1000GW, log screenings	68%
Maitland	IOB HWE = 91.83-0.3242 x malt 1000 GW	SKCS diameter, SKCS moisture, GH 48hrs, malt 1000GW, log screenings	40%
Brinkworth	IOB HWE = -14.4+6.87 x SKCS moisture + 0.0546 x GH 48 hours	SKCS diameter, SKCS moisture, GH steep, GH 24hrs, GH 48hrs, GH 72hrs, barley 1000GW, log screenings	51%
Tuckey	IOB HWE = 95.20-0.5282 x malt 1000 GW	SKCS diameter, SKCS moisture, barley 1000GW, malt 1000GW, log screenings	59%

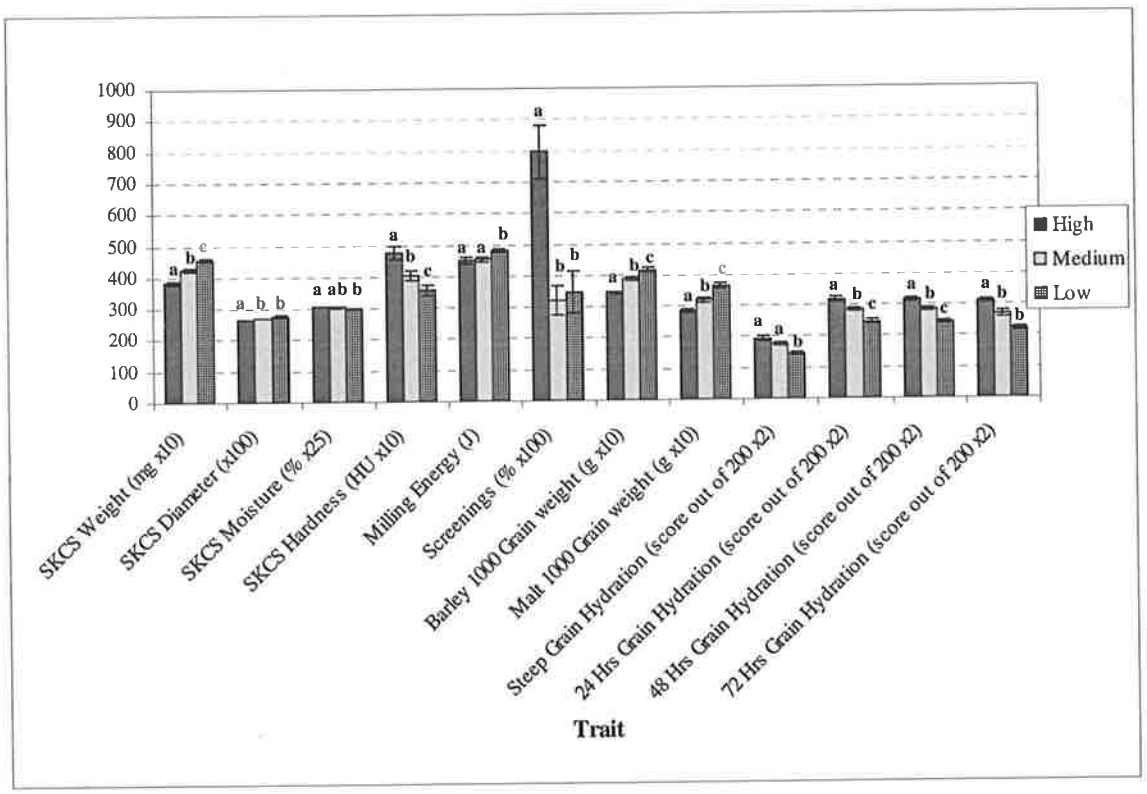


Figure 4.3.3: Graph showing the mean of the physical traits (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. [abc] Means with letter in common are not significantly different ($P<0.05$).

4.3.2.2 Components of Barley and Malt:

The Japanese variety Haruna Nijo had the lowest mean barley husk content, barley β-glucan and barley arabinoxylan content and the highest grain protein, while the variety Harrington had the lowest barley protein and highest barley starch content (Table 4.3.15). After malting Haruna Nijo still produced the lowest husk content, while Galleon produced the highest starch and β-glucan contents (Table 4.3.16).

Of all of the malt and barley components, the only two traits that showed consistent relationships with either IOB or EBC extract were barley husk content and malt β-glucan (Table 4.3.17). This was confirmed by dividing each trait into the three groups discussed in section 4.3.1.1. Barley husk content and malt β-glucan are the

only two traits where each group is significantly different ($P<0.05$) to the other groups (Figure 4.3.4). Barley husk content showed strong to moderate relationships with EBC HWE at all sites except Maitland. The relationship with barley husk content was stronger for EBC HWE than IOB HWE at most sites. Malt β-glucan showed moderate to strong relationships with IOB extract at all sites. EBC extract showed a slightly weaker correlation at all sites with poor to moderate relationships.

The change in the components of the barley and malt during the malting process were calculated by subtracting the amount of the component in the barley from the amount in the malt on a per grain weight basis (expressed as a percentage). This is shown graphically in Figure 4.3.5. During the malting process, approximately 27% of the protein was mobilized and discarded as rootlets. Additionally, approximately 15% of the husk, 35% of the starch, 85% of the β-glucan and 40% of the pentose were degraded and either remained in the malt as small molecules or were discarded as part of the rootlets. During the malting process, the high HWE varieties mobilized a significantly larger ($P<0.05$) percentage of the starch and β-glucan than the low varieties (Figure 4.3.5).

Multiple regression analysis was performed to compare HWE to the malt and barley components at all four sites (Table 4.3.18 and Table 4.3.19). Log of the malt β-glucan levels were used at Maitland and Tuckey due to skewing. Numerous traits from all sites showed poor relationships with HWE and were not considered in the analysis. Malt β-glucan was significant in the models at all four sites for EBC HWE and all sites except Pinery for IOB HWE. Barley husk content was included in the models at three sites for EBC HWE and two sites for IOB HWE. At Pinery the only trait included in the final model for EBC HWE was malt protein.

Table 4.3.15: The barley component traits, mean of four sites and three replicates

Variety	Husk (%db)	Protein (%db)	Starch (%db)	ß-Glucan (%db)	Pentose (%db)	Arabinoxylan (%db)
Alexis	9.8	11.0	61.6	4.1	6.1	5.6
Arapiles	10.0	10.7	63.4	3.7	5.8	5.0
Barque	12.1	10.9	60.6	3.7	6.4	5.3
Chariot	9.5	10.9	62.6	3.6	5.7	5.1
Chebec	11.0	10.6	61.4	3.3	6.1	5.3
Franklin	9.4	11.0	61.2	4.6	5.7	4.8
Galleon	10.5	11.1	62.8	3.5	6.1	5.3
Harrington	9.9	10.4	63.7	4.2	6.4	5.7
Haruna Nijo	8.1	12.5	63.0	3.0	6.0	4.5
Manley	11.6	11.6	61.9	3.8	6.5	5.4
Schooner	10.1	11.4	62.7	3.6	6.3	5.2
Sloop	10.1	11.2	61.9	3.1	6.3	4.9
SD	1.7	1.7	2.6	0.7	1.5	1.3
SE	0.1	0.1	0.2	0.1	0.1	0.1

Table 4.3.16: The malt component traits, mean of four sites and three replicates

Variety	Husk (%db)	Protein (%db)	Starch (%db)	ß-Glucan (%db)	Pentose (%db)	Arabinoxylan (%db)
Alexis	12.7	11.0	58.0	0.27	3.6	2.7
Arapiles	11.3	10.2	60.9	0.73	3.4	2.0
Barque	12.8	10.7	60.4	0.80	3.9	2.4
Chariot	12.3	10.6	61.3	0.26	3.2	1.5
Chebec	12.3	10.0	59.5	0.56	4.2	2.8
Franklin	11.8	10.7	59.0	0.35	3.5	2.6
Galleon	10.8	10.6	61.9	1.17	3.4	2.5
Harrington	12.5	10.1	57.5	0.46	3.7	2.1
Haruna Nijo	10.6	12.0	61.1	0.39	3.8	2.4
Manley	12.4	11.4	54.5	0.39	5.0	1.9
Schooner	11.2	11.0	61.7	0.43	3.2	2.1
Sloop	11.3	10.8	61.8	0.19	4.0	2.0
SD	1.4	1.5	3.6	0.4	1.1	1.1
SE	0.1	0.1	0.3	0.0	0.1	0.1

Table 4.3.17: Correlations between EBC HWE, IOB HWE and barley (B) and malt (M) components.

	EBC HWE				IOB HWE			
	Pinery	Maitland	Brinkworth	Tuckey	Pinery	Maitland	Brinkworth	Tuckey
B Husk	**-0.66**	-0.42	***-0.85***	**-0.60**	**-0.60**	-0.44	**-0.64**	-0.48
B Protein	-0.52	-0.18	0.17	0.00	-0.51	-0.18	0.19	-0.04
B Starch	0.33	0.29	0.37	**0.71**	0.24	0.33	0.07	0.43
B ß-glucan	0.14	-0.22	0.06	0.33	0.09	-0.32	-0.22	0.12
B Total Pentose	-0.31	0.11	-0.13	-0.36	-0.32	0.08	0.17	-0.33
B Arabinoxylan	-0.44	0.26	-0.03	0.16	-0.32	0.25	0.27	-0.06
M Protein	-0.57	-0.16	0.07	0.02	-0.53	-0.17	0.13	0.01
M Starch	0.33	-0.20	0.18	**-0.76**	0.50	-0.15	0.06	**-0.69**
M Husk	0.16	0.13	**-0.69**	0.20	0.21	0.04	**-0.71**	0.40
M ß-glucan	**-0.78**	***-0.80***	-0.39	-0.58	***-0.93***	***-0.86***	**-0.66**	***-0.90***
M Total Pentose	**-0.67**	0.05	0.11	0.50	**-0.73**	-0.02	0.26	0.45
M Arabinoxylan	-0.57	-0.26	0.09	0.33	-0.55	-0.37	0.13	0.46

\|r\|=0.8 –1 strong relationship, **\|r\|=0.6-0.8 moderate relationship**, \|r\|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.18: The model produced, using multiple regression analysis, to predict EBC HWE from the barley (B) and malt (M) components at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 96.56 – 0.640 x B husk – 0.955 M protein – 1.152 x M ß-glucan	B protein, B husk, B starch, B Arabinoxylan, M protein, M ß-glucan, M Arabinoxylan, M Pentose	79%
Maitland	EBC HWE = 80.419 – 3.177 x log M ß-glucan	B protein, B husk, B starch, M protein, log M ß-glucan	25%
Brinkworth	EBC HWE = 55.75 – 0.720 x B Husk – 2.457 x M β-glucan	B husk, B starch, M starch, M ß-glucan, M husk	61%
Tuckey	EBC HWE = 87.59 –0.467 B husk- 7.17 x log M ß-glucan	B husk, B starch, M starch, log M ß-glucan	39%

Table 4.3.19: The model produced using multiple regression analysis to predict IOB HWE from barley (B) and malt (M) components at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 1.169 + 0.9305 x M protein	B protein, B husk, B starch, B Arabinoxylan, M protein, M starch, M ß-glucan, M Arabinoxylan, M Pentose	90%
Maitland	IOB HWE = 77.171 – 3.915 x log M ß-glucan	B protein, B husk, B starch, B ß-glucan, log M protein, log M ß-glucan	45%
Brinkworth	IOB HWE = 80.67 – 1.346 x B Husk + 1.768 x B pentose – 2.55 x M β-glucan	B husk, B pentose, M ß-glucan, M husk	67%
Tuckey	IOB HWE = 87.32 –0.483 B husk - 20.58 x log M ß-glucan	B husk, B starch, M starch, log M ß-glucan, M pentose	75%

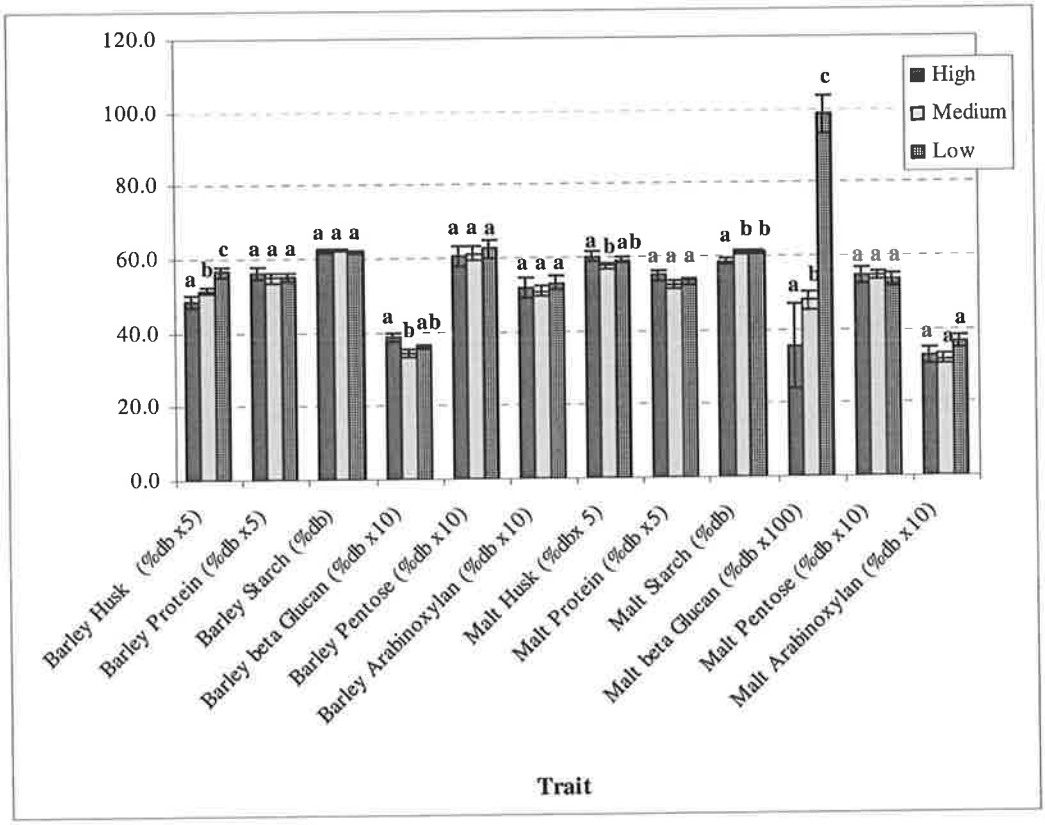


Figure 4.3.4: Graph showing the mean of the barley and malt components (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. ^{abc} Means with letter in common are not significantly different ($P<0.05$).

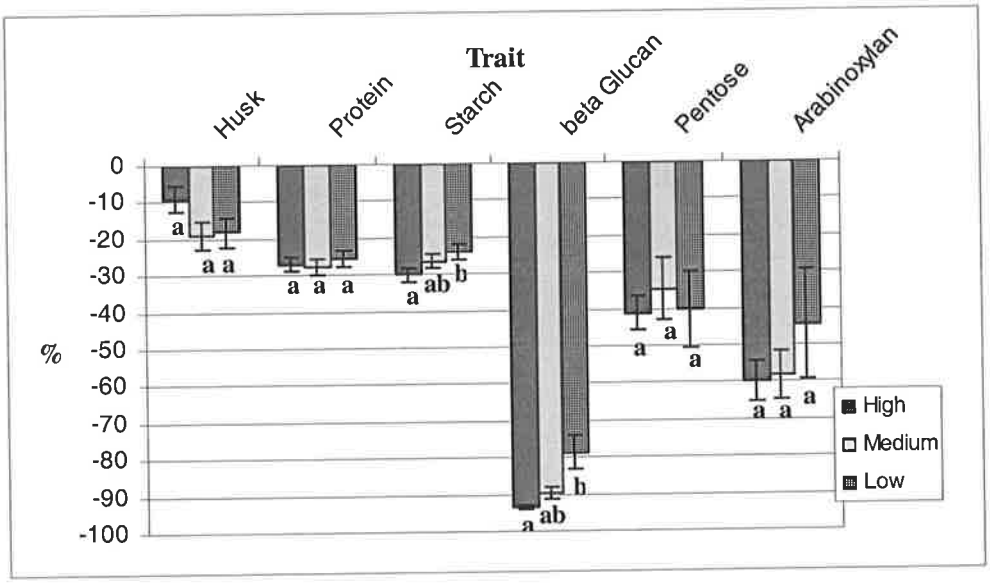


Figure 4.3.5: Graph showing the loss of the barley and malt components during the malting process divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. [abc] Means with letter in common are not significantly different ($P<0.05$).The change in the malting process was calculated by subtracting the amount of the component in the barley from the amount in the malt on a per grain weight basis as a percentage.

4.3.2.3 Starch and Cell Wall Degrading Enzymes

Galleon produced the lowest levels for all of the starch and cell wall degrading enzymes measured except β-amylase (Table 4.3.20). Assisted by high protein levels at Brinkworth (Table 4.3.2), Manley and Alexis produced high levels of all of the enzymes measured.

Moderate to strong correlations were found between both IOB and EBC HWE and the levels of all enzymes measured except β-amylase (Table 4.3.21). The site Brinkworth was an exception to this, as limit dextrinase was the only enzyme found to have a moderate relationship with either IOB HWE or EBC HWE. Brinkworth in 1997 was characterised by very dry conditions through October (Crop Harvest

Report, 1997/1998), which contributed to higher barley protein levels in a number of the late maturing overseas varieties (data not shown). The mean and range in barley protein at Brinkworth, in 1997, were generally higher than at the other three sites (Table 4.3.2). As enzyme levels within a variety are greatly influenced by the level of grain protein, the relationship with HWE may have been influenced atypically. In general the relationship between the starch and cell wall degrading enzymes and IOB HWE was stronger than the relationship between the enzyme levels and EBC HWE.

Figure 4.3.6 shows the mean for each starch and cell wall enzyme when divided into the three groups discussed in section 4.3.1.1. The means of the levels of DP, α-amylase and limit dextrinase for all three groups were statistically different ($P<0.05$). The means of the levels of β-amylase for the medium and low groups were statistically the same ($P<0.05$) and the mean of the levels of β-glucanase for the high and the medium groups were also statistically the same ($P<0.05$).

Multiple regression analysis was performed on the starch and cell wall degrading enzymes (Table 4.3.22 and Table 4.3.23). DP and β-amylase were highly correlated at all sites and therefore DP was omitted from the analysis. Limit dextrinase was the only significant enzyme in the final model for both IOB HWE and EBC HWE at all sites except EBC HWE at Brinkworth.

Table 4.3.20: Starch and cell wall degrading enzymes levels, mean of four sites and three replicates

Variety	Diastatic Power (μm/min/g)	α-amylase (μm/min/g)	ß-amylase (μm/min/g)	Limit Dextrinase (U/kg)	ß-Glucanase (U/kg)
Alexis	620	127	493	626	616
Arapiles	470	104	366	445	484
Barque	385	77	308	306	408
Chariot	568	99	469	567	571
Chebec	377	90	286	498	522
Franklin	581	118	463	497	478
Galleon	349	60	290	289	377
Harrington	526	134	392	575	543
Haruna Nijo	571	118	454	492	495
Manley	684	134	550	636	572
Schooner	403	89	314	504	544
Sloop	477	100	377	496	571
SD	135	117	29	125	110
SE	12	10	2	11	9

Table 4.3.21: Correlations between EBC HWE and IOB HWE and starch and cell wall degrading enzymes

	EBC HWE				IOB HWE			
	Pinery	Maitland	Brinkworth	Tuckey	Pinery	Maitland	Brinkworth	Tuckey
ß-Glucanase	***0.91***	0.37	0.19	0.56	***0.92***	0.45	0.35	**0.79**
Limit Dextrinase	**0.64**	***0.87***	**0.67**	**0.76**	**0.74**	***0.89***	**0.79**	***0.84***
Diastatic Power	**0.66**	**0.68**	0.12	0.58	**0.71**	**0.64**	0.15	**0.60**
α-amylase	***0.87***	**0.64**	0.29	**0.75**	***0.82***	0.56	0.46	***0.85***
ß-amylase	0.22	0.44	0.09	0.50	0.34	0.47	0.09	0.50

|r|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.22: The model produced, using multiple regression analysis, to predict EBC HWE from the starch and cell wall degrading enzymes at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 74.759 + 0.01099 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	75%
Maitland	EBC HWE = 78.197 + 0.0098 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	51%
Brinkworth	EBC HWE = 76.96 + 0.01633 x Limit Dextrinase – 0.0405 x α-amylase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	49%
Tuckey	EBC HWE = 78.0770 + 0.00861 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	42%

Table 4.3.23: The model produced using multiple regression analysis to predict IOB HWE from the starch and cell wall degrading enzymes at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 69.495 + 0.01629 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	80%
Maitland	IOB HWE = 72.598 + 0.01153 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	56%
Brinkworth	IOB HWE = 70.863 + 0.0159 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	61%
Tuckey	IOB HWE = 72.542 + 0.01674 x Limit Dextrinase	α-amylase, ß-amylase, Limit Dextrinase, ß-Glucanase	60%

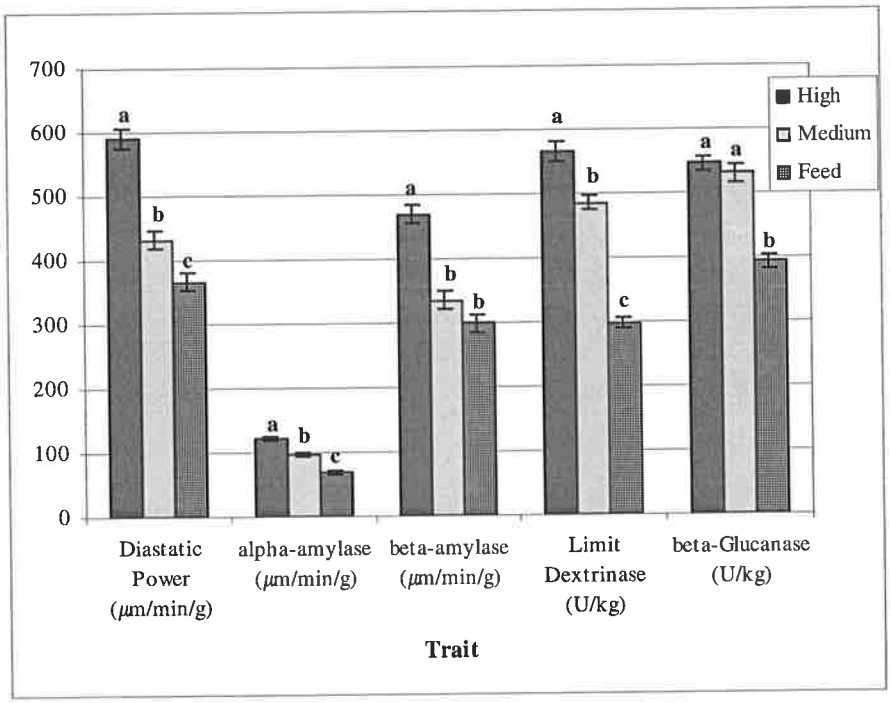


Figure 4.3.6: Graph showing the mean of the starch and cell wall degrading enzymes (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. [abc] Means with letter in common are not significantly different ($P<0.05$).

4.3.2.4 Starch Properties

Barque had the lowest DSC peak temperature and Franklin had the highest. Franklin and Alexis had the lowest peak height (Table 4.3.24). Chebec had the highest DSC peak area and height. The percentage of large starch granules (diameter>10μ) varied from 8 to 16% and the mean size varied from 14.9 to 19.4 microns (Table 4.3.25). Chariot had the largest mean large starch granule diameter at Pinery, while Harrington had the largest at Tuckey. Galleon recorded the smallest mean large starch granule diameter at both sites. The small starch granules (diameter<10μ) varied in size from 2.2 to 3.0μ. The RVA peak viscosity ranged between 19.0 and 54.1, with Harrington recording the lowest and Galleon the highest (Table 4.3.26).

Table 4.3.24: Malt DSC gelatinization temperatures for Tuckey (mean of 2 reps)

Variety	Onset (°C)	End (°C)	Peak (°C)	Peak Height (mW)	Peak Area (mJ)	Delta H (J/g)
Alexis	62.6	73.0	67.2	2.0	84.5	1.9
Arapiles	61.5	71.3	66.1	2.5	100.8	2.3
Barque	61.0	70.9	65.7	2.4	99.9	2.2
Chariot	61.4	72.0	66.1	2.3	103.9	2.3
Chebec	62.1	71.4	66.6	2.8	105.4	1.8
Franklin	63.2	74.1	68.3	2.0	85.9	2.1
Galleon	61.2	72.2	66.3	2.3	97.1	2.3
Harrington	61.8	71.4	66.4	2.5	97.1	2.2
Haruna Nijo	60.9	72.0	66.2	2.3	100.1	2.3
Manley	61.7	72.0	66.7	2.1	88.1	2.0
Schooner	62.0	72.6	67.1	2.3	100.9	2.3
Sloop	62.8	72.6	67.5	2.5	101.5	2.3
SD	0.8	1.0	0.8	0.3	8.4	0.3
SE	0.2	0.2	0.2	0.05	1.7	0.05

All of the starch properties measured by DSC produced poor correlations with both IOB and EBC HWE (Table 4.3.27). The percentage of large starch granules produced moderate correlations with EBC HWE at Pinery but not Tuckey (Table 4.3.27). The diameter of the large starch granules showed moderate to strong correlations with both EBC and IOB HWE and was the only trait to show significant differences ($P<0.05$) between all of the three groups described in section 4.3.1.1 (Figure 4.3.7). The size of the small starch granules only produced poor correlations with IOB and EBC HWE. This is in agreement with a number of studies that have also found good relationships between HWE and large starch granule diameter (Dunn *et al.*, 1996, Dunn *et al.*, 1997, Allan *et al.*, 1995).

Moderate to strong correlations were found between RVA peak viscosity and both IOB and EBC HWE (Table 4.3.27). However, when RVA peak viscosity was divided into the three groups described in Section 4.3.1.1, the groups were all statistically similar (Figure 4.3.7). RVA final viscosity produced a moderate relationship with

IOB HWE at Tuckey and RVA peak temperature produced a moderate correlation with EBC HWE at Maitland, but all other correlations for RVA traits were poor.

The diameter of the large starch granules was significant in the models produced by multiple regression analysis for both IOB HWE and EBC HWE at both of the sites measured, Pinery and Tuckey (Table 4.3.28 and Table 4.3.29). Other starch properties included in the models were RVA peak and final viscosity and the percentage of the large starch granules.

Table 4.3.25: Barley Starch Granule size and ratio for Tuckey and Pinery (mean of 2 reps)

Variety	*Mean small granule diameter (μ)		*Mean large granule diameter (μ)		*Percentage of large granules (%)	
	Pinery	Tuckey	Pinery	Tuckey	Pinery	Tuckey
Alexis	2.7	2.4	17.4	18.8	11	8
Arapiles	2.6	2.5	16.4	18.3	14	7
Barque	2.7	2.6	15.8	17.0	16	13
Chariot	2.4	2.5	18.0	18.3	8	14
Chebec	2.2	2.6	15.5	17.7	11	12
Franklin	2.5	3.0	17.0	17.3	9	11
Galleon	2.5	2.6	14.9	15.7	11	12
Harrington	2.3	2.5	16.8	19.4	8	8
Haruna Nijo	2.6	2.4	17.8	19.2	8	12
Manley		2.7		18.7		10
Schooner	2.5	2.4	15.8	16.6	15	8
Sloop	2.2	2.7	17.1	17.8	12	7
SD	0.2	0.2	1.0	1.1	3.7	3.2
SE	0.04	0.04	0.2	0.2	0.8	0.6

*Small granules <10μ, large granules >10μ,

Table 4.3.26: Malt RVA Starch Pasting Properties (mean of three sites)

	Peak Time (mins)	Peak Viscosity (RVA units)	Final Viscosity (RVA units)	Peak Temp (°C)
Alexis	3.1	24.0	0.9	72.2
Arapiles	3.1	31.1	1.5	72.3
Barque	3.2	47.7	12.6	73.8
Chariot	3.2	31.2	4.3	73.9
Chebec	3.2	38.4	13.4	73.7
Franklin	3.4	22.1	7.0	75.3
Galleon	3.3	54.1	21.4	74.3
Harrington	3.0	19.0	4.9	72.0
Haruna Nijo	3.1	29.4	5.5	72.9
Manley	3.2	23.4	4.0	73.2
Schooner	3.2	29.3	6.2	73.5
Sloop	3.1	28.6	2.8	72.8
SD	0.4	17.2	21.8	3.5
SE	0.04	1.8	2.2	0.35

Table 4.3.27: Correlations between EBC HWE and IOB HWE and starch properties

	EBC HWE			IOB HWE		
	Pinery	Maitland	Tuckey	Pinery	Maitland	Tuckey
DSC Onset			0.25			0.43
DSC End			0.18			0.24
DSC Peak			0.24			0.37
DSC Pk Ht			-0.15			-0.10
DSC Pk Area			-0.30			-0.19
DSC Delta H			-0.27			-0.35
Size Small Granules	-0.06		-0.06	-0.16		0.01
% Large Granules	**-0.72**		-0.19	-0.58		-0.17
Diameter Large Granules	***0.80***		***0.80***	***0.86***		**0.75**
RVA Peak Time	-0.02	-0.55	-0.18	-0.15	-0.52	-0.04
RVA Peak Viscosity	***-0.85***	**-0.74**	-0.58	***-0.88***	**-0.75**	**-0.69**
RVA Final Viscosity	0.18	-0.30	-0.48	0.08	-0.26	**-0.65**
RVA Peak Temp	-0.01	**-0.61**	-0.24	-0.18	-0.56	-0.11

|r|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.28: The model produced, using multiple regression analysis, to predict EBC HWE from the starch properties at three sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 67.35 + 0.969 x Diameter Large Granules – 0.1454 x % Large Granules – 0.0845 x RVA Peak Viscosity	Diameter Large Granules, RVA Peak Viscosity, % Large Granules	70%
Maitland	EBC HWE = 83.523 + 0.04275 x RVA Peak Viscosity	RVA Peak Viscosity	12%
Tuckey	EBC HWE = 63.08 + 1.009 x Diameter Large Granules	Diameter Large Granules, RVA Peak Time, RVA Peak Viscosity, log RVA Final Viscosity, RVA Peak Temp	55%

Table 4.3.29: The model produced using multiple regression analysis to predict IOB HWE from the starch properties at three sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 57.86 + 1.406 x Diameter Large Granules – 0.1716 x RVA Peak Viscosity	Diameter Large Granules, RVA Peak Viscosity, % Large Granules	73%
Maitland	IOB HWE = 82.564 -0.0906 x RVA Peak Viscosity + 0.0783 x Final Viscosity	RVA Peak Viscosity, Final Viscosity	41%
Tuckey	IOB HWE = 57.68 + 1.254 x Diameter Large Granules -0.0831 x RVA Peak Viscosity + 2.203 x log RVA Final Viscosity	DSC Onset Temp, DSC Peak Temp, Diameter Large Granules, RVA Peak Viscosity, log RVA Final Viscosity, RVA Peak Temp	78%

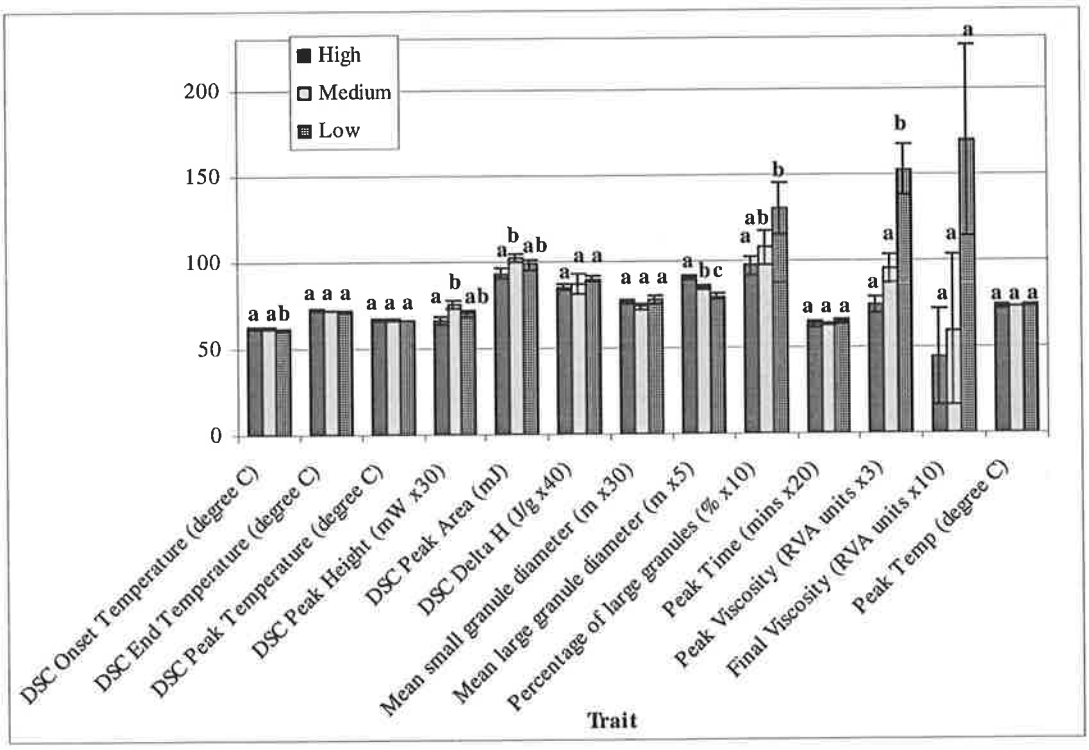


Figure 4.3.7: Graph showing the mean of the starch properties (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. ^{abc} Means with letter in common are not significantly different ($P<0.05$).

4.3.3 The influence of the wort and spent grain components on HWE

4.3.3.1 EBC Wort Components

The mean of the EBC wort components are listed in Table 4.3.30 and Table 4.3.31. Galleon produced the lowest levels of wort glucose, fructose, maltose, maltotriose, wort soluble protein, FAN and KI and the highest wort β-glucan, arabinoxylan and viscosity. After Galleon, Barque and Arapiles produced the lowest wort soluble protein, FAN and KI and the highest wort β-glucan and arabinoxylan levels.

Moderate correlations were formed between EBC HWE and β-glucan, arabinoxylan and KI at Pinery, Tuckey and Maitland (Table 4.3.32), while only poor correlations were formed at Brinkworth. The only two simple sugars that formed correlations with EBC HWE were glucose and fructose. Surprisingly, even though maltose is by far the

most abundant simple sugar in the wort, it did not relate to the level of EBC HWE. The levels of β-glucan, glucose and fructose showed significant differences ($P<0.05$) between all of the three groups described in section 4.3.1.1 (Figure 4.3.8).

Multiple regression analysis was performed for all four sites. The β-glucan levels at three of the sites were skewed and the log of β-glucan was used. At Brinkworth, where the simple sugar levels were not measured, β-glucan and viscosity produced a poor relationship with EBC HWE and were omitted from the analysis. The traits considered for the analysis for each site are shown in Table 4.3.33. A number of traits were significant in the final models for each site, including KI, soluble protein, log β-glucan, maltose, maltotriose and maltotetraose (Table 4.3.33).

Table 4.3.30: Mean results for EBC wort components, mean of four sites and three replicates

Variety	Soluble Protein (%)	FAN (mg/L)	ß-Glucan (mg/L)	Arabinoxylan (mg/L)	Viscosity (cP)	Kolbach Index (%)
Alexis	4.6	160	77	26	1.52	42.8
Arapiles	3.8	134	366	51	1.70	37.7
Barque	3.8	125	298	74	1.61	36.9
Chariot	4.2	143	56	14	1.51	40.3
Chebec	4.3	158	214	41	1.59	44.8
Franklin	4.5	155	175	32	1.64	42.8
Galleon	3.5	111	563	91	1.80	33.5
Harrington	5.2	197	121	20	1.57	51.5
Haruna Nijo	5.3	193	119	30	1.61	44.7
Manley	5.3	175	130	47	1.55	48.0
Schooner	5.1	181	127	47	1.60	43.0
Sloop	5.0	173	11	12	1.53	46.6
SD	0.8	36	226	52	0.14	8.7
SE	0.1	3	19	4	0.01	0.7

Table 4.3.31: Mean results for EBC wort simple sugars, mean of three sites and three replicates (mmols/L)

Variety	glucose	fructose	sucrose	maltose	maltotriose	maltotetraose	Total
Alexis	60	11	11	170	18	3.1	273
Arapiles	48	9	9	164	15	1.6	247
Barque	45	9	9	160	17	3.8	245
Chariot	54	11	8	160	18	2.8	254
Chebec	44	10	9	151	17	4.4	235
Franklin	48	10	9	153	16	3.2	239
Galleon	30	6	8	142	15	3.6	205
Harrington	62	12	6	143	17	2.3	242
Haruna Nijo	54	11	7	155	17	1.0	244
Manley	68	12	9	162	19	2.8	271
Schooner	46	10	9	155	18	4.1	241
Sloop	54	11	8	160	17	2.2	252
SD	0.6	0.1	0.1	0.8	0.2	0.1	
SE	0.1	0.0	0.0	0.1	0.0	0.0	

Table 4.3.32: Correlations between EBC HWE and EBC wort components

	Pinery	Maitland	Brinkworth	Tuckey
Soluble Protein	0.48	0.52	0.51	0.54
FAN	0.58	0.56	0.54	**0.71**
ß-Glucan	**-0.73**	**-0.68**	-0.26	-0.59
Arabinoxylan	**-0.67**	**-0.65**	-0.54	**-0.69**
Viscosity	-0.59	-0.58	0.10	-0.44
Kolbach	**0.73**	**0.70**	0.35	0.52
Glucose	***0.81***	**0.70**		**0.60**
Fructose	***0.84***	0.55		**0.66**
Sucrose	-0.01	-0.43		-0.18
Maltose	0.08	-0.26		0.24
Maltotriose	-0.48	0.57		0.34
Maltotetraose	-0.12	-0.56		-0.46
Total simple sugars	0.47	0.26		0.43

|r|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.33: The model produced using multiple regression analysis to predict EBC HWE from the EBC wort components at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 71.84 – 2.104 x SP + 0.4541 x KI	Soluble Protein, FAN, log ß-Glucan, Arabinoxylan, Viscosity, KI, Glucose, Fructose, Maltotetraose	57%
Maitland	EBC HWE = 84.608-1.078 x log ß-Glucan – 11.25 x maltotetrose	Soluble Protein, FAN, log ß-Glucan, Arabinoxylan, Viscosity, KI, Glucose, Fructose, Sucrose, Maltotriose, Maltotetraose	62%
Tuckey	EBC HWE = 80.78 – 1.008 x maltose – 16.28 x maltotetrose + 11.30 x maltotriose	Soluble Protein, FAN, log ß-Glucan, Arabinoxylan, Viscosity, Glucose, Fructose, Maltose, Maltotriose, Maltotetraose	44%
Brinkworth	EBC HWE = 69.94 + 0.2324 x KI	Soluble Protein, FAN, Arabinoxylan, KI	30%

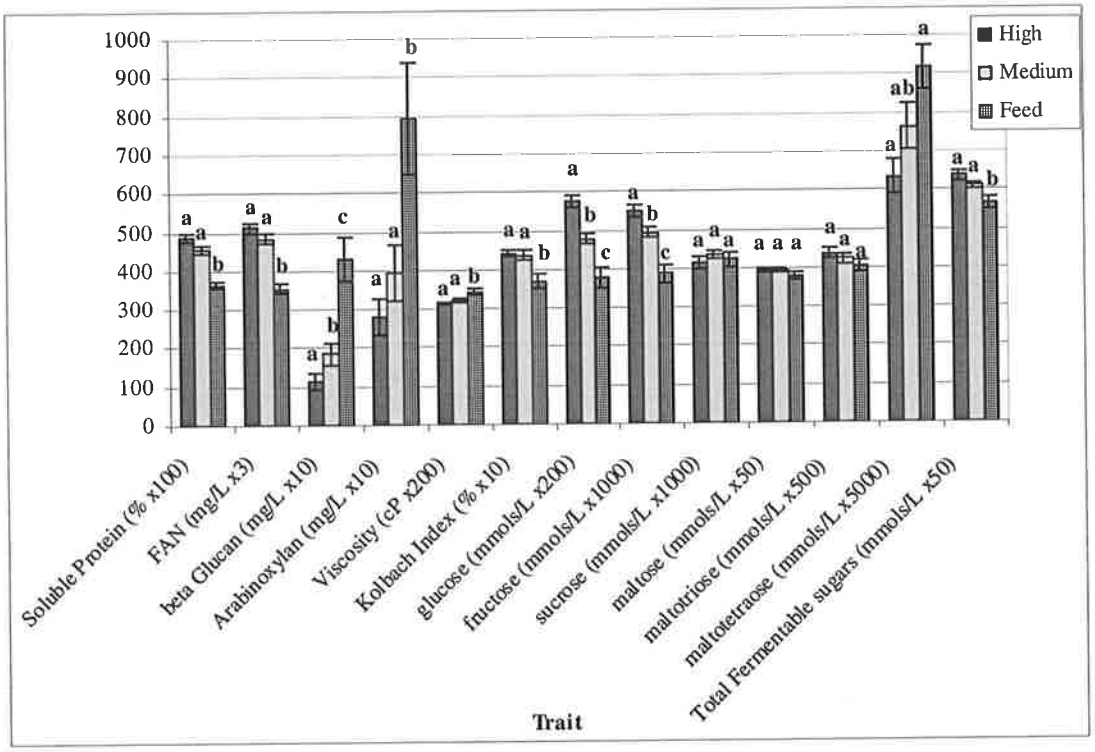


Figure 4.3.8: Graph showing the mean of the EBC wort components (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. [abc] Means with letter in common are not significantly different ($P<0.05$).

4.3.3.2 IOB Wort Components

The mean of the IOB wort components are listed in Table 4.3.34 and Table 4.3.35. Like EBC HWE Galleon had the lowest levels of wort glucose, fructose, maltose, maltotriose, soluble protein, FAN and KI and the highest wort β-glucan, arabinoxylan and viscosity. Again Galleon, Barque and Arapiles had the lowest wort soluble protein, FAN and KI and the highest wort β-glucan levels.

Moderate to strong correlations were formed between IOB HWE and soluble protein, FAN, β-glucan, arabinoxylan, viscosity and SP/TP (Table 4.3.36). Three sugars formed correlations with IOB HWE, namely glucose, fructose and maltotriose. The soluble protein, FAN, viscosity, glucose, fructose, sucrose and maltotetraose showed

significant differences (P<0.05) between all of the three groups suggested in section 4.3.1.1 (Figure 4.3.9).

The traits considered for the multiple regression analysis are shown in Table 4.3.37. The log of β-glucan was used at three of the sites due to skewing. The traits significant (P<0.05) in the final models for each site included SP/TP, soluble protein, log β-glucan, FAN, viscosity and glucose.

Table 4.3.34: Mean results for IOB wort components, mean of four sites and three replicates.

Variety	Soluble Protein (%)	FAN (mg/L)	ß-Glucan (mg/L)	Arabinoxylan (mg/L)	Viscosity (cP)	SP/TP (%)
Alexis	4.0	137	36	34	1.46	37.3
Arapiles	3.3	107	177	54	1.63	32.2
Barque	3.3	108	179	48	1.62	31.4
Chariot	3.6	119	22	10	1.46	34.8
Chebec	3.8	111	108	34	1.56	38.5
Franklin	3.9	132	78	53	1.49	37.0
Galleon	2.9	85	294	73	1.75	27.8
Harrington	4.4	155	73	32	1.53	43.3
Haruna Nijo	4.7	162	64	20	1.55	39.1
Manley	4.5	144	90	48	1.55	40.3
Schooner	4.0	143	79	60	1.57	36.8
Sloop	4.3	145	7	11	1.49	40.3
SD	0.7	35	122	52	0.12	7.5
SE	0.1	3	11	4	0.01	0.6

Table 4.3.35: Mean results for IOB wort simple sugars, mean of three sites and three replicates (mmols/L).

Variety	glucose	fructose	sucrose	maltose	maltotriose	maltotetraose	Total
Alexis	53	6.6	15	184	9	0.2	268
Arapiles	52	9.7	16	188	14	0.2	279
Barque	39	5.9	14	173	11	1.7	245
Chariot	50	7.4	15	185	10	0.0	268
Chebec	46	7.3	16	183	12	2.4	266
Franklin	53	7.5	17	190	9	0.0	277
Galleon	35	5.3	13	161	14	2.7	230
Harrington	64	8.9	17	182	10	0.0	281
Haruna Nijo	50	7.5	15	191	10	0.7	275
Manley	61	8.3	16	193	7	0.0	284
Schooner	46	7.1	14	181	12	2.5	263
Sloop	51	7.6	15	195	11	0.0	279
SD	0.7	0.2	0.1	0.9	0.3	0.1	0.7
SE	0.1	0.0	0.0	0.1	0.0	0.0	0.1

Table 4.3.36: Correlations between IOB HWE and IOB wort components

	Pinery	Maitland	Brinkworth	Tuckey
Soluble Protein	**0.67**	0.55	**0.65**	**0.73**
FAN	**0.67**	**0.62**	**0.78**	**0.72**
ß-Glucan	***-0.94***	**-0.74**	**-0.60**	***-0.88***
Arabinoxylan	-0.57	**-0.61**	-0.38	**-0.70**
Viscosity	***-0.91***	**-0.63**	-0.50	***-0.89***
SP/TP	**0.76**	**0.75**	***0.84***	***0.81***
Glucose	***0.86***	**0.65**		**0.72**
Fructose	***0.92***	0.52		***0.86***
Sucrose	0.05	-0.41		0.16
Maltose	0.12	-0.18		0.39
Maltotriose	-0.48	**0.60**		**0.61**
Maltotetraose	-0.12	-0.51		-0.30
Total simple sugars	0.52	0.29		**0.62**

\|r\|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.37: The model produced using multiple regression analysis to predict IOB HWE from the IOB wort components at four sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust).

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 67.65 + 0.3773 x SP/TP – 1.592 x log ß-Glucan	Soluble Protein, FAN, log ß-Glucan, Arabinoxylan, Viscosity, SP/TP, Glucose, Fructose, Sucrose, Maltose, Maltotriose	82%
Maitland	IOB HWE = 81.89 + 0.0519 x FAN – 1.445 x glucose – 1.251 x SP – 1.757 x log ß-Glucan	Soluble Protein, FAN, log ß-Glucan, Arabinoxylan, Viscosity, SP/TP, Glucose, Fructose, Sucrose, Maltotriose	71%
Tuckey	IOB HWE = 83.97 +1.377 x glucose – 8.59 x viscosity + 0.1074 x SP/TP	Soluble Protein, FAN, log ß-Glucan, log Arabinoxylan, Viscosity, SP/TP, Glucose, Fructose, Sucrose, Maltotriose, Maltotetraose	81%
Brinkworth	IOB HWE = 61.12 + 0.4619 x SP/TP	Soluble Protein, FAN, ß-Glucan, Arabinoxylan, Viscosity, SP/TP	57%

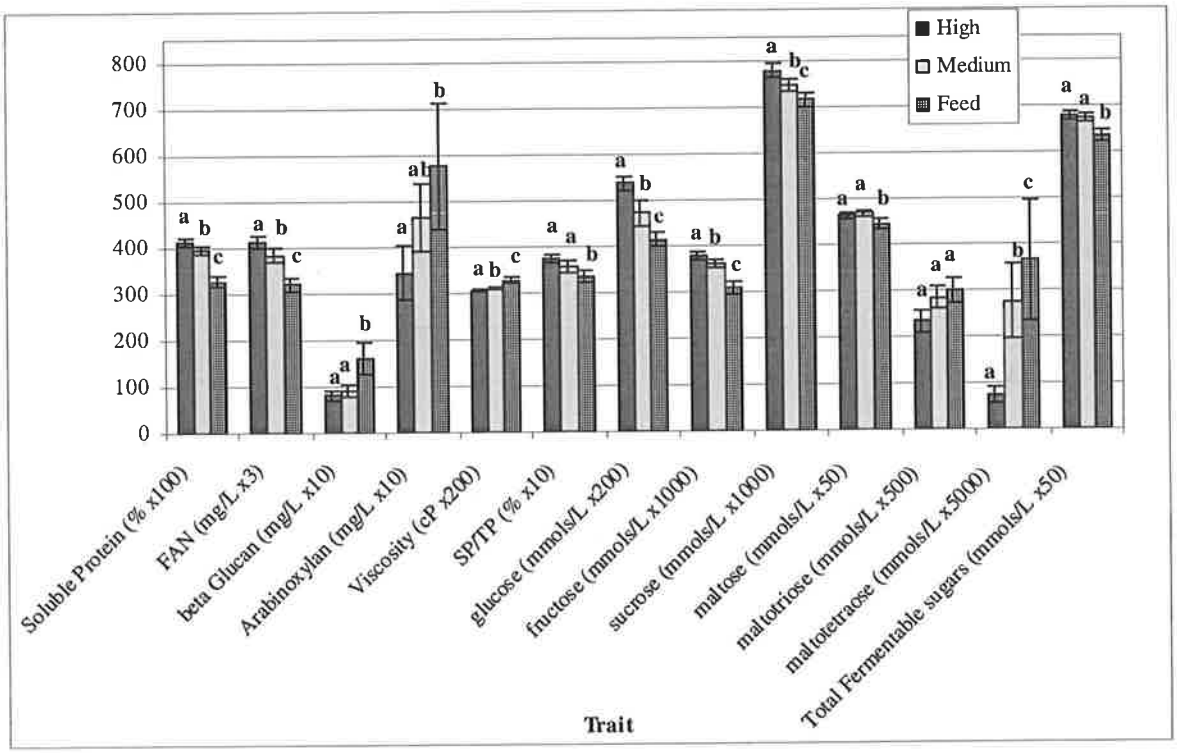


Figure 4.3.9: Graph showing the mean of the IOB wort components (scaled appropriately) divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. ^{abc} Means with letter in common are not significantly different ($P<0.05$).

4.3.3.3 Spent Grain Components

Spent grain is the solid waste material remaining after the mashing process. This was collected after filtering the wort produced by the EBC and IOB mashing procedures and the mean of the components remaining are shown in Table 4.3.38 and Table 4.3.39. Approximately 30% of the original malt remained in the spent grain after mashing. The spent grain contained approximately 20% protein. More protein remained in the spent grain from the under-modified Pinery site than the over-modified Maitland site. In most cases only small amounts of the β-glucan remained in the spent grain. The exception to this was Galleon from Pinery, produced by the IOB HWE method, which contained 4.3% β-glucan.

In general, only 1-2% of the spent grain consisted of starch. However the spent grain from Pinery, produced by the IOB HWE method, contained slightly larger amounts of starch, with Galleon in particular containing as much as 10%. A number of the samples were stained with iodine and observed under the microscope. Intact large and small granules were observed in all samples (Figure 4.3.10). In particular, the spent grain from Pinery, produced by the IOB HWE method, contained large numbers of intact starch granules. The numbers of granules observed were lower for Maitland than Pinery and lower for the EBC method than the IOB method.

The amount of malt remaining in the spent grain was strongly correlated with both IOB and EBC HWE (Table 4.3.40). IOB HWE formed strong to moderate correlations with β-glucan and starch content, while EBC HWE only formed moderate to weak correlations. In both cases, stronger correlations were formed between HWE and starch and β-glucan at Pinery than at Maitland. Arabinoxylan and protein content only formed poor correlations with HWE. However, only the total amount of material remaining in the spent grain showed significant differences ($P<0.05$) between all of the three groups described in section 4.3.1.1 (Figure 4.3.11).

Multiple regression analysis was performed on the spent grain from both the IOB HWE and EBC HWE methods at both sites. The β-glucan levels at all of the sites were skewed and the log of β-glucan was therefore used. When all of the traits were considered in the analysis, the percentage of the original grain was the only significant trait in all of the models except the IOB HWE model from Pinery. As it was considered important to assess which of the components of the spent grain were influencing HWE, the analysis was performed a second time with the exclusion of the percentage of the original grain. In the final models for EBC HWE, only log β-glucan was significant ($P<0.05$) at Maitland and no traits were found to be significant at Pinery (Table 4.3.41). The final models for IOB HWE included the traits protein, log β-glucan and starch (Table 4.3.42).

Table 4.3.38 Results of the analysis of the spentgrain produced from the EBC HWE method

Variety	% of original grain		Starch (%db)		β-Glucan (%db)		Arabinoxylan (%db)		Protein (%db)	
	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland
Alexis	29.5	26.7	1.1	1.3	0.29	0.15	13.6	15.2	21.8	16.7
Arapiles	31.7	29.5	2.3	2.1	1.09	0.60	13.6	9.6	21.4	22.7
Barque	34.8	31.4	2.2	1.3	0.83	0.34	14.8	10.8	22.5	19.2
Chariot	30.1	27.7	1.2	1.4	0.25	0.11	14.3	10.0	22.4	17.4
Chebec	32.9	28.9	1.9	1.7	0.46	0.16	14.5	13.5	21.1	17.2
Franklin	28.6	29.3	1.1	1.3	0.39	0.16	13.2	11.3	23.0	19.9
Galleon	35.3	29.4	1.9	1.7	1.68	0.43	15.0	9.3	25.1	16.7
Harrington	29.0	26.5	1.2	1.5	0.54	0.19	15.9	6.8	19.9	15.7
Haruno nijo	28.1	27.2	1.1	1.3	0.69	0.28	13.3	6.5	28.3	25.8
Schooner	30.7	29.0	1.0	1.5	0.43	0.19	13.9	11.0	20.3	18.9
Sloop	31.0	28.4	1.0	1.8	0.22	0.12	14.7	11.9	20.9	15.4
Mean	31.1	28.5	1.6	1.5	0.6	0.3	14.2	9.7	22.4	18.7
Standard deviation	2.5	1.5	0.5	0.4	0.4	0.2	2.0	2.8	2.6	3.5

Table 4.3.39 Results of the analysis of the spentgrain produced from the IOB HWE method

Variety	% of original grain		Starch (%db)		β-Glucan (%db)		Arabinoxylan (%db)		Protein (%db)	
	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland	Pinery	Maitland
Alexis	29.3	27.5	2.0	1.1	0.68	0.19	6.3	7.3	21.8	17.4
Arapiles	32.2	31.5	4.4	2.3	1.86	1.07	5.4	6.2	22.3	21.9
Barque	36.5	32.1	5.1	2.2	1.99	0.70	6.2	6.1	23.9	21.7
Chariot	30.1	28.3	2.0	1.2	0.47	0.19	5.5	5.7	24.6	20.8
Chebec	34.0	29.1	4.0	1.9	0.87	0.72	6.3	5.9	23.9	18.6
Franklin	30.0	27.9	3.3	1.1	0.70	0.23	5.8	5.3	23.5	23.5
Galleon	38.7	29.0	10.1	1.9	4.30	1.48	5.4	7.2	24.4	24.3
Harrington	30.2	26.5	3.1	1.2	1.50	0.26	7.2	7.1	20.5	16.7
Haruno nijo	28.0	29.1	2.2	1.5	1.71	0.49	6.1	5.5	28.2	19.3
Schooner	31.7	27.2	2.8	1.0	1.20	0.65	6.8	7.4	21.1	24.0
Sloop	30.5	28.6	1.5	1.0	0.40	0.29	4.5	7.6	21.9	19.5
Mean	31.9	28.7	3.7	3.3	1.4	0.6	5.9	6.3	23.3	20.7
Standard deviation	3.2	2.1	2.4	2.7	1.1	0.5	1.1	1.7	2.3	3.0

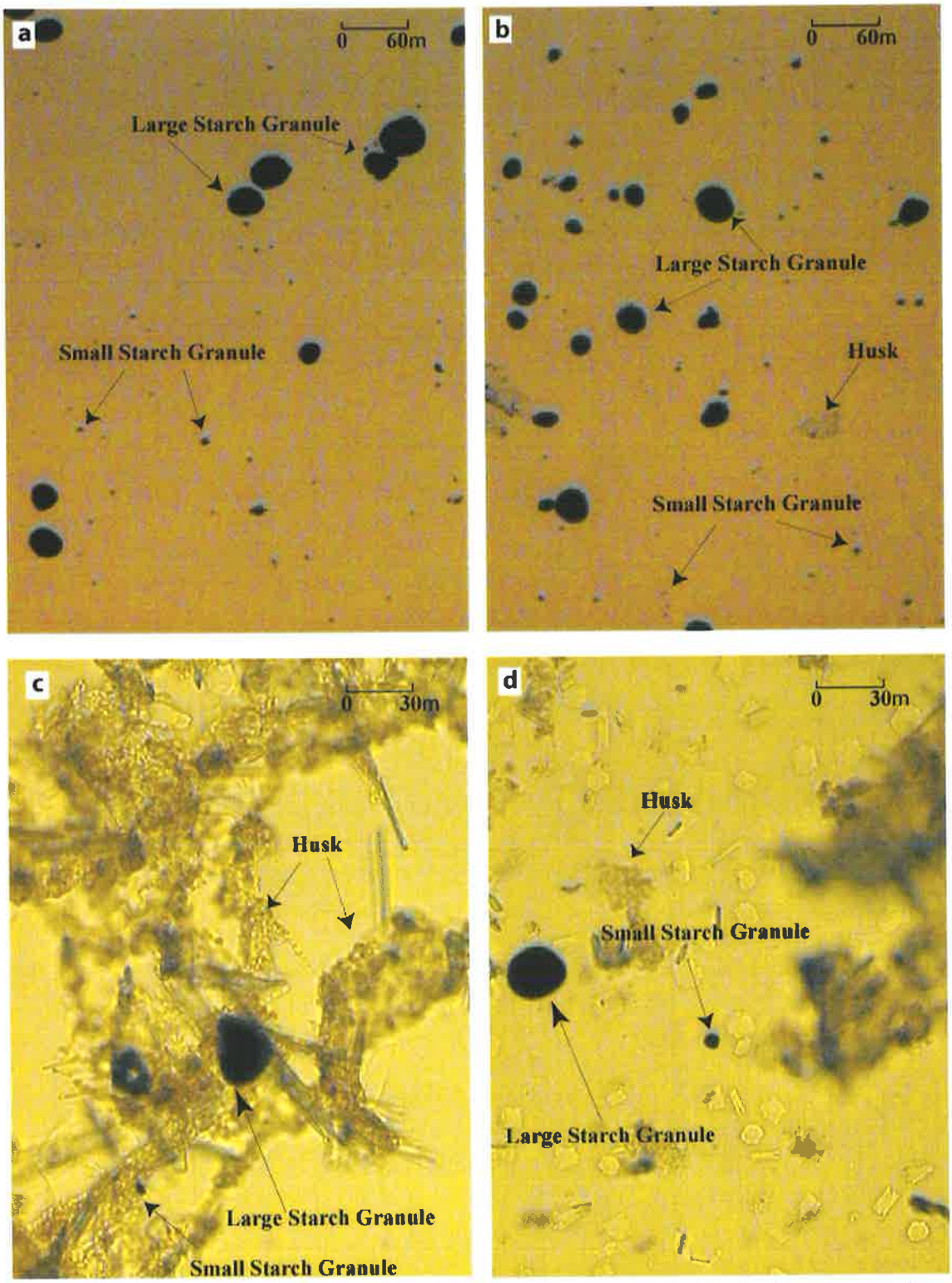


Figure 4.3.10 Images of starch granules stained with iodine. a: Chariot barley from Tuckey b: Barque barley from Tuckey c: Harrington spentgrain, EBC HWE method from Pinery d: Galleon spentgrain, IOB HWE method from Pinery

Table 4.3.40 Correlations between EBC HWE and EBC spent grain components and IOB HWE and IOB spent grain components.

	EBC HWE		IOB HWE	
	Pinery	Maitland	Pinery	Maitland
% of original malt	***-0.96***	***-0.93***	***-0.97***	**-0.78**
Starch	**-0.66**	-0.29	***-0.88***	**-0.72**
ß-Glucan	-0.57	-0.53	**-0.79**	**-0.64**
Arabinoxylan	-0.37	-0.19	0.17	0.01
Protein	0.04	-0.13	-0.06	-0.55

|r|=0.8 –1 strong relationship, **|r|=0.6-0.8 moderate relationship**, |r|<0.6 weak relationship (see section 4.2.3 for justification)

Table 4.3.41: The model produced using multiple regression analysis to predict EBC HWE from the EBC spent grain components at two sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust). The analysis was performed both with and without the percentage of original grain as one of the traits under consideration.

	Equation for EBC HWE	Traits included in analysis	Variance accounted for by model
Pinery	EBC HWE = 101.11 – 0.7023 x % of original grain	% of original malt, Starch, log ß-Glucan, Arabinoxylan, Protein	83%
Maitland	EBC HWE = 105.25 – 0.8070 x % of original grain	% of original malt, Starch, log ß-Glucan, Arabinoxylan, Protein	68%
Pinery (without % of original malt)	No traits significant	Starch, log ß-Glucan, Arabinoxylan, Protein	-
Maitland (without % of original malt)	EBC HWE = 80.191 – 3.01 x log ß-Glucan	Starch, log ß-Glucan, Arabinoxylan, Protein	21%

Table 4.3.42: The model produced using multiple regression analysis to predict IOB HWE from the IOB spent grain components at two sites. The method to decide which traits would be used in the analysis is discussed in Section 4.2.3. The final model and the variance accounted for in the model were calculated using the computer program GenStat® (fifth edition, release 4.2, Lawes Agricultural Trust). The analysis was performed both with and without the percetage of the original grain as one of the traits under consideration.

	Equation for IOB HWE	Traits included in analysis	Variance accounted for by model
Pinery	IOB HWE = 99.19 – 0.7161 x % of original grain –1.698 x log ß-Glucan	% of original malt, Starch, log ß-Glucan, Arabinoxylan, Protein	92%
Maitland	IOB HWE = 95.87 – 0.542 x % of original grain	% of original malt, Starch, log ß-Glucan, Arabinoxylan, Protein	42%
Pinery (without % of original malt)	IOB HWE = 80.191 – 1.010 x Starch	Starch, log ß-Glucan, Arabinoxylan, Protein	75%
Maitland (without % of original malt)	IOB HWE = 83.09 – 0.1800 x Protein – 2.969 x log ß-Glucan	Starch, log ß-Glucan, Arabinoxylan, Protein	56%

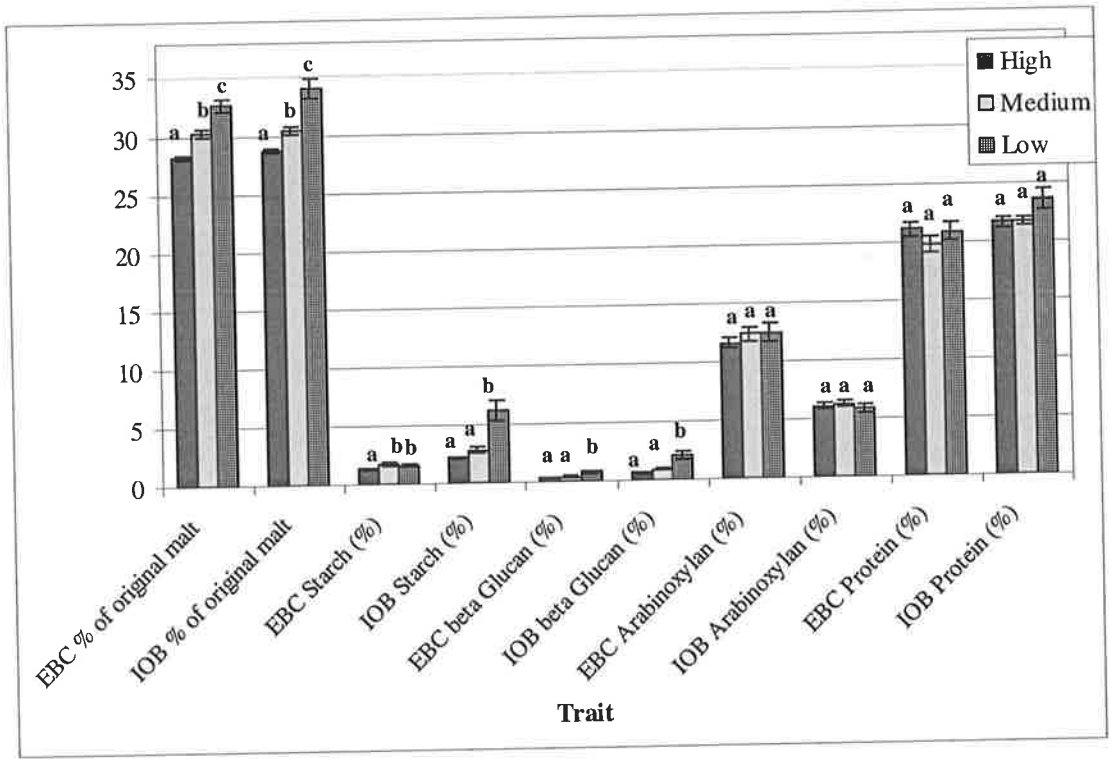


Figure 4.3.11: Graph showing the mean of the components in the spent grain remaining from the EBC and IOB HWE method divided into three groups based on the EBC common effect (Table 4.3.5) at four sites. High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque. [abc] Means with letter in common are not significantly different ($P<0.05$).

4.3.3.4 Comparison of EBC and IOB wort and spent grain components

The wort produced by the EBC HWE method contained significantly higher levels of protein, FAN, maltotriose, maltotetraose ($P<0.001$) and β-glucan ($P<0.05$) than the wort produced by the IOB HWE method (Table 4.3.43). It also had a higher soluble protein to total protein ratio and a higher wort viscosity. The IOB HWE method produced wort with significantly higher ($P<0.001$) levels of sucrose, maltose and total simple sugars.

Spent grain produced by the IOB HWE method contained significantly more ($P<0.05$) starch and β-glucan and significantly less ($P<0.05$) arabinoxylan than spent grain produced by the EBC HWE method (Table 4.3.44).

Table 4.3.43 The mean of 13 traits for the IOB and EBC wort characteristics

Trait	EBC	IOB	Trait	EBC	IOB
Soluble Protein (%)	4.6[a]	3.9[a]	glucose (mmols/L)	51	50
FAN (mg/L)	159[b]	129[b]	fructose (mmols/L)	10[f]	7[f]
ß-Glucan (mg/L)	188[c]	101[c]	sucrose (mmols/L)	9[g]	15[g]
Arabinoxylan (mg/L)	40.4	39.8	maltose (mmols/L)	156[h]	184[h]
Viscosity (cP)	1.60[d]	1.56[d]	maltotriose (mmols/L)	17[i]	11[i]
Kolbach Index	42.7[e]	36.6[e]	maltotetraose (mmols/L)	2.9[j]	0.9[j]
			Total sugars (mmols/L)	246[k]	268[k]

[cd]significantly different ($P<0.05$) [abefghijk]significantly different ($P<0.001$)

Table 4.3.44 Mean results for the components of spent grain formed from both the IOB and EBC HWE

Trait	EBC	IOB
% of original grain	29.8	30.4
Starch (%db)	1.5[a]	2.6[a]
β-Glucan (%db)	0.44[b]	1.00[b]
Arabinoxylan (%db)	12.4[c]	6.2[c]
Protein (%db)	20.6[d]	22.0[d]

[abcd]Significantly different ($P<0.05$)

4.4 Discussion

4.4.1 The relationship between site, variety and HWE

The varieties investigated in this study can be placed into three groups based on IOB and EBC HWE. The first group includes the high malting quality overseas varieties, Harrington, Haruna Nijo, Alexis, Chariot and Manley, and the Australian malting variety Franklin. The second group contains the other Australian malting varieties Sloop, Schooner and Arapiles, and the feed variety Chebec. The third group consists of the two Australian feed varieties Galleon and Barque. However, these groups are far from being fixed and overlaps between the three groups occur. In particular, Brinkworth performed quite differently to other sites. Brinkworth was characterized by a hot, dry finish to the growing season (Crop Harvest Report, 1997/1998). This impacted on the ability of the late maturing varieties to complete grain filling, thereby increasing their barley protein levels. These late maturing varieties tended to be the overseas varieties, which are less well adapted than the local varieties to the Australian growing conditions. Consequently a number of the overseas varieties had high barley protein levels at this site and therefore lower HWE results. The site Brinkworth highlights the influence the environment can have on traits such as HWE, especially traits that may be impacted by protein levels. The use of statistical tools such as BLUPS to calculate the common effect are useful to gauge how a variety will perform under average conditions. However, aberrations such as the results from Brinkworth must also be considered. Ideally in a study such as this one it would have been useful to analyse more sites to ensure that the differences found at Brinkworth were an unusual occurrence. Unfortunately the time and resource constraints of analyzing more sites made this impossible.

One of the biggest problems with a study of this nature is the influence that the use of a single micromalting schedule can have on the final outcomes of the study. Each variety modifies at a different rate. It is therefore unlikely, when selecting a micromalter protocol, that it will be suitable for all of the varieties in the study, and some varieties will be either under- or over-modified. An example of this is shown by the difference of the KI of Arapiles and Harrington (Table 4.3.3). Harrington is well known to modify quickly and consequently in this study produced a mean KI of

51.5% and ranged from 44.1 to 63.6% at the individual sites. On the other hand Arapiles, a slow modifier, had a mean KI of 37.7 and only produced a KI greater than 40 at one site. Therefore it is important, when assessing the results of this study, to consider the influence of modification on the traits that appear to relate to HWE, inorder to decide if these relationships are generated purely by differences in modification levels.

4.4.2 The relationship between the barley and malt components and HWE

Three methods were used in this chapter to compare HWE with the barley and malt traits, simple correlations between HWE and the barley and malt traits, multiple regression analysis and a comparison of the relationship between the three groups described in section 4.3.1 (shown graphically, eg Figure 4.3.3). These are summarized in Table 4.4.1. These three methods of analysis were used to determine the strength of the influence of a trait on HWE. Eight groups of traits or individual traits were shown to have the greatest influence on HWE (Table 4.4.2). While a number of other traits are mentioned in Table 4.4.1, they were only highlighted by one of the three methods and were therefore not considered to be as important. SKCS moisture formed strong to moderate correlations with HWE and was included in a number of the models. However, it was omitted from the final list of important traits since it is strongly influenced by the environment and unlikely to be a trait that breeders could manipulate.

Both malt and barley grain weight showed a relationship with HWE. This would partly be due to the selection of varieties. The European, Canadian and Japanese high HWE varieties were poorly adapted to the Southern Australian growing conditions, which could have resulted in small grain size. It could also be due to the relationship between grain hydration and grain size. Smaller grains require a smaller amount of time to hydrate and therefore modify faster, increasing HWE. By selecting for high malt quality it is possible that barley breeders have also been selecting for small grain size. However, large grain size is an important industry target for new barley varieties and in the future it will be important to change this relationship.

Milling energy and grain hardness did not relate to HWE. Whilst the high, medium and low varieties were statistically different for SKCS hardness, the relationship between it and HWE was positive and not negative as would be expected. Similarly Edmunds *et al.* (1993) found no significant relationship between milling energy and malt quality. This is, however, contradictory to a number of other reports (Alison *et al.*, 1979; Alison, 1986; MacLeod *et al.*, 1993; Alexander *et al.*, 1997) that have shown good relationships between both milling energy and grain hardness and HWE. Milling energy is used as a tool to screen early generation material for its malting quality throughout Europe. As the samples in both this study and that of Edmunds *et al.* (1993) were grown in Australia, it would suggest that from an Australian stand point the use of either milling energy or SKCS grain hardness to screen early generation material in breeding programs would lead to the rejection of some high HWE lines. For example, if this technique had been used on this particular group of varieties it would have led to the rejection of Franklin, one of Australia's highest HWE varieties. However Haruna Nijo would have been retained. This would suggest either a major difference between the germplasm used in Australian and European breeding programs or an aspect of the Australian environment that has caused these differences. Since European varieties have often been used in Australian breeding programs as sources of high malting quality, it is unlikely to be a difference in germplasm. On close inspection of the Australian varieties in the study it appears that the malting varieties that were best suited to the growing conditions were more likely to have lower milling energies. Australian malting varieties such as Franklin and Arapiles that are better adapted to wetter, cooler conditions produced the higher milling energy results. This was also shown with the variety Harrington, which produced the highest results. Milling energy may, therefore, only be useful to Australian breeding programs for varieties that are well adapted to the growing conditions. Hence, it would not be suitable as an early generation screening tool and should only be used to screen for HWE in later generations.

On the surface, the relationship shown between barley husk content and HWE is a fairly obvious one, as the more husk there is, the less of other barley components. If the assumption is made that the husk plays no part in the malting and mashing processes and ends up in the spent grain, essentially unchanged, it would be assumed that the thinner the husk, the more carbohydrates and protein there are available to be

used during malting and mashing. Unexpectedly, however, the relationship between HWE and the husk content of the malt is much weaker. This would indicate that the husk is playing a far more important role than purely a physical role. This relationship is to be discussed further in Chapter 6 and Chapter 8.

Malt β-glucan levels showed moderate to strong correlations with HWE at most sites, in particular IOB HWE. The levels of β-glucan in the barley showed only poor correlations with HWE, indicating that the amount of β-glucan initially present in the grain is unimportant in comparison to the changes that occur during the malting process. This confirms a number of studies in the literature (Allan *et al.,* 1995; Henry, 1985; Henry, 1986; Stuart *et al.,* 1988).

All of the enzymes investigated showed a relationship with HWE except β-amylase (Table 4.3.21). For many years, barley breeders have been selecting malting varieties with high HWE, DP, α-amylase and low levels of wort β-glucan, and subsequently high levels of β-glucanase. On the other hand, these traits are generally not important when selecting for feed quality varieties. It is possible that the relationship between HWE and a number of these enzymes is at least partly due to the indirect relationship caused by the selection criteria used to select for malting quality varieties compared to feed varieties.

During malting and mashing, α-amylase degrades insoluble intact starch granules and gelatinized starch molecules (MacGregor and Fincher, 1993), producing small soluble linear dextrins and highly branched dextrins (MacGregor and Morgan, 1986). Likewise, limit dextrinase attacks both soluble and insoluble branched dextrins producing smaller soluble dextrins (Longstaff and Bryce, 1993, Macri *et al.,* 1993). These soluble dextrins are further degraded by the action of β-amylase, which is not capable of attacking intact starch granules. Whilst the level of maltose in the wort is dependent on the level of β-amylase in the malt (Piendl, 1973: MacGregor and Fincher, 1993), β-amylase does not influence the overall level of soluble material in the wort. Since HWE is a measure of the soluble material in the wort, β-amylase does not have a major influence on it. It does, however, influence the quality of the wort produced.

Of these enzymes, the strongest relationship was found between limit dextrinase and HWE. Whilst high HWE is likely to be dependent on the levels of α-amylase, limit dextrinase and β-glucanase, only small amounts of limit dextrinase are available in the malt and it is therefore likely to be more limiting than the other enzymes. Traditionally, barley breeders have not selected for limit dextrinase as it is only a minor contributor, percent wise, to DP. However, the selection of varieties carrying high levels of limit dextrinase may subsequently increase the level of HWE.

None of the starch related traits that are based on the actual quantities of starch available (barley and malt starch content) showed consistent correlations with HWE. Likewise, the traits relating to the temperature at which the starch gelatinises (DSC peak temperature and RVA peak temperature and time) showed poor relationships with HWE. However both the diameter of the large starch granules and RVA peak viscosity showed moderate to strong correlations with HWE at all sites analysed. Both of these traits influence the accessibility of the starch to hydrolysis during mashing. During mashing, large starch granules are hydrolysed preferentially to small starch granules (Bathgate and Palmer, 1973). Smaller granules have adhering proteins (MacLeod and Wallwork, 1992) that hinder the access of the attacking enzymes, and have a higher gelatinization temperature, 64°C (MacGregor, 1980; MacGregor and Balance, 1980a), than large starch granules, making them less available to enzyme degradation during mashing. Additionally, more starch is available in the form of large starch granules as they make up approximately 90% of the total volume of the starch (Briggs *et al.*, 1981; Mäkelä *et al.*, 1982). Thus, the properties of the large starch granules are likely to exert a greater influence on HWE than the small granules.

The RVA peak viscosity of the malt is also likely to be influencing HWE by restricting the access of the enzymes to the gelatinising granules. The low HWE varieties tended to have a higher peak viscosity. The thicker solution would physically hinder the access of the enzymes to the starch granules by slowing the movement of the enzymes. It would also slow the removal of the products of the enzyme degradation from the immediate region of the starch granules. As β-amylase

and limit dextrinase are only capable of attacking the products of α-amylase, quick access to these products would be important before degradation of these enzymes.

Since the RVA peak viscosity was measured on the malt, the influences of other compounds in the malt also need to be taken into consideration. Silver nitrate was used to hinder enzyme activity. However, there would have been other carbohydrates and proteins in the solution. The low varieties tended to have higher malt β-glucan levels. β-Glucans are known to form viscous solutions (Bamforth, 1985) and therefore the low β-glucan varieties are likely to have higher RVA peak viscosity.

Table 4.4.1: Barley and malt traits that have either formed strong to moderate correlations at two or more sites with EBC HWE or IOB HWE or were significant in the final models for either EBC HWE or IOB HWE produced by multiple regression analysis. Also included is the significant difference ($P<0.05$) between the varieties when divided into three groups[a] based on the EBC common effect (Table 4.3.5) for each trait.

Cor: Correlation strength, M: moderate, S: strong, na: not analysed, +: positive correlation, -: negative correlation, GH: grain hydration, LSG: large starch granule, [a]High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque, [b]Model: traits with a tick were found to be significant ($P<0.05$) in the multiple regression models for either IOB or EBC HWE.

Trait	EBC HWE								IOB HWE								difference between groups
	Pinery		Maitland		Brinkworth		Tuckey		Pinery		Maitland		Brinkworth		Tuckey		
	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	Cor[a]	Model[b]	
SKCS Weight	S-		M-				M-		M-		M-				M-		all 3 different
Barley 1000 GW	S-		M-				M-	✓	M-		M-				M-		all 3 different
Malt 1000 GW	S-	✓	S-	✓	M-		M-		S-	✓	S-	✓			S-	✓	all 3 different
SKCS Moisture	S+	✓	M+			✓			M+		M+			✓			feed < high
SKCS Hardness																	all 3 different
GH 24 hours							S+								M+		all 3 different
GH 48 hours						✓	M+							✓	M+		all 3 different
GH 72 hours	S+	✓					S+		M+						S+		high >
Barley Husk	M-				S-	✓	M-	✓	M-				M-	✓		✓	all 3 different
Malt ß-glucan	M-	✓	S-	✓		✓		✓	S-		S	✓	M	✓	S-	✓	all 3 different
Malt Protein		✓								✓							all same
Barley Pentose														✓			all same
ß-Glucanase	S+								S+						M+		feed <
Limit Dextrinase	M+	✓	S+	✓	M+	✓	M+	✓	M+	✓	S+	✓	M+	✓	S+	✓	all 3 different
Diastatic Power	M+		M+						M+		M+				M+		all 3 different
α-amylase	S+		M+			✓	M+		S+						S+		all 3 different
Diameter LSG	S+	✓	na	na	na	na	S+	✓	S+	✓	na	na	na	na	M+	✓	all 3 different
% Large Granules	M-	✓	na	na	na	na					na	na	na	na			feed > high
RVA Peak Viscosity	S-	✓	M-	✓	na	na			S-	✓	M-	✓	na	na	M-	✓	feed >
RVA Final Viscosity					na	na						✓	na	na	M-	✓	all same

Table 4.4.2 A summary of the barley and malt traits found to have the strongest relationship with HWE as shown in Table 4.4.1 and the mean level of each trait produced in these trials for each of the three groups, high, medium and low.

	high	medium	low
grain size	SKSC Weight (mg) <39 Barley 1000 GW (g) <36 Malt 1000 GW (g) <30	39> SKSC Weight (mg) <44 36>Barley 1000 GW (g) <40 31>Malt 1000 GW (g) <34	SKSC Weight (mg) >44 Barley 1000 GW (g) <38 Malt 1000 GW (g) <34
grain hydration	grain hydration 72 hours >140	120< grain hydration 72 hours >140	grain hydration 72 hours <120
barley husk content	barley husk (%) <9.5	9.5< barley husk (%) <10.3	barley husk (%)>10.3
malt beta glucan	malt beta glucan (%) <0.4	0.4< malt beta glucan (%) <0.75	malt beta glucan (%) >0.75
limit dextrinase	limit dextrinase (U/kg) >500	500 > limit dextrinase (U/kg) >400	limit dextrinase (U/kg) <400
Other starch degrading enzymes	DP (μm/min/g) >500 alpha amylase (μm/min/g) >110	400<DP (μm/min/g) <500 110> alpha amylase (μm/min/g) >80	DP (μm/min/g) <400 alpha amylase (μm/min/g) <80
large starch granule size	large starch granule size (μ) >18	18> large starch granule size (μ) >17	large starch granule size (μ) <17
RVA peak viscosity	RVA peak viscosity (RVA units) <100	100< RVA peak viscosity (RVA units) <130	RVA peak viscosity (RVA units) >130

4.4.3 The relationship between the wort and spent grain components and HWE

A summary of the wort and spent grain components that formed moderate to strong correlations with either IOB or EBC HWE at two or more of the individual sites or traits that were found to be significant in the models produced by multiple regression analysis and the relationship between the high, medium and low varieties for each of these traits is shown in Table 4.4.3. Five groups of traits were shown to have the greatest influence on HWE (Table 4.3.4). Whilst sucrose, maltose, maltotriose and maltotetraose are all mentioned in Table 4.4.3, they were not found to relate to HWE in more than one of the methods of assessment and are therefore not considered to be important.

The wort protein related traits found to be associated with HWE include soluble protein, FAN and Kolbach index or SP/TP. All of these traits give an indication of the amount of the protein that has been degraded through the malting and mashing processes. This influences HWE in two ways. The first is by directly adding to the amount of dissolved material in the wort, thereby increasing the HWE. Secondly, the removal of proteins adhering to the starch granules in the endosperm allows greater access of the α-amylase to the starch granules, which would increase the breakdown of the starch granules and hence increase HWE.

Likewise, the removal of the cell wall from around the starch granules would allow the α-amylase access to the starch granules. The amount of β-glucan and arabinoxylan remaining in the wort gives an indication of the overall degradation of the cell wall material. The smaller the amount, the further the degradation has progressed, and the higher the levels of HWE. Additionally, the β-glucans and arabinoxylans contribute about nine percent of the material in barley (Table 4.3.15). Varieties that have the ability to degrade a greater proportion of this material will produce wort containing a larger amount of soluble matter and hence higher HWE.

The amount of the monosaccharides, fructose and glucose in the wort would both directly and indirectly influence HWE. The glucose and fructose would directly contribute to soluble material in the wort and therefore HWE levels. The majority of the glucose in the wort would be formed by the degradation of the starch and β-glucan during malting and mashing. High levels of glucose in the wort would indicate that a larger proportion of the starch and β-glucan would have been degraded during the malting and mashing processes.

The amount of material remaining in the spent grain and the components of the spent grain would influence HWE directly. A greater amount of material remaining in the spent grain would indicate that less material has solubilised during the mashing process and therefore HWE would be lower.

Table 4.4.3: Wort and spent grain traits that have either formed strong to moderate correlations at two or more sites with EBC HWE or IOB HWE or were significant in the final models for either EBC HWE or IOB HWE produced by multiple regression analysis Also included is the significant difference ($P<0.05$) between the varieties when divided into three groups[a] based on the EBC common effect (Table 4.3.5) for each trait.

Trait	EBC HWE									IOB HWE								
	Pinery		Maitland		Brinkworth		Tuckey		difference between groups	Pinery		Maitland		Brinkworth		Tuckey		difference between groups
	Cor	Model	Cor	Model	Cor	Model	Cor	Model		Cor	Model	Cor	Model	Cor	Model	Cor	Model	
Soluble Protein		✓							feed<	M+			✓	M+		M+		all 3 different
Wort FAN							M+		feed<	M+		M+	✓	M+		M+		all 3 different
Kolbach, SP/TP	M+	✓	M+			✓			feed<	M+	✓	M+		S+	✓	S+	✓	feed<
Wort ß-Glucan	M-		M-	✓					all 3 different	S-	✓	M-	✓	M-		S-		feed>
Wort Arabinoxylan	M-		M-				M-		feed>			M-				M-		high<feed
Wort Viscosity									feed>	S-		M-				S-	✓	all 3 different
Wort Glucose	S+		M+		na	na	M+		all 3 different	S+		M+	✓	na	na	M+	✓	all 3 different
Wort Fructose	S+				na	na	M+		all 3 different	S+				na	na	S+		all 3 different
Wort Sucrose									all same									all 3 different
Wort Maltose								✓	all same									feed<
Wort Maltotriose					na	na		✓	all same			M+		na	na	M+		all same
Wort Maltotetraose				✓				✓	high<feed									all 3 different
SG % of original grain	S-	✓	S-	✓	na	na	na	na	all 3 different	S-	✓	M-	✓	na	na	na	na	all 3 different
SG Starch	M-				na	na	na	na	high<	S-	✓	M-		na	na	na	na	feed<
SG β-glucan				✓	na	na	na	na	feed>	M-	✓	M-	✓	na	na	na	na	feed>
SG protein					na	na	na	na	all same				✓	na	na	na	na	all same

Cor: Correlation strength, M: moderate, S: strong, na: not analysed, +: positive correlation, -: negative correlation, GH: grain hydration, LSG: large starch granule, SG: spent grain, [a]High: Harrington, Haruna Nijo, Franklin, Alexis, Manley and Chariot; Medium: Sloop, Schooner, Arapiles and Chebec; Low: Galleon and Barque, [b]Model: traits with a tick were found to be significant ($P<0.05$) in the multiple regression models for either IOB or EBC HWE.

Table 4.4.4 A summary of the wort and spentgrain components found to have the strongest relationship with HWE as shown in Table 4.4.1 and the mean level of each trait produced in these trials for each of the three groups, high, medium and low.

	EBC			IOB		
	high	medium	low	High	medium	low
wort, protein modification	soluble protein (%) >4.9 FAN (mg/L) >170 KI (%) >47	4.9> soluble protein (%)>4.0 170> FAN (mg/L) >130 47> KI (%) >40	soluble protein (%) <4.0 FAN (mg/L) <130 KI (%) <40	soluble protein (%) >4.2 FAN (mg/L) >130 SP/TP (%) >47	4.2> soluble protein (%) >3.5 130> FAN (mg/L) >110 47> SP/TP (%) >40	soluble protein (%) <3.5 FAN (mg/L) <110 SP/TP (%) <40
wort, cell wall modification	ß-Glucan (mg/L) <150 Arabinoxylan (mg/L) <40 Viscosity (cP) <1.58	150 < ß-Glucan (mg/L) <250 40<Arabinoxylan (mg/L) <70 1.58< Viscosity (cP) <1.65	ß-Glucan (mg/L) >250 Arabinoxylan (mg/L) >70 Viscosity (cP) >1.65	ß-Glucan (mg/L) <80 Arabinoxylan (mg/L) <40 Viscosity (cP) <1.56	80 < ß-Glucan (mg/L) <150 40<Arabinoxylan (mg/L) <70 1.56< Viscosity (cP) <1.70	ß-Glucan (mg/L) >150 Arabinoxylan (mg/L) >70 Viscosity (cP) >1.70
wort, monosaccharides	glucose (mmols/L) >50 Fructose (mmols/L) >10.5	50> glucose (mmols/L) >40 10.5< fructose (mmols/L) >7	glucose (mmols/L) <40 fructose (mmols/L) <7	glucose (mmols/L) >49 fructose (mmols/L) >8	49> glucose (mmols/L) >40 8< fructose (mmols/L) >6	glocose (mmols/L) <40 fructose (mmols/L) <6
spent grain, % of original malt	% of original <29.5	29.5 < % of original <31	% of original >31	% of original <30	30 < % of original <33	% of original >33
spent grain components	Starch (%) <1.5 ß-Glucan (%) <0.55	1.5< Starch (%) <2.0 0.55<ß-Glucan (%) <1.0	Starch (%) >2.0 ß-Glucan (%) >1.0	Starch (%) <2.5 ß-Glucan (%) <0.6	2.5< Starch (%) <4.0 0.6< ß-Glucan (%) <2.0	Starch (%) >4.0 ß-Glucan (%) >2.0

4.4.4 The differences between the EBC and IOB methods for measuring HWE

The two methods of measuring HWE in the laboratory use quite different temperature regimes. A graphical representation of this is shown in Figure 4.4.1. The EBC HWE method involves an initial protein rest at 45°C, which allows the protease and β-glucanase to act. It also uses a fine grind, allowing easy access to the flour. However after this initial rest the temperature is rapidly increased at 1°C per minute to 70°C, where it is held for a further 60 minutes. This rapid increase in temperature has the effect of rapidly degrading the majority of the enzymes present. Optimum activity for β-amylase is 63°C (Piendl, 1973), but it is rapidly degraded at temperatures above this (Stenholm *et al.,* 1996). Thus the β-amylase is only given a short time to react before temperatures are too high and it is degraded. Since starch gelatinises around 65°C, the majority would be gelatinised at the temperatures used in this method. However, the rapid increase in temperature would only allow the β-amylase access to the gelatinized starch for a short period of time before it is completely degraded.

The IOB HWE method employs a constant temperature of 65°C. At this temperature the proteases and β-glucanases are rapidly degraded. The temperature is only slightly above the ideal temperature for β-amylase activity, allowing β-amylase time to react before it is degraded. However, at 65°C it is unlikely that all of the starch would be gelatinized (Table 4.3.24).

These differences in the two methods are likely to have resulted in the levels of both the proteins and the β-glucan to be significantly higher in the wort produced by the EBC HWE extract method than the IOB HWE extract. This was confirmed by the analysis of the spent grains. The EBC method produced spent grains that had significantly lower levels of β-glucan ($P<0.05$). Consequently the IOB method was influenced more by the levels of modification of both the β-glucan and the protein in the malt and wort. Malt and wort β-glucan showed much stronger relationship with IOB HWE than EBC HWE. This would be due to the rapid degradation of the β-glucanase with the IOB HWE method. Any β-glucan surrounding the starch granules would hinder the access of the amylolytic enzymes to the starch. In the EBC HWE

method these β-glucan would be rapidly removed in the initial protein rest and therefore would have little influence on the final HWE results.

Likewise, proteins adhering to the starch granules would be rapidly removed in the EBC HWE but not the IOB HWE method. Consequently protein modification traits such as KI, SP/TP and soluble protein had much stronger associations with IOB HWE than EBC HWE.

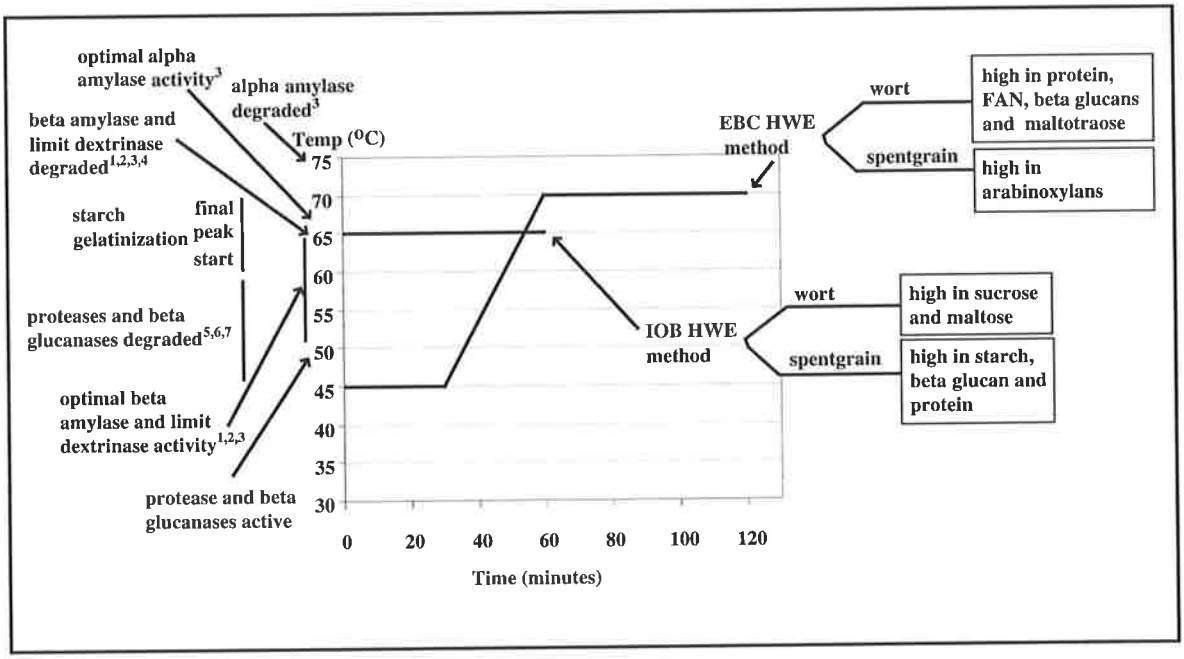
IOB HWE also showed a stronger relationship with wort viscosity levels. This is probably an indirect relationship based on the relationship with the wort β-glucan, which are known to form viscous solutions (Bamforth, 1985).

This complex relationship between the gelatinization of the starch and the deactivation of the enzymes is highlighted in the results for the wort sugars. The IOB HWE method produced significantly higher ($P<0.001$) levels of maltose but significantly lower levels of maltotriose and maltotetraose with a number of varieties having no detectable maltotetraose remaining in the wort. This would indicate that the β-amylase was more active in the IOB method, as evidenced by the degradation of most of the maltotetraose into maltose.

The spent grains produced by the EBC method had significantly lower levels of starch and fewer intact starch granules. This is likely to be due to the higher degree of gelatinization of the starch. In the case of the IOB HWE method, temperatures sufficient to completely gelatinize all of the starch granules would not be achieved (Table 4.3.24). This would be most evident in samples that were under-modified, such as the Galleon sample from Pinery shown in Figure 4.3.10. In the EBC HWE method, the temperature is held for an hour at 70°C, which would allow almost all of the starch granules to gelatinise. At this temperature, at least a portion of the α-amylase would be still be active (Stenholm, *et al.*, 1996; Inkerman *et al.*, 1997) and capable of breaking the starch into smaller dextrins. However the β-amylase would be rapidly degraded, preventing these dextrins from being converted into maltose. Hence, fewer starch granules would remain in the spent grain for the EBC HWE method, but maltose would be less likely to be formed due to the lack of β-amylase.

With the lower temperatures in the IOB HWE method, a larger proportion of intact starch granules would remain in the spent grain but once gelatinized, the starch would be more prone to attack by α-amylase and β-amylase and therefore more maltose would be formed (as shown in Table 4.3.31 and 4.3.35).

Figure 4.4.1: A schematic representation of the IOB and EBC methods for measuring HWE, showing the different temperatures and time involved in each method and the changes that occur during the mashing process to the components and enzymes of the malt.



[1]Piendl, 1975, [2]Stenholm *et al.*, 1996, [3]Inkerman *et al.*, 1997, [4]Sjoholm *et al.*, 1995, [5]Enari and Sopanen, 1986, [6]Loi *et al.*, 1987, [7]Barber *et al.*, 1994

4.5 Conclusion

This study indicated that the level of HWE was prone to vary between sites but the ranking between varieties stayed consistent over three of the four sites investigated. This would indicate that complex multi-site and year testing is not needed to select for malt extract and the measurement of samples from a few sites only would be more appropriate.

Eight groups of malt and barley traits and five wort and spent grain parameters were found to influence HWE. These included grain size, grain hydration during malting,

barley husk content, malt β-glucan, limit dextrinase activity, activity of other starch degrading enzymes, size of the large starch granules and RVA peak viscosity, protein modification (in the wort), cell wall modification (in the wort), wort monosaccharide levels, the percent of the original malt remaining in the spent grain and the components of the spent grain. This knowledge will enable breeders to have a greater understanding of appropriate areas to place selection pressure when selecting for high malt extract. These parameters are likely to be genetically linked to malt extract, either by the pleiotropic effects of a single gene, or the effects of gene 'clusters'. The link between the parameters found to be associated with malt extract in this Chapter and regions of the barley genome that are associated with malt extract and a number of these traits will be discussed in Chapter 8.

Chapter 5

Mapping of Chromosome Regions Associated with High Levels of Malt Extract

5.1: Introduction

As outlined in Section 2.6.1, twenty-four individual regions on the barley genome have been found to be associated with high malt extract (HWE), in eight different mapping populations developed around the world (Hayes *et al.,* 2001). If these regions are going to be useful to the Australian barley breeder, they need to be examined under Australian conditions and in genetic backgrounds suitable for use in Australian barley breeding programs.

A number of mapping populations have been established in Australia in conjunction with the National Barley Molecular Marker Program (NBMMP) for improving our understanding of the genetic basis of numerous malting quality, disease resistance and agronomic traits. Most of these populations have a high malt quality international parent and a good agronomic Australian parent, including the populations Galleon/ Haruna Nijo, Sloop/ Alexis and Sloop-sib/ Alexis. One of the aims of establishing these populations was to determine the genetic basis for high malt quality traits in backgrounds that are agronomically suitable to Australian growing conditions.

QTL associated with HWE have already been identified on chromosomes 1H and 5H in the Chebec/ Harrington population (Collins *et al.,* 2003). These correspond with regions associated with HWE in the population Harrington/ TR306 (Hayes *et al.*, 1997; Mather *et al.*, 1997).

The Galleon/ Haruna Nijo population was established in the 1990's by a group in the Department of Plant Science at the University of Adelaide (Karakousis *et al.*, 2003a). Galleon is a widely adapted Australian feed quality variety released in 1981. It is high yielding and Cereal Cyst Nematode (*Heterodera avenae*) resistant (Barr *et al.,* 1997).

Unfortunately it has very poor malting quality and particularly low levels of HWE (Section 4.3.1). Haruna Nijo is a malting quality variety from Japan with high HWE and high levels of diastatic enzymes (Section 4.3.1 and Section 4.3.2.3).

The other two populations mentioned above are the Sloop/ Alexis population and the Sloop-sib/ Alexis population. The South Australian Barley Improvement Program (SABIP) and the Malting Barley Quality Improvement Program (MBQIP) released the variety Sloop in 1997 (Plant Varieties Journal, 10(2), 1997). It is widely adapted to growing conditions in southern Australia. It produces good levels of diastatic enzymes and low levels of β-glucans in the wort but only moderate levels of HWE, well below those of a number of the leading malting varieties (Section 4.3.1, Section 4.3.2.2 and Section 4.3.3). Sloop was selected from a group of sister lines that included a line identified by the SABIP as WI2875-1. For the purposes of this discussion we have called this line Sloop-sib. A comparison of Sloop and its sister line, Sloop-sib, for a number of parameters is shown in Table 5.1.1. Sloop-sib produces higher levels of diastatic enzymes and lower levels of HWE than Sloop. Alexis is a high malting quality German variety that is grown in a number of European countries (Schildbach *et al.*, 2000).

For the purpose of this study, the Galleon/ Haruna Nijo, Sloop/ Alexis and Sloop-sib/ Alexis mapping populations were chosen to identify and investigate regions of the barley genome that are suitable for use in a barley breeding program to select for improved levels of HWE.

Table 5.1.1 Comparison of Sloop and Sloop-sib EBC HWE results (mean of three sites) (data sourced from the SABIP Barley Quality Report, 1994)

Variety	Grain Protein (%)	Malt Protein (%)	EBC HWE (%)	DP (μm/min/g)
Sloop-sib	11.8	13.3*	77.4**	777*
Sloop	11.2	12.0	79.2	618

*significantly different P<0.05, ** significantly different P<0.01

5.2: Materials and Methods

The Sloop/ Alexis and Sloop-sib/ Alexis mapping populations were produced, grown, mapped and analyzed under the coordination of the Australian National Barley Molecular Marker Program (NBMMP).

5.2.1: Genetic material

The Galleon/ Haruna Nijo population consists of 120 F_1 derived doubled haploid lines. A linkage map was generated using a total of 430 RFLP marker loci, covering the majority of the barley genome (Karakousis *et al.*, 2003a).

The Sloop/ Alexis population consists of 109 doubled haploid lines. A linkage map was constructed from 187 AFLP, 55 RFLP and 62 microsatellite markers spanning all seven chromosomes (Barr *et al.*, 2003b). The Sloop-sib/ Alexis population consists of 152 recombinant inbred lines. A linkage map was constructed from 177 AFLP, 54 RFLP and 51 microsatellite (Barr *et al.*, 2003b).

The Sloop/ Alexis and Sloop-sib/ Alexis linkage maps were combined using the computer software program Map Manager QT, version 3.0b28. The combined linkage map consisted of 167 AFLP, 48 RFLP and 46 microsatellite markers (Barr *et al.*, 2003b).

5.2.2: Field experiments

The Galleon/ Haruna Nijo mapping population was grown at Charlick Experimental Station, near Strathalbyn, South Australia, in 1996, 1998 and 1999 by the SABIP. 48, 64 and 85 doubled haploid lines were grown for each year respectively in single replicate trials.

The Sloop/ Alexis and Sloop-sib/ Alexis mapping populations were grown by SABIP near Strathalbyn, South Australia in 1998 and 1999 and by Agriculture Western Australian (AgWA) at Katanning and Wongan Hills, Western Australia in 1999. The

number of genotypes grown at each site is shown in Table 5.3.6. The samples were grown in single replicate trials in 1998 and double replicate trials in 1999.

5.2.3: Malt quality analysis

The Galleon/ Haruna Nijo mapping population was analyzed using the facilities of SABIP. Thirty grams of each sample were malted at 15°C and analyzed for EBC HWE (1996, 1998, 1999 season samples) and IOB HWE (1999 season) as described in Sections 3.1 and 3.2.

The Sloop/ Alexis and Sloop-sib/ Alexis mapping populations were malted and analyzed at the Victorian Institute of the Dryland Agriculture (VIDA), Horsham, Victoria as part of the NBMMP. Thirty grams of each sample were malted at 15°C, as described in Section 3.2. EBC HWE (all sites) and IOB HWE (Charlick, 1998) were measured as described in Section 3.2.

5.2.4: Statistical analysis

Simple regression and interval analyses were performed by the computer software program "Qgene" (Nelson, 1997), the latter by the method of Haley and Knott (1992). LOD scores greater than 3.0 were considered significant. For statistical purposes, the combined Sloop/ Alexis and Sloop-sib/ Alexis population was considered to be recombinant inbred lines (RIL) to allow for the presence of heterozygotes.

To take into account the variation in grain protein (GP) within trials, the HWE results for the Sloop/ Alexis and Sloop-sib/ Alexis populations were adjusted for protein as described in the SABIP Quality Report (1999 season). GP was plotted against HWE for each trial and a linear regression was fitted. Predicted HWE results were calculated from the protein results for each sample using the equation for the fitted line. The difference between the actual result and the calculated result gives an indication of how the result compares to the overall trend of the trial.

Frequency distributions were generated using the computer software program Jmp® (version 3.1.6, SAS Institute Inc., Figures 5.3.1 and 5.3.4). Least significant differences between means were calculated using the computer program Genstat 5 (release 4.1).

5.3: Results

5.3.1: Galleon/ Haruna Nijo

The mean HWE results for the Galleon/ Haruna Nijo mapping population and the parents for each of the years are shown in Table 5.3.1. The mean EBC HWE results for each year were significantly different ($P<0.001$) with the highest EBC HWE being recorded in 1996 and the lowest in 1998. The frequency distribution of the EBC HWE results is shown graphically in Figure 5.3.1. For the EBC HWE, between six and 29% of the lines in the population produced EBC HWE results significantly lower ($P<0.05$) than Galleon. Only 6% of lines produced significantly higher ($P<0.05$) EBC HWE than Haruna Nijo in 1999 and no lines produced significantly higher ($P<0.05$) HWE than Haruna Nijo in 1996 and 1998. The IOB HWE analysis showed only 2% of lines significantly higher ($P<0.05$) than Haruna Nijo and no lines significantly lower than Galleon.

Table 5.3.1: EBC HWE analysis from three years (1996, 1998, 1999) and IOB HWE analysis from one year (1999) of the Galleon/ Haruna Nijo mapping population and the parents, grown near Strathalbyn, South Australia.

Year	Galleon		Haruna Nijo		Population			
	Mean	SD	Mean	SD	Mean	SD	Range	No
1996	79.4	0.6	82.8	1.0	*80.3	1.6	76.6-83.4	48
1998	75.5	0.6	80.0	0.4	*75.9	2.0	70.0-79.4	64
1999	77.4	1.5	80.6	0.6	*77.7	2.4	71.4-82.7	85
1999[a]	67.0	1.4	77.5	0.5	73.7	2.7	66.5-78.6	85

[a]IOB HWE results, *significantly different ($P<0.001$)

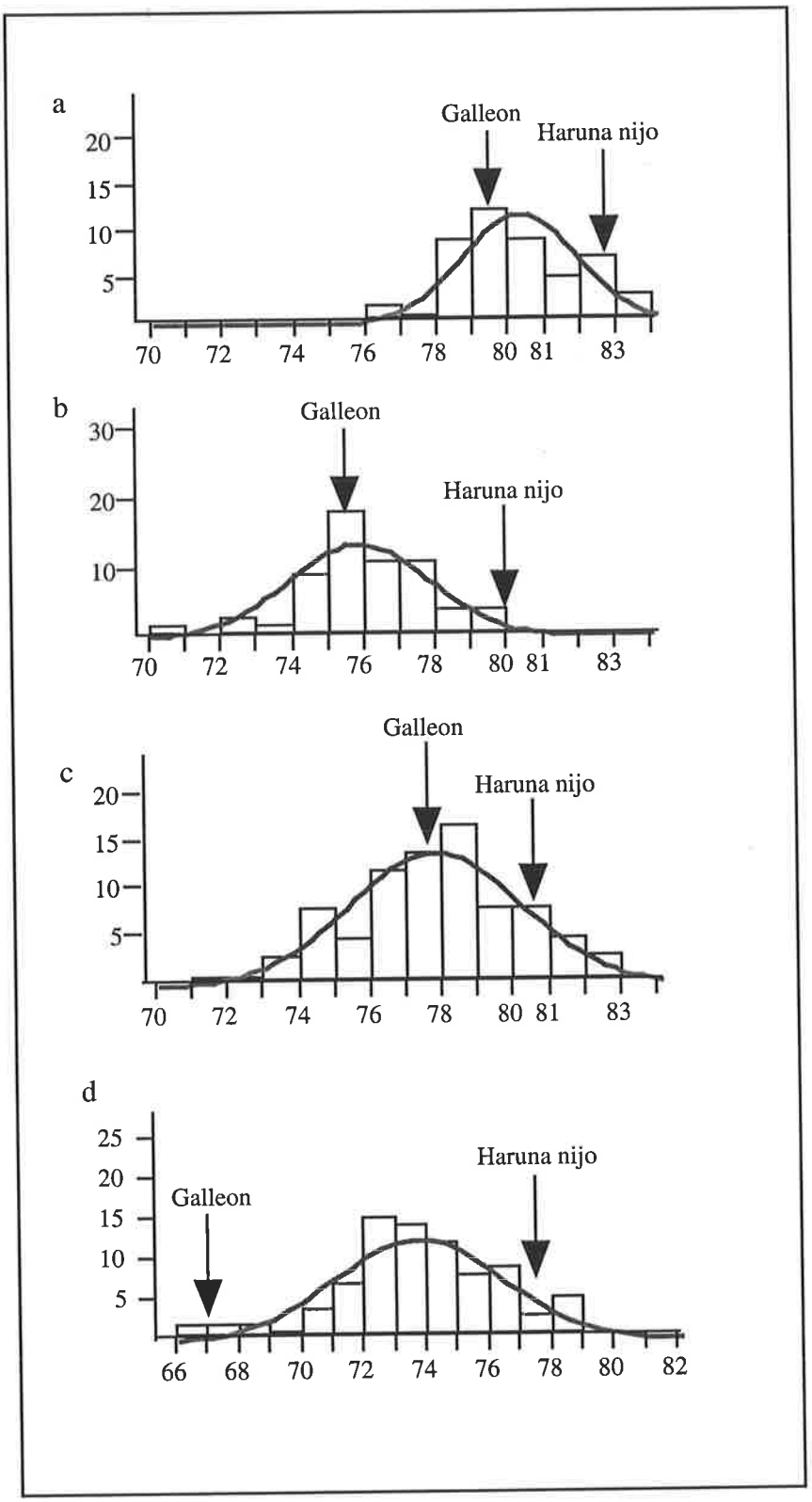


Figure 5.3.1 Frequency distribution graphs of the EBC HWE and IOB HWE results for the Galleon/ Haruna Nijo mapping population for each year. a:1996 EBC, b:1998 EBC, c:1999 EBC, d:1999 IOB. The results for the parents, for each year are indicated graphically by an arrow.

Interval analysis revealed a significant association between a region on the short arm of chromosome 2H and EBC HWE in all years and IOB HWE in 1999 (Figure 5.3.2). This region accounted for between 16% and 28% of the total variation in HWE. LOD scores are shown in Table 5.3.2. One of the markers in this region is *Xpsr108* and the mean HWE for the two marker allele groups for this marker are shown in Table 5.3.3. The difference in the mean HWE between the lines carrying the Haruna Nijo allele and the Galleon allele at this marker locus ranged from 1.7% to 2.4%.

Three other regions were found to be weakly associated with HWE (Figure 5.3.3). These were on chromosome 2H (long arm), chromosome 5H and 6H (Table 5.3.2). The region on the long arm of chromosome 2H associated with marker *Xmwg503* is likely to be coincident with a region found to be associated with HWE in the Calicuchuma-sib/ Bowman mapping population (Hayes *et al.*, 1996). Kleinhofs and Han (2002) developed a BinMap based on the linkage map of the Steptoe/ Morex mapping population by dividing each barley chromosome into regions, called bins and assigning a number of markers to each bin. The markers associated with the region on chromosome 5H (*Xawbma32 and Xawbma33*) have been placed in bin 4. Previously a region associated with HWE has also been assigned to bin 4 in the mapping population of Dicktoo/ Morex (Hayes *et al.*, 2001).

These three QTL identified had LOD scores that were close to, but below the given threshold level for significance (3.0) in this study. However, when the effect of marker alleles on HWE was assessed it was found that lines carrying the Haruna Nijo allele at all four loci produced 5.5% higher ($P<0.001$) EBC HWE (mean of three sites) than the lines carrying the Galleon allele at all four loci (Table 5.3.4). This was an average of 1.5% higher EBC HWE than the lines carrying the Haruna Nijo allele at *Xpsr108* only. This suggests that the locus on the short arm of chromosome 2H has a major effect on HWE and that other minor loci, possibly including the long arm of chromosome 2H, short arm of 5H and the short arm of 6H, could have minor but additive effects on HWE. The limited population size prevented critical assessment of the effect of each possible allele combination.

Table 5.3.2: Regions of the barley genome found to be associated with HWE in the Galleon/ Haruna Nijo mapping population.

Chromosome	Region	Year	LOD	r^2	P	Marker[b]
2H short	*xKsuA3(a)-Xawbma28*	1996	3.12	0.27	0.002	***Xbcd175***
		1998	4.46	0.28	0.000	*Xabg397*
		1999	4.91	0.24	0.000	***Xpsr108***
		1999[a]	3.11	0.16	0.000	***Xpsr108***
2H long	*Xmwg503*	1998	1.43[c]	0.10	0.012	*Xmwg503*
		1999	1.91[c]	0.10	0.004	*Xmwg503*
		1999[a]	1.76[c]	0.10	0.005	*Xmwg503*
5H short	*Xcdo989-Xabg497(a)*	1999	1.82[c]	0.11	0.005	*Xawbma33*
		1999[a]	2.44[c]	0.13	0.001	*Xawbma32*
6H short	*XksuA3(b)-Xpsr666*	1996	1.51[c]	0.16	0.011	*Xnar7(b)*
		1998	1.19[c]	0.09	0.022	*Xnar7(b)*
		1999	1.75[c]	0.10	0.005	*Xnar7(b)*

[a] IOB HWE method, [b]most significant marker, [c]LOD score not significant

Table 5.3:3: Mean EBC HWE from three years and IOB HWE from one year for the lines carrying either the Haruna Nijo or the Galleon allele at the marker *Xpsr108*.

Year	Haruna Nijo allele			Galleon allele			Difference
	Number	Mean HWE	SD	Number	Mean HWE	SD	
1996	15	81.5	1.8	29	79.8***	1.2	1.7
1998	24	77.1	1.7	39	75.1***	1.8	2.0
1999	33	79.1	2.1	50	76.7***	2.1	2.4
1999[a]	32	75.0	2.5	50	72.8***	2.4	2.2
Mean[c]		79.2			77.2		

[a]IOB HWE results, [c]Mean of three years for the EBC HWE results, ***Haruna Nijo and Galleon alleles are significantly different (P<0.001)

Table 5.3.4: Mean EBC HWE results for the Galleon/ Haruna Nijo mapping population separated into groups based on the alleles at each of four marker loci.

2H	2H	5H	6H			EBC HWE (%db)			
Xpsr108	*Xmwg503*	*Xawbma32*	*Xnar7*	No of lines	Group	1996	1998	1999	mean[1]
*G	G	G	G	6	1	78.2	72.8	74.6	75.2
G	G	G	#H	5	2	79.5	75.2	76.0	76.9
G	G	H	G	2	3	77.8	73.1	74.3	75.0
G	H	G	G	3	4	78.7	75.2	76.0	76.6
H	G	G	G	3	5	-	77.2	78.3	77.7
G	G	H	H	0	6	-	-	-	-
G	H	G	H	9	7	80.3	75.3	77.0	77.5
H	G	G	H	0	8	-	-	-	-
G	H	H	G	10	9	79.7	75.0	77.5	77.4
H	G	H	G	0	10	-	-	-	-
H	H	G	G	3	11	82.0	78.7	77.2	79.3
G	H	H	H	10	12	80.5	76.5	78.3	78.4
H	G	H	H	1	13	79.6	75.5	79.5	78.2
H	H	G	H	7	14	82.5	77.1	79.4	79.4
H	H	H	G	3	15	82.3	77.7	77.8	79.3
H	H	H	H	7	16	82.9	78.4	80.7	80.7

*Galleon allele, #Haruna Nijo allele

[1]Group 1 is statistically different (P<0.05) to group 7,9,11,12,14,15,16

Group 2 is statistically different (P<0.05) to group 12,14,16

Group 3 is statistically different (P<0.05) to group 12,14,15,16

Group 4 is statistically different (P<0.05) to group 16

Group 5 is statistically different (P<0.05) to group 16

Group 7 is statistically different (P<0.05) to group 16,1

Group 9 is statistically different (P<0.05) to group 16,1

Group 11 is statistically different (P<0.05) to group 1

Group 12 is statistically different (P<0.05) to group 1,2,3

Group 14 is statistically different (P<0.05) to group 1,2,3

Group 15 is statistically different (P<0.05) to group 1,3

Group 16 is statistically different (P<0.05) to group 1,2,3,4,5,7,9

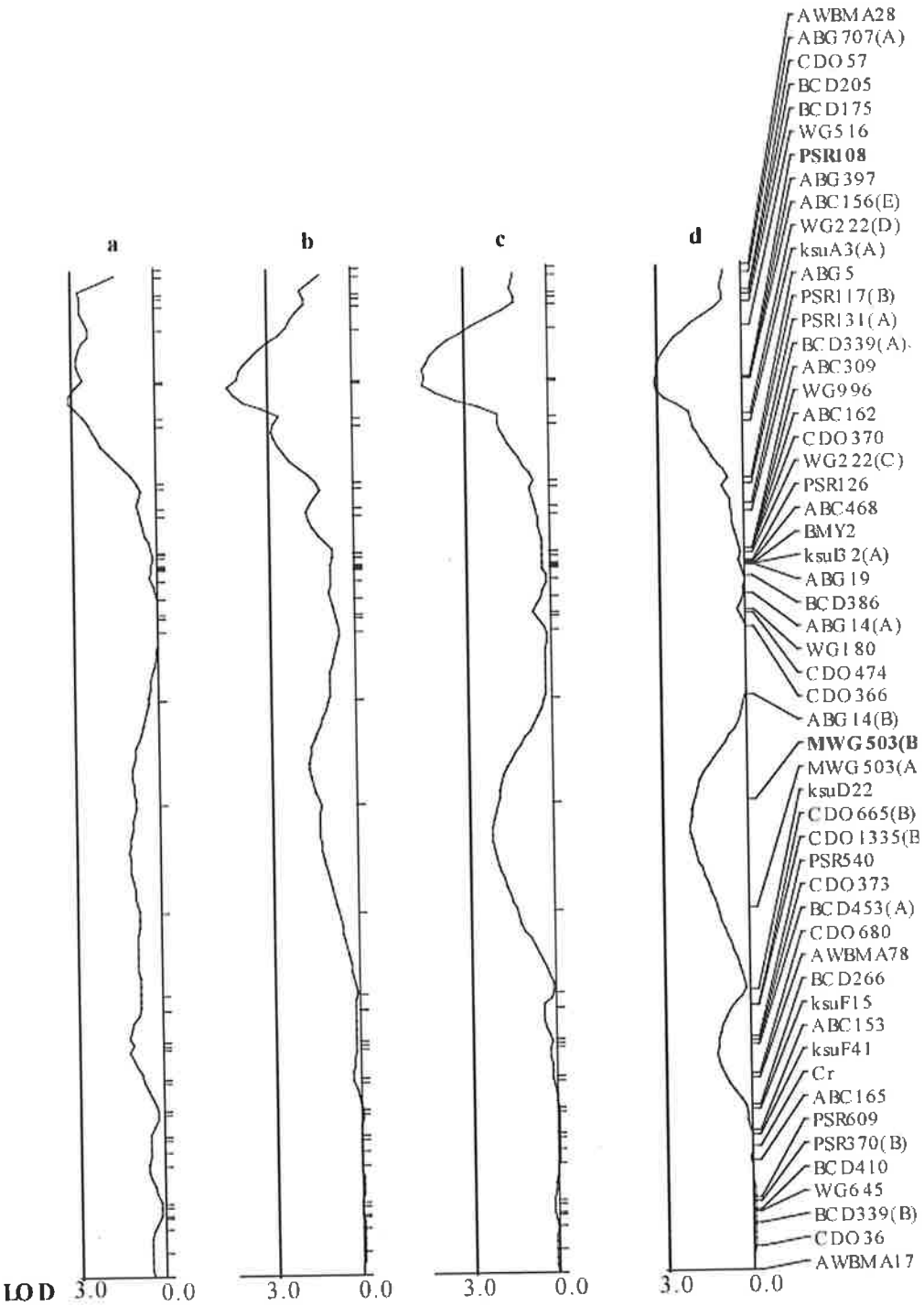


Figure 5.3.2: Interval maps of chromosome 2H for HWE in the Galleon/ Haruna Nijo mapping population with LOD score plotted on the x axis. a: 1996, EBC HWE, b:1998, EBC HWE, c:1999, EBC HWE, d: 1999, IOB HWE method.

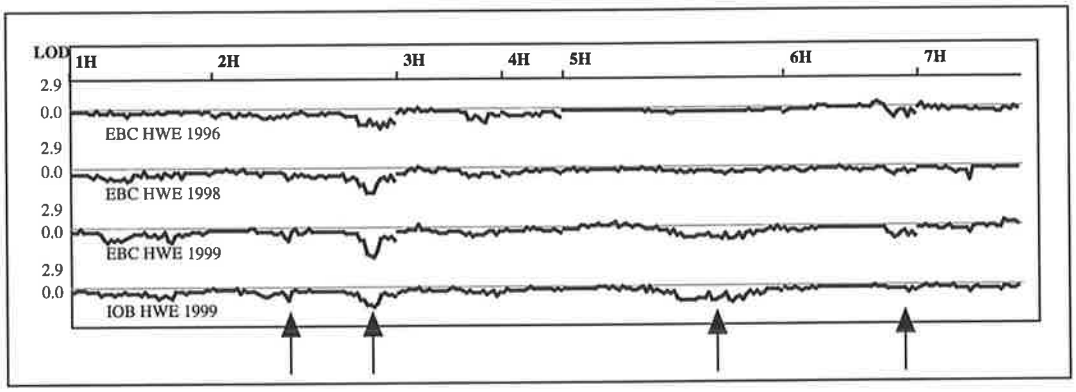


Figure 5.3.3 Regression analysis for HWE in the Galleon/ Haruna Nijo mapping population against F value score. The arrows indicate the four regions associated with HWE

Figure 5.3.3 Regression analysis for HWE in the Galleon/ Haruna Nijo mapping population against LOD score. The arrows indicate the four regions associated with HWE

5.3.2: Sloop/ Alexis and Sloop-sib/ Alexis

The mean HWE results for the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations and the parents Alexis and Sloop are shown in Tables 5.3.5 and 5.3.6. Overall, mean EBC HWE results for Charlick in 1999 and Wongan Hills in 1999 were significantly higher ($P<0.001$) than Charlick in 1998 and Katanning in 1999. The frequency distribution of HWE from the two mapping populations is shown graphically in Figures 5.3.4 and 5.3.5. For Charlick, in 1999, the Alexis and Sloop controls produced the same mean results. In the Sloop/ Alexis population between 18 and 60% of the lines produced HWE results significantly lower ($P<0.05$) than Sloop and four to 26% significantly higher ($P<0.05$) than Alexis. In the Sloop-sib/ Alexis population between 37 and 59% of the lines produced HWE results significantly lower ($P<0.05$) than Sloop and six to 17% produced results significantly higher ($P<0.05$) than Alexis. In general, the Sloop-sib/ Alexis population produced more lines with HWE results lower than Sloop and fewer lines higher than Alexis.

HWE QTL, as identified by interval analysis, varied between sites and populations. HWE was found to be associated with regions on five chromosomes of the barley genome in both the individual Sloop/ Alexis, Sloop-sib/ Alexis mapping populations and the combined mapping population (Table 5.3.7, Table 5.3.8 and Table 5.3.9) although these regions were not identical for the three populations and none of the regions were significant across all sites and seasons. Alexis donated the higher HWE allele at all loci except for the regions on chromosomes 3H and 4H.

EBC HWE was significantly associated with a region on chromosome 1H, flanked by the markers *Xksud14B* and *XBmac0154* (Figure 5.3.6 and Figure 5.3.7). This region was found to be significantly associated with HWE in both the individual populations when grown at Wongan Hills, in 1999 and in the Sloop/ Alexis population only, when grown at Charlick in 1998. It was also found to be associated with IOB HWE at Charlick in 1998. However, in the combined map this region was found to be significantly associated with HWE at all sites and accounted for a significant difference in mean EBC HWE of 0.7 to 0.8% ($P<0.001$) between the lines carrying the allele donated by Alexis and the allele donated by either Sloop or Sloop-sib (Table 5.3.7).

The region on chromosome 2H, flanked by the markers *Xcdo665A and Xbmag0518* was found to be significantly associated with EBC HWE and with grain protein-adjusted EBC HWE in the Sloop-sib/ Alexis mapping population and the combined mapping population when grown at Charlick in 1999, and with IOB HWE at Charlick in 1998 (Figure 5.3.8). In the Sloop/ Alexis mapping population this region was only found to be significantly associated with grain protein adjusted EBC HWE at Charlick, in 1999. However in this population the region extends to marker *XAC_CCA270.* For all three populations the LOD scores were much higher for the grain protein-adjusted results than for the raw data (Table 5.3.7, Table 5.3.8 and Table 5.3.9) indicating that the locus is strongly influenced by the effects of the environment on protein.

Markers *Xabg705b* and *Xbmag0225* flank a region significantly associated with EBC HWE in all three mapping populations at Wongan Hills and Katanning on chromosome 3H (Figure 5.3.9). While the LOD scores for EBC HWE in this region from the combined mapping population at Charlick in 1998 and 1999 were not significant, the differences in mean EBC HWE between the lines carrying the Alexis allele and the lines carrying the Sloop or Sloop-sib allele were significant ($P<0.05$) (Table 5.3.10). Sloop and Sloop-sib marker alleles were associated with higher HWE values.

Two regions were found to be associated with HWE on chromosome 4H in the combined mapping population. The first of these regions, flanked by the markers *Xbmag0419* and *Xwg719,* was found to be associated with both EBC HWE and IOB HWE at Charlick in 1998.

Three regions were found to be associated with HWE on chromosome 5H (Figure 5.3.9 and Figure 5.3.10). The markers *XAA_CCA193* and *XAG_ CTA69* flank the first of these. This region was significantly associated with EBC HWE in the Sloop/ Alexis population when grown at Charlick in 1999, the Sloop-sib/ Alexis population when grown at Charlick in 1998 and Wongan Hills in 1999 and the combined mapping population when grown at Charlick in 1998 and 1999 and Wongan Hills in

1999. It was also significantly associated with IOB HWE in the Sloop-sib/ Alexis population and the combined mapping population at Charlick in 1998.

The second region of chromosome 5H, flanked by the markers *XAG_CTA69* and *XAT_CAC195*, was significantly associated with EBC and IOB HWE in the Sloop/ Alexis population at all sites except Katanning. In the Sloop-sib/ Alexis population, the region was significantly associated with EBC HWE at Charlick in 1998, Wongan Hills in 1999 and Charlick in 1999 when the results were adjusted for protein. This region was also associated with IOB HWE at Charlick in 1998. In the combined mapping population this region was significantly associated with both EBC and IOB HWE at all sites and seasons measured.

The markers *XAT_CAC195* and *XAC__CCT118* flank the third region on chromosome 5H found to be associated with HWE. This region was significantly associated with EBC HWE in the Sloop/ Alexis mapping population, when grown at Wongan Hills only. In the Sloop-sib/ Alexis population the region was significantly associated with EBC HWE at Charlick in 1998 and 1999 and IOB HWE at Charlick in 1998. In the combined mapping population this region was significantly associated with HWE at all sites except Katanning.

Table 5.3.5: Mean EBC HWE for Alexis and Sloop at one site in 1998 and three sites in 1999 and mean IOB HWE at one site in 1998.

Year	Site	EBC HWE				Protein			
		Alexis		Sloop		Alexis		Sloop	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1998	Charlick	75.0	1.2	73.9	0.7	15.4	0.6	15.6	0.7
1999	Charlick	77.3	0.9	77.3	0.8	14.5	1.3	13.2	0.8
1999	Katanning	75.5	1.0	74.9	1.3	13.2	0.5	13.2	0.5
1999	Wongan Hills	77.2	0.4	76.9	0.7	12.6	0.6	13.4	0.4
1998	*Charlick	69.5	1.2	67.6	0.9	15.4	0.6	15.6	0.7

*IOB HWE method

Table 5.3.6: Mean EBC HWE for the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations at three sites in 1999 and one site in 1998 and mean IOB HWE at one site in 1998.

Year	Site	Sloop/ Alexis					Sloop-sib/ Alexis				
		Mean HWE	SD	Range	No	Mean Protein	Mean HWE	SD	Range	No	Mean Protein
1998	Charlick	74.1^{ace}	1.3	71.2-77.0	92	15.5	73.8^{adf}	1.1	70.3-77.0	147	15.5
1999	Charlick	76.4^{cd}	1.5	73.1-79.3	102	14.2	76.4^{ab}	1.2	72.9-79.4	128	14.2
1999	Katanning	74.6^{bde}	1.5	70.9-77.5	81	13.5	74.4^{bcf}	1.5	71.3-77.8	127	13.4
1999	Wongan Hills	76.2^{ab}	1.1	74.0-78.8	80	13.4	76.2^{cd}	1.0	73.8-78.4	127	13.2
*1998	Charlick	68.3	2.5	61.5-72.8	92	15.5	67.6	2.7	63.1-72.5	146	15.5

*IOB HWE method, ^{a,b,c,d,f} in common: mean EBC HWE significantly different (P<0.001),

^{e} in common: mean EBC HWE significantly different (P<0.05)

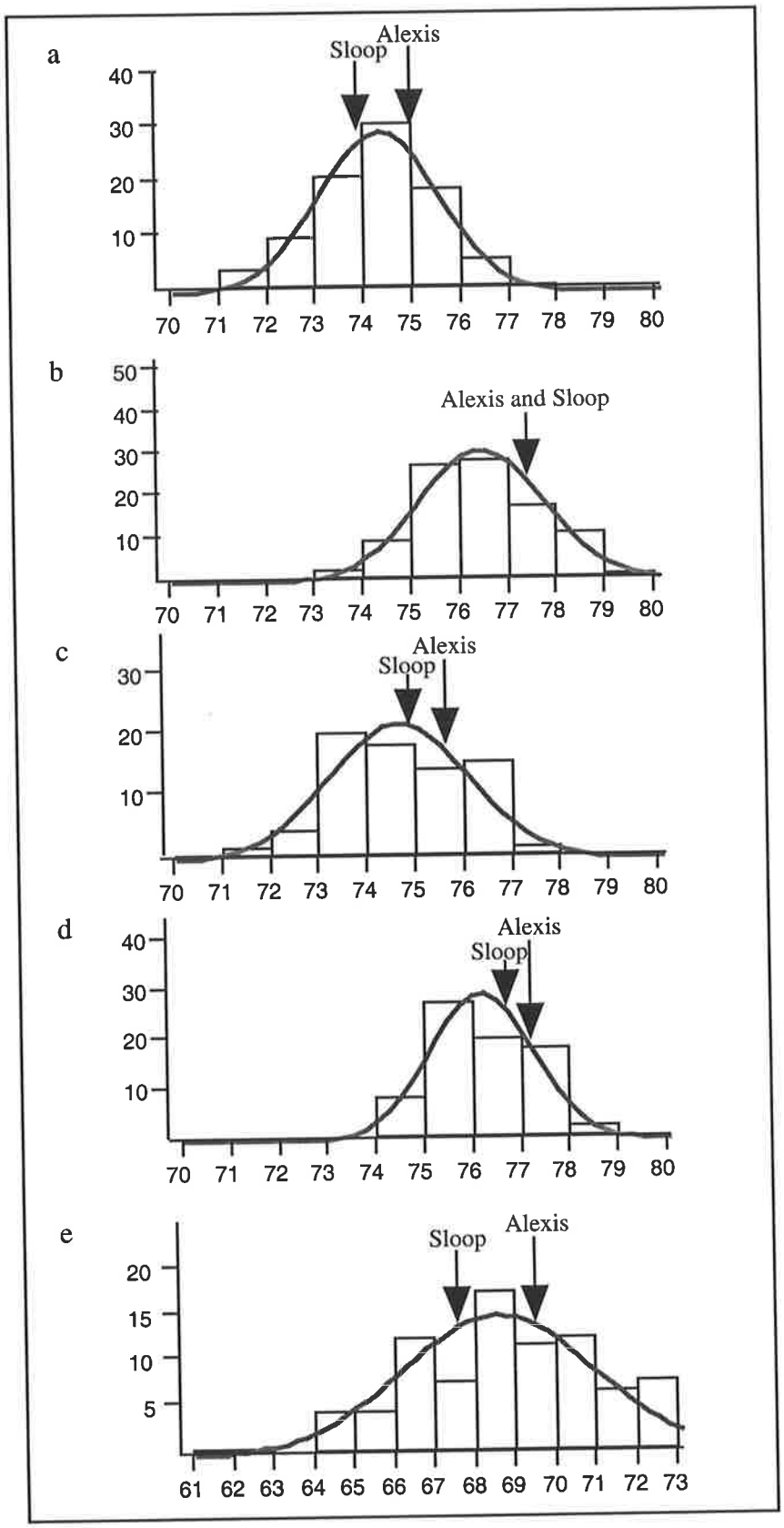


Figure 5.3.4 Frequency distribution graphs of the EBC HWE and IOB HWE results for the Alexis/ Sloop mapping population for each year. a:1998 Charlick EBC, b:1999 Charlick, c: 1999 Katanning, d: 1999 Wongan Hills, e:1998 Charlick IOB. The results for the parents for each year are indicated graphically by an arrow.

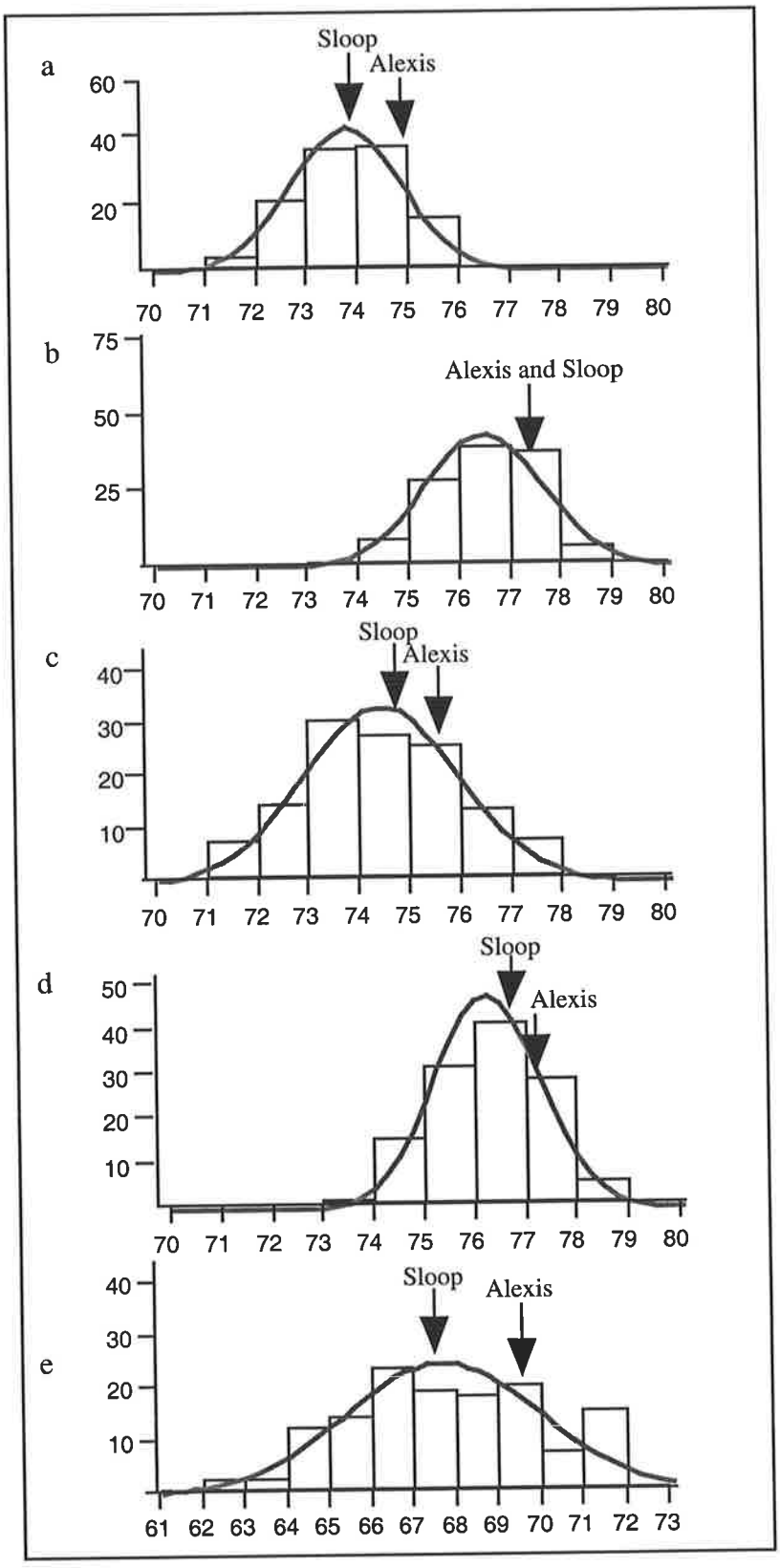


Figure 5.3.5 Distribution graphs of the EBC HWE and IOB HWE results for the Alexis/ Sloop-sib mapping population for each year. a:1998 Charlick EBC, b:1999 Charlick, c: 1999 Katanning, d: 1999 Wongan Hills, e:1998 Charlick IOB. The results for the parents for each year are indicated graphically by an arrow.

Table 5.3.7 Chromosome regions of the barley genome found to be associated with HWE in the Alexis/ Sloop mapping population.

Chromosome	Region	Year	Site	HWE			HWE adjusted for Protein			[b]Marker	Parent donating the higher allele
				LOD	$r^{2\,c}$	P^c	LOD	$r^{2\,c}$	P^c		
1H	*Xksud14B-XBmac0154*	1998	Charlick	5.4	0.27	0.000	4.4	0.24	0.000	*XEBmac501*	Alexis
		1999	Wongan Hills	3.0	0.14	0.001	1.3	0.07	0.018	*XEBmac501*	Alexis
		1998	[a]Charlick	3.4	0.18	0.000	2.8	0.15	0.001	*XEBmac501*	Alexis
2H	*Xcdo665A-Xabg2*	1999	Charlick	2.6	0.11	0.001	5.6	0.23	0.000	*XEBmac0684*	Alexis
3H	*Xabg705b-Xbmag0225*	1999	Katanning	5.1	0.26	0.000	5.6	0.28	0.000	*XBmag0013*	Sloop
		1999	Wongan Hills	5.2	0.25	0.000	6.6	0.31	0.000	*XAG_CAT331*	Sloop
5H	*XAA_CCA193-XAG_CTA69*	1999	Charlick	4.5	0.19	0.000	5.8	0.24	0.000	*XAG_CCA193*	Alexis
5H	*XAG_CTA69- XAT_CAC195*	1998	Charlick	3.2	0.19	0.000	3.6	0.21	0.000	*XSerpinZ7*	Alexis
		1999	Charlick	5.6	0.29	0.000	5.3	0.28	0.000	*XAG_CGA131*	Alexis
		1999	Wongan Hills	3.5	0.19	0.000	4.5	0.24	0.000	*XAT_CAC195*	Alexis
		1998	[a]Charlick	3.3	0.19	0.000	3.5	0.21	0.000	*XSerpinZ7*	Alexis
5H	*XAT_CAC195-XAC_CC118*	1998	Wongan Hills	4.4	0.23	0.000	3.9	0.21	0.000	*Xabg3*	Alexis

[a]IOB HWE method, [b]most significant marker, [c]based on the most significant marker in that region

Table 5.3.8 Interval and regression analysis of the significant regions for HWE in the Alexis/ Sloop-sib mapping population.

Chromosome	Region	Year	Site	HWE			HWE adjusted for Protein			[b]Marker	Parent donating the higher allele
				LOD	r^{2c}	P^c	LOD	r^{2c}	P^c		
1H	*Xksud14B-XBmac0154*	1999	Wongan Hills	3.3			2.7				Alexis
		1998	[a]Charlick	3.0	0.09	0.000	3.4	0.10	0.000	*XEBmac501*	Alexis
2H	*Xcdo665A-Xbmag0518*	1999	Charlick	4.9	0.12	0.000	9.0	0.28	0.000	*Xcdo474*	Alexis
		1998	[a]Charlick	4.8	0.14	0.000	4.8	0.14	0.000	*Xabg14*	Alexis
3H	*Xabg705b-Xbmag0225*	1999	Katanning	3.5	0.12	0.000	4.5	0.15	0.000	*Xbcd131a*	Sloop
		1999	Wongan Hills	3.7	0.13	0.000	4.6	0.16	0.000	*Xbcd131a*	Sloop
5H	*XAA_CCA193-XAG_CTA69*	1998	Charlick	5.4	0.18	0.000	6.7	0.23	0.000	*XAG_CTT421*	Alexis
		1999	Wongan Hills	5.1	0.18	0.000	4.7	0.18	0.000	*XAA_CCA193*	Alexis
		1998	[a]Charlick	6.5	0.22	0.000	6.9	0.23	0.000	*XAG_CTT421*	Alexis
5H	*XAG_CTA69- XAT_CAC195*	1998	Charlick	3.2	0.11	0.000	4.0	0.15	0.000	*XAG_CTA191*	Alexis
		1999	Wongan Hills	6.7	0.25	0.000	6.2	0.24	0.000	*XAG_CTA191*	Alexis
		1998	[a]Charlick	3.9	0.13	0.000	4.4	0.15	0.000	*X AT_CAC197*	Alexis
5H	*XAT_CAC195-XAC_CC118*	1998	Charlick	4.5	0.14	0.000	6.0	0.19	0.000	*XGMS01*	Alexis
		1999	Charlick	2.5	0.09	0.000	3.7	0.13	0.000	*X AG_CTT224*	Alexis
		1998	[a]Charlick	3.6	0.14	0.000	4.8	0.19	0.000	*X AG_CTG388*	Alexis

[a]IOB HWE method, [b]most significant marker, [c]based on the most significant marker in that region

Table 5.3.9 Interval and regression analysis of the significant regions for HWE in the combined Alexis/ Sloop and Alexis/ Sloop-sib mapping population.

Chromosome	Region	Year	Site	HWE			HWE adjusted for Protein			[b]Marker	Parent donating the higher allele
				LOD	$r^{2\,c}$	P^c	LOD	$r^{2\,c}$	P^c		
1H	*Xksud14B-XBmac0154*	1998	Charlick	6.3	0.12	0.0000	6.4	0.13	0.0000	*XEBmac501*	Alexis
		1999	Charlick	4.3	0.07	0.0000	5.0	0.09	0.0000	*XEBmac501*	Alexis
		1999	Katanning	3.6	0.07	0.0002	2.7	0.06	0.0008	*XEBmac501*	Alexis
		1999	Wongan Hills	5.2	0.09	0.0000	3.6	0.06	0.0008	*XEBmac501*	Alexis
		1998	[a]Charlick	6.2	0.13	0.0000	5.9	0.13	0.0000	*XEBmac501*	Alexis
2H	*Xcdo665A-Xabg2*	1999	Charlick	5.3	0.10	0.0000	13.5	0.20	0.0000	*XAT/CTG111*	Alexis
		1998	[a]Charlick	5.5	0.10	0.0000	6.1	0.11	0.0000	*Xabg14*	Alexis
3H	*Xabg705b-Xbmag0225*	1999	Katanning	7.7	0.16	0.0000	9.3	0.19	0.0000	*Xbcd131A*	Sloop
		1999	Wongan Hills	7.6	0.14	0.0000	10.1	0.19	0.0000	*Xabg4*	Sloop
4H	*XBmag0419-Xwg719*	1998	Charlick	3.6	0.07	0.0004	3.8	0.08	0.0002	*XAA/CAC119*	Sloop
		1998	[a]Charlick	3.6	0.06	0.0006	3.5	0.06	0.0006	*XAA/CAC119*	Sloop
4H	*XAG/CAA252-XAA/CAC307*	1998	[a]Charlick	2.7	0.06	0.0006	4.3	0.09	0.0000	*XEbmac0906*	Alexis
5H	*XAA_CCA193-XAG_CTA69*	1998	Charlick	4.3	0.14	0.0000	5.2	0.15	0.0000	*XAG/CCA199*	Alexis
		1999	Charlick	4.6	0.09	0.0000	6.4	0.11	0.0000	*XAG/CCA199*	Alexis
		1999	Wongan Hills	4.2	0.09	0.0000	4.0	0.09	0.0000	*XAA/CCA193*	Alexis
		1998	[a]Charlick	7.2	0.16	0.0000	7.8	0.17	0.0000	*XAC/CAT298*	Alexis
5H	*XAG_CTA69-XAT_CAC195*	1998	Charlick	6.5	0.12	0.0000	7.6	0.15	0.0000	*XAG/CTA191*	Alexis
		1999	Charlick	5.4	0.11	0.0000	7.4	0.14	0.0000	*Xabc310*	Alexis
		1999	Katanning	2.8	0.06	0.0010	4.6	0.09	0.0000	*XAG/CTA191*	Alexis
		1999	Wongan Hills	8.5	0.22	0.0000	9.7	0.24	0.0000	*XAG/CTA191*	Alexis
		1998	[a]Charlick	6.5	0.14	0.0000	7.3	0.16	0.0000	*XAT/CAC197*	Alexis
5H	*XAT_CAC195-XAC_CCT118*	1998	Charlick	6.0	0.12	0.0000	6.7	0.13	0.0000	*Xabg712*	Alexis
		1999	Charlick	4.5	0.10	0.0000	6.4	0.12	0.0000	*XAT/CAG171*	Alexis
		1999	Wongan Hills	5.9	0.12	0.0000	7.2	0.11	0.0000	*Xcdo400*	Alexis
		1998	[a]Charlick	4.1	0.08	0.0000	4.5	0.09	0.0000	*XAT/CAG171*	Alexis

[a]IOB HWE method, [b]most significant marker, [c]based on the most significant marker in that region

Table 5.3:10: Mean HWE for the lines carrying either the Alexis or the Sloop or Sloop-sib allele at a number of markers in the combined Sloop/ Alexis and Sloop-sib/ Alexis mapping populations.

Chromosome	Marker	Parent donating allele	Charlick 1998	Charlick 1999	Katanning 1999	Wongan Hills 1999	Charlick 1998*	Mean EBC HWE
1H	*XEBmac0501*	**Alexis**	**[a]74.3**	**[a]76.8**	**[a]74.9**	**[a]76.6**	**[a]68.8**	**[a]75.6**
		#Sloop	73.5	76.1	74.1	75.9	67.1	74.9
2H	*Xabg14*	**Alexis**	**[b]74.1**	**[a]76.7**	**74.5**	**76.2**	**[a]68.6**	**[c]75.4**
		#Sloop	73.6	76.1	74.4	76.1	67.1	75.1
3H	*Xbcd131a*	Alexis	[c]73.7	[c]76.2	[a]73.9	[a]75.9	67.8	[a]74.9
		#Sloop	**74.0**	**76.6**	**75.0**	**76.5**	**67.9**	**75.5**
4H	*XEBmac0906*	Alexis	73.7	76.3	74.4	76.1	[a]67.4	75.2
		#Sloop	**74.1**	**76.5**	**74.6**	**76.3**	**68.6**	**75.4**
4H	*XAA_CAC119*	Alexis	[a]73.6	[b]76.2	74.5	76.2	[b]67.4	75.1
		#Sloop	**74.2**	**76.7**	**74.5**	**76.3**	**68.5**	**75.4**
5H	*Xabg3*	**Alexis**	**[a]74.1**	**[a]76.7**	**[a]74.7**	**[a]76.5**	**[b]68.3**	**[a]75.5**
		#Sloop	73.5	76.0	74.1	75.8	67.3	74.8
5H	*XAG_CTA191*	**Alexis**	**[a]74.3**	**[a]76.8**	**[b]74.8**	**[a]76.7**	**[a]68.7**	**[a]75.6**
		#Sloop	73.5	76.0	74.1	75.7	67.0	74.8
5H	*XAG_CCA199*	**Alexis**	**[a]74.2**	**[a]76.8**	**74.7**	**[a]76.5**	**[a]68.9**	**[a]75.6**
		#Sloop	73.6	76.1	74.3	75.9	67.1	75.0

*IOB HWE method, #Sloop/Sloop-sib, [a] mean HWE significantly different (P<0.001), [b] mean HWE significantly different (P<0.01), [c] mean HWE significantly different (P<0.05), **bold**: parent carrying higher allele

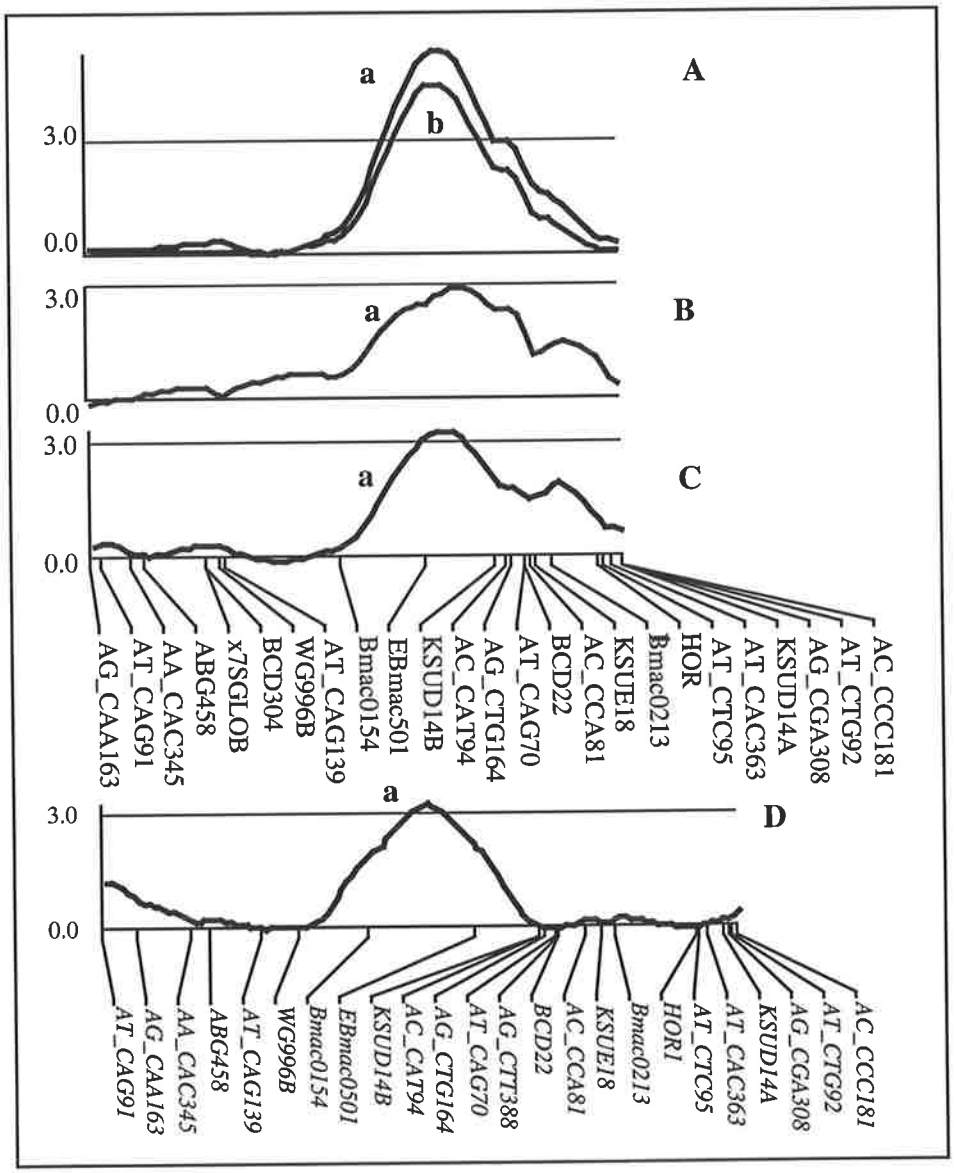


Figure 5.3.6 Interval map of Chromosome 1H for HWE in the Alexis/ Sloop and Alexis/ Sloop-sib mapping populations. LOD score is plotted on the y axis. a: raw data, b: data adjusted for protein, A: Alexis/ Sloop, EBC HWE, Charlick, 1998, B: Alexis/ Sloop, EBC HWE, Wongan Hills, C: Alexis/ Sloop, IOB HWE, Charlick, 1998, D: Alexis/ Sloop-sib, EBC HWE, Wongan Hills

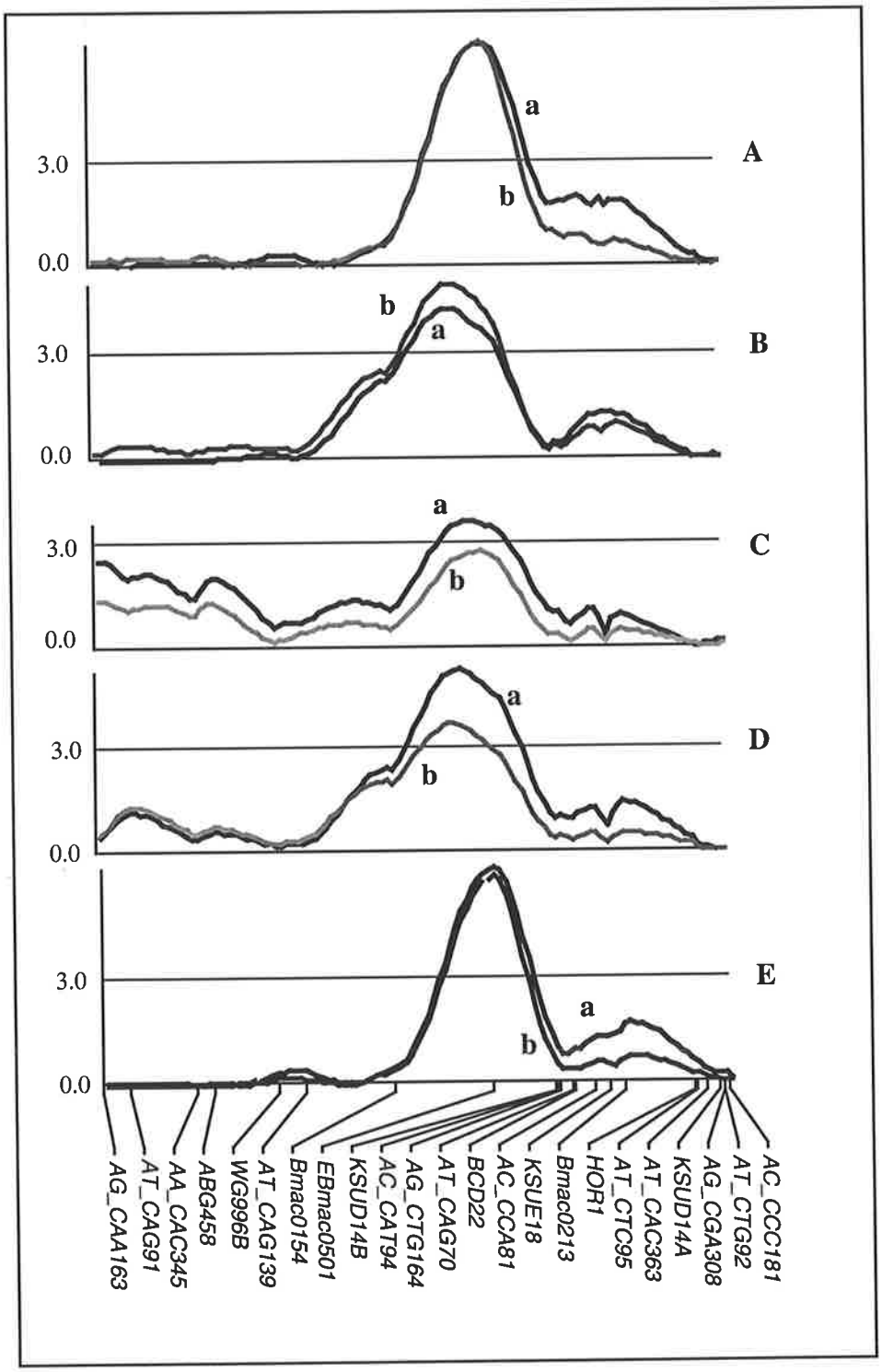


Figure 5.3.7 Interval map of Chromosome 1H for HWE in the Combined Alexis/ Sloop and Alexis/ Sloop-sib mapping population. LOD score is plotted on the y axis. a: raw data, b: adjusted data, A: EBC HWE, Charlick, 1998, B: EBC HWE, Charlick, 1999, C: EBC HWE, Katanning, 1998, D: EBC HWE, Wongan Hills, E: IOB HWE, Charlick, 1998

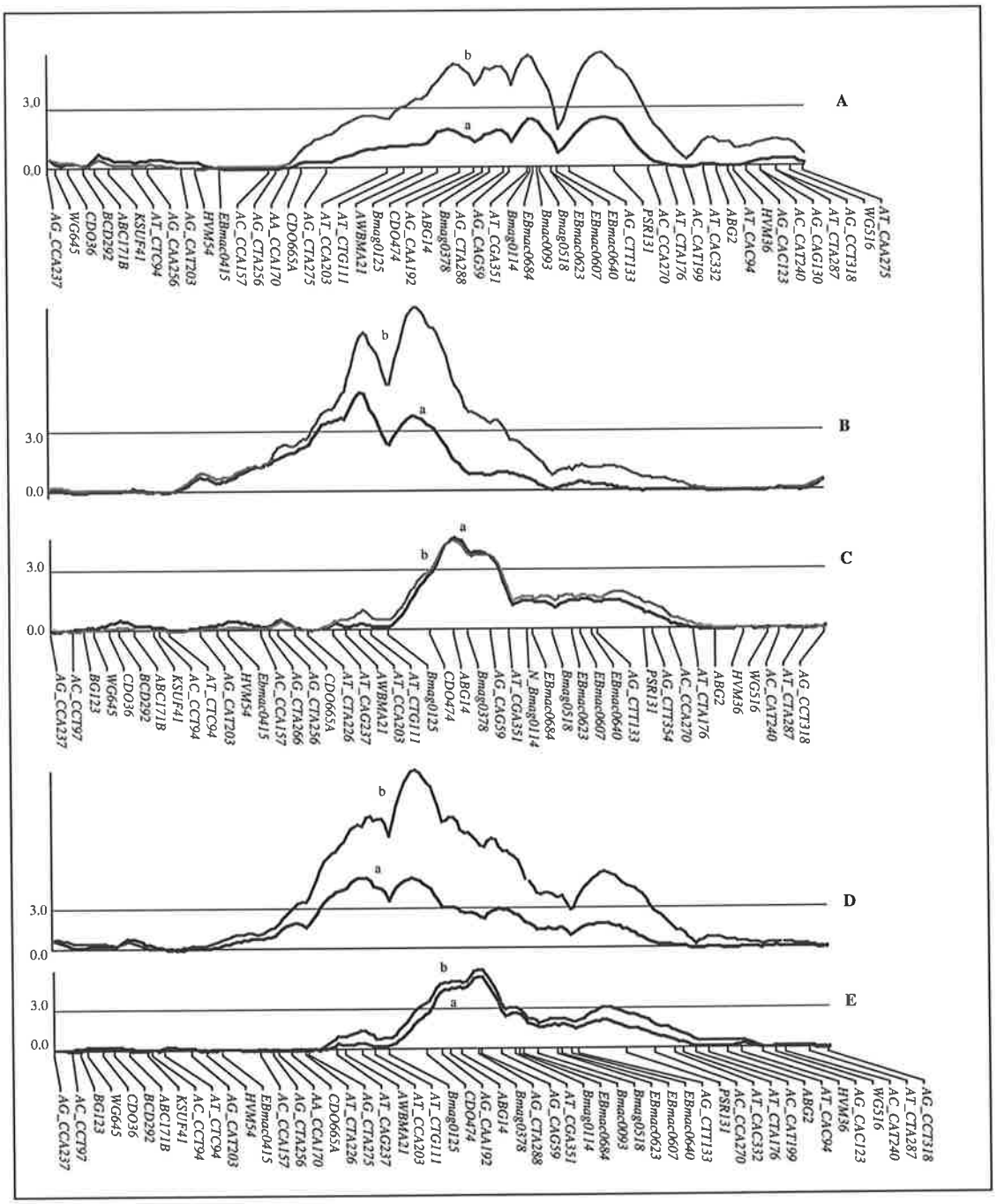


Figure 5.3.8 Interval map of Chromosome 2H for HWE in the Alexis/ Sloop and Alexis/ Sloop-sib mapping populations and the Combined Alexis/ Sloop and Alexis/ Sloop-sib mapping population. LOD score plotted on the y-axis. a: raw data, b: adjusted data, A: Alexis/ Sloop, EBC HWE, Charlick, 1999, B: Alexis/ Sloop-sib, EBC HWE, Charlick, 1999, C: Alexis/ Sloop-sib, IOB HWE, Charlick, 1998, D: Combined map, EBC HWE, Charlick, 1999, E: Combined map, IOB HWE, Charlick, 1998

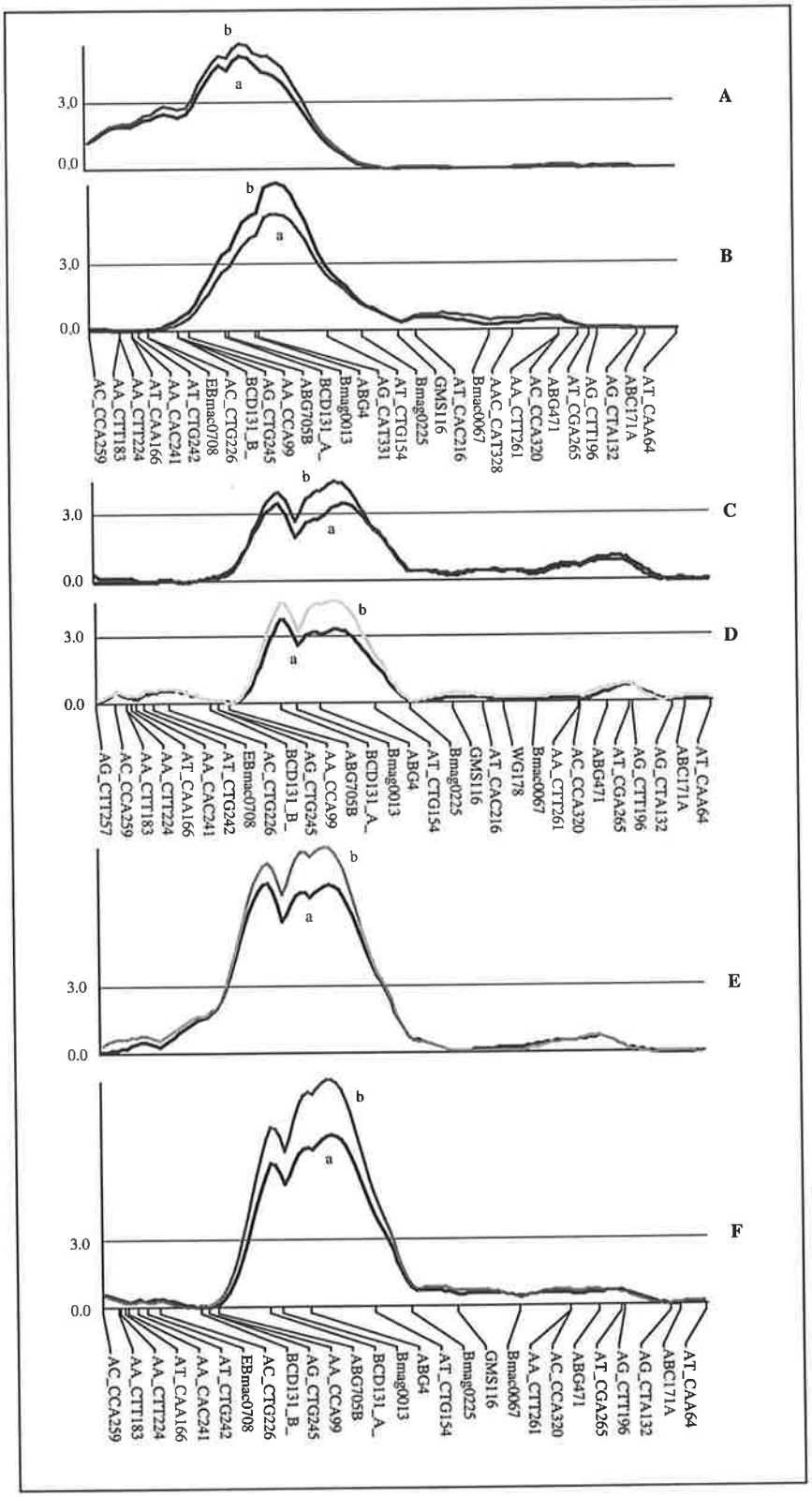


Figure 5.3.9 Interval map of Chromosome 3H for EBC HWE in the Alexis/ Sloop and Alexis/ Sloop-sib mapping populations and the Combined Alexis/ Sloop and Alexis/ Sloop-sib mapping population. LOD score plotted on the y axis. a: raw data, b: adjusted data, A: Alexis/ Sloop, Katanning, 1999 B: Alexis/ Sloop, Wongan Hills, 1999, C: Alexis/ Sloop-sib, Katanning, 1999, D: Alexis/ Sloop-sib, Wongan Hills, 1999, E: Combined map, Katanning, 1999, F: Combined map, Wongan Hills, 1999

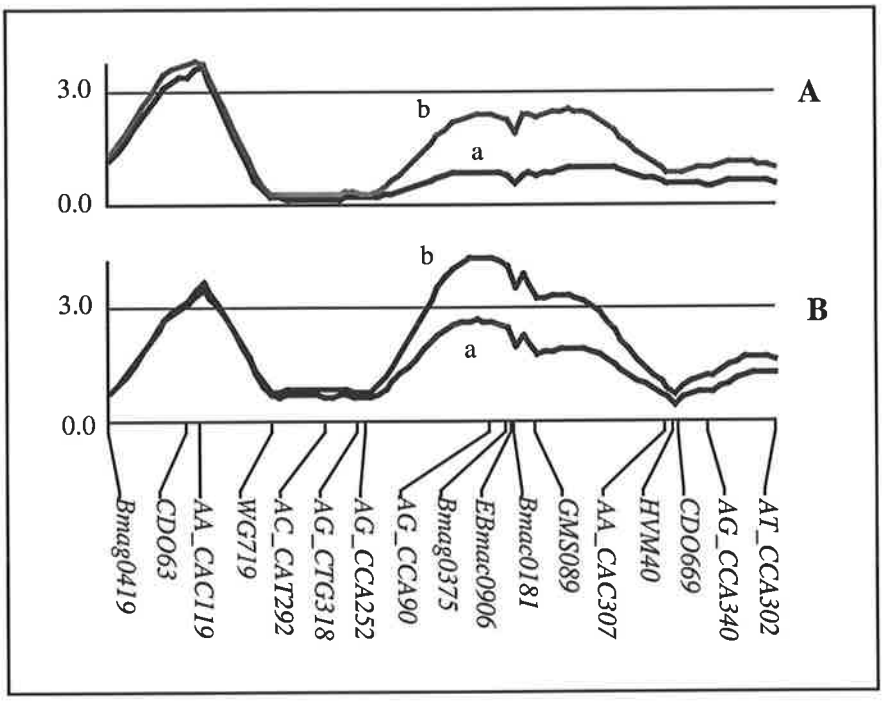


Figure 5.3.10 Interval map of Chromosome 4H for HWE in the Combined Alexis/ Sloop and Alexis/ Sloop-sib mapping population. LOD score is plotted on the y-axis. a: raw data, b: adjusted data, A: EBC HWE, Charlick, 1998, B: IOB HWE, Charlick, 1998,

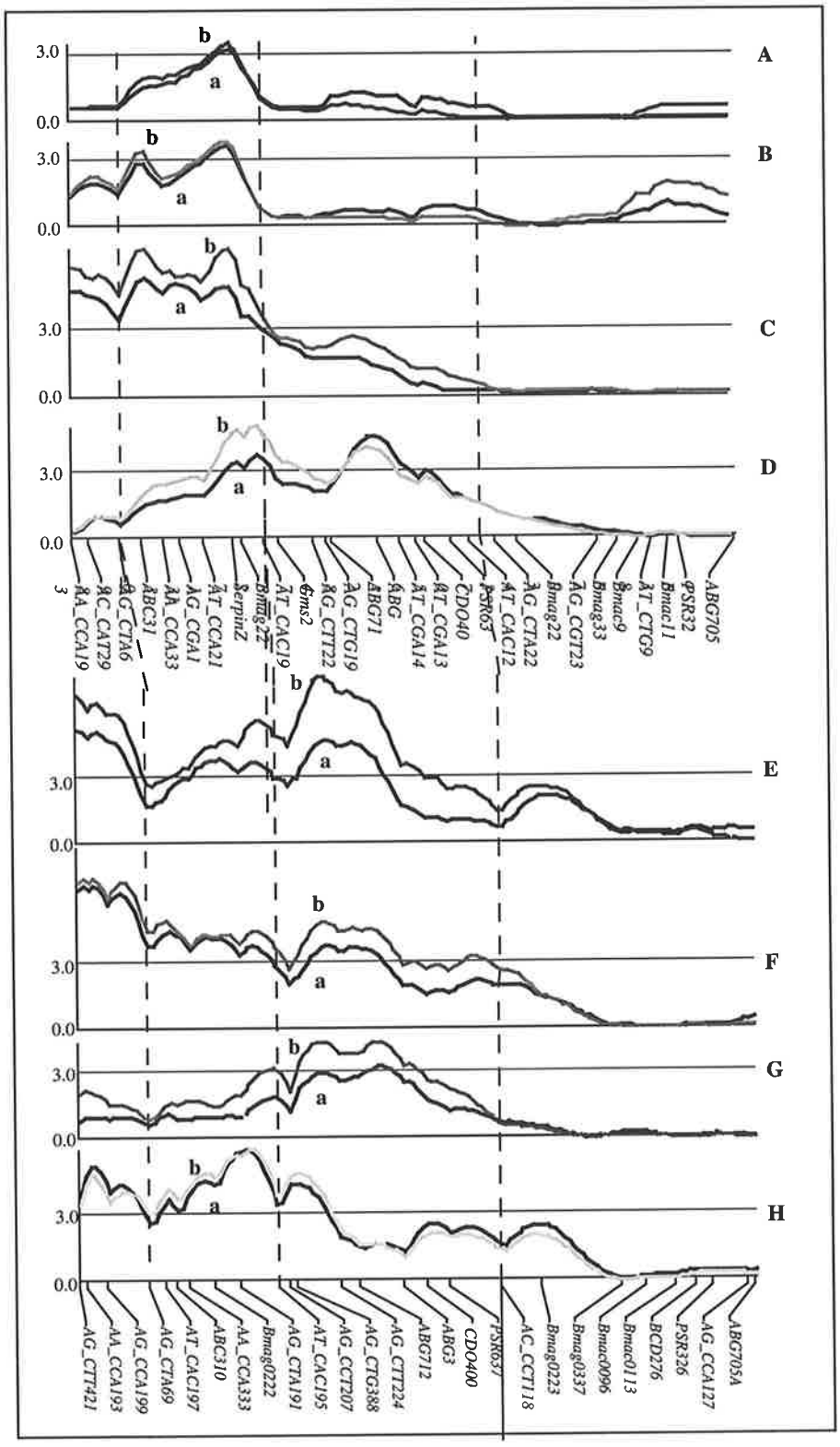


Figure 5.3.11 Interval map of Chromosome 5H for HWE in the Alexis x Sloop and Alexis x Sloop-sib mapping populations. LOD score is plotted on the y axis. a: raw data, b: data adjusted for protein, A: Alexis x Sloop, EBC HWE, Charlick, 1998, B: Alexis x Sloop, IOBHWE, Charlick, 1998, C: Alexis x Sloop, EBC HWE, Charlick, 1999, D: Alexis x Sloop, EBC HWE, Wongan Hills, E: Alexis x Sloop-sib, EBC HWE, Charlick, 1998, F: Alexis x Sloop-sib, IOBHWE, Charlick, 1998, G: Alexis x Sloop-sib, EBC HWE, Charlick, 1999, H: Alexis x Sloop-sib, EBC HWE, Wongan Hills

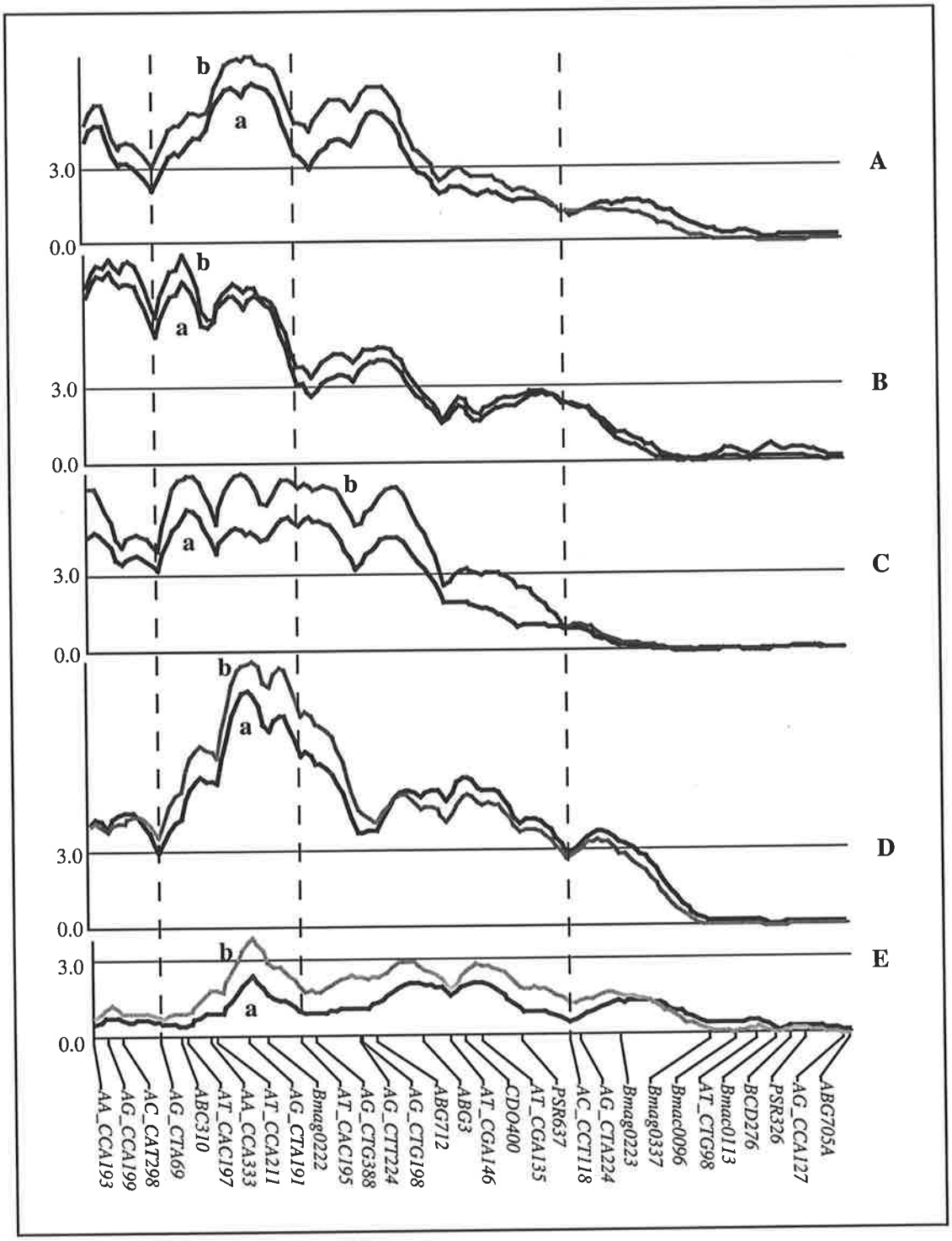


Figure 5.3.12 Interval map of Chromosome 5H for HWE in the Combined Alexis x Sloop and Alexis x Sloop-sib mapping populations. LOD score is plotted on the y axis. a: raw data, b: adjusted data, A: EBC HWE, Charlick, 1998, B: IOBHWE, Charlick, 1998, C: EBC HWE, Charlick, 1999, D: EBC HWE, Wongan Hills, E: EBC HWE, Katanning, 1998,

5.4: Discussion

Kleinhofs and Han (2002) developed a BinMap based on the linkage map developed from the Steptoe/ Morex mapping population where markers from each barley chromosome have been assigned to regions, called bins. Hayes *et al.* (2001) used this BinMap to relate numerous traits, including HWE using both the EBC and IOB methods of analysis, from previous studies to bins. The regions found to be associated with HWE in this study have also been allocated to bins in an attempt to relate them to regions found in previous studies. Eight regions of the barley genome were found to be associated with HWE. These are summarized in Table 5.4.1. A number of these regions had been identified previously in alternate populations (Hayes *et al.* 1993; Hayes *et al.,* 1996; Oziel *et al.* 1996; Thomas *et al.* 1996; Bezant *et al.* 1997a; Hayes *et al.* 1997; Mather *et al.,* 1997; Powell *et al.* 1997; Ullrich *et al.* 1997; Marquez-Cedillo *et al.* 2000; Hayes *et al.,* 2001; Collins *et al.,* 2003; Panozzo *et al.,* in preparation).

5.4.1. Galleon/ Haruna Nijo mapping population

5.4.1.1 QTL for HWE on the short arm of Chromosome 2H

The region on the short arm of chromosome 2H found to be significantly associated with HWE in the Galleon/ Haruna Nijo population can be placed in bins 2 to 4. QTL for HWE, located in bins 2 to bin 4 on chromosome 2H, have been identified in a number of other mapping populations (Hayes and Jones, 2000). HWE was found to be associated with bin 2 in the populations of Blenheim/ E224/3 and Blenheim/ Kym. These populations are the only two in the published literature where HWE was measured using the IOB method (Bezant *et al.* 1997a; Powell *et al.* 1997). Bins 3 and 4 have been shown to be associated with HWE in the populations of Steptoe/ Morex, Harrington/ Morex and Dictoo/ Morex. In all of these cases either Blenheim or Morex were donating the higher HWE allele (Hayes *et al.*, 1993; Oziel *et al.,* 1996; Bezant *et al.*, 1997a; Hayes *et al.*, 1997; Powell *et al.*, 1997; Ullrich *et al.*, 1997; Marquez-Cedillo *et al.*, 2000). This indicates that bins 2 to 4 on chromosome 2H contain a major region associated with HWE derived from at least three different parental sources, namely Blenheim, Morex and Haruna Nijo. Whether a single or densely linked group of genes are responsible for influencing HWE in this region is unknown.

The difference in HWE between the lines carrying the Haruna Nijo allele at the marker locus *Xpsr108* on the short arm of chromosome 2H and those carrying the Galleon allele was approximately 2% (EBC method). Australian breeders are looking for an improvement in HWE of approximately 2% over Schooner and Sloop. For a feed variety such as Galleon, which has particularly low levels of HWE (Section 4.3.1), an improvement of at least 4% in HWE would be required for it to compete with the current world malting varieties. MAS for this QTL alone may only result in bringing a variety such as Galleon up to the standard of Schooner and Sloop. Selection for other regions will be required to make the gains in HWE necessary to raise a variety such as Galleon to malting standard.

5.4.1.1 QTL for HWE on the long arm of Chromosome 2H, short arm of Chromosome 5H and short arm of Chromosome 6H

The three regions associated with HWE in the Galleon/ Haruna Nijo mapping population on the long arm of chromosome 2H, short arm of chromosome 5H and short arm of chromosome 6H with LOD scores that were not significant may well have been significant with a larger population size. The precision in the detection of QTL in the Galleon/ Haruna Nijo mapping population would have been limited by the low number of lines in the population and the number of lines phenotyped, particularly in 1996 when only 48 lines were grown. Nonetheless, within the literature, QTL for HWE in barley were all identified in mapping populations consisting of less than 180 lines (Hayes *et al.* 1993; Hayes *et al.* 1996; Oziel *et al.*, 1996; Thomas *et al.* 1996; Bezant *et al.* 1997a; Hayes *et al.* 1997; Mather *et al.* 1997; Powell *et al.* 1997; Ullrich *et al.* 1997; Marquez-Cedillo *et al.* 2000). Ideally, for the detection of QTL of small effect, population sizes of approximately 350 are more appropriate (Lande and Thompson, 1990). Melchinger *et al.* (1998) compared QTL mapping results from two populations, varying in size, produced from the testcross progeny of F_2 plants in maize. They identified three times as many QTL in the larger of the two populations (344 lines versus 107 lines). When using composite interval mapping, a population of 107 individuals and a minimum LOD score of 2.5, the minimum phenotypic variance that can be detected for significant QTL is 10%.

However, with 344 individuals, significant QTL can be detected that account for 3% of the phenotypic variance (Melchinger *et al.* 1998).

Whilst large population sizes would theoretically be advantageous for the discovery of QTL, in practical terms this rarely occurs due to the large cost involved in producing, genotyping and phenotyping mapping populations. It is therefore unlikely that QTL having small effects on HWE will be discovered until larger populations are developed or better consensus maps are produced. As HWE has been shown to be a highly complex trait, influenced by many factors, it is likely that a large number of QTL remain to be discovered.

5.4.2 Sloop/ Alexis and Sloop-sib/ Alexis mapping populations

As discussed in the previous section for Haruna Nijo/ Galleon, the varying population sizes between the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations would have had an influence on the differences between the two populations in the number of QTL found. Whilst there were four regions significantly associated with HWE identified in both of the populations, the QTL were more consistently identified across sites in the Sloop-sib/ Alexis population than the Sloop/ Alexis population. QTL with small effects cannot be identified in small populations. At two of the sites the Sloop/ Alexis population had only approximately 80 lines (Table 5.3.6), which would exclude the detection of any QTL that did not explain at least, approximately 15% of the variation, based on the equation:

$$r^2=1-e^{-LOD/(0.2171*N)}$$

for composite interval mapping (Melchinger *et al.* 1998) from the work of Haley and Knott (1992), and a LOD score of 3.0. In contrast, the Sloop-sib/ Alexis population consisted of between 127 and 147 lines, allowing detection of QTL theoretically accounting for nine and 10% of the variation, respectively. The combined population consisted of 255 lines, allowing detection of QTL theoretically accounting for as little as five percent of the variation. Hence, using the combined map, two extra regions on chromosome 4H were found to be associated with HWE. Additionally the regions on Chromosomes 1H and 5H were found to be significant at all sites investigated.

Table 5.4.1 A summary of the regions of the barley genome found to be associated with malt extract in this study, the bin number (Kleinhofs and Han, 2002) and other populations where QTL for malt extract (using both the EBC and IOB methods of analysis) have also been identified in previous studies.

Chromosome	Population	Location	bin	Other populations	Reference
1H	Sloop/ Alexis, Sloop-sib/ Alexis	centromere	5-7	Harrington /Morex, Arapiles/ Franklin	Marquez-Cedillo *et al.*, 2000; Hayes *et al.*, 2001; Panozzo *et al.*, in preparation
2H	Galleon/ Haruna Nijo	Short arm	2-4	Blenheim/ E224/3, Blenheim/ Kym, Dicktoo/ Morex, Harrington /Morex, Steptoe/ Morex	Hayes *et al.*, 1993; Oziel *et al.*, 1996; Bezant *et al.*, 1997a; Hayes *et al.*, 1997; Powell *et al.*, 1997; Ullrich *et al.*, 1997; Marquez-Cedillo *et al.*, 2000; Hayes *et al.*, 2001
2H (putative)	Sloop/ Alexis, Sloop-sib/ Alexis Galleon/ Haruna nijo	Long arm	9-11	Calicuchuma-sib/ Bowman	Hayes *et al.*, 1996; Hayes *et al.*, 2001
3H	Sloop/ Alexis, Sloop-sib/ Alexis	Long arm	12-13	Blenheim/ Kym	Bezant *et al.*, 1997; Hayes *et al.*, 2001
4H	Sloop/ Alexis, Sloop-sib/ Alexis	Long arm	11	none	
4H	Sloop/ Alexis, Sloop-sib/ Alexis	Short arm	unassigned		
5H	Sloop/ Alexis, Sloop-sib/ Alexis	Long arm	11-15	Harrington/ TR306, Dicktoo/ Morex, Blenheim/ E224/3, Chebec/ Harrington	Oziel *et al.*, 1996; Thomas *et al.*, 1996; Mather *et al.*, 1997; Powell *et al.*, 1997; Hayes *et al.*, 2001; Collins *et al.*, 2003
5H (putative)	Galleon/ Haruna nijo	Short arm	4	Dicktoo/ Morex	Oziel *et al.*, 1996; Hayes *et al.*, 2001
6H (putative)	Galleon/ Haruna nijo	Short arm	1-2	none	

The differences between the EBC HWE levels in Sloop and Sloop-sib are shown in Table 5.1.1. Sloop produces higher HWE results then Sloop-sib. Consequently, it could be assumed that the genetic differences between Alexis and Sloop-sib contributing to the variation in HWE would be greater in number than the genetic differences between Alexis and Sloop. However, the genetic differences between Sloop and Sloop-sib appear to have had little impact on the number of regions found to be associated with HWE in the two populations, with six regions found in both populations (Table 5.3.7 and Table 5.3.8).

5.4.2.1 QTL for HWE on the centromere region of Chromosome 1H

The markers Xksud14B and XBmac0154 flank the region found to be associated with HWE on chromosome 1H. This region corresponds to bins 5 to 7 according to Kleinhofs and Han (2002). Bin 5 has previously been found to be associated with HWE in the mapping population Harrington/ Morex (Hayes *et al.*, 2001) and bin 7 is associated with HWE in the Arapiles/ Franklin mapping population (Panozzo *et al.*, in preparation).

5.4.2.2 QTL for HWE on the long arm of Chromosome 2H

The region on chromosome 2H, flanked by markers Xcdo665A and Xbmag0518, can be placed in bins 8 to 11 according to Kleinhofs and Han (2002). This region corresponds with the region discussed in Section 5.3.1 found to be weakly associated with HWE on chromosome 2H in the Galleon/ Haruna Nijo population. QTL have also been identified that are associated with HWE in the region of chromosome 2H assigned to bin 10 in the population Calicuchuma-sib/ Bowman (Hayes *et al.*, 2001).

In most cases, adjusting HWE for differences in grain protein levels had little impact on the significance of regions found to be associated with HWE with the LOD scores changing by less than 1.0. However, adjusting the results for grain protein had a large influence on the LOD score for the region found on chromosome 2H. This region falls into bins 9 to 11 according to Kleinhofs and Han (2002). These bins have also been associated with grain protein in the populations of Harrington/ Morex and

Calicuchuma-sib/ Bowman (Hayes *et al.*, 2001) which may explain the large influence of protein on the LOD scores found in this region. Whether the genes in this region are pleotropically influencing HWE and grain protein individually or influencing HWE by influencing grain protein remains to be discovered.

5.4.2.3 QTL for HWE on the long arm of Chromosome 3H

The region found to be associated with HWE on chromosome 3H was only significant at two sites, Katanning and Wongan Hills. Both of these sites are located in Western Australia and are likely to have had similar growing conditions. However, the mean HWE for both of the populations at these two sites were significantly different ($P<0.001$) while the protein levels were similar (Table 5.3.6). It is likely that an environmental factor such as day length, which would be similar at the two sites, may have had an influence on an agronomic trait that is associated with genes in this region and this trait caused the differing HWE levels.

5.4.2.4 QTL for HWE on the long and short arm of Chromosome 4H

The two regions found to be associated with HWE on chromosome 4H were only found in the combined mapping population. These two regions each only accounted for about 6% of the total variation in HWE at the sites at which they were found. This highlights the importance of using large populations to identify regions that only have a minor influence on a trait.

5.4.2.5 QTL for HWE on the long arm of Chromosome 5H

Three regions were found to be significantly associated with HWE on chromosome 5H (Section 5.3.2). These regions are poorly defined and extend from bin 11 to bin 15 (Kleinhofs and Graner, 2002). It is likely that a number of genes influencing HWE in these populations are present in these regions.

In bins 11 to 15 three regions have been associated with HWE in other mapping populations (Hayes *et al.*, 2001). Bin 11 has been associated with HWE, measured by

the IOB method in the population of Blenheim/ E224/3. Bin 13 is associated with a QTL for EBC HWE in the Dicktoo/ Morex population and bin 15 contains a significant QTL for EBC HWE in the populations of Harrington/ TR306 and Chebec/ Harrington (Hayes *et al.*, 2001; Collins *et al.*, 2003).

5.5: Conclusion

Nine regions of the barley genome were found to be associated with HWE in this study, four major regions and five minor regions. The major regions were found on chromosome 1H, 2H (short arm), 2H (long arm) and 5H (long arm). These regions have also been found to be associated with HWE in numerous populations in previous studies (Hayes *et al.*, 1993; Hayes *et al.*, 1996; Oziel *et al.*, 1996; Thomas *et al.*, 1996; Bezant *et al.*, 1997a; Hayes *et al.*, 1997; Mather *et al.*, 1997; Powell *et al.*, 1997; Ullrich *et al.*, 1997; Marquez-Cedillo *et al.*, 2000; Hayes *et al.*, 2001; Collins *et al.*, 2003; Panozzo *et al.*, in preparation). The five minor regions were found on chromosome 3H (long arm), 4H (long and short arm), 5H (short arm) and 6H (short arm). These regions all had low significance or were found at limited sites. The regions on chromosome 4H (long arm) and 6H were not found in any previous studies and may offer a source of new genes to improve the levels of HWE.

Whilst the four major regions found to be associated with HWE have the potential to be useful to breeders wishing to use MAS to select for improved levels of HWE further investigation of these regions is required to assess the suitability of these regions for MAS in alternate genetic backgrounds. Also, the knowledge of how the manipulation of the genes in these regions are likely to influence other traits pleiotropically linked to these genes is important and will be investigated in Chapter 6.

Chapter 6

Malt Extract Loci Effects on Other Important Malt and Barley Quality Traits

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6.1: Introduction

Whilst an investigation of the biochemistry of barley, malt and wort can lead to an understanding of the reasons for differences between varieties in the levels of malt extract (HWE) (Chapter 4), discovering the chromosomal regions influencing malt extract, and other related traits would, not only considerably enhance this understanding but also enable us to manipulate such differences.

Quantitative trait loci (QTL) are regions of the genome that are statistically associated with a trait using various regression models. This statistical association can only occur when the parents of the mapping population differ in the genetic control of the trait, usually indicated by a difference in the levels of the trait under investigation. Often a single region of the genome influences a number of different traits (Section 2.6.3). These regions may contain a single gene that has pleiotropic effects or a gene 'cluster' may exist where a number of individual genes controlling each trait separately are closely linked. Useful information about how a trait of interest is influenced by a region can be gained by investigating other traits that are also associated with that region. For example, while a structural gene for an enzyme would be responsible for the synthesis of that enzyme, there could be a completely different genomic region influencing the level of that enzyme in the malt, such as the levels of a particular hormone.

Whilst the function of only a few genes controlling malting quality in barley have been discovered, many QTL have been found. Hayes and Jones (2000) reported on 181 QTL for 29 phenotypes. These are summarized by Hayes *et al.*, (2001) on the web site http:/www.css.orst.edu/barley/nabgmp/qtlsum.htm. Since then, a number of

other QTL have also been reported (Barr *et al.*, 2003b; Karakousis *et al.*, 2003a; Moody *et al.*, unpublished; Pallotta *et al.*, 2003; Panozzo *et al.*, in preparation)

This chapter investigates the effect of alleles at a number of regions of the barley genome, shown previously to be important in influencing HWE under Australian conditions, on other aspects of malt quality. These include regions that have been associated with HWE in the mapping populations Galleon/ Haruna Nijo, Sloop/ Alexis, Sloop-sib/ Alexis (Chapter 5), Chebec/ Harrington (Collins *et al.*, 2003) and Amaji Nijo/ WI2585 (Pallotta *et al.*, 2003)

6.2: Materials and Methods

6.2.1: Genetic material

The Galleon/ Haruna Nijo mapping populations were produced and mapped as described in section 5.2.1.

6.2.2: Field experiments

The Galleon/ Haruna Nijo mapping population was grown in 1996, 1998 and 1999 by the South Australian Barley Improvement Program (SABIP). 48, 64 and 85 doubled haploid lines, respectively, were grown near Strathalbyn, South Australia each year in single replicate trials.

6.2.3: Barley, malt and wort analysis

Barley husk content, DP, β-amylase, soluble and malt protein and FAN were measured following the methods outlined in chapter 3 (section 3.5.4, section 3.6.1, section 3.6.1.2, section 3.6.1 and section 3.6.5). The number of seeds germinated in petri dishes containing 4 and 8mls of water was measured after 24, 48 and 72 hours as outlined in section 3.3.8. Measurements were taken six months after harvest as it was assumed that by this stage all primary dormancy would be broken. The level of skinned grains was measured as described in section 3.3.6. The components of

skinning are defined in the Australian Barley Board classification manual (ABB, 1998), as follows:

Side or back skinning: "one third or more of the husk is missing from the side or back"

Germ exposed: "husk is removed from the germ end or the germ has been removed"

Chipped: "approximately one third of the grain has been removed at the awn end"

Pearled: "all the husk has been removed and the aleurone layer is exposed"

Split skirts: "the husk is split along the center or side ridges on the back of the grain, at the germ end"

Split backs: "the husk is split along the length of the centre ridge on the back of the grain"

Awn skinning: "greater then half of the husk from the awn end towards the centre of the grain has been removed".

6.2.4: Statistical analysis

Statistical analysis was performed as outlined in section 5.2.4. The computer software program "Qgene" (Nelson, 1997) was used for all simple regression and interval analysis. LOD scores greater than 3.0 were considered significant. Least significant differences between means were calculated using the computer program Jmp® (version 3.1.6, SAS Institute Inc).

6.3: Results

6.3.1: Galleon/ Haruna Nijo

6.3.1.1: Short arm of Chromosome 2H

The mean barley husk content for the Galleon/ Haruna Nijo mapping population and the parents is shown in Table 6.3.1. In 1996, Galleon produced 1.6% higher mean husk content than Haruna Nijo ($P<0.05$). The mean husk content of the Galleon/ Haruna Nijo mapping population was significantly higher in 1996 than in 1998 ($P<0.001$).

The percentage of germinated seeds after 24, 48 and 72 hours incubation with 4mLs of water for the Galleon/ Haruna Nijo mapping population and the parents is shown in Table 6.3.1. No significant difference was found between the parents, Haruna Nijo and Galleon, at any of the time points for either year except after 24 hours in 1998 ($P<0.01$). However, the population means were significantly different at all time points measured between the two years at ($P<0.001$). A significant increase was observed ($P<0.05$) in the percentage of Haruna Nijo seeds germinated in 8mLs of water than Galleon at all time points for both years (Table 6.3.1).

Interval regression analysis revealed a significant region on the short arm of chromosome 2H associated with barley husk content, 4 and 8mL germinations and the level of skinnings in the Galleon/ Haruna Nijo population (Figure 6.3.1). This is the same region that was found to be significantly associated with HWE in this population (Chapter 5). It accounted for 48% and 28% of the variation in husk content in 1996 and 1998 respectively, and 27% and 40% of the variation in the level of skinnings (Table 6.3.2). The different components of skinnings were also compared in the Galleon/ Haruna Nijo mapping population and it was found that only the components side or back skinning, split skirts and germ exposed were significantly associated with this region.

This region on the short arm of chromosome 2H was also found to be significantly associated with the number of grains germinated after 24 hours in 4mLs of water in both years and the number of grains germinated in 8mLs of water in both years after 24, 48 and 72 (1999 only) hours (Table 6.3.2, Figure 6.3.1). After 48 hours, for the 4mL germinations, the majority of the grains had completely germinated, removing any variation in the data.

A large number of the lines in the population showed high levels of water sensitivity, as indicated by the 8mL germination test. Galleon itself was very water sensitive with less than 40% of the seeds germinating after 72 hours. In all of these cases, Haruna Nijo donated the less water sensitive allele.

6.3.1.2: Long arm of Chromosome 5H

The mean malt protein, soluble protein, DP and β-amylase activities for the Galleon/ Haruna Nijo mapping population and the parents are shown in Table 6.3.1. The mean DP and β-amylase activity was significantly higher for Haruna Nijo than Galleon in both 1998 and 1999 ($P<0.05$, $P<0.001$). The Galleon/ Haruna Nijo mapping population produced much higher malt protein in 1998 than 1999 ($P<0.001$) but lower soluble protein in 1998 ($P<0.001$). However, DP and β-amylase activity were not significantly different between the two years.

A significant region on the long arm of chromosome 5H, in the Galleon/ Haruna Nijo population was found by interval regression analysis, to be associated with DP and β-amylase activity in 1998 and 1999, and malt protein and soluble protein in 1999 (Figure 6.3.2). It accounted for 26% and 25% of the variation in DP and β-amylase activity in 1998 and 1999 respectively, and 19% and 22% of the variation in the levels of malt protein and soluble protein in 1999 (Table 6.3.2). This is the same region that was found to be significantly associated with HWE in the Alexis/ Sloop and Alexis/ Sloop-sib mapping populations (Chapter 5).

Table 6.3.1 The mean, standard deviation and range of a number of traits for the Galleon/ Haruna Nijo mapping population and the parents, grown near Strathalbyn, South Australia for three years.

Trait		Year	Galleon		Haruna Nijo		Population			
			Mean	SD	Mean	SD	Mean	SD	Range	No
Husk Content (%db)		96	12.0*	1.0	10.4	1.3	11.7###	1.1	9.6-14.0	41
		98	8.4	1.8	6.3	1.7	9.7	2.0	5.0-17.4	67
Germination (%) 4mL	24 hrs	98	19**	2	81	5	48###	24	3-87	63
		99	60	13	78	16	70	15	31-97	86
	48 hrs	98	98	1	98	3	92###	8	64-100	63
		99	97	3	98	3	97	3	85-100	86
	72 hrs	98	100	0	98	2	98###	2	89-100	63
		99	100	0	99	1	99	1	97-100	86
Germination (%) 8mL	24 hrs	98	12*	5	63	13	43#	23	4-92	64
		99	13***	3	74	11	52	20	10-94	86
	48 hrs	98	31**	6	88	3	76	19	27-99	64
		99	32***	4	90	7	71	20	17-100	86
	72 hrs	98	39**	3	94	0	83##	18	31-100	64
		99	37***	4	94	5	75	20	18-100	86
Skinnings (%)	Total skinning	98	8	0	28	8	23	13	2-56	64
		99	6***	3	19	3	12	9	1-41	86
	Side or back skinning	98	4*	1	11	2	8###	5	0-21	64
		99	2*	1	5	3	4	3	0-11	86
	Germ exposed	98	2*	2	13	0	6###	5	0-21	64
		99	1*	1	3	3	2	2	0-8	86
	Chipped	98	0	0	0	0	1###	1	0-3	64
		99	0	0	0	0	0	0	0-2	86
	Pearled	98	0	0	2	3	1###	1	0-5	64
		99	0	0	0	0	0	0	0-2	86
	Split Skirts	98	3	2	2	2	4	4	0-19	64
		99	2	2	8	8	4	5	0-27	86
	Split Backs	98	0	0	1	1	1	1	0-6	64
		99	1	1	3	3	1	4	0-21	86
	Awn skinning	98	1	1	0	0	4###	4	0-14	64
		99	0	0	0	0	0	0	0-1	86
Soluble Protein (%)		98	3.9	0.0	5.2	0.9	4.6###	0.6	3.7-7.3	66
		99	4.3***	0.1	6.4	0.4	5.2	0.7	4.1-7.6	85
Malt Protein (%)		98	14.0	0.3	14.1	0.3	15.0###	2	11.4-21.4	68
		99	11.9	1.3	12.7	1.2	12.9	2.1	9.4-19.6	84
DP[a]		98	550*	19	898	85	623	156	344-1024	66
		99	425***	68	757	54	600	237	262-1494	84
β-amylase[a]		98	484*	19	785	68	532	147	237-909	66
		99	352***	67	634	56	497	232	166-1379	84

*Haruna Nijo and Galleon are significantly different (P<0.05), **Haruna Nijo and Galleon are significantly different (P<0.01), ***Haruna Nijo and Galleon are significantly different (P<0.001), ###The population means for the different years are statistically different (P<0.001), ##The population means for the different years are statistically different (P<0.01), #The population means for the different years are statistically different (P<0.05), [a]micromoles of maltose equivalents released per minute per gram dry weight.

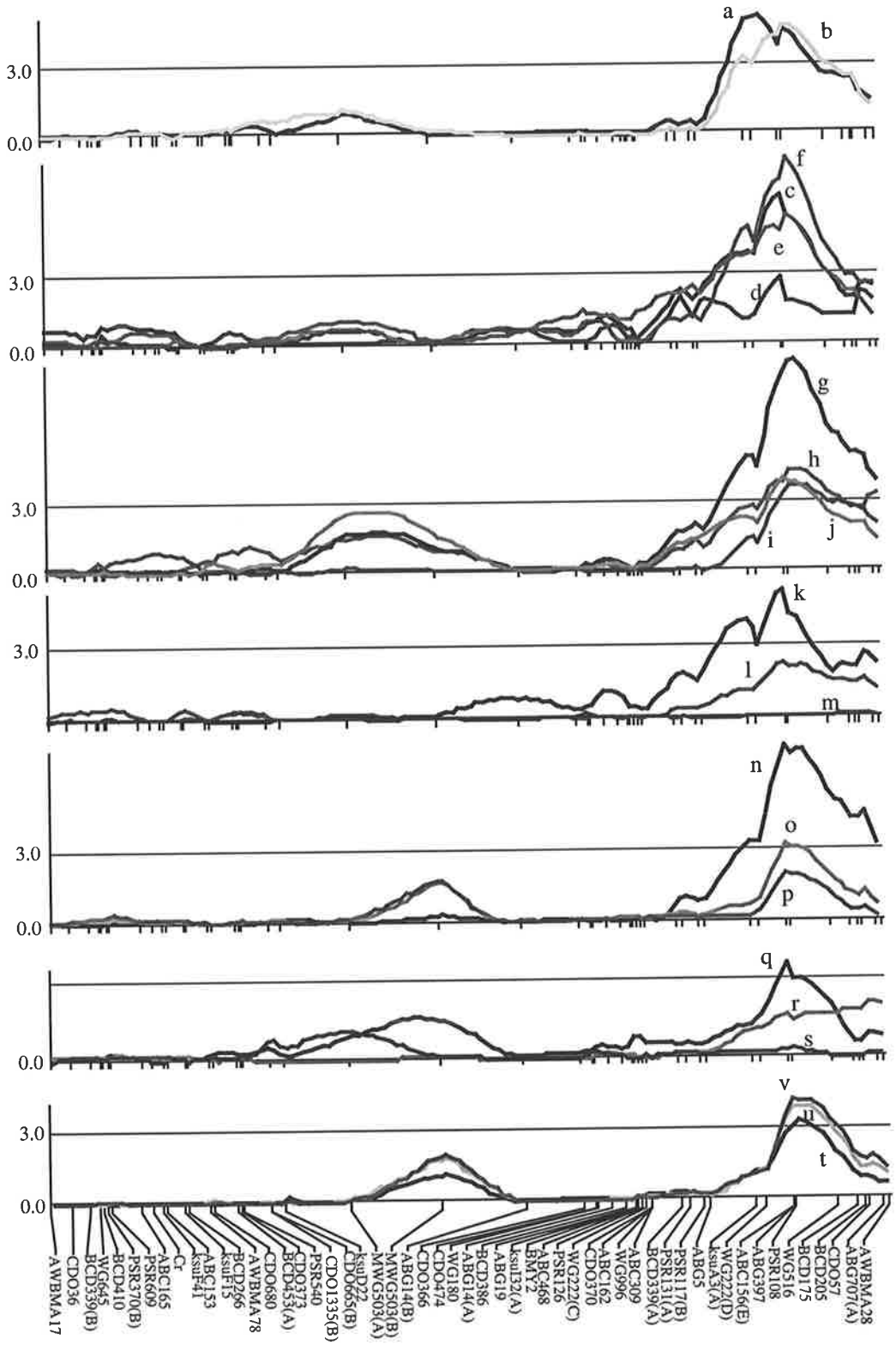


Figure 6.3.1: 11 Interval map of Chromosome 2H for a number of traits in the Galleon x Haruna nijo mapping population. LOD score is plotted on the y axis.

a: Husk Content (%), 1996, b: Husk Content (%), 1998, c: Total Skinnings, 1998,
d: Side and back skinnings, 1998, e: Germ exposed, 1998, f: Split skirt, 1998, g: Total Skinnings, 1999
h: Split skirt, 1999, i: Germ exposed, 1999, j: Side and back skinnings, 1999,
k: Total Germinated (%), 24hours, 4mL water, 1998, l: Total Germinated (%), 48hours, 4mL water, 1998,
m: Total Germinated (%), 72hours, 4mL water, 1998, n: Total Germinated (%), 24hours, 8mL water, 1998,
o: Total Germinated (%), 48hours, 8mL water, 1998, p: Total Germinated (%), 72hours, 8mL water, 1998,
q: Total Germinated (%), 24hours, 4mL water, 1999, r: Total Germinated (%), 48hours, 4mL water, 1999,
s: Total Germinated (%), 72hours, 4mL water, 1999, t: Total Germinated (%), 24hours, 8mL water, 1999,
u: Total Germinated (%), 48hours, 8mL water, 1999, v: Total Germinated (%), 72hours, 8mL water, 1999,

Table 6.3.2: Regions of the barley genome found to be associated, by interval analysis, with a number of traits in the Galleon/Haruna Nijo mapping population.

Trait	Chromosome	Region	Parent donating higher allele	1996			1998			1999		
				LOD	r^2	Marker	LOD	r^2	Marker	LOD	r^2	Marker
Husk	2H	*Xpsr117(B)-Xawbma28*	Galleon	5.0	0.48	*XKsuA3(A)*	4.5	0.28	*Xpsr108*			
Skinnings: Total	2H	*Xpsr117(B)-Xawbma28*	Haruna Nijo				6.6	0.37	*Xabc156(E)*	9.1	0.40	*Xpsr108*
-Side and back							2.8	0.18	*Xabc156(E)*	4.0	0.21	*Xpsr108*
-Germ exposed							8.1	0.44	*Xpsr108*	3.7	0.17	*Xpsr108*
-Chipped							0.3	0.01	*Xabc156(E)*	1.0	0.01	*Xpsr108*
-Pearled							1.3	0.07	*Xabc156(E)*	0.0	0.00	*Xpsr108*
-Split skirt							5.6	0.34	*Xpsr108*	4.4	0.21	*Xpsr108*
-Split back							0.3	0.01	*Xabc156(E)*	1.1	0.07	*Xpsr108*
-Awn skinning							0.7	0.00	*Xabc156(E)*	0.8	0.01	*Xpsr108*
Germinations	2H	*Xpsr117(B)-Xawbma28*	Haruna Nijo									
-4ml , 24hrs							5.4	0.33	*Xabc156(E)*	3.6	0.17	*Xabc156(E)*
-4ml, 48hrs							2.3	0.24	*Xabg397*	2.1	0.09	*Xabg397*
-4ml, 72hrs							0.4	0.01	*Xabc156(E)*	1.0	0.01	*Xabc156(E)*
-8ml , 24hrs							7.3	0.39	*Xpsr108*	3.3	0.16	*Xpsr108*
-8ml, 48hrs							3.2	0.20	*Xabc156(E)*	3.9	0.19	*Xabc156(E)*
-8ml, 72hrs							2.0	0.13	*Xabc156(E)*	4.2	0.20	*Xabc156(E)*
DP	5H	*Xbcd298-Xbcd508*	Haruna Nijo				3.7	0.26	*Xpsr637*	5.5	0.25	*Xabg712*
β-amylase	5H	*Xbcd298-Xbcd508*	Haruna Nijo				3.7	0.26	*Xpsr637*	5.3	0.25	*Xabg712*
Soluble protein	5H	*Xbcd298-Xbcd508*	Haruna Nijo				1.1	0.16	*Xpsr426*	4.6	0.22	*Xcdo504*
Malt protein	5H	*Xbcd298-Xbcd508*	Haruna Nijo				2.8	0.20	*Xpsr426*	3.8	0.19	*Xabg712*

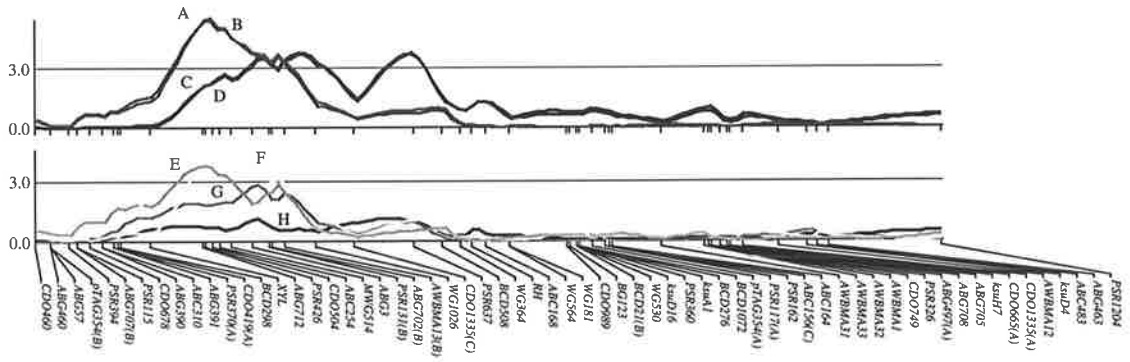


Figure 6.3.2: Interval map of Chromosome 5H for a number of traits in the Galleon/ Haruna Nijo mapping population. LOD score is plotted on the y-axis. A: DP 1999, B: β-amylase 1999, C: DP 1998, D: β-amylase 1998, E: malt protein 1999, F: soluble protein 1999, G: malt protein 1998, H: soluble protein 1998

6.4: Discussion

Eight regions of the barley genome have been found to be associated with HWE in the Australian environment (Figure 6.4.1). These include five regions found to be associated with HWE in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations on chromosomes 1H, 2H, 3H, 4H and 5H (Section 5.3.2). The region on the long arm of chromosome 2H was found to be coincident with a minor region found in the Galleon/ Haruna Nijo mapping population (Section 5.3.1). Another region was also found to be associated with HWE on the short arm of chromosome 2H in the Galleon/ Haruna Nijo population (Section 5.3.1). Two regions, on chromosomes 1H and 5H, were identified in the Chebec/ Harrington mapping population (Collins *et al.*, 2003) and the Harrington/ TR306 mapping population (Hayes *et al.*, 1997; Mather *et al.*, 1997). The final region was found in the Amagi Nijo/ WI2585 mapping population (Pallotta *et al.* 2003) on chromosome 2H. These QTL have been included in a schematic diagram based on the BinMap developed by Kleinhofs and Han (2002) (Figure 6.4.1). Hayes *et al.* (2001) have associated a large number of traits with the markers on this BinMap and these are shown on the diagram. These include numerous malt quality and agronomic traits. Also included are a number of traits, not mentioned in the Hayes *et al.* (2001) summary, that were extracted from alternate sources (Li *et al.*, 1996; Zale *et al.*, 2000, Barr *et al.*, 2003a; Barr *et al.*, 2003b;

Collins *et al.,* 2003; Karakousis *et al.*, 2003a; Moody *et al.*, unpublished; Pallotta *et al.,* 2003; Panozzo *et al.*, in preparation; Asamaya, unpublished).

6.4.1: Chromosome 1H, marker locus *XEbmac501*

A region on chromosome 1H was found to be associated with HWE in the Sloop/ Alexis and Sloop-sib/ Alexis populations (section 5.3.2). This region was also found to be associated with wort viscosity, free amino nitrogen and diastatic power in these populations (Panozzo *et al.*, in preparation). Whilst the level of modification of the grain during the malting process has an influence on all of these traits, other important modification related traits, such as kolbach index and soluble protein were not found in this region for these populations (Panozzo *et al.*, in preparation). However, kolbach index was found to be significantly associated with this region in the Franklin/ Arapiles mapping population (Panozzo *et al.*, in preparation). Wort β-glucan levels and malt α-amylase levels were also found to be associated with this region in the Franklin/ Arapiles mapping population (Panozzo *et al.*, in preparation). All of these traits are related to modification and it is likely that a gene or genes are located in this region that influence the level of modification of the grain.

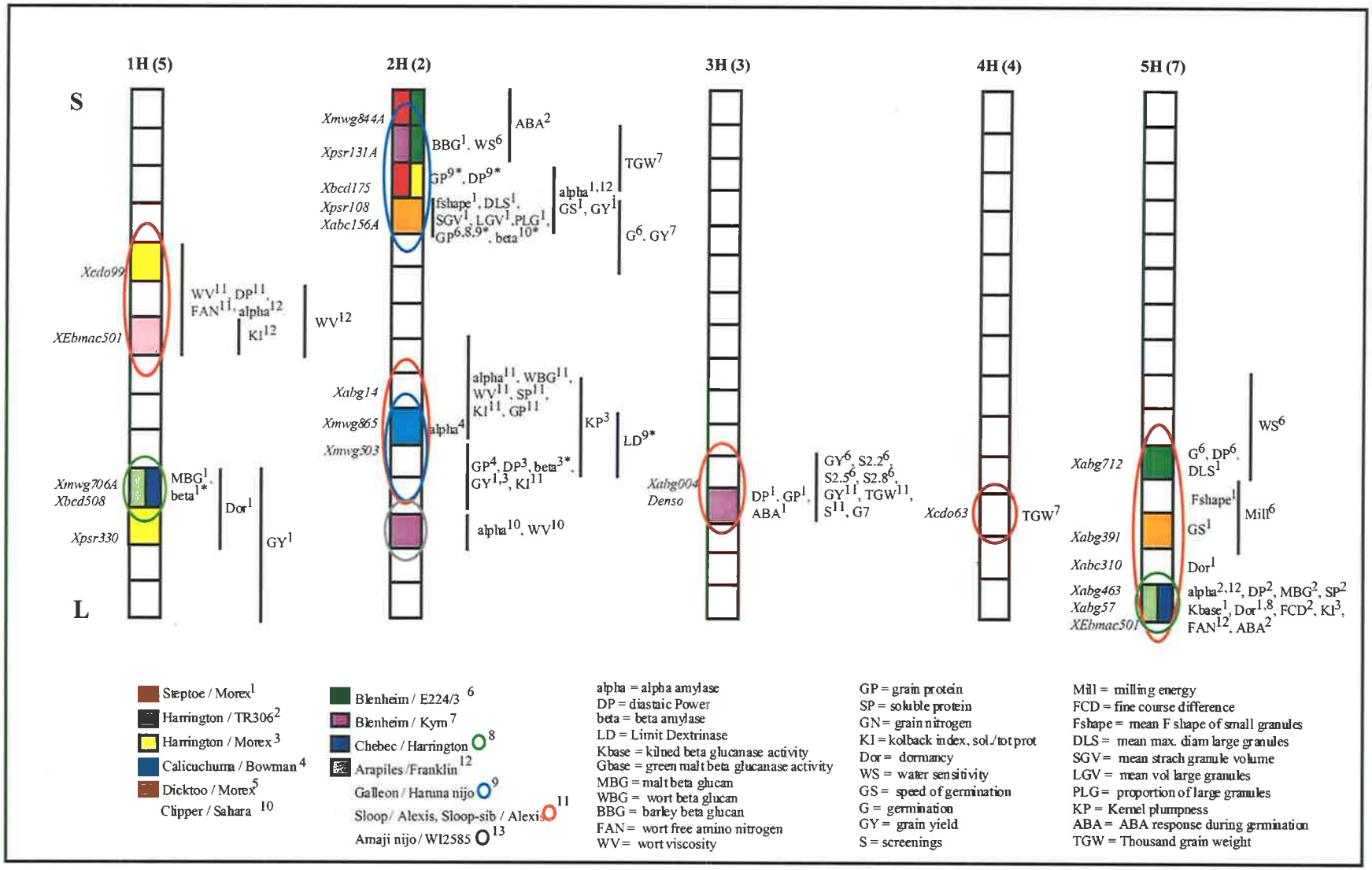
6.4.2: Chromosome 1H, marker locus *Xbcd508*

A region on the long arm of chromosome 1H was found to be associated with HWE in the two populations Chebec/ Harrington and Harrington/ TR306 (Panozzo *et al.*, in preparation, Hayes *et al.*, 1997; Mather *et al.*, 1997). In both populations, Harrington was found to be donating the lower HWE allele. While this region was found to be associated with grain yield, dormancy, malt β-glucan levels and β-amylase activity in the Steptoe/ Morex mapping population (Hayes *et al.,* 1993; Han *et al.,* 1995; Oberthur *et al.,* 1995; Zwickert-Menteur *et al.,* 1996; Ullrich *et al.,* 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001), no other malting quality associations were found in either the Chebec/ Harrington or Harrington/ TR306 populations (Li *et al.,* 1996; Mano and Takeda, 1997; Mather *et al.,* 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001; Barr *et al.*, 2003a; Collins *et al.,* 2003). The underlying cause of the differences in HWE at this locus remains to be discovered.

Figure 6.4.1: A schematic diagram of a number of regions found to be associated with HWE in Australian mapping populations. Each chromosome is separated into a number of regions called Bins as described by Kleinhofs and Han (2002). Coloured squares represent regions found in mapping populations around the world. Circles represent regions investigated in this study. Markers are listed left of each chromosome. Listed right of the chromosome are other traits found to be associated with each region.

References

1. Hayes *et al.*, 1993; Han *et al.*, 1995; Oberthur *et al.*, 1995; Zwickert-Menteur *et al.*, 1996; Han *et al.*, 1997; Larson *et al.*, 1996; Mano and Takeda, 1997; Ullrich *et al.*, 1997; Borem *et al.*, 1999; Zale *et al.*, 2000; Hayes *et al.*, 2001
2. Mano and Takeda, 1997; Mather *et al.*, 1997; Zale *et al.*, 2000; Hayes *et al.*, 2001
3. Marquez-Cedillo *et al.*, 2000; Zale *et al.*, 2000; Hayes *et al.*, 2001
4. Hayes *et al.*, 1996; Hayes *et al.*, 1997; Zale *et al.*, 2000; Hayes *et al.*, 2001
5. Oziel *et al.*, 1996; Zale *et al.*, 2000; Hayes *et al.*, 2001
6. Thomas *et al.*, 1995; Thomas *et al.*, 1996; Powell *et al.*, 1997; Zale *et al.*, 2000; Hayes *et al.*, 2001
7. Bezant *et al.*, 1997a; Bezant *et al.*, 1997b; Zale *et al.*, 2000; Hayes *et al.*, 2001
8. Li *et al.*, 1996; Zale *et al.*, 2000; Barr *et al.*, 2003a; Collins *et al.*, 2003
9. Li *et al.*, 1996; Zale *et al.*, 2000; Karakousis *et al.*, 2003a
10. Zale *et al.*, 2000; Karakousis *et al.*, 2003c
11. Barr *et al.*, 2003b; Panozzo *et al.*, in preparation
12. Moody *et al.*, unpublished; Panozzo *et al.*, in preparation
13. Asamaya, unpublished; Pallotta *et al.*, 2003


1H (5)
2H (2)
3H (3)
4H (4)
5H (7)
S
L
Xcdo99
XEbmac501
Xmwg706A
Xbcd508
Xpsr330
WV11, DP11, FAN11, alpha12
KI12
WV12
MBG1, beta1*
Dor1
GY1
Xmwg844A
Xpsr131A
Xbcd175
Xpsr108
Xabc156A
BBG1, WS6
ABA2
GP9*, DP9*
fshape1, DLS1, SGV1, LGV1, PLG1, GP6,8,9*, beta10*
alpha1,12
GS1, GY1
TGW7
G6, GY7
Xabg14
Xmwg865
Xmwg503
alpha4
alpha11, WBG11, WV11, SP11, KI11, GP11
KP3
LD9*
GP4, DP3, beta3*, GY1,3, KI11
alpha10, WV10
Xabg004
Denso
DP1, GP1, ABA1
GY6, S2.26, S2.56, S2.86, GY11, TGW11, S11, G7
Xcdo63
TGW7
Xabg712
Xabg391
Xabc310
Xabg463
Xabg57
XEbmac501
G6, DP6, DLS1
WS6
Fshape1
GS1
Mill6
Dor1
alpha2,12, DP2, MBG2, SP2
Kbase1, Dor1,8, FCD2, KI3, FAN12, ABA2
Steptoe / Morex1
Harrington / TR3062
Harrington / Morex3
Calicuchuma / Bowman4
Dicktoo / Morex5
Clipper / Sahara 10
Blenheim / E224/3 6
Blenheim / Kym 7
Chebec / Harrington 8
Arapiles /Franklin 12
Galleon / Haruna nijo 9
Sloop/ Alexis, Sloop-sib / Alexis 11
Amaji nijo / WI2585 13
alpha = alpha amylase
DP = diastaic Power
beta = beta amylase
LD = Limit Dextrinase
Kbase = kilned beta glucanase activity
Gbase = green malt beta glucanase activity
MBG = malt beta glucan
WBG = wort beta glucan
BBG = barley beta glucan
FAN = wort free amino nitrogen
WV = wort viscosity
GP = grain protein
SP = soluble protein
GN = grain nitrogen
KI = kolback index, sol./tot prot
Dor = dormancy
WS = water sensitivity
GS = speed of germination
G = germination
GY = grain yield
S = screenings
Mill = milling energy
FCD = fine course difference
Fshape = mean F shape of small granules
DLS = mean max. diam large granules
SGV = mean strach granule volume
LGV = mean vol large granules
PLG = proportion of large granules
KP = Kernel plumpness
ABA = ABA response during germination
TGW = Thousand grain weight

6.4.3: Chromosome 2H, marker locus *Xpsr108*

The region on the short arm of chromosome 2H has been shown to be associated with numerous traits in the mapping population Galleon/ Haruna Nijo (Figure 6.3.1, Table 6.3.2). In general, the lines with higher husk content have lower levels of HWE. As husk content is measured as a percentage of the final grain weight, it stands to reason that the more husk on a seed, the less protein and starch there is available and therefore the lower the level of HWE.

Since the majority of lines had close to 100% germination in the 4ml test after 72 hours, it can be assumed that primary dormancy was broken by the time the analysis was performed. The differences that occurred after 24 hours were an indication of the differences in the speed of germination between the various lines of the population. It is likely that the higher husk content of the Galleon type lines in the population either physically or chemically hindered the water uptake and therefore the speed of germination of these lines. This could result in the incomplete modification of the protein and cell wall material, which would hinder the access of the amylolytic enzymes to the starch granules, resulting in lower HWE. Therefore, the differences observed at this locus are due to the physical and chemical barriers caused by the thickness of the husk in the Galleon type lines. This barrier has the effect of slowing the speed of germination, reducing the level of modification, and hence, the level of HWE.

The high levels of water sensitivity remaining in a number of the lines accentuated the differences in the speed of germination between the lines, thereby causing the LOD scores to be greater for the 8mL test, which gives an indication of water sensitivity, than the 4mL test. This region has also been associated with water sensitivity and the speed of germination in the mapping populations Blenheim/ E224/3 and Steptoe/ Morex (Powell *et al.*, 1997, Mano and Takeda, 1997).

As mentioned previously (section 5.4.1) this region on chromosome 2H can be placed in bins 2 to 4 according to the BinMap developed by Kleinhofs and Han (2002) based on the Steptoe/ Morex mapping population. Numerous other traits have been found to be associated with these bins (Figure 6.4.1), including barley beta glucan

levels, alpha amylase activity and numerous starch traits in the Steptoe/ Morex population (Han *et al.*, 1995, Zwickert-Menteur *et al.*, 1996, Borem *et al.*, 1999, Hayes and Jones, 2000). These bins cover approximately 30cM of the chromosome and it is likely that a number of important genes for malt quality and plant development are located in this region.

In this same region of chromosome 2H, interval analysis detected a significant region for skinnings (Figure 6.3.1, Table 6.3.2). This has far reaching implications for the breeder, as selection for high HWE at this locus would also select for higher levels of skinnings. However, Roumeliotis *et al.* (1999) investigated the complex relationship between HWE, husk content, skinnings and hull adherence on a number of promising experimental lines, all of which had Haruna Nijo as a parent, in the South Australian Barley Improvement Program. They concluded that it was possible to produce lines with high malt extract, low husk content, yet low levels of skinnings.

6.4.4: Chromosome 2H, marker locus *Xabg14, Xmwg508 and Xksud22*

HWE was found to be associated with the long arm of chromosome 2H in four Australian mapping populations, Sloop/ Alexis, Sloop-sib/ Alexis, Galleon/ Haruna Nijo (Chapter 5) and Amagi Nijo/ WI2585 (Pallotta *et al.*, 2003). The region found to be associated with HWE in the Sloop/ Alexis and Sloop-sib/ Alexis populations is coincident with Bins 8 to 11, the minor region found in the Galleon/ Haruna Nijo population with Bins 10 to 11 and the region found in the Amagi Nijo/ WI2585 population can be placed in Bins 12 to 13 (Figure 6.4.1).

A number of other malting quality traits were also found to be associated with HWE in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations, including alpha amylase, wort β-glucan, wort viscosity, soluble protein, kolbach index and grain protein (Figure 6.4.1, Panozzo *et al.*, in preparation). Limit dextrinase activity was also found to be associated with this region in the Chebec/ Harrington population (Li *et al.*, 1996).

6.4.5: Chromosome 3H, marker loci *Xabg004*

The region found to be associated with HWE on chromosome 3H in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations is coincident with the semi-dwarfing genes, *sdw1* (Coventry *et al.*, 2003b). The *sdw1.denso* allele of this gene is responsible for conferring a dwarfism plant type (Hellewell *et al.*, 2000). Numerous plant development traits have been found to be associated with the region close to these genes. The *sdw1.d* gene causes a dwarfism phenotype resulting from a deficiency in the plant growth hormones, the gibberellins, caused by a partial block in the gibberellin biosynthesis pathway (Spielmeyer *et al.*, 2002). This produces late maturing plants with stiff straw and more small grains per spike. The Sloop/ Alexis and Sloop-sib/ Alexis mapping populations are segregating for the *sdw1.denso* gene with the Alexis plant types carrying the *sdw1.denso* allele. This region has been found to be associated with yield, grain weight and screenings in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations (Coventry *et al.*, 2003b), with Sloop and Sloop-sib conferring the favorable alleles for these traits. Sloop and Sloop-sib are also responsible for the higher HWE allele in this region. Increased HWE would be the result of a cascade of favourable plant development traits caused by the presence of the non-dwarfism allele of this gene in the Sloop and Sloop-sib type plants.

6.4.6: Chromosome 4H, marker loci *Xcdo63*

A minor QTL was found to be associated with HWE in the mapping populations Sloop/ Alexis and Sloop-sib/ Alexis (section 5.3.2). This region was only significant when the two populations were combined. No other malting quality traits were found to be located in this region of chromosome 4H (Figure 6.4.1, Zale *et al.*, 2000). Due to this it is unlikely that this region will be of high importance to the barley breeder when selecting for HWE or any other malting quality trait.

6.4.7: Chromosome 5H, marker loci *Xabg57, XGms01, Xabg712* and *Xabc310*

A region on chromosome 5H, bin 15, was found to be associated with HWE in the Chebec/ Harrington mapping population (Collins *et al.*, 2003) and the Harrington/ TR306 mapping population (Hayes *et al.*, 1997; Mather *et al.*, 1997). This region has been found to be associated with many other traits in both of these populations

including diastatic power, alpha amylase, wort free amino nitrogen, wort β-glucan levels, soluble protein levels, fine coarse difference, dormancy, preharvest sprouting and abscisic acid (ABA) response during germination (Figure 6.4.1) (Li *et al.,* 1996; Mano and Takeda, 1997; Mather *et al.,* 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001; Barr *et al.*, 2003a, Li *et al.,* 2003). Harrington typically carries very low levels of dormancy compared to Chebec and this lack of dormancy is responsible for preharvest sprouting under certain environmental conditions. Apart from dormancy, preharvest sprouting and ABA response during germination all of the traits are related to the level of modification achieved during the malting process and are therefore likely to be hormonally driven. It is possible that this region contains a gene that is responsible for the rapid breakdown of ABA. Alternatively, and most probably it contains a gene that is responsible for either the levels or the speed of release of one or more of the gibberellin group of hormones (GA). GA and ABA have opposing influences on germination. ABA induces dormancy, while GA induces germination. The release of GA would not only serve to reduce dormancy and hence increase the chances of preharvest sprouting but also increase the speed of germination and therefore the level of modification and HWE. This opposing relationship would also explain the ABA response during germination QTL found in this region (Mano and Takeda, 1997).

The long arm of chromosome 5H was also found to be associated with HWE in the mapping populations Sloop/ Alexis and Sloop-sib/ Alexis (Chapter 5). In these populations, the region was poorly defined and extended from bin 11 to bin 15. It is likely that a number of genes are present in this region that are influencing HWE in these populations. Located in bin 11 is the vernalization gene *sgh2* (Kleinofs and Han, 2002). Whilst it is unknown whether Alexis, Sloop or Sloop-sib carry this gene this gene or its alternative alleles may be influencing the development of the grain and therefore influencing malt quality. It will not be possible to determine the underlying causes of the differences in HWE associated with this region, in these two populations, until further investigations are carried out to more accurately define these HWE QTL.

6.5 Conclusion

Eight regions of the barley genome, found to influence HWE, have been discussed in this Chapter. By analyzing other traits that have also been found to be associated with these regions it is possible to gain a greater understanding of the underlying physical or biochemical bases of elevated HWE. Differences in the levels of HWE due to four of these regions could be directly attributed to differences in the levels of modification of the grain during germination. In all cases the differences in these modification levels were most likely due to differences in the speed of germination caused by either a physical property of the grain, such as husk content or a hormonally based difference caused by a gene or genes in the region. The differences found due to one of the regions could be attributed to the development of the plant causing a cascade of differences in plant types that influenced the overall level of HWE.

Chapter 7

Validation of Malt Extract Markers

7.1: Introduction

A number of key chromosome regions associated with malt extract (HWE) have been identified in Australian mapping populations (Chapter 5). A number of other chromosome regions have also been identified to be associated with malt extract (Section 2.5.1) (Hayes *et al.*, 1993; Hayes *et al.*, 1996; Thomas *et al.*, 1996; Bezant *et al.*, 1997a; Hayes *et al.*, 1997; Mather *et al.*, 1997; Powell *et al.*, 1997; Ullrich *et al.*, 1997; Marquez-Cedillo *et al.*, 2000). The use of molecular markers linked to these regions by barley breeders utilizing marker-assisted selection (MAS) has the potential to increase the efficiency of selection of new material in breeding programs dramatically. However, before markers can be successfully used for routine screening purposes, the influence of the genes located at the regions associated with the markers on the malting quality of lines in breeding populations needs to be assessed. This is the process known as 'validation' and is the subject of the experiments reported in this chapter.

To date only a few studies in the literature have investigated the effectiveness of QTL introgressed into alternate genetic backgrounds in either wheat or barley (Jefferies *et al.*, 2000, Coventry *et al.*, 2003a). In particular, few studies have investigated the usefulness of molecular markers when selecting for malting quality traits in backgrounds genetically different to the mapping population in which they were found (Coventry *et al.*, 2003a).

After regions have been identified in mapping populations that are associated with the trait of interest, markers from that region should be validated in breeder's populations to ensure their suitability for use as early generation screening tools. The process of validation used in this study involved four major steps:

1. The first of these was the selection and development of a suitable population. The populations were chosen from breeder's populations, with each

population having a high malting quality variety as a parent. In a number of cases the variety that carried the superior allele for the trait of interest in the original mapping population was chosen as one of the parents.

2. The second step involved the screening of the population with a polymorphic marker in the region of interest and dividing the population into two groups, the group carrying the allele from the superior parent and the group carrying alternate alleles.
3. Simultaneously the population was screened for the trait of interest.
4. Finally the trait scores for the two allelic groups were statistically compared.

In this study the influence of QTL associated with superior malt quality traits has been investigated when selected in genetic backgrounds other than the mapping population used to identify these QTL. It aims to assess the usefulness of these regions to barley breeders for marker assisted selection.

7.2: Materials and Methods

7.2.1 Genetic Material and Field experiments

In this study six regions were investigated, having been previously assessed for their relationship to HWE. Details of relevant QTL and associated markers are provided in Table 7.2.1. A schematic of the approximate chromosomal locations of these regions is shown in Figure 7.2.1. Two HWE QTL were identified in the Chebec/ Harrington mapping population on chromosomes 1H and 5H (Collins *et al.*, 2003). Both regions have also been found in the mapping population Harrington/ TR306 (Hayes *et al.*, 1997; Mather *et al.*, 1997). In both populations the region on chromosome 5H had the superior allele donated by the variety Harrington. The region on the long arm of chromosome 1H had the inferior allele donated by Harrington.

Pallotta *et al.* (2003) investigated malting quality traits in the mapping population of Amagi Nijo/ WI2585. A region was found to be associated with HWE on chromosome 2H. Amagi Nijo is the source of the superior allele.

Four regions found to be associated with HWE in the mapping populations of Alexis /Sloop and Alexis /Sloop-sib were discussed in Chapter 5.3.2. Three of these regions had the superior allele coming from the parent Alexis and were therefore chosen for validation purposes. These regions are on chromosomes 1H, 2H and 5H. A further region was shown to be associated with HWE in the mapping population of Galleon/ Haruna Nijo on chromosome 2H (Chapter 5.3.1).

Markers shown in Table 7.2.1 were selected on the basis of their proximity to the center of a significant QTL. DNA extraction, RFLP and microsatellite analysis followed the methods outlined in Section 3.8. The restriction endonucleases used for southern blot analysis of RFLP markers are listed in Table 7.2.3. Initially the parents of each population were screened for marker allele polymorphisms. If a polymorphism could be identified the full population was subsequently screened with that marker.

Details of the six populations chosen for validation purposes, including parents, number of lines, year and site at which they were grown are provided in Table 7.2.2. All six populations were F_3 derived populations. The populations were grown in single replicate experimental plots by the South Australian Barley Improvement Program.

7.2.3 Malt analysis

The samples in each population were malted at 15°C using the malting schedule described in Section 3.2. EBC HWE was measured according to Section 3.3.2

7.2.4 Statistical analysis

The lines of each population were separated into two groups, consisting of those carrying either the high or low malt quality parent marker allele, omitting heterozygotes. Least square means were calculated from an ANOVA using the marker allele class as the single factor. Statistical analysis was performed using JMP (v3.1.6, SAS Institute Inc, 1995).

Table 7.2.1: The markers used in the study to validate regions found to be associated with HWE in Australian mapping populations.

Population from which QTL were identified	Parent donating higher allele	Chromosome	Marker used in validation
Chebec/ Harrington[a]	Chebec	1H	*Xbcd508*
Chebec/ Harrington[a]	Harrington	5H	*Xabg57*, *XGMS01*
Galleon/ Haruna Nijo[b]	Haruna Nijo	2H	*Xpsr108*
Alexis /Sloop, Alexis /Sloop-sib[b]	Alexis	2H	*Xabg14*
Alexis /Sloop, Alexis /Sloop-sib[b]	Alexis	5H	*Xabg712*
Alexis /Sloop, Alexis /Sloop-sib[b]	Alexis	5H	*Xabc310*
Amaji Nijo/ WI2868[c]	Amaji Nijo	2H	*Xksud22*
Alexis /Sloop, Alexis /Sloop-sib[b]	Alexis	1H	*XEbmac501*

[a]Collins *et al.*, 2003, [b]Chapter 5, [c]Pallotta *et al.*, 2003,

Table 7.2.2: The mean HWE (% dry basis) for the populations used to validate QTL.

Population	No of lines	Year	Site	Mean HWE	HWE SD
Barque/Harrington	61	1996	Charlick	78.9	1.4
Barque/Haruna Nijo	45	1996	Charlick	78.3	2.4
Sloop/Harrington//VB9623	51	1998	Charlick	77.6	1.6
Sloop/Harrington//VB9624	43	1998	Charlick	79.3	1.2
Sloop-sib/Alexis//VB9624	45	1998	Charlick	77.6	1.4
DH115/Sloop-sib//Amaji Nijo/Alexis	89	1998	Charlick	77.2	1.4

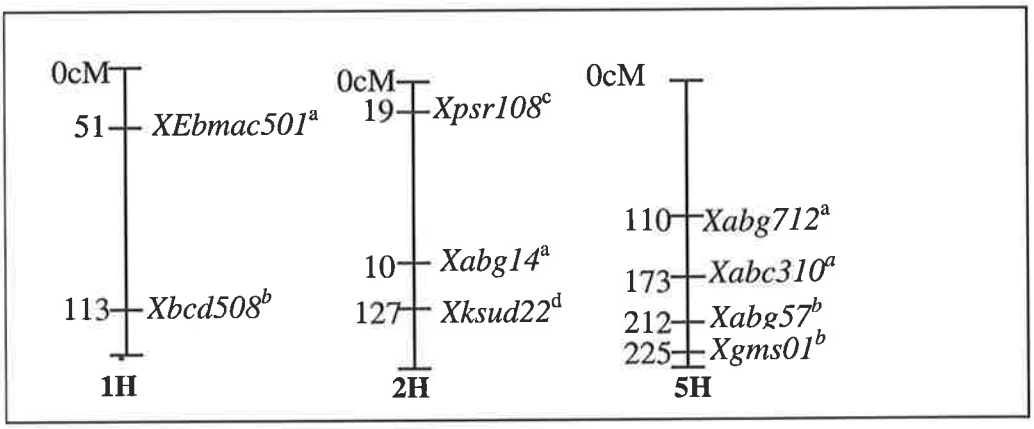


Figure 7.2.1: A schematic diagram of the location of markers found to be associated with a number of malting quality traits in Australian mapping populations. [a]Barr *etal.* 2003b, [b]Barr *et al.,* 2003a, [c]Karakousis *et al.,* 2003a, [d]Pallotta *et al.,* 2003

Table 7.2.3: Restriction endonucleases chosen for southern blot analysis of each population and each RFLP marker, following the method of analysis outlined in Section 3.8.

Population	Marker	Enzyme*
Barque/Harrington	*Xbcd508*	*EcoRV*
	Xabg57	*DraI*
	Xpsr108	*EcoRV*
Barque/Haruna Nijo	*Xabg57*	*DraI*
	Xpsr108	*EcoRV*
Sloop/Harrington//VB9623	*Xbcd508*	*HindIII*
	Xabg57	*HindIII*
	Xpsr108	*HindIII*
Sloop/Harrington//VB9624	*Xbcd508*	*HindIII*
	Xabg57	*HindIII*
	Xpsr108	*HindIII*
Sloop-sib/Alexis//VB9624	*Xabg14*	*BamHI*
	Xabg712	np
	Xabc310	*EcoRI*
DH115/Sloop-sib//Amaji	*Xabg14*	*BamHI*
Nijo/Alexis	*Xabg712*	*XbaI*
	Xabc310	*EcoRI*
	Xksud22	np

*Restriction endonucleases, np: no polymorphism

7.3 Results

Individuals in the six breeding populations were screened with markers associated with various HWE QTL as outlined in Table 7.2.3. No useful polymorphism could be found between the parents in the population Sloop-sib/Alexis//VB9624 when screened with the marker *Xabg712* or the population DH115/Sloop-sib//Amaji Nijo/Alexis when screened with the marker *Xksud22*.

7.3.1: Chromosome 2H, marker locus *Xpsr108*

The marker *Xpsr108* on chromosome 2H was shown previously to be significantly associated with HWE in the Galleon/ Haruna Nijo mapping population (Section 5.3.1). This observation was confirmed by the results presented in Table 7.3.1. The mean HWE for the lines carrying either the Haruna Nijo allele or the Barque allele for the marker locus *Xpsr108* in the population Barque/Haruna Nijo are shown in Table 7.3.1. The lines carrying the Haruna Nijo allele were on average 3% higher in HWE then the lines carrying the Barque allele ($P<0.001$).

To assess whether this QTL confers superior HWE in a source of high HWE other than Haruna Nijo, three populations not involving Haruna Nijo as a parent were assessed (Table 7.3.1). These three populations all contained Harrington as the superior HWE parent. Lines carrying the Harrington allele at this marker locus produced significantly higher HWE (0.8%, $P<0.05$) than lines carrying the Barque allele in the population of Barque/ Harrington, despite the fact that no significant HWE QTL in this region were identified in the Chebec/ Harrington mapping population (Collins *et al.*, 2003). The lines carrying the Harrington allele were not significantly different to the lines carrying the alternate alleles in the other two populations, Sloop/Harrington//VB9623 and Sloop/Harrington//VB9624.

Table 7.3.1: The mean HWE[c] (% dry basis) for the individuals in four populations carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker locus *Xpsr108* (chromosome 2H).

Population	Superior allele[e]			Alternate allele[f]		
	No.[d]	HWE[c]	SD	No.[d]	HWE[c]	SD[d]
Barque/Harrington	29	79.2[a]	1.5	26	78.4[a]	1.4
Barque/Haruna Nijo	14	80.4[b]	1.7	28	77.1[b]	2.1
Sloop/Harrington//VB9623	30	77.5	1.7	21	77.6	1.4
Sloop/Harrington//VB9624	6	79.4	1.6	38	79.2	1.2

[a]significantly different (P<0.05), [b]significantly different (P<0.001), [d]number lines carrying the allele, [e]lines carrying the Harrington or Haruna Nijo allele, [f]lines carrying the alternate allele

7.3.2: Chromosome 5H, marker loci *Xabg57* and *XGms01*

The markers *Xabg57*and *Xgms01* are located within the region of chromosome 5H found to be significantly associated with HWE in the mapping populations of Chebec/ Harrington (Collins *et al.*, 2003) and Harrington/ TR306 (Mather *et al.*, 1997). Marker *Xabg57* is a RFLP marker and *Xgms01* is a SSR marker. These marker loci have been estimated to be 13cM apart (Barr *et al*, 2002) and therefore differences are likely to occur between the lines contained within the allelic groups in the validation populations.

Lines carrying the Harrington allele produced significantly higher HWE than the lines carrying the alternate alleles for *Xabg57* in the populations of Barque/ Harrington (P<0.05) and Sloop/Harrington//VB9623 (P<0.01) (Table 7.3.2). Similarly, the lines carrying the Harrington allele at *XGms01* in the population of Sloop/Harrington//VB9623, produced significantly higher (P<0.01) HWE than the lines carrying other alleles. However, in the third population, Sloop/Harrington//VB9624, the lines carrying the Harrington allele were not significantly different for HWE to the lines carrying the other allele for either marker. Lines in the population Barque/ Haruna Nijo were also screened with the marker *Xabg57* but there was no significant difference in HWE between the two allelic groups.

In both the populations Sloop/Harrington//VB9624 and Sloop/Harrington//VB9623 there was only a single line that carried the Harrington allele at the marker locus *Xgms01* and the alternate allele at the marker locus *Xabg57* or vice versa.

Table 7.3.2: The mean HWE[c] (% dry basis) for the individuals in four populations carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker loci *Xabg57* and *Xgms01* (chromosome 5H).

Population	Marker locus	Superior allele[e]			Alternate allele[f]		
		No.[d]	HWE[c]	SD	No.[d]	HWE[c]	SD
Barque/Harrington	*Xabg57* (5H)	20	79.5[a]	1.5	41	78.7[a]	1.3
Barque/Haruna Nijo	*Xabg57* (5H)	28	77.9	2.5	17	78.8	2.3
Sloop/Harrington//VB9623	*Xabg57* (5H)	14	78.6[b]	1.4	34	77.1[b]	1.5
	Xgms01 (5H)	13	78.8[b]	1.2	28	77.1[b]	1.4
Sloop/Harrington//VB9624	*Xabg57* (5H)	15	79.6	1.4	26	79.1	1.1
	Xgms01 (5H)	16	79.6	1.4	24	79.1	1.2

[a]significantly different (P<0.05), [b]significantly different (P<0.01), [d]number lines carrying the allele, [e]lines carrying the Harrington or Haruna Nijo allele, [f]lines carrying the alternate allele

7.3.3: Chromosome 1H, marker locus *Xbcd508*

Marker *Xbcd508* is located within the region on chromosome 1H found to be associated with HWE in two populations containing Harrington as a parent, Chebec/ Harrington (Collins *et al.*, 2003) and Harrington/ TR306 (Mather *et al.*, 1997). In these two populations, the Harrington allele was associated with lower HWE. No significant difference was identified between the lines carrying the Harrington allele and the lines carrying the other allele in the three breeding populations screened (Table 7.3.3). In the Sloop/Harrington//VB9623 population, there were no lines found to be carrying the Harrington allele. The populations used in this study were non-random routine breeding populations. Therefore it is possible that a QTL for an agronomic trait is co-located within this region causing the plants carrying an undesirable plant type to be culled through early phenotypic selection.

Table 7.3.3: The mean HWE[a] (% dry basis) for the individuals in three populations carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker locus *Xbcd508* (chromosome 1H).

Population	Harrington allele[c]			Alternate allele[d]		
	No.[b]	HWE[a]	SD	No.[b]	HWE[a]	SD
Barque/Harrington	27	79.1	1.7	30	78.7	1.3
Sloop/Harrington//VB9623	51	77.6	1.6	0	-	-
Sloop/Harrington//VB9624	17	79.4	1.1	25	79.1	1.3

[b]number lines carrying the allele, [c]lines carrying the Harrington allele, [d]lines carrying the alternate allele

7.3.4: Interaction effects between the HWE QTL on Chromosome 2H (marker locus *Xpsr108)* and 5H (marker locus *Xabg57*)

The effect of the interaction between QTL on chromosome 5H (*Xabg57*) and 2H (*Xpsr108*) on HWE was also investigated (Table 7.3.4). Lines from the four populations were divided into the four alternate allele groups based on results from screening with the two markers. In the population Barque/Harrington the lines carrying the Harrington allele at both marker loci had significantly ($P<0.01$) higher HWE than all other groups. Lines carrying the Harrington alleles at both marker loci (Table 7.3.4) produced 1.1% higher HWE than those carrying the Harrington allele at *Xabg57* only (Table 7.3.2).

This result was not confirmed in the other two populations containing Harrington as a parent. In the population Sloop/Harrington//VB9623 the difference between the lines carrying the Harrington allele at both marker loci (*Xabg57* and *Xpsr108,* Table 7.3.5) and those carrying the Harrington allele at *Xabg57* only (Table 7.3.2) was just 0.2% in mean HWE. The mean HWE of the lines carrying the Harrington alleles at both marker loci were not significantly higher than the mean HWE of the lines carrying the alternate allele at both marker loci (Table 7.3.5).

The third population Sloop/Harrington//VB9624 only had one individual that had the Harrington allele at both marker loci, *Xabg57* and *Xpsr108* (Table 7.3.5). Despite this

line producing very high HWE (82%), inadequate population size prevented statistical assessment.

In the population Barque/ Haruna Nijo, the lines carrying the Haruna Nijo allele at *Xabg57* and *Xpsr108* produced significantly higher (P<0.05) HWE than the lines carrying the Barque allele at both marker loci (Table 7.3.4). However, there was no difference in HWE between the lines carrying the Haruna Nijo allele at both marker loci and those carrying Haruna Nijo at *Xpsr108* only.

Table 7.3.4: The mean HWE[g] (% dry basis) for lines carrying the superior malting quality parent allele (High) or alternative allele (other) for *Xpsr108* (chromosome 2H) and *Xabg57* (chromosome 5H) in two populations containing Harrington or Haruna Nijo as a parents.

Allele type		Barque/Harrington			Barque/Haruna Nijo		
Xpsr108 (2H)	*Xabg57* (5H)	No.[h]	HWE[g]	SD	No.[h]	HWE[g]	SD
other	other	15	78.3	1.5	12	78.0[bd]	1.9
other	high	10	78.7	1.2	16	76.5[ef]	2.0
high	other	19	78.7	1.2	5	80.7[be]	2.0
high	high	8	80.6[a]	1.3	9	80.1[df]	1.6

[a]significantly different (P<0.01) to all groups for that population, [bd]alleles with letters in common are significantly different (P<0.05) for that population, [ef]alleles with letters in common are significantly different (P<0.001) for that population, [h]number of lines carrying the allele

Table 7.3.5: The mean HWE[b] (% dry basis) for lines carrying the Harrington (Harrington) malting quality or alternative (other) parent allele for *Xpsr108* (chromosome 2H) and *Xabg57* (chromosome 5H) in two populations containing Harrington as a parent.

Allele type		Sloop/Harrington// VB9623			Sloop/Harrington// VB9624		
Xpsr108 (2H)	*Xabg57* (5H)	No.[c]	HWE[b]	SD	No.[c]	HWE[b]	SD
other	other	14	77.1	1.3	13	79.3	0.9
other	Harrington	7	78.3	1.3	15	79.4	1.3
Harrington	other	20	77.0[a]	1.6	4	78.8	1.2
Harrington	Harrington	7	78.8[a]	1.5	1	82.0	-

[a]alleles with letters in common are significantly different (P<0.05) for that population,
[c]number of lines carrying the allele

7.3.5: Chromosome 2H, marker locus *Xabg14*

Marker *Xabg14* located within a region of chromosome 2H, was shown in Section 5.3.2 to be associated with HWE in the mapping populations Alexis /Sloop and Alexis /Sloop-sib. The lines carrying the Alexis allele for marker *Xabg14* in the population Sloop-sib/Alexis//VB9624, produced HWE that was 1% (P<0.05) higher then the lines carrying the alternate allele (Table 7.3.6). However, there was no significant difference in HWE between the lines carrying the Alexis allele for marker *Xabg14* and the lines carrying the alternate allele in the population DH115/Sloop-sib//Amaji Nijo/Alexis.

Table 7.3.6: The mean HWE[c] (% dry basis) for the individuals in two populations carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker locus *Xabg14* (chromosome 2H).

Population	Alexis allele[d]			Alternate allele		
	No.[c]	HWE[b]	SD	No.[c]	HWE[b]	SD
Sloop-sib/Alexis// VB9624	11	78.4[a]	1.1	33	77.4[a]	1.4
DH115/Sloop-sib// Amaji Nijo/Alexis	18	77.6	1.1	64	76.9	1.4

[a]significantly different (P<0.05), [c]number of lines carrying the allele, [d]lines carrying the Alexis allele, [e]lines carrying the alternate allele

7.3.6: Chromosome 5H, marker loci *Xabg712* and *Xabc310*

In section 5.3.2, three regions on chromosome 5H that were found to be associated with HWE in the mapping populations Alexis /Sloop and Alexis /Sloop-sib, were discussed. Two marker loci, *Xabg712* and *Xabc310* from these regions were selected for validation purposes.

The lines carrying the Alexis *Xabc310* allele in the population DH115/Sloop-sib//Amaji Nijo/Alexis produced 1% higher mean HWE ($P<0.05$) than the lines carrying the alternate allele (Table 7.3.7). In the population Sloop-sib/Alexis//VB9624, however, there was no significant difference between the two allele classes for this marker. In this population there was no polymorphism between the parents at the marker locus *Xabg712*. Also in the population DH115/Sloop-sib//Amaji Nijo/Alexis, no significant difference in HWE was found between the lines carrying the Alexis *Xabg712* allele and the lines carrying the alternate allele (Table 7.3.7).

The interaction effects between both marker loci were also investigated (Table 7.3.8). The mean HWE of the lines carrying the Alexis allele at both marker loci was significantly higher ($P<0.05$) than the mean HWE of the lines carrying the alternate allele at both marker loci. However, the mean HWE for the lines carrying the Alexis allele at both marker loci was the same as the mean of the lines carrying the Alexis allele at *Xabc310* only.

Table 7.3.7: The mean HWE[c] (% dry basis) for the individuals in two populations carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker loci *Xabg712* and *Xabc310* (chromosome 5H).

Population	Marker locus	Alexis allele[e]			Alternate allele[f]		
		No.[c]	HWE[b]	SD	No.[c]	HWE[b]	SD
Sloop-sib/Alexis// VB9624	*Xabg712* (5H)	[d]np					
	Xabc310 (5H)	9	78.3	1.5	36	77.4	1.3
DH115/Sloop-sib// Amaji Nijo/Alexis	*Xabg712* (5H)	37	77.3	1.3	48	77.0	1.4
	Xabc310 (5H)	14	77.9[a]	1.1	57	76.9[a]	1.5

[a]significantly different (P<0.05), [c]number lines carrying the allele, [d]no polymorphism, [e]lines carrying the Alexis allele, [f]lines carrying the alternate allele

Table 7.3.8: The mean HWE[a] (% dry basis) for lines carrying the Alexis (Alexis) malting quality or alternative (other) parent allele for *Xabc310* and *Xabg712* (chromosome 5H) in the DH115/Sloop-sib//Amaji Nijo/Alexis population.

Xabc310	*Xabg712*	No.[b]	HWE[a]	SD
Other	Other	35	76.7[a]	1.5
Other	Alexis	22	77.2	1.4
Alexis	Other	4	78.0	0.4
Alexis	Alexis	10	77.9[a]	1.3

[a]significantly different (P<0.05), [b]number lines carrying the allele

7.3.7: Interaction effects between the HWE QTL on Chromosome 2H (marker locus *Xabg14)* and 5H (marker locud *Xabc310*)

The interaction effects between the two QTL with the marker loci, *Xabg14* and *Xabc310* were also assessed in the two populations (Table 7.3.9). The population Sloop-sib/Alexis//VB9624 contained no lines that carried the Alexis allele for both markers. In the population DH115/Sloop-sib//Amaji Nijo/Alexis, there was no significant difference between allele classes. This was possibly due to the small number of lines carrying the Alexis allele at both marker loci.

Table 7.3.9: The mean HWE[c] (% dry basis) for lines carrying the Alexis (Alexis) malting quality or alternative (other) parent allele for *Xabc310* (chromosome 5H) and *Xabg14* (chromosome 2H) in two populations containing Alexis.

allele type		DH115/Sloop-sib//Amaji Nijo/Alexis			Sloop-sib/Alexis//VB9624		
Xabg14	*Xabc310*	No.[b]	HWE[c]	SD	No.[b]	HWE[c]	SD
Other	Other	47	76.7	1.5	24	77.1[a]	1.3
Other	Alexis	10	77.8	1.3	9	78.3	1.5
Alexis	Other	9	77.2	1.2	11	78.3	1.1
Alexis	Alexis	4	78.1	0.5	0	-	-

[a]significantly different (P<0.05) to the other groups, [b]number lines carrying the allele

7.3.8: Chromosome 1H, marker locus XEbmac501

In the mapping populations Alexis /Sloop and Alexis /Sloop-sib, a region of chromosome 1H was shown to be associated with HWE (Section 5.3.2). The marker chosen for validation purposes in this region was *XEbmac501* (Table 7.3.10). In the population DH115/Sloop-sib//Amaji Nijo/Alexis the mean HWE for the different parental allele classes, Alexis and the alternate allele, were not significantly different (Table 7.3.10). No polymorphism could be found between the parents in the population Sloop-sib/Alexis//VB9624 for this marker.

Table 7.3.10: The mean HWE[a] (% dry basis) for the individuals carrying the superior malt quality parent marker allele and those carrying an alternative marker allele at the marker locus *XEbmac501* (chromosome 1H) in the population DH115/Sloop-sib// Amaji Nijo/Alexis.

	Allele	
	Alexis	Alternate
HWE[a]	77.5	77.0
No.[b]	14	71
SD	1.7	1.3

[b]number lines carrying the allele

7.4 Discussion

7.4.1 The response to selection for alleles from superior malt quality parents in validation populations

A summary of the marker loci, examined is shown in Table 7.4.1. To assess the impact of each locus an indication of the response to selection for the allele donated from the high malt quality parent is shown. Overall there were ten cases where the allele from the high quality parent produced a significant positive effect while there were no instances where a significant negative effect was observed. It would appear that the risks, in terms of malt quality, of selecting for any of the regions assessed would be low and there is most likely to be a overall positive effect on malt extract. A practical strategy for the implementation of MAS for improved malt quality therefore, would be to genotype progeny at all of the key loci and select for the allele from the high quality parent in all cases. By removing the lines carrying the allele from the poor malt quality parents, the overall population mean for the traits examined would probably increase and at worst remain the same. In particular the marker loci *Xpsr108* and *Xabg57* have been shown to be quite robust. When crossing Harrington, Alexis and Haruna Nijo with Australian germplasm, such as Chebec, Sloop, Galleon, Barque and Skiff derived lines, strong responses to selection could be expected for these loci. To more fully test the effectiveness of MAS, a strategy to combine the promising alleles from Haruna Nijo, Harrington and Alexis into a single alternate germplasm using markers to select the loci discussed could be designed and implemented.

One of the challenges for a plant breeder is to efficiently select the elite recombinants from among the very large populations required to achieve the complex objectives set for the breeding program. Families should be selected for heritable traits as soon as possible to minimize the number of lines to be tested in expensive multi-location yield trials. Therefore any techniques available to the plant breeder for early generation trait screening are of huge importance. One of the aims of MAS is to increase the frequency of desirable alleles in the breeding population for the particular trait under assessment by removing any lines that are likely to be particularly poor in that trait. The use of these markers has the potential to remove lines from the breeding population that have particularly poor levels of HWE.

Table 7.4.1: The response in levels of HWE to selection from choosing the superior allele from three high malting quality donors when tested in six different validation populations.

Chrom	marker locus	HWE		
		Harrington	Haruna Nijo	Alexis
1H	*XEbmac501*			(+)
	Xbcd508	(+),(+)		
2H	*Xpsr108*	+, =, =	+++	
	Xabg14			+, (+)
5H	*Xabg712*			(+)
	Xabc310			(+), +
	Xabg57	+,++, (+)	(-)	
	Xgms01	++,(+)		

+++ significant P<0.001, ++ significant P<0.01, + significant P<0.05, (+) not significant but positive, = not significant, (-) not significant but negative

7.4.2 Effectiveness of the validation strategy

One of the greatest risks involved in the strategy suggested above would be the removal of lines carrying genes from alternate high quality parents in the cross under investigation. For example, in the population Sloop/Harrington//VB9623, Harrington would be considered to be the high malt quality parent for HWE. However, VB9623 was originally selected from the cross between Franklin and Chebec. In Section 4.3.1, Franklin was shown to produce high levels of HWE. As Franklin is likely to have a number of different regions of the genome controlling high HWE, selecting only the lines carrying regions associated with HWE in Harrington is likely to exclude lines that are carrying the Franklin alleles at other unknown locations on the genome.

A likely hindrance to the successful implementation of MAS into breeding programs is the lack of marker allele polymorphisms found within a cross. In this study, there were two regions that could not be validated due to a lack of marker allele polymorphisms. To ultimately overcome this problem, the exact location and sequence of the actual genes involved in the control of these traits will need to be known. This would allow the use of diagnostic markers for desirable alleles such as single nucleotide polymorphisms (SNP's) to be used. However, such information will

come at considerable cost and in the absence of this investment, greater saturation of key regions with RFLP's and SSR's is likely to be a more practical strategy.

7.4.3: Chromosome 2H, marker locus *Xpsr108*

The marker allele from the high HWE parent (Harrington and Haruna Nijo) at the marker locus *Xpsr108* was found to be associated with significantly higher HWE in two breeding populations including the population Barque/ Harrington. In contrast to this Morex was the donor of the marker allele associated with superior HWE in Harrington/ Morex (Marquez-Cedillo *et al.*, 2000). Additionally, this region of chromosome 2H was found to be associated with HWE derived from at least three different parental sources, namely Blenheim, Morex and Haruna Nijo (Section 5.4.1.1). This indicates that this region of chromosome 2H contains a major region associated with HWE derived from at least three different parental sources namely, Blenheim, Morex and Haruna Nijo with the possibility that there are at least three different alleles in this region, namely Morex, Harrington and Barque with different levels of expression of HWE. This highlights the problem in some crosses of deciding which of the parents is the high malt quality donor. In the case of Harrington/ Morex, both parents would be considered to be high malt quality.

7.5 Conclusion

Markers from four of regions of the barley genome found to be associated with HWE in Chapter 5 and two regions found to be associated with HWE in the Chebec/ Harrington mapping population (Collins *et al.*, 2003) were investigated using a number of breeding populations with a high HWE variety as a parent. The allele from the high HWE parent was found to be associated with a significant increase in HWE in four regions, two regions on chromosome 2H and two regions on chromosome 5H. The use of markers from these regions for MAS would increase the overall mean HWE of the breeding population and is therefore a valuable tool to barley breeders.

Chapter 8

General Discussion

In this thesis, the biochemical and genetic factors influencing high levels of HWE were investigated. The research presented here has contributed to a greater understanding of the barley, malt, wort and spentgrain parameters that are influencing HWE. A number of regions of the barley genome have been shown to be suitable to be used in MAS by barley breeders to select for new high HWE varieties. The influence of these regions on HWE has also been examined.

8.1 The relationship between the biochemical and genetic bases of HWE

Malt extract was shown to be influenced by 13 different parameters involving either an individual trait or a group of traits from barley, malt, wort and spent grain (Chapter 4). These included grain size, grain hydration during malting, barley husk content, malt β-glucan, limit dextrinase activity, activity of other starch degrading enzymes, size of the large starch granules and RVA peak viscosity, protein modification (in the wort), cell wall modification (in the wort), wort monosaccharide levels, the percent of the original malt remaining in the spent grain and the components of the spent grain. In Chapter 6, six regions of the barley genome that influence HWE under Australian conditions were investigated and a number of the related traits identified in Chapter 4 were also shown to be associated with these regions. A number of these traits have been studied in barley mapping populations and regions of the barley genome have been identified that are associated with them (Hayes *et al.,* 1993; Han *et al.,* 1995; Oberthur *et al.,* 1995; Thomas *et al.*, 1995; Hayes *et al.,* 1996; Li *et al.,* 1996; Oziel *et al.*, 1996; Zwickert-Menteur *et al.,* 1996; Bezant *et al.*, 1997a; Han *et al.,* 1997; Hayes *et al.,* 1997; Larson *et al.,* 1997; Mano and Takeda, 1997; Mather *et al.,* 1997; Powell *et al.*, 1997; Ullrich *et al.,* 1997; Borem *et al.,* 1999; Marquez-Cedillo *et al.*, 2000; Zale *et al.,* 2000; Hayes *et al.,* 2001; Barr *et al.*, 2003a; Barr *et al.*, 2003b; Karakousis *et al.*, 2003a; Pallotta *et al.,* 2003; Asamaya, unpublished; Panozzo *et al.,* in preparation). HWE and the 13 parameters are likely to be linked genetically by

either the pleiotropic effects of a single gene or the effects of gene 'clusters' where individual genes controlling each trait are closely linked and therefore identified through QTL mapping as a single region. An example of this was discussed in Chapter 7, where both barley husk content and HWE were found to be associated with a region on the short arm of chromosome 2H. It was found that husk content influenced HWE both directly, by diluting the amount of starch in the grain, and indirectly, by providing a physical or chemical barrier to water uptake, thereby lowering modification and hence HWE. The following discussion investigates the linkage between HWE and the 13 parameters.

Grain weight, in both barley and malt, was shown to negatively influence HWE. The selection of varieties used in Chapter 4 would have intensified this relationship. The European, Canadian and Japanese high HWE varieties were poorly adapted to the Southern Australian growing conditions, which would have resulted in small grain size. However, in general, low extract feed varieties tend to have a larger grain size than higher extract malting varieties. Large grain size is an important industry target for new barley varieties and there is a need therefore, to breed varieties with both high HWE and large grain size. Three regions of the barley genome have favorable alleles for the expression of both HWE and barley 1000 grain weight (the most commonly used assessment method for grain size). Two regions were found to be associated with HWE and barley 1000 grain weight on chromosome 2H (bins 2-3 and 12-14) in the mapping population Blenheim/ Kym (Bezant *et al.,* 1997a; Bezant *et al.,* 1997b; Hayes *et al.,* 2001). One of these had Kym donating the favorable alleles for both traits and the other had Blenheim. Additionally a region on the long arm of chromosome 3H was found to be associated with the Sloop or Sloop-sib allele for both traits in the mapping populations Sloop/Alexis and Sloop-sib/Alexis (Coventry *et al.,* 2003a; Chapter 6). The exploitation of these regions by breeders may lead to the production of barley varieties with a combination of high HWE and larger grain size.

The level of cell wall modification in both the malt, as indicated by the malt β-glucan content, and in the wort, as indicated by the wort β-glucan content and wort viscosity, were found to be negatively associated with HWE. Whilst malt β-glucan has not been

measured in the populations used in this project, it has been measured in the Steptoe/ Morex population (Han *et al.,* 1995; Han *et al.,* 1997; Ullrich *et al.,* 1997; Hayes *et al.,* 2001). Three regions of the barley genome, donated from Morex, were found to be associated with increased levels of HWE and decreased levels of malt β-glucan on chromosomes 7H and 4H (Han *et al.,* 1995; Ullrich *et al.,* 1997). Additionally, the regions of the genome found to be associated with HWE on chromosomes 1H and 2H in the Sloop/ Alexis and Sloop-sib/ Alexis populations (Chapter 5) were found to be associated with traits relating to the level of modification of the cell wall material, wort β-glucan and wort viscosity. The manipulation of genes to increase the level of degradation of the cell walls is a viable method of improving HWE. Due to the problems associated with high levels of cell wall material in the wort during the lautering and filtration processes, increased modification of the cell walls is an important breeding target. Regions of the genome that influence the modification and degradation of the cell walls are an important source of genes for overall malting quality.

The diameter of the large starch granules was shown to be positively associated with HWE (Chapter 4). QTL influencing the diameter of the large starch granules were found in the Steptoe/ Morex population on chromosomes 2H and 5H (Borem *et al.,* 1999; Hayes *et al.,* 2001). Whilst no QTL for HWE was found in these regions in the Steptoe/ Morex population, QTL for HWE were found on chromosome 2H in the Dicktoo/ Morex population (Oziel *et al.*, 1996; Hayes *et al.,* 2001) and the Galleon/ Haruna Nijo population (Chapter 5) and on 5H in the Blenheim/ E224/3 population (Thomas *et al.*, 1995; Powell *et al.*, 1997; Zale *et al.,* 2000; Hayes *et al.,* 2001) and the Sloop/ Alexis and Sloop-sib/ Alexis populations (Chapter 5). The large starch granules make up approximately 90% of the total volume of the starch in the barley and therefore contribute the greatest proportion of carbohydrates to the wort. Large starch granules are hydrolysed preferentially to small starch granules during mashing (Bathgate and Palmer, 1973) and have a lower gelatinisation temperature (MacGregor, 1980; MacGregor and Balance, 1980a), making them more available to enzyme degradation during mashing. Whilst the measurement of large starch granule diameter is both difficult and time consuming, further investigation of this trait in other populations may lead to useful information for the improvement of malt quality in general.

In Chapter 4 the activities of the starch degrading enzymes were found to be associated with HWE. In particular the limit dextrinase activity was found to be strongly associated with HWE. Limit dextrinase has been investigated in only a limited number of mapping populations. Whilst the gene encoding limit dextrinase, *LD*, is on chromosome 7H (Li *et al.,* 1999; Hayes and Jones, 2000), a number of QTL for limit dextrinase activity have been found elsewhere on the barley genome (Li *et al.,* 1996). One of these regions is associated with the marker *Xmwg503(b)* on chromosome 2H (Li *et al,* 1996) in the mapping population Galleon/ Haruna Nijo. This region was also putatively found to be associated with HWE in the Galleon/ Haruna Nijo population (Chapter 5). The attention given to the more abundant starch degrading enzymes, alpha and beta amylase, has often overshadowed the importance of limit dextrinase to overall malting quality. This study has shown that selecting new varieties with increased levels of limit dextrinase should lead to an increase in HWE. To assist barley breeders with selection strategies for high limit dextrinase, further mapping studies to locate regions conferring high limit dextrinase will be important.

Kolbach index (KI), soluble protein and free amino nitrogen (FAN), all give an indication of the level of the modification and degradation of proteins during the malting and mashing processes and were found to be positively associated with HWE. In the Sloop/ Alexis and Sloop-sib/ Alexis populations, two regions of the genome were found to be associated with HWE and FAN, and one region was found to be associated with HWE and KI (Barr *et al.,* 2003b; Panozzo *et al.,* in preparation). All regions had Alexis donating the higher allele. The increase in the degradation of the proteins during malting and mashing leads to higher levels of HWE by increasing the accessibility of the starch granules to starch degrading enzymes. However, a large amount of soluble protein in the wort is not necessarily advantageous as it can lead to haze problems in the final beer, reducing the shelf life of the product. Most breweries have strict limits on the level of soluble protein remaining in the wort. The release of new malting varieties with improved levels of HWE combined with high levels of soluble protein may not acceptable to the end user.

To this author's knowledge, RVA peak viscosity, grain hydration and wort monosaccharides have not been mapped. Likewise, the properties of the spent grain

have not been mapped. Few studies have investigated either the quantity or the components of the spent grain. Generally when spent grain has been investigated it has been in relation to the influence of the spent grain on the lautering process (Kano and Karakawa, 1979). In depth analysis of the starch and cell wall material in the spent grain may lead to a better understanding of the reasons these materials remain in the spent grain and hence, methods of improving their release earlier in the process.

8.2 The use of MAS for improvement of HWE

Four regions of the barley genome were found to significantly influence HWE in breeders' populations (Chapter 7). These include two regions on chromosome 2H and two regions on chromosome 5H. All of these regions have been shown to be "hot spots", influencing numerous malt quality traits (Figure 6.4.1). To fully assess the impact of using these regions for MAS, knowledge of other traits that are likely to be controlled by genes located in these regions and how these traits influence HWE will be important.

The region on the short arm of chromosome 2H found to be associated with HWE was also found to be associated with husk content, speed of germination (Chapter 6), starch granule properties (Borem *et al.,* 1999), grain weight (Bezant *et al.,* 1997b) and the level of starch degrading enzymes (Li et al., 1996; Zwickert-Menteur et al. 1996; Zale et al., 2000; Panozzo et al., unpublished). These traits all correlated with HWE (Chapter 4). Hence, by selecting for the high HWE allele, it is likely that the breeder will be selecting a variety with a relatively thin husk, large starch granules, high levels of starch degrading enzymes, low grain weight and rapid germination. Additionally, the variety is likely to produce high levels of skinned grains due to the loosely adhering husk.

The region associated with HWE on the long arm of chromosome 2H in the Sloop/ Alexis and Sloop-sib/ Alexis mapping populations was also found to be associated with a number of traits relating to the modification of both proteins and cell wall material (Figure 6.4.1), and also the levels of a number of starch degrading enzymes. While this region will be a useful tool in selecting for HWE, care will need to be

taken to ensure the varieties that are produced do not over modify proteins and therefore cause problems during the brewing process.

The region on the long arm of chromosome 5H (marker locus *Xabg57*) was found to be associated with HWE in the Chebec/ Harrington mapping population (Collins *et al.*, 2003) and the Harrington/ TR306 mapping population (Hayes *et al.*, 1997; Mather *et al.*, 1997). This region has also been found in both of these populations to be associated with many other traits, linked to the speed of germination and the modification of the grain, including DP, α-amylase, wort FAN, wort β-glucan, soluble protein and fine/coarse difference (Figure 6.4.1; Li *et al.*, 1996; Mano and Takeda, 1997; Mather *et al.*, 1997; Zale *et al.*, 2000; Hayes *et al.*, 2001; Barr *et al.*, 2003a). It has also been associated with low levels of dormancy and preharvest sprouting (Figure 6.4.1; Oberthur *et al.*, 1995; Li *et al.*, 2003). Preharvest sprouting is an undesirable trait to the malting industry. In moderate cases it damages the starch in the grain, while in extreme cases it renders the seed unviable. Selection for high HWE alleles here will only be appropriate for varieties that will be grown in areas that experience both, very little rain and low humidity during harvest. The challenge for breeders is to find other loci which modify the effects of the low levels of dormancy caused by the high HWE allele at this locus, allowing a short period of dormancy to allow harvest to be safely negotiated.

Distal to this region on the long arm of chromosome 5H is another region found to be associated with HWE in the mapping populations Sloop/ Alexis and Sloop-sib/ Alexis. Two marker loci were validated within this region (*Xabg712* and *Xabc310*). Whilst other traits such as DP, the speed of germination and mean diameter of the large starch granules were also found in this region in different mapping populations, (Thomas *et al.*, 1996; Mano and Takeda, 1997; Powell *et al.*, 1997; Borem *et al.*, 1999; Hayes *et al.*, 2001), the region was poorly defined. Before it can be assessed for its potential for MAS, fine mapping is required to more thoroughly understand the nature of the QTL in this region.

MAS has the potential to be a very useful tool. When used in conjunction with other early screening methods, it can dramatically decrease the time taken to produce a new

variety of barley. By not only mapping, but also validating and assessing the influence of regions of the barley genome on HWE, this study has successfully shown that MAS will be a valuable aid for the selection of high HWE varieties. Breeders can not only use these regions to select for high HWE but can also understand how these regions will influence the grain quality as a whole. This will enable them to release varieties tailored to the needs of the target environments and markets.

Bibliography

ABB (1998). Australian Barley Board Classification Manual. **Version 2.0**.

Alexander, A., Buitendag, C. and Meijering, I. (1997). Single Kernel Analyses of Malt and Barley Quality - A Potential Tool for Screening. 8th Australian Barley Technical Symposium, Gold Coast, Queensland, Australia.

Allan, G. R., Chrevatidis, A., Sherkat, F. and Stuart, I. M. (1995). The Relationship Between Barley Starch and Malt Extract For Australian Barley Varieties. Institute of Brewing Conference, Central and South African Section, Victoria Falls.

Allison, M. J. (1986). "Relationships Between Milling Energy and Hot Water Extract Values of Malts From Some Modern Barley's and Their Parental Cultivars." Journal of the Institute of Brewing **92**: 604-605.

Allison, M. J., Cowe, I. A., Borzucki, R., Bruce, F. and McHale (1979). "Milling Energy of Barley." Journal of the Institute of Brewing **85**: 262-264.

Anderson, F. B. (1966). "Further Studies on the Foam-Stabilizing Substances in Beer." Journal of the Institute of Brewing **72**: 384-387.

Anderson, J. A., Sallans, H. R. and Meredith, W. O. S. (1940). "Varietal Differences in Barleys and Malts XII. Summary of Correlations Between 18 Major Barley, Malt, and Malting Properties." Canadian Journal of Research **19**(C): 278-291.

Arends, A. M., Fox, G. P., Henry, R. J., Marschke, R. J. and Symons, M. H. (1995). "Genetic and Environmental Variation in the Diastatic Power of Australian Barley." Journal of Cereal Science **21**: 63-70.

ASBC Methods of Analysis(1987). American Society of Brewing Chemists **8th edition**.

Atwell, W. A., Hood, L. F., Lineback, D. R., Varriano-Marston, E. and Zobel, H. F. (1988). "The Terminology And Methodology Associated With Basic Starch Phenomena." Cereal Foods World **33**(3): 306, 308, 310-311.

Ballance, G. M. and Meredith, W. O. S. (1976). "Purification and Partial Characterization of an Endo-β1,3-Glucanase from Green Malt." Journal of the Institute of Brewing **82**: 64-67.

Bamforth, C. W. (1985). "Cambridge Prize Lecture, Biochemical Approaches to Beer Quality." Journal of the Institute of Brewing **91**: 154-160.

Bamforth, C. W. and Barclay, A. H. P. (1993). Chapter 7: Malting Technology and the Uses of Malt. Barley: Chenistry and Technology. A. M. a. R. Bhatty. St Paul, MN, American Association of Cereal Chemists, Inc,.

Barber, M. G., Jackson, E. A. and Smith, D. B. (1994). "Total and Individual Barley (1-3), (1-4)-b-D-Glucanase Activities in Some Green and Kilned Malts." Journal of the Institute of Brewing **100**: 91-97.

Barley Genetics Newsletter (1996). "Recommendations for the Designation of the Barley Chromosomes and Their Arms." Barley Genetics Newsletter **27**(July).

Barr, A. R., Chalmers, K. S., Karakousis, A., Kretschmer, J. M., Manning, S., Lance, R. C. M., Lewis, J., Jeffries, S. P. and Langridge, P. (1997). "RFLP Mapping of a New Cereal Cyst Nematode Resistance Locus in Barley." Plant Breeding **117**: 185-187.

Barr, A. R., Jefferies, S. P., Broughton, S., Chalmers, K. J., Kretschmer, J. M., Collins, H. M., Roumeliotis, S., Logue, S. J., Coventry, S. J., Moody, D., Read, B., Poulsen, D., Lance, R., Platz, G., Park, R., Panozzo, J., Karakousis, A., Lim, P., Verbyla, A. and Eckerman, P. (2003b). "Mapping and QTL analysis of the barley population Alexis x Sloop." Australian Journal of Agricultural Research **in press**.

Barr, A. R., Karakousis, A., Lance, R. C. M., Logue, S. J., Manning, S., Chalmers, K., Kretschmer, J. M., Collins, H. M., Roumeliotis, S., Coventry, S., Moody, D. B., Read, B. J., Poulsen, D., Li, C. D., Platz, G. J., Inkerman, A., Panozzo, J. F., Cullis, B. R., Smith, A. B., Lim, P., Langridge, P. and Boyd, W. J. R. (2003a). "Mapping and QTL analysis of the barley population Chebec x Harrington." Australian Journal of Agricultural Research **in press**.

Barrett, J. and Kirsop, B. H. (1971). "The Relative Contributions to Wort Nitrogen of Nitrogenous Substances Solubilized During Malting and Mashing." Journal of the Institute of Brewing **77**: 39-42.

Bathgate, G. N., Clapperton, J. F. and Palmer, G. H. (1973). The Significance of Small Starch Granules. 14th Congress of the European Brewery Convention.

Bathgate, G. N. and Palmer, G. H. (1972). "A Reassessment of the Chemical Structure of Barley and Wheat Starch Granules." Starch-Starke **24**: 336-341.

Bathgate, G. N. and Palmer, G. H. (1973). "The In Vivo and IN Vitro Degradation of Barley and Malt Starch Granules." Journal of the Institute of Brewing **79**: 402-406.

Bertoft, E. and Kulp, S. E. (1986). "A Gel Filtration Study on the Action of Barley a-Amylase Isoenzymes on Granular Starch." Journal of the Institute of Brewing **92**: 69-72.

Bezant, J. H., Laurie, D. A., Pratchett, N., Chojecki, J. and Kearsey, M. J. (1997a). "Mapping of QTL Controlling NIR Predicted Hot Water Extract and Grain Nitrogen Content in a Spring Barley Cross Using Marker-Regression." Plant Breeding **116**(2): 141-145.

Bezant, J. H., Laurie, D. A., Pratchett, N., Chojecki, J. and Kearsey, M. J. (1997b). "Mapping QTL controlling yield and yield components in a spring barley (*Hordeum vulgare* L.) cross using marker regression." Molecular Breeding **3**: 29-38.

Borem, A., Mather, D. E., Rasmusson, D. C., Fulcher, R. G. and Hayes, P. M. (1999). "Mapping quantitative trait loci for starch granule traits in barley." Journal of Cereal Science **29**(2): 153-160.

Brandt, A. (1976). "Endosperm Protein Formation During Kernel Development of Wild Type and High-Lysine Barley Mutants." Cereal Chemistry **53**: 890-891.

Briggs, D. (1964). "Origin and Distribution of a-Amylase in Malt." Journal of the Institute of Brewing **70**: 14-24.

Briggs, D., Hough, J., Stevens, R. and Young, T. (1981). Chapter 2, 9. Malting and Brewing Science. London, Chapman and Hall. **Volume 1, Malt and Sweet Wort**.

Brunswick, P., Manners, D. J. and Stark, J. R. (1987). "The Development of ß-D-Glucanases During the Germination of Barley and the Effect of Kilning on Individual Isoenzymes." Journal of the Institute of Brewing **93**: 181-186.

Buckee, G., Hickman, E. and Brown, D. (1976). "Evaluation of Mills for Malt Analysis." Journal of the Institute of Brewing **82**: 299-303.

Collins, H. M., Panozzo, J., Logue, S. J., Jefferies, S. P. and A.R., Barr. (2003). "Mapping and Validation of Chromosome Regions Associated with High Malt Extract in barley (Hordeum *vulgare* L.)." Australian Journal of Agricultural Research **in press**.

Coventry, S. J., Collins, H. M., Barr, A. R., Jefferies, S. P., Chalmers, K. J., Logue, S. J. and Langridge, P. (2003a). "Use of putative QTL and Structural genes in marker assisted selection for Diastatic Power in malting Barley (*Hordeum vulgare* L.)." Australian Journal of Agricultural Research **in press**.

Coventry, S. J., McDonald, G. K. M., Barr, A. R. and Eglington, J. K. (2003b). "Genome locations of loci influencing grain weight and size in Australian and world mapping populations of barley (*Hordeum vulgare* L.)." Australian Journal of Agricultural Research **in press**.

Crop Harvest Report (1997/1998). Primary Industries and Resoursces SA**:** 7-8.

Devore, J. and Peck, R. (1996). Statistics: the exploration and analysis of data. Belmont, Wadsworth Publishing Company**:** 111.

Dunn, C. A., Bonnici, M. J., Logue, S. J., Long, N. R., Allan, G. R. and Stuart, I. M. (1996). An Assessment of the Physical and Chemical Properties of Barley Starch to Produce Malt Quality. 24th Institute of Brewing Conference, Asia Pacific Section.

Dunn, C. A., Trinh, M. L. T., Bonnici, M. J. and Stuart, I. M. (1997). The Effect of Viscometric and Gelatinisation Properties of Barley on Malt Quality. 47th Cereal Chemistry Conferece, Perth, Australia.

Edmunds, M. D., Kenn, D. A. and Stuart, I. M. (1993). An Investigation of Milling Energy to Predict the Malting Potential of Australia Barley. 6th Australian Barley Technical Symposium.

Enari, T. M. and Sopanen, T. (1986). "Centenary Review: Mobilisation of Endospermal Reserves During the Germination of Barley." Journal of the Institute of Brewing **92**: 25-31.

EBC Analytica (1998). European Brewing Convention Analytica, Brauerei und Gertränke Rundschau, Zurich.

Fincher, G. B. (1975). "Morphology and Chemical Composition of Barley Endosperm Cell Walls." Journal of the Institute of Brewing **81**: 116-122.

Georing, K. and DeHaas, B. (1974). "A Comparison of the Properties of Large- and Small- Granule Starch Isolated from Several Isogenic Lines of Barley." Cereal Chemistry **51**: 573-578.

Georing, K. J., Fritts, D. H. and Eslick, R. F. (1973). "A Study of Starch Granule Size and Distribution in 29 Barley Varieties." Starch-Starke **25**: 297-302.

GRDC Yearbook (2003). Australian Grain Yearbook. Report to Industry, GRDC: 24-25.

Guerin, J. R., Lance, R. C. M. and Wallace, W. (1992). "Release and Activation of Barley Beta--Amylaes by Malt Endopeptidases." Journal of Cereal Science **15**: 5-14.

Haley, C. S. and Knot, S. A. (1992). "A Simple Regression Method for Mapping Quantitative Trait Loci in Line Crosses Using Flanking Markers." Heredity **69**: 315-324.

Hammerton, R. W. and Ho, T. H. D. (1986). "Hormonal Regulation of the Development of Protease and Carboxypeptidase Activities in Barley." Plant Physiology **80**: 692-697.

Han, F., Ullrich, S. E., Chirat, S., Menteur, S., Jestin, L., Sarrafi, A., Hayes, P. M., Jones, B. L., Blake, T. K., Wesenberg, D. M., Kleinhofs, A. and Kilian, A. (1995). "Mapping of Beta-Glucan Content and Beta-Glucanase Activity Loci in Barley Grain and Malt." Theoretical & Applied Genetics **91**(6-7): 921-927.

Han, F., Ullrich, S. E., Kleinhofs, A., Jones, B. L., Hayes, P. M. and Wesenberg, D. M. (1997). "Fine Structure Mapping of the Barley Chromosome-1 Centromere Region Containing Malting-Quality QTLs." Theoretical & Applied Genetics **95**(5-6): 903-910.

Han, J. Y. and Schwarz, P. B. (1996). "Arabinoxylan Composition in Barley, Malt, and Beer." American Society of Brewing Chemists **54**(4): 216-220.

Harris, G., Hall, R. D. and MacWilliam, I. C. (1955). "A Contibution to the Assessment of the Carbohydrates of Malt and Wort." European Brewery Convention: 26-36.

Hayes, P. M., Castro, A., Marquez-Cedillo, L., Corey, A., Henson, C., Jones, B., Kling, J., Mather, D., Matus, I., Rossi, C. and Sato, K. (2001). "A summary of published barley QTL reports.", http://www.css.orst.edu/barley/nabgmp/qtlsum.htm

Hayes, P. M., Cerono, J., Witsenhoer, H., Kuiper, M., Zabeau, Sato, K., Kleinhofs, A., Kudrna, D., Kilian, A., Saghai-Maroof, M., Hoffman, D. and North_American_Barley_Genome_Mapping_Project (1997). "Characterizing and Exploiting Genetic Diversity and Quantitative Traits in Barley (*Hordeum vulgare*) using AFLP Markers." Journal of Quantitative Trait Loci **2**(3).

Hayes, P. M., Iyarnabo, O. and North_American_Barley_Genome_Mapping_Project (1994). "Summary of QTL Effects in the Steptoe × Morex Population." Barley Genetics Newsletter **23**: 98-143.

Hayes, P. M. and Jones, B. L. (2000). "Malting Quality from a QTL Perspective." 8th International Barley Genetic Symposium **1**: 99-106.

Hayes, P. M., Liu, B. H., Knapp, S. J., Chen, F., Jones, B., Blake, T., Franckowiak, J., Rasmusson, D., Sorrells, M., Ullrich, S. E., Wesenberg, D. and Kleinhofs, A. (1993). "Quantitative Trait Locus Effects and Environmental Interaction in a Sample of North American Barley Germ Plasm." Theoretical & Applied Genetics **87**(3): 392-401.

Hayes, P. M., Prehn, D., Vivar, H., Blake, T., Comeau, A., Henry, I., Johnston, M., Jones, B., Steffenson, B., St Pierre, C. A. and Chen, F. (1996). "Multiple Disease Resistance Loci and Their Relationship to Agronomic and Quality Loci in a Spring Barley Population." Journal of Quantitative Trait Loci(2).

Hellewell, K. B., Rasmusson, D. C. and Gallo-Meagher, M. (2000). "Enhancing yield of semidwarf barley." Crop Science **40**: 352-358.

Henry, R. J. (1985). "A Comparative Study of the Total β-Glucan Contents of Some Australian Barleys." Australian Journal of Experimental Agriculture **25**: 424-427.

Henry, R. J. (1986). "Genetic and Environmental Varietion in the Pentosan and β-Glucan Contents of Barley, and their Relation to Malting Quality." Journal of Cereal Science **4**: 269-277.

Inkerman, P. A., Curtis, A., Hettariachchi, M., Osman, A. M., Healy, P., de Jersey, J. and Hamilton, S. (1997). Behaviour of Starch Degrading Enzymes under Symulated Australian Mashing Conditions. 8th Australian Barley Technical Symposium, Gold Coast, Queensland, Australia.

IOB Recommended Methods of Analysis (1991). Institute of Brewing. London.

Janes, P. W. and Skerritt, J. H. (1993). "High Performance Liquid Chromatography of Barley Proteins: Relative Quantities of Hordein Fractions Correlate with Extract." Journal of the Institute of Brewing **99**: 77-84.

Jefferies, S. and Wheeler, R. (1991). South Australian Field Crop Evaluation Report, Depatrment of Primary Industries.

Jefferies, S. P., Pallotta, M. A., Paull, J. G., Karakousis, A., Kretschmer, J. M., Manning, S., Islam, A., Langridge, P. and Chalmers, K. J. (2000). "Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (Triticum aestivum)." Theoretical & Applied Genetics **101**(5-6): 767-777.

Jones, N., Ougham, H. and Thomas, H. (1997). "Matkers and Mapping: We are all Geneticists Now." New Phytologist **137**: 165-177.

Kano, Y. and Karakawa, T. (1979). "Importance of the Residual Starch in the Spent Grains for Wort Run-off." Bulletin of Brewing Science **25**: 1-7.

Karakousis, A., Barr, A. R., Kretschmer, J. M., Manning, S., Jefferies, S. P., Islam, A. K. M., Langridge, P. and Chalmers, K. J. (2003c). "Mapping and QTL analysis of the barley population Clipper x Sahara." Australian Journal of Agricultural Research **in press**.

Karakousis, A., Barr, A. R., Kretschmer, J. M., Manning, S., Logue, S. J., Roumeliotis, S., Collins, H. M., Chalmers, K. J., Li, C. D., Lance, R. C. M. and Langridge, P. (2003a). "Mapping and QTL analysis of the barley population Galleon x Haruna Nijo." Australian Journal of Agricultural Research **in press**.

Karakousis, A., Gustafson, J. P., Chalmers, K. J., Barr, A. R. and Langridge, P. (2003b). "A Consensus Map of Barley Integrating SSR, RFLP, and AFLP Markers." Australian Journal of Agricultural Research **in press**.

Kleinhofs, A. and Han, F. (2002). Molecular mapping of the barley genome. Barley Science - recent advances from molecular biology to agronomy of yield and quality. Slafter GA, Molina-Cano JL, Savin R, Araus JL and R. I**:** 31-63.

Kristensen, M., Svensson, B. and Larsen, J. (1993). Purification and Characterization of Barley Limit Dextrinase During Malting. 24th Congress of the European Brewery Convention.

Landau, J. B., Chandra, G. S. and Proudlove, M. O. (1995). Grain Hydration and Modification: A Key to Malt Quality. 7th Barley Technical Symposium,, Perth, Australia.

Lande, R. and Thompson, R. (1990). "Efficiency of marker-assisted selection in the improvement of quantitative traits." Genetics **124**(3): 743-756.

Larson, S., Bryan, G., Dyer, W. E. and Blake, T. (1996). "Evaluating Gene Effects of a Major Barley Seed Dormanvy QTL in Reciprocal Backcross Populations." Journal of Agricultural Genomics **2**.

Lee, R. (1996). Evaluation of (1,3), (1,4)-ß-Glucan and Arabinoxylan in Barley, Malt and Wort. Ballarat, Honours Thesis, University of Ballarat.

Lee, R., Allan, G. R., Greig, R., Dunn, C. A. and Stuart, I. M. (1998). The Effect Of (1-3,1-4)-β-Glucan and Arabinoxylan on Malt Quality and Brewhouse Performance. 25th Convention of the Institute of Brewing- Asia Pacific Section, Perth, Western Australia.

Li, C. D., Langridge, P., Lance, R. C. M., Xu, P. and Fincher, G. B. (1996). "Seven Members of the (1-3)-β-glucanase Gene Family in Barley (*Hordeum vulgare*) are Clustered on the Long Arm of Chromosome 3 (3HL)." Theoretical & Applied Genetics **92**: 791-796.

Li, C. D., Tarr, A., Lance, R., Harasymow, S., Uhlmann, J., Westcot, S., Young, K., Grime, C., Cakir, M., Broughton, S., Loughman, R. and Appels, R. (2003). "A major QTL controlling seed dormancy and pre-harvest sprouting/Grain alpha-amylase in two-rowed barley (Hordeum vulgare L.)." Australian Journal of Agricultural Research **in press**.

Li, C. D., Zhang, X. Q., Eckstein, P., Rossnagel, B. G. and Scoles, G. J. (1999). "A polymorphic microsatellite in the limit dextrinase gene of barley (Hordeum vulgare L.)." Molecular Breeding **5**(6): 569-577.

Logue, S. J. (1997). South Australian Barley Improvement Program, Barley Quality Report. Adelaide, Department of Plant Science, University of Adelaide.

Loi, L., Barton, P. A. and Fincher, G. B. (1987). "Survival of Barley (1-3, 1-4)-β-Glucan Isoenzymes during Kilning and Mashing." Journal of Cereal Science **5**: 45-50.

Longstaff, M. A. and Bryce, J. H. (1991). Levels of Limit Dextrinase Activity in Malting Barley. 23rd EBC Congress, Lisbon.

Longstaff, M. A. and Bryce, J. H. (1993). "Development of Limit Dextrinase in Germinated Barley (Hordeum vulgare L.)." Plant Physiology **101**: 881-889.

MacGregor, A. W. (1978). "Changes in α-Amylase Enzymes During Germination." Journal of the American Society of Brewing Chemists **36**: 1-5.

MacGregor, A. W. (1980). "Action of Malt a-Amylase on Barley Starch Granules." Master Brewing Association of America Technical Quarterly **17**(4): 215-221.

MacGregor, A. W. and Ballance, D. L. (1980a). "Hydrolysis of Large and Small Granules from Normal and Waxy Barley Cultivars by Alpha-Amylases from Barley Malt." Cereal Chemistry **57**(6): 397-402.

MacGregor, A. W. and Ballance, D. L. (1980b). "Quantitative Determination of a-Amylase Enzymes in Germinated Barley After Separation by Isoelectricfocusing." Journal of the Institute of Brewing **86**: 131-133.

MacGregor, A. W. and Fincher, G. B. (1993). Chapter 3: Carbohydrates of the Barley Grain. Barley: Chenistry and Technology. A. MacGregor and R. Bhatty. St Paul, MN, American Association of Cereal Chemists, Inc.

MacGregor, A. W. and Marchylo, B. A. (1986). "a-Amylase Components in Excised, Incubated Barley Embryos." Journal of the Institute of Brewing **92**: 159-161.

MacGregor, A. W. and Morgan, J. E. (1986). "Hydrolysis of Barley Starch Granules by Alpha-Amylases from Barley Malt." Cereal Foods World **31**: 688-693.

MacLeod, L. C. and Wallwork, M. A. B. (1992). Barley Starch Composition and Malting Quality. 23rd Institute of Brewing Conference, Australia and New Zealand Section.

MacLeod, L. C., Wallwork, M. A. B. and Giles, L. C. (1993). Malting Quality - The Importance of Barley and Malt Characteristics. 6th Australian Barley Technical Symposium.

Macri, L. J., Macgregor, A. W., Schroeder, S. W. and Bazin, S. L. (1993). "Detection of a limit dextrinase inhibitor in barley." Journal of Cereal Science **18**(2): 103-106.

Maeda, I., Kiribuchi, S. and Nakamura, M. (1978). "Digestion of barley Starch Granules by the Combined Action of α- and β-Amylases Purified from Barley and Barley Malt." Agricultural and Biological Chemistry **42**: 259-267.

Mäkelä, M., Korpela and Laakso, S. (1982). "Studies of Starch Size and Distribution in 33 Barley Varieties with a Celloscope." Starch-Starke **34**: 329-334.

Manners, D. J. and Yellowlees, D. (1973). "Studies on the Debranching Enzymes. Part I The Limit Dextrinase Activity of Extracts of Certain Higher Plants and Commercial Malts." Journal of the Institute of Brewing **79**: 377-385.

Mano, Y. and Takeda, K. (1997). "Mapping Quantitative Trait Loci for Salt Tolerance at Germination and the Seedling Stage in Barley (Hordeum Vulgare L)." Euphytica **94**(3): 263-272.

Marquez-Cedillo, L. A., Hayes, P. M., Jones, B. L., Kleinhofs, A., Legge, W. G., Rossnagel, B. G., Sato, K., Ullrich, E. and Wesenberg, D. M. (2000). "QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups." Theoretical & Applied Genetics **101**(1-2): 173-184.

Mather, D. E., Tinker, N. A., Laberge, D. E., Edney, M., Jones, B. L., Rossnagel, B. G., Legge, W. G., Briggs, K. G., Irvine, R. B., Falk, D. E. and Kasha, K. J. (1997). "Regions of the Genome That Affect Grain and Malt Quality in a North American Two-Row Barley Cross." Crop Science **37**(2): 544-554.

MBIBTC (2001). Industry Guidelines for Australian Malting Barley, Malting and Brewing Industry Barley Technical Committee.

McCleary, B. V., Gibson, T. S., Solah, V. and Mugford, D. C. (1994). "Total Starch Measurement in Cereal Products - Interlaboratory Evaluation of a Rapid Enzymic Test Procedure." Cereal Chemistry **71**(5): 501-505.

Melchinger, A. E., Utz, H. F. and Schon, C. C. (1998). "Quantitative Trait Locus (QTL) Mapping Using Different Testers and Independent Population Samples in Maize Reveals Low Power of QTL Detection and Large Bias in Estimates of QTL Effects." Genetics **149**(1): 383-403.

Merritt, N. R. (1967). "A new Strain of Barley with Starch of High Amylose Content." Journal of the Institute of Brewing **73**: 583-586.

Molina-Cano, J. L., Rubio, A., Igartua, E., Gracia, P. and Montoya, J. L. (2000). "Mechanisms of malt extract development in barleys from different European regions: I. Effect of environment and grain protein content on malt extract yield." Journal of the Institute of Brewing **106**(2): 111-115.

Morgan, A. G., Gill, A. A. and Smith, D. B. (1983a). "Some Barley Grain and Green Malt Properties and Their Influence on Malt Hot-Water Extract I. ß-Glucan, ß-Glucan Solubilase and Endo-ß-Glucanase." Journal of the Institute of Brewing **89**: 283-291.

Morgan, A. G., Gill, A. A. and Smith, D. B. (1983b). "Some Barley Grain and Green Malt Properties and Their Influence on Malt Hot-Water Extact II. Protein, Proteinase and Moisture." Journal of the Institute of Brewing **89**: 292-298.

Morrison, W. R., Milligan, T. P. and Azudin, M. N. (1984). "A Relationship Between the Amylose and Lipid Contents of Starches from Diploid Cereals,." Journal of Cereal Science **2**: 257-271.

Nelson, J. C. (1997). "Qgene - Software for Marker-Based Genomic Analysis and Breeding." Molecular Breeding **3**(3): 239-245.

Nilan, R. A. and Ullrich, S. E. (1993). Chapter 1: Barley: Taxonomy, Origin, Distribution, Production, Genetics, and Breeding. Barley: Chenistry and Technology. A. MacGregor and R. Bhatty. St Paul, MN., American Association of Cereal Chemists, Inc.

Oberthur, L., Blake, T., Dyer, W. E. and Ullrich, S. E. (1995). "Genetic Analysis of Seed Dormancy in Barley (*Hordeum valugare L.*)." Journal of Agricultural Genomics **1**: Paper 5.

Oliveira, A. B., Rasmusson, D. C. and Fulcher, R. G. (1994). "Genetic Aspects of Starch Granule Traits in Barley." Crop Science **34**(5): 1176-1180.

Oziel, A., Hayes, P. M., Chen, F. Q. and Jones, B. (1996). "Application of Quantitative Trait Locus Mapping to the Development of Winter-Habit Malting Barley." Plant Breeding(115): 43-51.

Pallotta, M., Asayama, S., Reinheimer, J., Davies, P., Barr, A., Jefferies, S., Chalmers, K., Lewis, J., Collins, H., Roumeliotis, S., Logue, S., Coventry, S., Lance, R., Karakousis, A., Lim, P., Verbyla, A. and Eckerman, P. (2003). "Mapping and QTL analysis of the barley population Amagi Nijo x WI2585." Australian Journal of Agricultural Research **in press**.

Palmer, G. H. and Bathgate, G. N. (1976). Malting and Brewing. Advances in Cereal Science and Technology. Y. Pomeranz. St Paul, Minnesota, American Association of Cereal Chemists. **I**.

Panozzo, J., Lim, P., Eckermann, P. and Cullis, B. (in preparation). "The analysis of quantitative traits for barley quality in the Arapiles x Franklin Doubled Haploid Population."

Panozzo, J. F. and Ratcliffe, M. (1997). Appplication of the Single Kernel Characterisation System and its Relationship with Barley Quality. 8th Australian Barley Technical Symposium, Gold Coast, Queensland, Australia.

Piendl, A. (1973). "Malt Modification and Mashing Conditions as Influencing Factors on the Carbohydrates of Wort." The Brewers Digest, **48**(9): 58-84.

Powell, W., Thomas, W. T. B., Baird, E., Lawrence, P., Booth, A., Harrower, B., McNicol, J. W. and Waugh, R. (1997). "Analysis of Quantitative Traits in Barley By the Use of Amplified Fragment Length Polymorphisms." Heredity **79**(Part 1): 48-59.

Rogowsky, P. M., Guidet, F. L. Y., Langridge, P., Shepherd, K. W. and Koebner, R. M. D. (1991). "Isolation and Characterization of Wheat-Rye Recombinants Involving Chromosome Arm 1DS of Wheat." Theoretical & Applied Genetics **82**: 537-544.

Roumeliotis, S., Collins, H. M., Logue, S. J., Willsmore, K. L., Jefferies, S. P. and A.R., Barr. (1999). Implications of Thin Husk in Barley. 9th Australian Barley Technical Symposium, Melbourne, Australia.

Schildbach, R., Rath, F., Burbidge, M. and Walsamos, G. (2000). "Barley varieties and production in the EBC- and some other countries." Brauwelt International **1**: 60-66.

Sissons, M. J. and Macgregor, A. W. (1994). "Hydrolysis of Barley Starch Granules By Alpha-Glucosidases From Malt." Journal of Cereal Science **19**(2): 161-169.

Sjoholm, K., Macri, L. J. and MacGregor, A. W. (1995). Is There a Role for Limit Dextrinase in Mashing? 25th Congress of the European Brewery Convention.

Slack, P. T., Baxter, E. D. and Wainwright, T. (1979). "Inhibition by Hordein of Starch Degradation." Journal of the Institute of Brewing **85**: 112-114.

Slade, A. M., HØj, P. B., Morrice, N. A. and Fincher, G. B. (1989). "Purification and Characterization of Three (1→4)-ß-D- Xylan endohydrolases from Germinating Barley." European Journal of Biochemistry **185**: 533-539.

Smith, D. B. and Gill, A. A. (1986). "Quantitative Changes in the Concentration of the Major Components of Four Genotypes of Barley Grains During Malting and Mashing." Journal of the Institute of Brewing **92**: 360-366.

Snedecor, G. W. and Cochran, W. G. (1989). Statistical Methods. Ames, Iowa, Iowa State University Press**:** 187, 473.

Sopanen, T. and Laurière, C. (1989). "Release and Activity of Bound ß-Amulase in a Germinating Barley Grain." Plant Physiology **89**: 244-249.

Spielmayer, W., Ellis, M. H. and Chandler, P. M. (2002). Cloning sd-1, the semidwarfing gene in rice and developing 'perfect markers' for breeding.

Proceending of the 12th Australasian Plant Breeding Conference, Perth, West Australia.

Stark, J. R. and Yin, X. S. (1986). "The Effect of Physical Damage on Large and Small Barley Starch Granules." Starch-Starke **38**: 369-374.

Stenholm, K., Home, S., Pietila, K., Macri, L. J. and Macgregor, A. W. (1996). "Starch Hydrolysis in Mashing." Proceedings of the 24th Institute of Brewing Convention Asia Pacific Section: 142-145.

Stuart, I. M., Loi, L. and Fincher, G. B. (1988). "Varietal and Environmental Variations in (1-3)(1-4)-ß-Glucan Levels and (1-3)(1-4)-ß-Glucanase Potential in Barley: Relationships to Malting Quality." Journal of Cereal Science **7**: 61-71.

Stuart, J. F. (1997). Better Malting, A Users Guide to Better Malting Techniques, New South Wales Grains Board**:** 9.

Sun, Z. and Henson, C. A. (1990). "Degradation of Native Starch Granules by α-Glucosidase." Plant Physiology **94**: 320-327.

Tester, R. F., South, J. B., Morrison, W. R. and Ellis, R. P. (1991). "The Effects of Ambient Temperature During the Grain-filling Period on the Composition and Properties of Starch from Four Barley Genotypes." Journal of Cereal Science **13**: 113-127.

IOB Recommended Methods of Analysis (1991). The Institute of Brewing. London.

Thomas, W. T. B., Powell, W., Swanston, J. S., Ellis, R. P., Barua, U. M., Chalmers, K. J., Jack, P., Lea, V., Forster, B. P., Waugh, R. and Smith, D. B. (1996). "Quantitative trait loci for germination and malting quality characters in spring barley cross." Crop Science **36**: 265-273.

Thomas, W. T. B., Powell, W., Waugh, R., Chalmers, K. J., Barua, U. M., Jack, P., Lea, V., Forster, B. P., Swanston, J. S., Ellis, R. P., Hanson, P. R. and Lance, R. C. M. (1995). "Detection of Quantitative Trait Loci For Agronomic, Yield, Grain and Disease Characters in Spring Barley (Hordeum Vulgare L)." Theoretical & Applied Genetics **91**(6-7): 1037-1047.

Ullrich, S. E., Han, F. and Jones, B. L. (1997). "Genetic Complexity of the Malt Extract Trait in Barley Suggested By Qtl Analysis." Journal of the American Society of Brewing Chemists **55**(1): 1-4.

Van Den Berg, R. and Gheeraert, F. A. C. (1991). "Potential Quality of Barley." Ferment **4**(4): 232-234.

Weselake, R. J., Macgregor, A. W. and Hill, R. D. (1985). "Effect of endogenous Barley α-amylase Inhibitor on Hydrolysis of Starch Under Various Conditions." Journal of Cereal Science **3**: 249-259.

Zale, J. M., Clancy, J. A., Ullrich, S. E., Jones, B. L., Hayes, P. M. and North_American_Barley_Genome_Mapping_Project (2000). "Summary of Barley Malting Quality QTLs Mapped in Various Populations." Barley Genetics Newsletter **30**: 44-54.

Zwickert-Menteur, S., Jestin, L. and Branlard, G. (1996). "Amy2 Polymorphism As a Possible Marker of Beta-Glucanase Activity in Barley (Hordeum Vulgare L)." Journal of Cereal Science **24**(1): 55-63.