Genetic control of grain quality in bread wheat (*Triticum aestivum* L.) grown under a range of environmental conditions

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Adelaide

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List of Abbreviations

AFLP : Amplified fragment length polymorphism

CIM : Composite interval mapping

DArT : Diversity array technology™

DH : Doubled haploid

GMP : Gluetenin macropolymer

GSP : Grain softness protein

Ha locus : Hardness locus

HMW-GS : High molecular weight-glutenin subunits

LMW-GS : Low molecular weight-glutenin subunits

LOD : Logarithm of the odds

MAGIC : Multiparent advanced generation inter cross

MAS : Marker assisted selection

MVWGAIM : Multivariate whole genome average interval mapping

NaCl : Sodium chloride

NAM : Nested association mapping

NIR : Near infrared reflectance

PCR : Polymerase chain reaction

PSI : Particle size index

QTL(s) : Quantitative trait locus/loci

RAPD : Randomly amplified polymorphic DNA

RIL : Recombinant inbred line

RFLP : Restriction fragment length polymorphism

SDS : Sodium dodecyl sulphate

SE-HPLC : Size-exclusion high-performance liquid chromatography

SIM : Simple interval mapping

SNP : Single nucleotide polymorphism

SSR : Simple sequence repeats

TPP : Total polymeric protein

UPP : SDS-unextractable polymeric protein in total polymeric protein

WGAIM : Whole genome average interval mapping

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Abiotic stresses including high temperatures and moisture deficit are detrimental to bread wheat production. Under abiotic stresses, characteristics such as yield, growth rate, gene expression and quality are affected and responses might involve interaction of many genes. Most studies on the impact of abiotic stresses such as high temperatures and moisture deficit have concentrated on effects on yield and agronomic traits with less work being done on grain quality. This project focussed on the end-use quality of wheat grain produced under a range of field production conditions including high temperatures and water shortages, using two mapping populations, Gladius/Drysdale and RAC875/Kukri. Gladius, Drysdale and two pairs of backcross derivatives having Wyalkatchem and RAC1262A as recurrent parents were also studied under normal and heat stress conditions in a glasshouse experiment. Of the backcross derivatives, one line of each pair has a *Gpc-B1* (high grain protein content) gene introgression and the other does not. Field trials were conducted in Australia and Mexico and the glasshouse experiment was conducted in Australia. For the glasshouse experiment, Gladius showed more heat tolerance with no significant decrease in grain weight compared to Drysdale. The backcross derivatives with the introgression segment had higher grain protein content, percentage unextractable polymeric protein and accelerated senescence than ones without the segment. Grain weight and senescence were severely affected by heat stress. Quality analysis of field grown material involved sequential assessment of grain, flour, dough and baked product characteristics. Stress conditions increased protein content, decreased yield, grain thickness, width and increased dough development time compared to the control. The exposure to heat stress resulted in an increase in loaf volume compared to the control experiment. Genetic linkage maps were constructed for the Gladius/Drysdale population and used for quantitative trait loci (QTL) analysis. Quantitative trait loci analysis detected several genomic regions associated with quality traits under a range of conditions including drought and heat stress in both populations. Some of the traits were associated with known phenology and quality genes, some QTLs detected have been reported in other studies but some QTLs were novel and had not been detected elsewhere. The novel QTLs detected under conditions involving heat and drought stress present opportunities for selection of lines that are able to maintain quality under these adverse conditions.

Declaration

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Chapter 1

General Introduction

Bread wheat (*Triticum aestivum* L.) is hexaploid with three genomes A, B and D, inbreeding and normally self pollinates. It is a widely grown cereal crop with millions of tonnes consumed worldwide every year. Wheat grain has multiple end uses, all with different quality specifications. Soft bread wheat is used mainly for cookies, cakes and crackers whilst hard wheat is used for production of pan bread, flat bread and noodles. The commercial value of wheat is determined by its end use quality. Overall, wheat quality is dependent on genotype, environment and genotype-by-environment interactions.

Wheat is cultivated over a wide range of climates with differing temperature regimes and rainfall patterns. Wheat production and quality are severely affected by a range of abiotic stresses such as high temperatures and water deficit (Altenbach, 2012). In Mediterranean climates such as in most wheat producing regions of Australia, it is common for the wheat crop to experience drought and heat stress in spring (Panozzo and Eagles, 1999). This period coincides with grain filling and huge yield and quality losses can be incurred. High temperatures and drought conditions usually occur concurrently in the field and possibly interact with high temperatures increasing transpiration and uptake and loss of water from the soil. In practical situations, drought can be eliminated through irrigation where there is sufficient water and heat can be avoided through early sowing to ensure plants mature before the onset of high temperatures. However, early sowing comes with the risk of potential frost damage in some wheat growing regions.

Heat and drought stress constrain grain filling in cereal crops, potentially compromising grain quality, with the impact depending on the plant growth stage (Blumenthal et al., 1991). Extreme temperatures can even prematurely terminate grain filling (Panozzo and Eagles, 1999). Younger crops generally tend to be more susceptible to drought and heat stresses than more developed older crops. This might be because older plants have well developed defence mechanisms and more extensive root systems which enable them to tap into stored soil moisture at depth. Heat has been shown to reduce starch deposition, total grain weight, milling yield and increase protein content (Altenbach et al., 2003; Dias and Lidon, 2009; Gooding et al., 2003; Hurkman et al., 2009; Labuschagne et al., 2009) and drought has been shown to reduce starch accumulation, cause grain shrivelling, and increase grain hardness and

protein content (Gooding et al., 2003; Jiang et al., 2009; Weightman et al., 2008). Grain hardness decreases under heat stress but increases under drought (Li et al., 2012). Weakening of dough due to heat stress during grain filling has also been reported, and has been attributed to changes in protein composition (Blumenthal et al., 1991).

One challenge for wheat breeders is to produce genetically superior genotypes that inherently maintain quality even under differing environmental conditions. Different wheat varieties may respond differently to heat and drought stress conditions, making it possible to select tolerant varieties. Quantitative trait locus (QTL) analysis can be used to identify genomic regions that control quality traits under heat and drought stress conditions thus making it possible to identify and select lines with favourable alleles. Through genetic studies it is possible to look for tolerance genes from wild relatives and landraces and introgress favourable segments into modern varieties to improve quality (Distelfeld et al., 2007; Vaccino et al., 2010).

The aim of this study was to investigate the genetic control of wheat quality under a range of environments involving drought and/or heat stress conditions. The study used two mapping populations, a recombinant inbred line (RIL) population derived from a cross between two wheat cultivars, Gladius and Drysdale, and a doubled haploid (DH) population derived from a cross between a breeding line RAC875 and a cultivar Kukri. In addition, Gladius, Drysdale, and a set of backcross derivatives with and without the *Gpc-B1* grain-protein locus were evaluated under heat stress in a glasshouse experiment. The thesis will review literature (Chapter 2), then report on the challenges faced in constructing the Gladius/Drysdale genetic linkage maps (Chapter 3), on genetic control of Gladius/Drysdale quality traits under a range of different environmental conditions (Chapters 4 and 5), on genetic control of quality traits in the RAC875/Kukri population grown in water-limited environments (Chapter 6), and on the effect of heat stress on Gladius, Drysdale and *Gpc-B1* backcross derivatives (Chapter 7). It will conclude with a general discussion and encompassing the main conclusions (Chapter 8).

Chapter 2

Literature Review

2.1 Introduction

Wheat is an important cereal crop grown worldwide and has various end uses. Wheat breeding and quality improvement programs focus on developing genetically superior, high yielding, acceptable quality and disease resistant cultivars that are adapted to different growth environments. The quality of new varieties has to meet both domestic and international market standards. Both yield and quality can be compromised by both abiotic and biotic stresses. The most prominent abiotic stresses are high temperatures and moisture deficit and these shorten the duration of grain development and affect grain quality (Altenbach, 2012).

The impact of environmental changes, driven by global warming, on food production necessitates the need to produce more food on limited cultivation area. Field environments are unpredictable, cannot be controlled and various environmental factors interact and affect quality. It is expected that processing quality will vary for a given genotype from location to location and from season to season. Genotypes also respond differently to variation in environmental conditions. Although heat stress is difficult to control in the field, early and late sowings are often used to anticipate heat stress. Drought can be controlled through irrigation.

A number of methods are employed to assess wheat grain and processing quality. In early breeding stages, little grain is available and only predictive tests are done, but in later generations, full scale tests including baking are conducted. For some quality traits, genetic loci have been mapped and in some cases specific genes have been isolated and this makes it possible for wheat breeders to integrate molecular selection tools into their quality improvement programs.

2.2 Wheat yield

Yield is an important parameter in the wheat industry and is a target of selection during wheat improvement. It is a function of grain number and grain weight. Yield related traits include grain number, thousand grain weight, test weight (hectolitre mass) and percentage screenings. Some cultivars are inherently higher yielding than others but yield is also influenced by the

growth environment. Yield and yield components are complex traits and their genetic control involves many interacting genes.

Various quantitative trait loci (QTLs) with both major and minor effects, for yield and yield components have been detected in many genomic regions under a range of environments (Bennett et al., 2012b; Bennett et al., 2012c; Groos et al., 2003; Kirigwi et al., 2007; Snape et al., 2007). Comparative genomics, collinearity of genes and synteny with others cereals (e.g rice which has a completely sequenced genome) has facilitated the development of diagnostic markers linked to grain weight and yield in wheat. A marker *TaGW2* on chromosome 6A which is associated with increases in cell numbers and grain milk filling rate results in increased grain weight through increasing grain width and slightly increasing grain length (Su et al., 2011; Yang et al., 2012). A cell wall invertase gene *TaCwi-A1* on chromosome 2A involved in sink tissue development and carbon partitioning increases grain weight (Ma et al., 2012) and a sucrose synthatase gene *TaSus2-2B* on chromosome 2B involved in starch biosynthesis, is also associated with grain weight (Jiang et al., 2011). The cytokinin oxidase/dehydrogenase (CKX) gene *TaCKX6-D1* on chromosome 3D is associated with grain weight but the *TaCKX2* gene versions on chromosomes 7B and 7D are not associated with grain weight (Zhang et al., 2012).

Yield and related traits are also heavily influenced by cold temperatures (vernalisation requirement), day length (photoperiod sensitivity) and intrinsic earliness (earliness per se) (Eagles et al., 2009; Eagles et al., 2010; Fischer, 2011; Liu et al., 2012), through the effect of these genomic regions on time to heading, and by dwarfing genes (*Rht*) (Ellis et al., 2002; Wang et al., 2012a). It is therefore necessary to avoid the potentially confounding effects of these plant development genes when searching for genes that control complex traits such as wheat yield and yield components. In addition to the genetic makeup of crops, abiotic stresses such as drought and heat stresses reduce yield and yield components to varying levels depending on the plant growth stage at which the crops are stressed (Barnabas et al., 2008; Gooding et al., 2003; Pinto et al., 2010).

2.3 Wheat quality parameters

In addition to yield, quality assessment involves testing the grain, flour, dough and baked product. Wheat quality is mainly determined by the desired end use of the grain. Most quality

tests on grain and flour are attempting to predict the quality of the end product. A number of quality parameters are tested to determine overall quality and usability of wheat grain. Before their release to the market, cultivars are subjected to rigorous processing quality tests to ascertain if they meet the market's expectations. In industry quality is a function of cultivar, production environment and chemical alteration/blending (genotype-by-environment-by-technology) interactions (Békés, 2012).

2.3.1 Grain shape

Grain shape dimensions can be easily measured using a ruler, vernier calipers or digital image analysis. Commonly measured dimensions are length, width and thickness, with aspect ratio, roundness and area then calculated based on these dimensions. Both mathematical models and experimental studies indicate that grain shape is related to flour extraction (Berman et al., 1996; Marshall et al., 1984; Marshall et al., 1986). Round and plump grain yield more flour than thin and shrivelled grain. Breeding and selection has resulted in shorter and wider grain in modern cultivars compared to their progenitors and to landraces (Gegas et al., 2010). Growing conditions affect grain dimensions with drought and heat stress resulting in thin and shrivelled grains which are difficult to mill. Water deficit reduces grain width and thickness resulting in overall small grains (Konopka et al., 2007).

Various QTL studies have shown that the different grain dimensions are largely under different genetic control mechanisms and exhibit QTL-by-QTL and genotype-by-environment interactions (Bergman, 2000; Breseghello and Sorrells, 2007; Campbell et al., 1999; Dholakia et al., 2003; Gegas et al., 2010; Prashant et al., 2012; Sun et al., 2009; Xiao et al., 2011). Though the genes controlling grain shape dimensions have not been identified, candidate genes have been proposed. The candidate genes include *TaGW2* on chromosome 6A (Su et al., 2011) for grain width and sucrose synthase 2 gene (*TaSus2-2B*) on chromosome 2B also for grain width (Jiang et al., 2011).

2.3.2 Grain hardness

Grain hardness is a measure of the resistance of the grain to mechanical crushing or particle size distribution on ground grain. Hard grain is difficult to crush compared to soft one. The single kernel characterisation system (SKCS) (Perten Instruments, Springfield, IL) and Foss near infrared reflectance (NIR) instrument (FOSS NIR Systems, Inc., Laurel, MD) are

routinely used to estimate grain hardness. The NIR estimates particle size index (PSI) with hard grained cultivars having lower PSI values than soft grained ones.

Hexaploid bread wheat is broadly categorised into hard and soft market classes based on grain texture. However, within class classifications exists though variation tends to be small in these sub-classifications. Grain hardness is heritable and it is possible to turn a hard wheat into a soft one and a soft wheat into a hard through backcrossing (Symes, 1965). A major gene and to some extent minor modifying genes are involved in the inheritance of hardness (Sourdille et al., 1996). Hard and soft wheats differ mainly at one major locus called the hardness (*Ha*) locus on the short arm of chromosome 5D (Morris, 2002). The *Ha* locus contains puroindoline A and B genes (*Pina-D1* and *Pinb-D1*) and *GSP-1* encoding grain softness protein (Chantret et al., 2005; Morris and Bhave, 2008). The exact function of *GSP-1* in determining grain hardness is not clearly defined (Bhave and Morris, 2008; Mohler et al., 2012). The allele combination at the *Ha* locus determines the grain texture, which ranges from soft grain, through moderately hard to extremely hard grain (Cane et al., 2004; Capparelli et al., 2003; Chen et al., 2012; Morris and Beecher, 2012; Morris, 2002). The common haplotypes are *Pina-D1a* and *Pinb-D1a* that confers soft phenotype, *Pina-D1a* and *Pinb-D1b* that result in intermediate hardness and *Pina-D1b* and *Pinb-D1a* that result in hard phenotype (Cane et al 2004).

Mutations at the *Pinb-D1* gene result in altered puroindoline protein content causing variation in hardness and overall quality (Bhave and Morris, 2008). Cultivars carrying the *Pinb-D1d* allele has been shown to have poor sedimentation volume, gluten index and loaf volume (Mohler et al., 2012). The *Ha* locus might also control endosperm vitreosity (glassy physical appearance) with hard phenotypes being more vitreous (Morris and Beecher, 2012). The location of the *Ha* locus on a group D chromosome means that durum wheat cannot produce puroindoline proteins. Due to the absence of puroindolines, durum wheat has extremely hard grains.

A new puroindoline gene family (Puroindoline b-2 genes) has been detected on all group 7 chromosomes (Chen et al., 2010; Geng et al., 2012a). Association between this new family of puroindolines and grain hardness has been observed through QTL mapping (Wilkinson et al 2008). Various allelic variants exist for this gene family (Mohler et al., 2012; Ramalingam et al., 2012). Though puroindolines genes on the short arm of chromosome 5D and on group 7 chromosomes largely control grain hardness, QTLs for hardness have been detected on other

genomic regions including on chromosomes 4B and 4D, close to *Rht* genes (Wang et al., 2012a).

The *Ha* locus has also been shown to be associated with other quality traits. Some QTL studies have reported that the *Ha* locus can control protein content (Turner et al., 2004; Zhang et al., 2009). Proteomic studies have shown that increased grain hardness might be due to increased protein folding, protein matrix formation and faster endosperm development in hard lines (lacking *Pina-D1*) than in soft ones (Lesage et al., 2012). The molecular weight of storage proteins is higher in the harder than in the softer lines and this might suggest that puroindolines influence grain texture through protein aggregation (Lesage et al., 2011). Loci controlling grain hardness have also been shown to be associated with flour colour, with harder grain having a more yellow and less bright colour (Peterson et al., 2001; Tsilo et al., 2011).

2.3.3 Milling yield

Milling yield is the amount of flour obtained from milling a certain amount of grain and it can be affected by growing conditions, but it also differs between cultivars. Milling serves to separate the endosperm from the germ and to obtain refined wheat flour from the endosperm. Grain shrivelling and shape, which are heavily influenced by growth conditions, have an effect on milling yield and quality of flour obtained (Marshall et al., 1986). Commonly used mills in research are the Bühler MLU-202 laboratory test mill (Bühler AG, Uzwil, Switzerland) and Quadrumat Junior test mill (Brabender, Germany).

Martin et al. (2001) found that within the hard wheats category the soft textured sub-group gave more flour and lower ash content than the hard textured sub-group. Though, there are no clear differences between the hard and soft classes in terms of milling yield, soft wheats are difficult to mill, require long sieving time and generally have low water absorption. In hard wheats the starch granules and protein matrix are tightly bound, thus requiring more energy to mill resulting in more starch damage and coarse flour (Békés, 2012).

QTLs controlling grain hardness tend to co-locate with ones controlling milling yield in many regions including the *Ha* locus (Tsilo et al., 2011; Wang et al., 2012a). The serpin genes have also been shown to affect milling yield (Cane et al., 2008). In addition to the *Ha* locus and serpins, QTLs associated with milling yield have been detected in many other genomic regions (Campbell et al., 2001; Carter et al., 2012; Kuchel et al., 2006; Parker et al., 1999).

2.3.4 Flour colour

The colour of flour and of the end product is an important criterion in the marketing of wheat. White colour is desirable for bread baking and yellow colour is preferred for yellow alkaline noodle production. The CIE colour space measures brightness (L*) with higher values for brighter colour, redness-greenness (a*) with positive values indicating redness, and blueness-yellowness (b*) with positive values indicating yellowness. The colour of the baked product depends on initial flour colour and reactions during the baking process. While flour colour is mainly under genetic control, presence of bran and germ in flour during milling also affects flour colour.

Genomic regions on chromosomes 2A, 2B, 2D, 3B, 4B, 5B, 7A and 7B have been shown to be associated with colour traits (Bordes et al., 2011; Howitt et al., 2009; Kuchel et al., 2006; Mares and Campbell, 2001; Parker et al., 1998). However, about 75% of the variation in flour yellowness is explained by variation at the phytoene synthase (PSY1) genes on group 7 chromosomes (Ravel et al., 2012). Phytoene synthase, is involved in the biosynthesis of carotenoids which give flour a yellowish creamy colour, and novel alleles have been detected in homoeologous copies of *Psy-1* (*Psy-A1*, *Psy-B1* and *Psy-D1*) in all the group 7 chromosomes (Crawford et al., 2011; He et al., 2008; He et al., 2009; Ravel et al., 2012). Multiple alleles of *Psy-A1* have been identified and cause variation in flour colour ranging from white to yellow (Crawford et al., 2011; He et al., 2008; Howitt et al., 2009). While some of the QTLs associated with flour yellowness mapped on chromosomes 7A and 7B correspond with *Psy-A1* and *Psy-B1* genes, Crawford et al. (2011) further showed that some QTLs detected on chromosome 7A were not directly linked to *Psy-A1* in some populations. This shows that additional genes close to *Psy-A1* are associated with variation in flour colour. Phylogenetic relationships and evolutionary origins of *Psy-A1* alleles show that most might have originated from *T. urartu* (Crawford et al., 2011).

Additional candidate genes controlling flour colour have been reported in other genomic regions. On chromosome 3B, yellow pigment was shown to be influenced by ε-cyclase (ε-LCY), a gene involved in lutein synthesis (Howitt et al., 2009). Carotene desaturase activity (ZDS) genes *TaZds-A1* and *TaZds-D1* on chromosomes 2A and 2D have been cloned and shown to be related to carotenoid synthesis and yellow pigment (Dong et al., 2012; Zhang et al., 2011a). Polyphenol oxidase (PPO) and lipoxygenase (LOX) activity can also influence the colour of flour or wheat based products to some extent and functional markers to detect

allelic variants of these genes are available (Geng et al., 2012b; He et al., 2007; Sun et al., 2005). Taken together, the different studies show that candidate genes controlling most of the variation in flour colour have been detected.

2.3.5 Protein quantity

Protein is an important constituent of wheat grain and both protein quantity and quality play a major role in flour quality. Total protein content is used for pricing purposes in the wheat industry and it can be quickly estimated with the NIR instrument (FOSS NIR Systems, Inc., Laurel, MD). Protein content is about 1% less in milled flour than in the grain (Miles et al., 2012). Protein content is more influenced by genotype and genotype-by-environment interactions than protein quality that is more dependent on the genotype. Nitrogen fertilisation increases protein content and also affects the polymeric to monomeric ratio and the proportion of high molecular weight glutenin subunits (HMW-GS) in glutenin polymers (Békés, 2012). Heat and drought stresses are known to increase total protein content (Gooding et al., 2003; Weightman et al., 2008) and this often results in dough with very long development and mixing times (Blumenthal et al., 1991; Guttieri et al., 2001).

Many QTLs associated with protein content have been detected across the genome (Mann et al., 2009; Perretant et al., 2000; Prasad et al., 2003; Turner et al., 2004; Wang et al., 2012a). A genetic segment, originally from wild emmer wheat *Triticum turgidum* ssp. *dicoccoides*, containing the *Gpc-B1* locus on the short arm of chromosome 6B has been introgressed into modern wheat varieties and shown to increase total protein content through increased nitrogen remobilisation (Brevis and Dubcovsky, 2010; Uauy et al., 2006a; Waters et al., 2009). The gene is a No Apical Meristem (*NAM*) gene belonging to the NAC transcription factor family and is called *NAM-B1* (Uauy et al., 2006b). A study on a global collection of wheat shows that the functional and non-functional alleles of this gene might have originated from different geographic areas, and still display a geographic distribution pattern with the high grain protein content allele predominantly in environments with shorter growing seasons (Hagenblad et al., 2012).

2.3.6 Protein composition and dough properties

Wheat proteins are generally classified based on their solubilities as albumins, globulins, gliadins and glutenins. Albumins are water soluble, globulins are insoluble in pure water but

soluble in dilute sodium chloride (NaCl) solution but insoluble at high NaCl concentrations, gliadins are soluble in 70% ethy alcohol and glutenins are soluble in dilute acid or sodium hydroxide solutions (MacRitchie and Lafiandra, 1997). Glutenins are complex polymeric proteins comprised of HMW-GS and low molecular weight glutenin subunits (LMW-GS) while gliadins are simple monomeric proteins and occur in the alpha, beta, gamma and omega types (MacRitchie and Lafiandra, 1997).

The HMW-GS are mainly responsible for dough strength, LMW-GS are mainly responsible for dough extensibility and gliadins are mainly responsible for dough viscosity (Békés, 2012 and references therein). Glutenins have more influence on baking performance than gliadins (Barak et al., 2012). The function of wheat storage proteins can be effectively measured by incorporating them into rice base flour because rice does not contain prolamin type protein and hence there will be no interaction with other prolamins (Oszvald et al., 2011). Dough formulation (adding or removing gluten protein) from flour results in flour with different dough and baking quality and can be used to measure the effects of individual proteins (Marchetti et al., 2012). The LMW-GS and gliadins have not been extensively used in quality predictions compared to HMW-GS mainly due to limited understanding of the function of individual proteins and their similar molecular weights. The similar molecular weights make it difficult to distinguish these classes of proteins (Yang et al., 2011) and thus limit their use in processing and baking quality improvement.

The gliadins and glutenins which comprises 70-80% of total grain protein form a gluten complex with water (Altenbach, 2012). Ability to bake bread from wheat is due to its unique gluten proteins which gives dough its viscoelastic properties. The gluten complex plays a role in trapping carbon dioxide produced during dough fermentation resulting in leavened bread. Poor baking quality flour has more gliadins and less glutenins than better quality one (Marchetti et al., 2012). The gluten index, a measure of gluten quality and quantity, is not correlated with loaf volume and hence it cannot be used as predictor of baking quality (Bonfil and Posner, 2012).

Variation in dough properties is largely associated with polymorphism of known genes at the glutenin and gliadin loci on chromosome groups 1 and 6 (Gale, 2005; Ma et al., 2005; Payne et al., 1987). The glutenin loci *Glu-A1*, *Glu-B1* and *Glu-D1* on the long arms of chromosomes 1A, 1B and 1D encode HMW-GS (Payne et al., 1982; Payne et al., 1987) and the glutenin loci *Glu-A3*, *Glu-B3* and *Glu-D3* on the short arms of chromosomes 1A, 1B and 1D encode

LMW-GS (Gupta and Shepherd, 1987). Gliadin loci are closely linked to glutenin loci encoding LMW-GS on the short arms of group 1 chromosomes, but additional gliadin loci are present on the short arms of group 6 chromosomes. Allele combinations at the glutenin loci can be used to predict dough properties (Eagles et al 2002; 2004; Payne et al 1987).

Glutenin quantity is directly related to dough properties, for example, duplication at the *Glu-B1* locus results in over-expression of glutenin proteins and is accompanied by increased dough strength and mixing time (Butow et al., 2003; Eagles et al., 2004; Mann et al., 2009; Vawser and Cornish, 2004). At least two *Glu-B1* over-expression alleles have been characterised (Gao et al., 2012; Ragupathy et al., 2008). QTLs associated with glutenin quantity have been detected (Mann et al., 2009; Ravel et al., 2006; Zhang et al., 2011b) and largely co-locate with known glutenin, secalin (*Sec1* on chromosome 1B) and *Ha* locus indicating that these genes influence dough properties through affecting actual quantity of glutenins (Zhang et al., 2011b).

Dough properties are also influenced by the molecular weight distribution of glutenins and can be predicted by measuring the amount of polymerised protein (Ciaffi et al., 1996; Don et al., 2003). It has been shown that cultivars with the 5 + 10 at *Glu-D1* (7 + 9 at *Glu-B1*) subunits polymerise earlier and have higher percentage unextractable polymeric protein (% UPP) (also referred to as insoluble polymeric protein and similar to glutenin macropolymer (GMP)) than those with 2+12 (20x +20y) subunits (Naeem and MacRitchie, 2005; Naeem et al., 2012). Percentage UPP is a measure of dough strength.

Both small scale and large scale dough testing methods can be used to assess dough properties (Békés et al., 2001; Mann et al., 2008; Mares and Mrva, 2008). Dough mixing properties can be measured using a mixograph or a Brabender farinograph depending on the amount of available grain. Indices measured with a mixograph are the peak time, which is a measure of optimum time for dough consistency and development; the rate of dough development; mixing tolerance, which measures the resistance to breakdown of dough; mixograph height and width which are measures of dough strength. At peak mixing time the gluten network is completely assembled (Martinant et al., 1998).

When there is sufficient flour, a Brabender farinograph can be used to measure dough development time, stability, breakdown, mixing and water absorption characteristics. Dough development time tends to increase with increasing protein content (Fu et al., 2008). Short dough development time indicate weak flour with poor bread making quality, whilst very

long time requires more energy and are therefore might be undesirable in industry. The baking industry is interested in knowing how much amount of water to add to flour in order to create dough of a certain consistency. Flour from hard textured wheat generally have a slightly higher water absorption capacity than that from softer ones. Within class variations are small and Martin et al. (2001) could not find differences in the water absorption between the flour of softer and harder textured sub-groups of hard wheat.

2.3.7 Baking tests

The most commonly measured output of baking tests is loaf volume, which is a quantitative measure of baking quality and can be measured using rapeseed displacement method. Qualitative scores can also be assigned for loaf score, texture and structure. Different types of baking methods are available and the choice of the method can depend on protein content of the flour and on dough strength. The methods commonly used in research are the long fermentation method, straight dough bulk fermentation, mechanical dough development (MDD), Chorleywood bread making process (CBP), activated dough development (ADD) processes and sponge and dough method. Sponge and dough method differs from the others in that it is a two-stage process and involves firstly preparing a sponge and after a period of fermentation dough is prepared. An optimum proving time is required to give acceptable loaf volume and form ratio (height/width) with an increase in proving time leading to an increase in loaf volume and a decrease in form ratio (Tronsmo et al., 2003).

Many genomic regions have been shown to be associated with loaf properties (Carter et al., 2012; Groos et al., 2007; Kuchel et al., 2006; Mann et al., 2009). Loaf volume and crumb colour display genotype-by-environment interaction (Elangovan et al., 2008; Law et al., 2005). Genomic regions controlling grain hardness (including the *Ha* locus on chromosome 5D) have been shown to be associated with loaf properties mainly through their influence on water absorption (Campbell et al., 2001; Cane et al., 2004; Groos et al., 2007; Martin et al., 2001; Nelson et al., 2006; Perretant et al., 2000). Both protein quantity and quality impact on baking performance and these in turn are influenced by genotype and by environmental conditions. High protein content increases dough strength and results in higher loaf volume. The HMW-GS composition influences baking quality (Huang et al., 2006). The 5+10 HMW-GS that is encoded by the allele *Glu-D1d* contributes positively to baking quality relative to other HMW-GS (Payne et al., 1987). Baking performance can be predicted from dough and gluten behaviour (Kieffer et al., 1998). Since, baking tests are time consuming and require

large amounts of wheat samples, which are not available in early generations, use of rheological tests to predict baking outcome is often used and is justifiable.

2.4 Abiotic stresses

Heat and drought stress, at times in combination with other abiotic stress such as hostile soils or frost affect overall wheat yield and quality. High temperatures and moisture deficit usually coincide in many wheat growing regions and can be detrimental to quality especially when wheat plants become exposed at the sensitive grain filling stage. It is difficult to separate the effects of heat stress from those of drought as they often occur together.

2.4.1 Heat stress

Plants that can or are exposed to heat stress respond either through escape or tolerance. Escape involves reaching physiological maturity before the onset of stress thus avoiding unfavourable conditions. Tolerance means the ability to yield successfully even under heat stress. If high temperature episodes occur late in the season, early maturing lines may escape heat stress because they would reach physiological maturity before the onset of extremely high temperatures. Heat tolerance is a quantitative trait and can be measured by heat tolerance index of traits such as grain weight, yield, grain filling duration and canopy temperature. Genomic regions associated with heat tolerance have been detected on chromosomes 2B, 7B and 7D (Paliwal et al., 2012) and 4A (Pinto et al., 2010). A region on chromosome 7A was shown to be associated with quality maintenance under heat stress (Beecher et al., 2012). It is possible to try to impose temperature profiles by anticipating sowing date to attain favourable growth temperatures (Borghi et al., 1995). For research on the performance of lines under heat stress, narrowing the maturity window ensures that all lines are exposed to heat stress at the same time and this minimizes the confounding effects of different developmental stages (Pinto et al., 2010).

Cultivars generally vary with respect to their sensitivity to high temperatures. For example, European ones are the most sensitive followed by Australian ones and tropical region ones being the least sensitive, during the grain filling period (Jenner, 1994). Even within the same cultivar there can be variation in response to heat stress, with a cultivar being susceptible with respect to the yield components but tolerant with regard to quality components (Stone and Nicolas, 1994).

Heat stress shortens the duration of grain filling and results in decreased grain weight and starch content, and increased total protein content (Altenbach et al., 2003; Altenbach, 2012; Gooding et al., 2003). It is widely accepted that heat stress activates heat shock elements which are located close to gliadin genes, and it has been suggested that this leads to increased gliadin synthesis (Blumenthal et al., 1991). Increased gliadin synthesis would increase the gliadin:glutenin ratio and this has been cited as a possible cause of weak dough properties (Blumenthal et al., 1991; Blumenthal et al., 1994). However, Stone and Nicholas (1994) observed both increases and decreases in gliadin synthesis under heat stress depending on cultivar, possibly due to different duration of exposure, different growth stage of the crop or even recovery after exposure to heat.

It has been suggested that heat stress affects the rate and degree of protein polymerisation (Ciaffi et al., 1996). Glutenin macropolymer (GMP) represents the glutenin fraction that is insoluble in 1.5% sodium dodecyl sulphate (SDS) and plays a major role in wheat quality. In one study, GMP was increased by heat shock but only for crops initially grown at 18/13°C (day/night) (Spiertz et al., 2006). In contrast, there was no noticeable difference in GMP for plants initially grown at 25/20°C when exposed to heat shock (Spiertz et al., 2006). This might mean the plants already exposed to higher temperatures of 25/20°C acquired some tolerance and were then less affected by heat stress. There is also variation in the response of % UPP, another dough strength estimator which is similar to GMP, to heat stress with some studies reporting decreases (Irmak et al., 2008; Naeem and MacRitchie, 2005) and another reporting increases (Balla et al., 2011) in amount of % UPP in heat stressed plants. The differences may be due to stage of exposure, with early exposure resulting in increased % UPP and late exposure resulting in decreased percentages.

2.4.2 Drought stress

Drought stress affects grain development, yield and quality (Altenbach, 2012). Drought is complex and there can be many types of drought experienced in different production zones and years. These include cyclic drought, which involves periods of drought that are interspersed by periods of water availability. There can also be terminal drought that occurs towards the end of the plant growing season. Continuous drought from sowing to harvest can be another form of drought but this will not permit crop production at all. The regions in which this thesis research was conducted experience cyclic drought and receive most of the rain in the early season with amount of water decreasing as the season progresses. The

decrease in water supply is accompanied by increases in temperature. The stage at which the wheat crop is subjected to water shortage is critical. Moisture stress prior to pollination leads to a decrease in photosynthesis and this is accompanied by a reduction in stomatal conductance but both recover upon reintroduction of water supply (Rajala et al., 2009). The decrease in the rate of photosynthesis results in decreased starch accumulation. Plants accumulate assimilates such as water soluble carbohydrates during favourable conditions which they can later remobilise to developing grain during periods of drought stress (Mir et al., 2012).

Pre-anthesis drought often leads to reduced grain set resulting in decreased grain number and yield. In contrast, moisture stress after anthesis usually affects grain size, weight and quality. Reduced water supply affects grain appearance and shape resulting in shrivelled grain (Gooding et al., 2003; Smith and Gooding, 1999). Grain shrivelling due to drought stress has been shown to affect milling yield (Gaines et al., 1997). Decreased water supply can result in reduction of flour extraction and increased mixograph peak time to varying extents depending on the genotype's response to moisture stress (Guttieri et al., 2001). Total protein content increases under drought conditions probably due to reduced leaching and loss of nitrogen (Weightman et al., 2008) in addition to the dilution effect due to decreased starch content. The increase in protein content can be the cause of increased mixograph peak time. Under water shortage, protein accumulates a bit earlier than starch and stops accumulating earlier than would be expected under normal conditions (Altenbach et al., 2003).

2.5 Genetic mapping and molecular markers

Wheat exhibits low level of polymorphism compared to other cereals, and polymorphism also varies amongst the genomes, with the D-genome being the least polymorphic (Akhunov et al., 2010). The low polymorphism has in turn slowed genetic mapping studies in wheat compared to other cereals as the level of polymorphism affects marker density (Fleury et al., 2012). The complexity of the wheat genome further complicates genetic mapping, analysis, genome sequencing and gene discovery (Edwards et al., 2012). The complexity and large genome size of wheat necessitates the use comparative genomics with species such as rice whose genome is fully sequenced because gene order is mostly conserved in the absence of chromosomal rearrangements (Fleury et al., 2012).

Genetic mapping employs marker technology to understand the underlying genes or loci controlling important traits such as quality traits. Molecular markers are simple assays that detect polymorphic sites in genomic DNA. They differentiate genotypes and can enhance the efficiency and precision of plant breeding. Molecular markers can also aid breeding through selecting for traits that are difficult to select phenotypically. Molecular markers are either polymorphic or monomorphic, with polymorphic ones able to detect genetic differences between different lines. Polymorphic markers are divided into dominant and co-dominant markers, with co-dominant markers capable of distinguishing homozygotes from heterozygotes. Both dominant and co-dominant markers have been extensively used in genetic mapping in bread wheat (Chalmers et al., 2001; Crossa et al., 2007; Sherman et al., 2010; Uphaus et al., 2007). Numerous molecular markers designed based on known sequence polymorphisms in specific genes for which the functions have been studied are routinely used in genotyping of wheat mapping populations (Liu et al., 2012) for example, markers are available for multiple alleles of vernalisation, photoperiod, puroindoline, glutenin, dwarfing and serpin genes.

Earlier studies used mainly restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and sequence based DNA markers and each type has its advantages and disadvantages (Collard et al., 2005; Langridge et al., 2001). In recent years, diversity arrays technology (DArT) markers (Akbari et al., 2006; Wenzl et al., 2004) have been commonly used for linkage map construction in wheat. Lately, single nucleotide polymorphism (SNP) markers (Akhunov et al., 2009), KASPar based genotyping (Allen et al., 2011) and genotyping by sequencing (Elshire et al., 2011) have been gaining prominence in wheat genetic studies. Next generation sequencing platforms that circumvert the vector cloning step, thus reducing costs and time, are also being employed in genetic studies and result in the generation of enormous amount of molecular data, presenting challenges in handling the data and in phenotyping (Fleury et al., 2012).

Molecular markers are used in the construction of linkage maps. Linkage maps show the position of the markers on the genome. Genetic distances between loci on linkage maps reflect frequencies of recombination between loci as opposed to the number of base pairs in physical maps (Collard et al., 2005; Narain, 2010). Different linkage maps constructed from

different genetic backgrounds can be merged into a consensus map (Fleury et al., 2012; Somers et al., 2004). Linkage maps are used in QTL analysis and mapping.

2.5.1 Quantitative trait loci (QTL)

QTL studies are performed to understand the underlying genetic architecture of complex traits such as quality traits. QTL analysis involves developing mapping populations, identifying markers that are polymorphic between the parents and genotyping the population with them, constructing genetic linkage maps and phenotyping traits of interest. The genotypic data and corresponding phenotypic data are then used to detect QTL-trait associations. High marker density in the linkage map gives good genetic resolution and helps accurately estimate the QTL position on the chromosome. Advances in marker technology and gene sequencing are increasing marker density and at slightly lower costs. Segregation distortion of markers can cause a bias in marker-trait associations (Narain, 2010).

The methods used to conduct QTL analysis include single marker analysis (Edwards et al., 1987) which tests the association at each locus, simple interval mapping (Lander and Botstein, 1989) which tests the association within marker intervals, composite interval mapping (Zeng, 1994) which tests the association within intervals but taking into account the effect of background markers and inclusive composite interval mapping (ICIM) (Li et al., 2007; Li et al., 2008) which goes further and tests for the epistatic interactions between genomic regions. Univariate whole genome average interval mapping (WGAIM) (Verbyla et al., 2007) and multivariate whole genome average interval mapping (MVWGAIM) (Verbyla and Cullis, 2012) include all linkage map intervals simultaneously in the analysis. Genome wide association mapping based on linkage disequilibrium can also be used to investigate marker trait associations (Gouis et al., 2012; Neumann et al., 2011; Reif et al., 2011). In all these methods a threshold logarithm of the odds (LOD) score is calculated using permutation tests to indicate the significance of the QTL (Churchill and Doerge, 1994). Various computer programs are used to conduct QTL analysis such as GenStat (Payne et al., 2009), QGENE (Nelson, 1997), QTL cartographer (Wang et al., 2012b), QTLNetwork (Yang et al., 2008) and MapManager (Manly et al., 2001). Both single environment and multi-environment QTL analysis can be conducted.

Linkage analysis has mostly used bi-parental populations and hence two alleles at any locus can be studied at a time. Recombinant inbred lines (RILs) and doubled haploid (DH) are the mostly used population types for QTL studies and these represent permanent resources that can be replicated over sites and years (Collard 2008). Lately, multiparent advanced generation inter cross (MAGIC) populations involving crossing multiple parents in order to increase genetic diversity (Cavanagh et al., 2008) and nested association mapping (NAM) populations involving crossing parental lines to a reference line (McMullen et al., 2009) are gaining prominence.

The ability to detect a QTL depends on map quality (marker density), population size, and the heritability of the trait. Association of QTLs with traits can be due to many genes with small effects or few genes with large effects. The QTLs can interact with environments (QTL-by-environment interaction), interact with each other (epistatic interactions) and they can affect more than one trait (pleiotropy). Co-location of QTLs can be either through pleiotropic effect of single genes or the influence of two closely linked genes. Detection of QTLs is an important step towards candidate gene identification and gene discovery. Knowledge gained from genetic studies can aid in gene pyramiding, that is combining many genes in one genotype.

Validation QTLs across different environments and backgrounds is necessary if a marker is to be of value in breeding (Gupta et al., 2010; Langridge et al., 2001). Validated QTLs can be fine mapped and underlying genes can be cloned (Fleury et al., 2012). The stable and validated QTLs can be used, in addition to candidate gene search, in marker assisted selection (MAS), a field that offers great potential in accelerating plant breeding (Collard and Mackill, 2008). In MAS, reliable and validated molecular markers are used to predict the phenotype and select for the trait of interest. It offers advantages in that molecular assays are faster and are not subject to environmental effects. It also allows genotypic selection at any plant tissue and at any developmental stage, combines and analyses many genes at the same time offering prospects of speeding the selection process at early generation (Collard et al., 2005).

Since most methods used for assessing grain quality traits are relatively expensive, time consuming and require large samples of grain, quality traits are good candidates for MAS. Quality traits such as grain protein content, hardness, flour colour, tolerance to pre-harvest sprouting, gluten strength and bread making quality have been targeted for MAS and MAS

has facilitated the release of cultivars with enhanced quality (Gupta et al., 2010). The reliability of MAS is anchored in the use of accurate mapping data, flanking markers, large population sizes, reliable phenotypic results, varying and diverse genetic backgrounds, different seasons and validation (Collard and Mackill, 2008; Young, 1999). However, despite its adoption and extensive use by some wheat breeding programmes, the limitations of MAS include lack of reliable markers especially markers developed from germplasm that is not adapted to local production environments, cost though they are decreasing, the complexity of the wheat genome including presence of desirable QTLs in alien segments introgressed into wheat (Gupta et al., 2010).

2.6 Aims of the thesis research

Wheat quality in most wheat producing regions is adversely affected by high temperatures and water deficiency. The aims of the research conducted for this thesis were to investigate the performance of two segregating populations (Gladius/Drysdale and RAC875/Kukri) under a range of conditions involving heat and drought stresses and to understand the genetic control of quality in wheat produced under these stresses. The research also aimed to characterise the performance of Gladius and Drysdale, a set of backcross derivatives and their recurrent parents under controlled heat stress conditions. This thesis seeks to answer the following questions:

1. Are quality traits affected by the same or different QTLs in Gladius/Drysdale and RAC875/Kukri?
2. Do the QTLs for these quality traits interact with environments, especially environments that differ with respect to heat and drought stress?
3. Do the QTLs for quality traits correspond with any known genes for grain quality traits or any previously detected QTLs for grain quality traits?
4. Do these QTLs correspond with QTLs for agronomic performance under heat stress or drought stress?
5. What is the effect of heat stress on plant senescence, grain weight, protein content and unextractable polymeric protein (% UPP)?
6. What is the effect of the *Gpc-B1* introgression segment in Australian germplasm?

Information contained in Chapters 3 to 5 will be combined into two manuscripts which will be submitted for publication at a later stage. Information contained in Chapter 3 will introduce the linkage map which was used for QTL analysis for quality traits in Chapters 4 and 5. Information contained in Chapter 6 has already been published and information in Chapter 7 is almost ready for submission for publication.

Chapter 3

Construction of genetic linkage maps using random and non-random samples of recombinant inbred lines from a cross between two cultivars of bread wheat

3.1 Introduction

Bread wheat (*Triticum aestivum* L.) is allohexaploid and has a complex genetic architecture. It has a total genome size of approximately 16,000 Mbp, compared to only 5,200 Mbp for barley (*Hordeum vulgare* L.) and 430 Mbp for rice (*Oryza sativa* L.) (Arumuganathan and Earle, 1991; Sasaki and Burr, 2000). These features can complicate the application of molecular methods and genetic mapping in wheat.

For genetic mapping in wheat and other self-pollinated species, researchers often develop populations of doubled haploid (DH) or recombinant inbred lines (RILs) from the F_1 generation of a cross between two contrasting parents. Direct development of F_1-derived DH lines can be faster and less expensive, but RILs offer more opportunities for recombination and for fine mapping.

Variation in phenological traits (e.g., sensitivity to differences in photoperiod or response to vernalisation) within a mapping population can confound assessment of other traits of interest, complicating the mapping of quantitative trait loci. Attempts have been made to minimise the confounding effects by using parents that do not differ at loci that are known to affect responses to vernalisation and photoperiod (Pinto et al., 2010; Reynolds et al., 2009). However, this approach can severely constrain the choice of parental combinations that can be used for genetic mapping. An alternative approach is to make crosses among parents that differ at loci affecting phenology, generate random samples of lines, and then select among these lines to obtain a non-random subset with relatively uniform phenology. With intense selection, this approach could even fix some of the loci that directly affect phenology, and also some of the genomic regions adjacent to those loci thus effectively excluding them from the construction of genetic linkage maps and from QTL analysis.

In bread wheat, the transition from the vegetative to the reproductive phase and overall crop adaptation are controlled mainly by three groups of loci, those affecting photoperiod responses, vernalisation requirements and earliness *per se*. Photoperiod genes regulate response to day length (Beales et al., 2007; Eagles et al., 2009; Worland et al., 1994) and vernalisation genes regulate requirements for exposure to cold temperatures (Eagles et al.,

2009; Fu et al., 2005; Trevaskis et al., 2007). Photoperiod genes are mainly located on group 2 chromosomes and vernalisation loci are predominantly located on group 5 chromosomes. Earliness *per se* loci affect phenological development when all photoperiod and vernalisation requirements have been satisfied. Quantitative trait loci for possible earliness *per se* have been mapped in numerous genomic regions (Griffiths et al., 2009; Hanocq et al., 2004; Hanocq et al., 2007).

Another factor to be considered in the construction of genetic linkage maps is the type (or types) of molecular marker to be used. Numerous marker technologies are now available, and data representing several marker types with different properties may need to be integrated with each other for linkage mapping. During the course of a mapping initiative, changes in technology, resource limitations and/or technical difficulties may result in not all lines being genotyped with the same numbers and types of markers, presenting an additional challenge for linkage map construction. Recently, large numbers of SNP markers have become available for wheat (Akhunov et al., 2009) and genotyping-by-sequencing technology (Elshire et al., 2011) has become feasible.

This chapter describes and discusses a linkage mapping process undertaken using two sets of RILs from a phenotypically diverse mapping population derived from a cross between the wheat cultivars Gladius and Drysdale. The two sets of lines were selected for different purposes and were genotyped at different times using different technologies, presenting some challenges for construction of a useful genetic map. The first set of lines was selected at random. The second set is comprised of lines having similar heading dates in order to minimise confounding effects of phenological differences on QTL mapping.

3.2 Materials and Methods

3.2.1 Population development

The Australian cultivars Gladius and Drysdale were used as parents to develop a population of 5000 RILs. Gladius (http://pbr.ipaustralia.plantbreeders.gov.au/) was derived from a complex cross involving derivatives of RAC875, Krichauff, Excalibur and Kukri. Drysdale (http://pbr.ipaustralia.plantbreeders.gov.au/) has the pedigree Hartog*3/Quarrion. Gladius and Drysdale were chosen as parents based on their performance under drought and heat stress conditions (Fleury et al. 2010). Gladius is more drought and heat tolerant and Drysdale

was chosen based on carbon isotope discrimination, an indicator of water use efficiency (Fleury et al., 2010).

3.2.2 Subsets of lines

From among the 5000 RILs, two subsets of lines were selected at random, one consisting of 205 lines (the 'random subset') and one consisting of 360 lines (the 'additional lines') (Figure 3.1). Within each of these subsets, lines that headed between 110 and 120 days in irrigated trials conducted at Urrbrae, South Australia in 2010 (AusUrr10_SI_CS_1 and AusUrr10_SI_CS_2, Table 3.1) were selected to form a 'mid-maturity subset of 134 lines, consisting of 46 lines from the 'random subset' and 88 of the 'additional lines'. There were some common lines between the subsets, the "common subset", whose linkage map serves as the link between the maps of the random and mid-maturity subsets (Figures 3.2, 3.3 3.4 and 3.5) but no analysis was done for this subset.

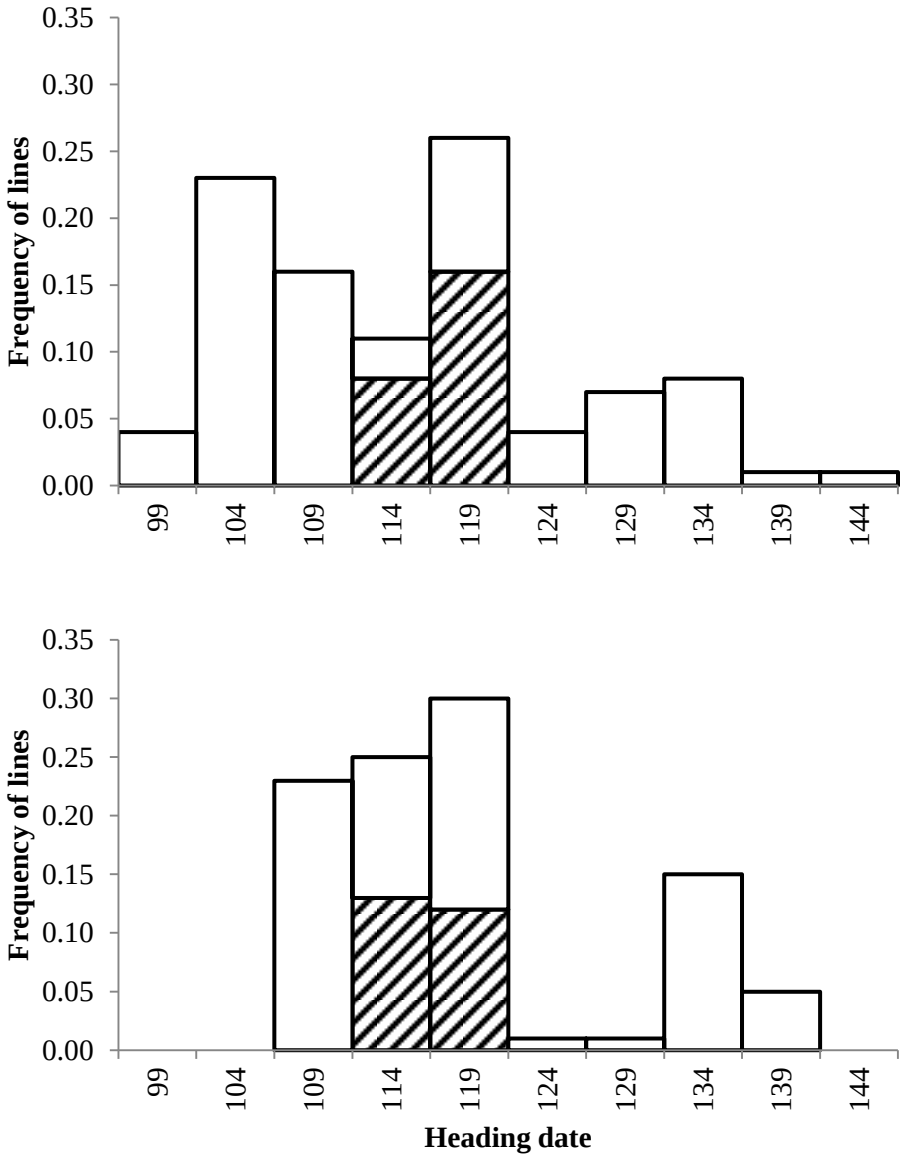

Figure 3.1 Frequency distributions of heading dates (time from sowing to ear emergence) for a 'random subset' of 205 Gladius/Drysdale recombinant inbred lines (above) and 360 "additional lines" (below), grown in irrigated trials at Urrbrae, South Australia in 2010. The hatched pattern designates the 'mid-maturity subset' of 134 lines (46 from the random subset and 88 from the additional lines). The absence of very early lines in the additional subset might possibly be because the scoring started slightly late as different investigators were involved. The very early lines might have been scored days after heading.

3.2.3 Field trials

Field trials were conducted in a total of 11 environments but QTL analysis was done for nine of these environments (Table 3.1). The other two environments had very few lines to conduct a QTL analysis.

3.2.3.1 Moderate drought experiment at Roseworthy

The 205 RILs were grown in the 2009 season at Roseworthy, South Australia. The experiment was randomised using a nearest neighbour design with two replicates of each RIL, parent and five control cultivars (Kukri, RAC875, Excalibur, Espada and Krichauff) and the plot area was 6.3 m^2. The growing season rainfall was 347 mm which can be classified as moderate drought and yield was 3331 kg/ha. Plants were scored once before anthesis using the Zadoks cereal development scale (Zadoks et al., 1974).

3.2.3.2 Heat experiments at Yanco

The 205 RILs, their parents (Gladius and Drysdale) and 11 control cultivars (Kukri, Excalibur, Mace, Espada, Krichauff, Ventura, Waagan, Gregory, Janz, Diamondbird and Sokoll) were grown in field experiments at Yanco, New South Wales in the 2009 and 2010 seasons. The experimental design was a spatially optimised incomplete block design, consisting of two replicates of each line, parent and control cultivars and the plot area was 7.5 m^2. There were two experiments in each season, an early sown experiment designed to escape heat stress and a late sown experiment designed to be exposed to heat stress during grain filling. Both experiments were irrigated to avoid moisture stress, with moisture sensors fitted in the soil used to monitor water levels and thus schedule irrigation. In 2009, plants were scored once before anthesis using the Zadoks growth scale. In 2010, the date of heading (number of calendar days from sowing to ear emergence) was recorded for each plot as the date on which spikes had emerged from the boot on 50% of the plants. Unfortunately, the 2010 season late sown experiment was flooded and severely weather damaged. Grain from that experiment had very low falling number, indicating post-harvest sprouting, and was not used for quality analysis reported in this thesis.

3.2.3.3 Drought experiment at Ciudad Obregon

A drought experiment was conducted at Ciudad Obregon, Mexico in the 2010 season. The field design was a two-replicate alpha lattice design with 205 RILs, their parents and 11

control cultivars (Kukri, Excalibur, Mace, Espada, Krichauff, Ventura, Waagan, Gregory, Janz, Diamondbird and Sokoll). The area of each plot was 1.6 m^2. Plants were irrigated by drip irrigation for three months from sowing and received a total amount of 150 mm of water. Thereafter, irrigation was withheld. During the period between the end of the irrigation and harvest, there was no rainfall. The date of heading was recorded for each plot.

3.2.3.4 Heat and drought experiments at Urrbrae

Four experiments were conducted at Urrbrae, South Australia in 2010. In each of the experiments, each experimental unit consisted of a single row of 12 plants, with 10 cm between plants within the row and 10 cm between rows. Two of these experiments were sown in a polyurethane tunnel (polytunnel) and the other two were sown under netting. In the two polytunnel experiments, the entries included 60 Gladius/Drysdale RILs that had been selected as being of intermediate maturity based on their Zadoks scores in the moderate drought experiment conducted at Roseworthy in 2009 described in 3.2.3.1 above, their parents (Gladius and Drysdale) and 11 control cultivars (Kukri, Excalibur, Mace, Espada, Krichauff, Ventura, Waagan, Gregory, Janz, Diamondbird and Sokoll). These experiments were arranged in completely randomised designs, with two replications of each Gladius/Drysdale RIL and four replications of each parent or control cultivar. The two experiments conducted under netting were arranged in partially replicated designs, with two replications of each of the above-mentioned 60 RILs, one replication of each of the remaining 145 RILs and four replications of each parent or control cultivar. Prior to booting (Zadoks stage 4.1) all the experiments were at ambient temperature and were irrigated daily to full soil moisture capacity.

For polytunnel experiments, when the first plant reached booting stage (Zadoks stage 4.1), the walls of the polytunnel were lowered in order to elevate the temperature. Once the polytunnel walls were closed, the temperature inside was roughly 10°C higher than ambient temperature. For drought experiments (one in the polytunnel and the other under netting), cyclic drought was imposed when the first plant reached booting stage (Zadoks stage 4.1) by withholding irrigation, allowing soil moisture levels to decrease to a critical level (-9 bar), at which the plots were re-watered to full capacity. The drought trial under netting was covered with an overhead rain cover and moisture sensors were fitted in the soil and were connected to a weather station (MWS model, Hunter Industries, Australia) that monitored moisture content, air temperature, wind speed and direction. The control experiment at ambient

temperature under netting was irrigated daily. Weeds were removed from all the fields and Aquosol multi-nutrient fertiliser (nitrogen, phosphorus, potassium, sulfur, magnesium, manganese, copper, iron, zinc, boron and molybdenum) was applied twice during the vegetative phase (two months after planting and at booting). Heading date was recorded in all the experiments but only the heading date of the set of 205 lines was used for analysis in this thesis Chapter.

An additional set of 360 lines (from the original 5000 RILs) was also grown in 2010 under irrigated conditions at Urrbrae, South Australia (AusUrr10_SI_CS_2). Each plot was a single 1.2 m long row consisting of 12 plants spaced 10 cm apart, with the rows also 10 cm apart. Dates of heading and anthesis were recorded.

In the 2011 season, the 134 mid-maturity lines were grown at Urrbrae, South Australia in experiment AusUrr11_SI_LS that was sown late (15 July 2011) in order to increase the chances of exposure to heat during grain filling. Each plot consisted of four 0.8 m long rows spaced 7.5 cm apart, each with eight plants and with the rows 10 cm apart. Heading date was recorded for each line.

Table 3.1 Description of the environments in which the experiments were conducted showing location, year in which experiment was conducted, the type of subset analysed and the genetic analysis performed. Plant development was measured using the Zadoks system in two environments and heading date was recorded in the other environments.

Environment[a]	Location[b]	Year	Latitude	Longitude	Altitude (M)	Lines tested	Zadoks scored	Heading date recorded	MEA[c]
AusRos09_SI_CS	Roseworthy (SA)	2009	34° S	138° E	68	Random set of 205 RILs	yes	no	yes
AusYan09_FI_CS	Yanco (NSW)	2009	34° S	146° E	136	Random set of 205 RILs	yes	no	yes
MexObr10_D_LS	Ciudad de Obregon	2010	27° N	109° W	38	Random set of 205 RILs	no	yes	yes
AusYan10_FI_CS	Yanco (NSW)	2010	34° S	146° E	136	Random set of 205 RILs	no	yes	yes
AusYan10_FI_LS	Yanco (NSW)	2010	34° S	146° E	136	Random set of 205 RILs	no	yes	yes
AusUrr10_D_CS	Urrbrae (SA)	2010	34° S	138° E	225	Random set of 205 RILs	no	yes	yes
AusUrr10_SI_CS_1	Urrbrae (SA)	2010	34° S	138° E	225	Random set of 205 RILs	no	yes	yes
AusUrr10_SI_CS_2	Urrbrae (SA)	2010	34° S	138° E	225	Additional lines	no	yes	yes
AusUrr10_H_SI_CS	Urrbrae (SA)	2010	34° S	138° E	225	Subset of 60 RILs	no	yes	no
AusUrr10_HD_CS	Urrbrae (SA)	2010	34° S	138° E	225	Subset of 60 RILs	no	yes	no
AusUrr11_SI_LS	Urrbrae (SA)	2011	34° S	138° E	225	Mid-maturity subset	no	yes	yes

[a]Aus for Australia, Mex for Mexico, D for drought, H for heat, HD for heat and drought, CS for conventional sowing, LS for late sowing, FI for flooding irrigation, SI for sprinkler irrigation

[b]SA for South Australia, NSW for New South Wales

[c]MEA for multiple environment QTL analysis

3.2.4 DNA extraction and genotyping

DNA was extracted from 2.0 g of bulked leaf tissue (3-6 plants per line) of eight week old plants using a mini prep ball bearing extraction method (Rogowsky et al., 1991) with some modifications (Pallotta et al., 2000). DNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies). A total of 293 lines (the random subset of 205 and the selected set of 88) were genotyped with markers for *Vrn-A1*, *Vrn-D1*, *Ppd-B1* and *Ppd-D1*. In addition, the random subset of 205 RILs was genotyped with DArT markers, SSR markers, and markers for *Pina-D1*, *Pinb-D1*, *Glu-D3* and *TaGW2*. Later, the mid-maturity subset, consisting of 134 similar maturing lines, was genotyped with SNP markers.

3.2.4.1 DArT genotyping

For each line to be genotyped with DArT markers, about 100 ng/µl DNA (30 µl) was sent to Triticarte Pty Ltd (Akbari et al., 2006; Wenzl et al., 2004). For each line, each DArT marker was scored based on the presence or absence of hybridisation.

3.2.4.2 SSR genotyping

Polymerase chain reaction (PCR) amplification, marker analysis and scoring was done using Multiplex-Ready technology (Hayden et al., 2008). Dye-labelled tag primers and locus-specific primers for four fluorescent dyes FAM, VIC, NED and PET were used in PCR amplification. The PCR products were de-salted using ultra-filtration plates AcroPrep 384 Filter Plate with 10-kD Omega membrane, short tips natural, 100 µl well (PALL Life Sciences) and run on a DNA fragment analyser ABI3730.

3.2.4.3 Gene-based marker genotyping

For *Vrn-A1*, the primer pair VRN1AF-F/VRN1-INTR (Yan et al., 2004) was used to identify variation in the promoter region, with the PCR reaction mixture and program described by Eagles et al. (2009). For *Vrn-D1*, intron-1 alleles were detected using a mixture of primer pairs Vrn-D1 Intr1/D/F; Intr1/D/R3 and vrn-D1 Intr1/D/F; Intr1/D/R4 (Fu et al., 2005), with the PCR reaction mixture and program described by Eagles et al. (2009). For *Ppd-B1*, the method described by Díaz et al. (2012) was used to detect the *a* allele carried by Gladius. For *Ppd-D1*, alleles of the pseudo-response regulator *Ppd-D1* on chromosome 2D were detected using the primers Ppd-D1_F, Ppd-D1_R1 and Ppd-D1_R2 (Beales et al., 2007), using the

PCR protocol described by Eagles et al. (2009). For *Pina-D1* and *Pinb-D1*, the PCR reaction mixture and program were as described by Cane et al. (2004) and the primers used were those described by Gautier et al. (1994) and Giroux and Morris (1997). For *Glu-D3*, a multiplex PCR was carried out using the primer pair M2F12/ M2R12 (Zhao et al., 2007a) to amplify the *a* allele carried by Gladius and primer pair M4F3/M4R3 (Zhao et al., 2007b) amplifying the *g* allele carried by Drysdale with the PCR reaction mixture and program described by Appelbee et al. (2009). For *TaGW2*, the primer pair and reaction mix used for amplification were those described by Su et al. (2011). The reaction was run on a LightCycler 480 High Resolution Melting instrument (Roche Diagnostics Australia Pty Limited) with the RIL alleles scored based on their melting curves and their relation to the parental melting curve.

3.2.4.4 SNP genotyping

For each line to be genotyped with SNP markers, approximately 100 ng/µl DNA (30 µl) was sent to Department of Primary Industries, Victoria to be assayed on an Infinium 9K SNP assay as described by Akhunov et al. (2009).

3.2.5 Linkage map construction

For each marker, distortion from a segregation ratio of 1:1 was tested using a chi-square test ($p < 0.01$). All markers, except phenology genes, showing significant segregation distortion were excluded from map construction. Marker data were also visually inspected to identify lines with large numbers of markers with missing data or heterozygous scores and these lines were excluded. Markers were grouped into linkage groups using the program Multipoint (http://www.multiqtl.com/). A LOD score threshold of three was set as the minimum threshold to indicate linkage between markers. Marker order within each linkage group and map orientation were obtained by comparing with published maps available on GrainGenes (http://wheat.pw.usda.gov/) and Triticarte (www.triticarte.com.au/) web sites and with a wheat consensus map (Somers et al., 2004). Final marker order was obtained using the program RECORD (Isidore et al., 2003). Map distances were calculated using R/qtl (Broman et al., 2003) and maps were drawn using MapChart (Voorrips, 2002).

3.2.6 Statistical and genetic analysis

Significance of differences in Zadoks score and time to heading between the parents were tested using analysis of variance. For the random subset of 205 lines, normality in all the

experiments was tested using a Shapiro-Wilk test for normality (Shapiro and Wilk, 1965) with GenStat 14 (Payne et al., 2009).

For both the random and mid-maturity subsets, a multi-environment QTL analysis was done. A two-step process was used for QTL analysis for Zadoks scores and heading date with GenStat 14 (Payne et al., 2009). The first step was to generate best linear unbiased estimates (BLUEs) by incorporating block, row and range effects into the analysis (Gilmour et al., 1997). The second step was QTL analysis using the BLUEs as trait values. The best variance-covariance model was automatically selected based on Schwarz information criterion. Single trait linkage analysis was done by first conducting an initial scan using simple interval mapping, then the selected candidate QTL were used as co-factors, with a minimum co-factor proximity of 30 cM using composite interval mapping and a final QTL model was selected. The maximum step size along the genome was set at 10 cM and the genome wide significance level was $\alpha = 0.05$.

3.3 Results

3.3.1 Zadoks score and days to heading

No significant differences were observed between the parents in either of the environments in which development was assessed based on Zadoks scores (Table 3.2). The heading dates of Gladius and Drysdale differed significantly ($p<0.05$) only in the AusUrr10_D_CS experiment (Table 3.2) in which Gladius headed four days later than Drysdale. In the late-sown experiments (AusYanco10_FI_LSand AusUrr11_SI_LS), the RILs headed much sooner after sowing than in other experiments. Heritability estimates for Zadoks scores and heading dates were high, ranging from 86% (Zadoks at AusYan09_FI_CS) to 98% (Zadoks at AusRos09_SI_CS) (Table 3.2).

For heading date, there were significant deviations from normality in all experiments, with p values of 0.023 in the AusYan10_FI_CS experiment, 0.005 in the AusYan10_FI_LS experiment, and less than 0.001 in MexObr10_D_LS, AusUrr10_SI_CS_1 and AusUrr10_D_CS experiments.

Table 3.2 Mean Zadoks score and number of days from sowing to heading for Gladius, Drysdale and the Gladius/Drysdale recombinant inbred lines in the environments in which the experiments were conducted.

	Parents			Recombinant inbred lines					
Environment	Gladius	Drysdale	p-value	mean Zadoks score	mean days to heading	minimum	maximum	standard deviation	Broad sense heritability (%)
AusRos09_SI_CS	54	58	0.11	50	-	32	69	11.6	98
AusYan09_FI_CS	50	54	0.06	48	-	39	60	7.4	86
MexObr10_D_CS	112	111	0.61	-	112	98	127	6.6	93
AusYan10_FI_CS	113	112	0.67	-	111	100	122	5.0	94
AusYan10_FI_LS	76	74	0.48	-	76	65	85	4.5	95
AusUrr10_D_CS	112	108	0.01	-	108	96	133	9.9	92
AusUrr10_SI_CS_1	119	114	0.13	-	114	97	135	9.8	87
AusUrr10_SI_CS_2	119	114	0.13	-	115	110	120	2.2	-
AusUrr10_H_SI_CS	109	104	0.09	-	108	101	122	3.9	64
AusUrr10_HD_CS	104	100	0.06	-	105	94	119	3.8	55
AusUrr11_SI_LS	89	89	1.00	-	89	78	100	4.0	-

3.3.2 Frequency of alleles at the phenology loci

Some loci (*Ppd-D1* and *Vrn-D1*) did not deviate significantly from the expected 1:1 ratio in either subset (Table 3.3). One locus (*Ppd-B1*) did not deviate significantly from expectations in the random subset but was significantly distorted in the mid-maturity subset in favour of Gladius allele for photoperiod insensitivity. The locus *Vrn-A1* locus was significantly distorted in the random subset and even more distorted in the mid-maturity subset in favour of the Drysdale winter allele.

Table 3.3 Frequencies of alleles from Gladius and Drysdale in two sets of Gladius/Drysdale recombinant inbred lines at four phenology loci and p-values from chi-square tests for goodness-of-fit to a 1:1 ratio.

		Proportions of parental alleles		
Locus	Subset of lines	Gladius	Drysdale	p-value
Ppd-B1	Random	0.53	0.47	0.549
	Mid-maturity	0.69	0.31	<0.001
Ppd-D1	Random	0.52	0.48	0.689
	Mid-maturity	0.59	0.41	0.072
Vrn-A1	Random	0.40	0.60	0.046
	Mid-maturity	0.36	0.64	0.005
Vrn-D1	Random	0.53	0.47	0.549
	Mid-maturity	0.55	0.45	0.317

3.3.3 Allele combinations at phenology loci

Across the four loci there are 16 possible allele combinations and analysis of variance showed that selection significantly shifted time to heading in five of these combinations (Table 3.4). Of these five allele combinations, only the presence of both alleles for photoperiod insensitivity at the photoperiod loci delayed time to heading in the mid-maturity subset compared to the random subset. A combination of winter alleles at vernalisation loci and an allele for photoperiod sensitivity at *Ppd-D1* locus decreased time to heading in the mid-maturity subset compared to the random subset.

Table 3.4 Comparison of days to heading between recombinant inbred lines in the random and mid-maturity subsets for 16 allele combinations at four phenology loci and p-values from one-way analysis of variance.

Allele combination at phenology loci[a]				Subset of lines		
Ppd-B1	*Ppd-D1*	*Vrn-A1*	*Vrn-D1*	Random	Mid-maturity	p-value
a	*a*	*a*	*a*	110	115	0.262
a	*a*	*v*	*v*	110	114	0.068
a	*a*	*a*	*v*	107	110	0.425
a	*a*	*v*	*a*	106	113	<0.001
b	*b*	*a*	*a*	131	118	0.044
b	*b*	*v*	*v*	128	118	0.083
b	*b*	*a*	*v*	131	115	<0.001
b	*b*	*v*	*a*	129	116	0.037
a	*b*	*a*	*a*	117	115	0.232
a	*b*	*v*	*v*	118	116	0.003
a	*b*	*a*	*v*	119	116	0.140
a	*b*	*v*	*a*	116	115	0.257
b	*a*	*a*	*a*	112	115	0.554
b	*a*	*v*	*v*	113	114	0.830
b	*a*	*a*	*v*	112	114	0.352
b	*a*	*v*	*a*	111	114	0.081

[a]At *Ppd-B1* and *Ppd-D1*, *a* is for the allele for photoperiod insensitivity and *b* is for the allele for photoperiod sensitivity. At *Vrn-A1* and *Vrn-D1*, *a* is for the spring allele and *v* is for the winter allele.

3.3.4 Linkage maps constructed

Genetic linkage maps were generated for the random and mid-maturity subsets. These maps have different types of markers, numbers of markers, chromosome lengths and numbers of linkage groups (Table 3.5; Appendix 1 and Appendix 2).

Table 3.5 The distribution of marker types, the numbers of markers, linkage groups and the lengths of the linkage groups in the random and mid-maturity genetic maps.

Random subset					Mid-maturity subset			
	numbers and type of markers					numbers and type of markers		
Linkage group	DArT	SSR	gene-based	Length (cM)	Linkage group	SNP	gene-based	Length (cM)
1A	13	1	1	121.8	1A	67		65.3
1A2	29			12.4	1B	234		116.6
1B	29	2	1	213.8	1D	38		39.9
1D	15	5	1	132.2	2A	13		15.6
1D2	19			9.9	2A1	236		95.8
2A	43	10		169.1	2B	246	1	158.1
2B	35	10	1	260.2	2D	56	1	116.1
2D	6	4	1	198.2	3A	248		167.9
2D2	17	1		35.8	3B	208		148.4
3A	14	7		246.7	3D	13		8.0
3A2	13	1		63.5	4A	188		223.6
3B	47	10		335.8	4B	110		107.3
3D	14	3		39.9	5A	151	1	128.2
4A	54	1		146.0	5A1	24		16.4
4B	13	2		200.1	5B	285		169.6
5A	9	3	1	260.4	5D	26	1	157.1
5B	44	5		197.1	6A	265		105.4
5D			2	0.1	6B	350		87.5
5D1	3	1		11.4	6D	9		17.9
5D2	1	1	1	43.8	7A	265		169.9
6A	24	3	1	142.1	7B	146		122.2
6A2	5	5		109.2	7D	17		28.1
6B	55			133.0				
6B1	15			19.3				
7A	14	5		176.4				
7A2	19	1		37.4				
7B	68	6		288.3				
7D		5		41.3				
Total	618	92	10	3645.1		3195	4	2264.9

3.3.5 QTLs for Zadoks score and time from sowing to heading

In the random subset, Zadoks score was associated with photoperiod loci *Ppd-B1* and *Ppd-D1* on chromosomes 2B and 2D (Table 3.6; Figures 3.2 and 3.3). At both loci, the allele for photoperiod sensitivity delayed development, reducing Zadoks scores. Using the random subset, QTLs for time to heading were detected at or near known phenological loci: at the SSR marker locus *gwm0429* on chromosome 2B, which is close to *Ppd-B1*, at *Ppd-D1* on chromosome 2D, at *Vrn-A1* on chromosome 5A and at *Vrn-D1* on chromosome 5D (Table 3.6; Figures 3.2, 3.3, 3.4 and 3.5). At the photoperiod loci, the alleles for photoperiod sensitivity prolonged time to heading. At both vernalisation loci, the winter alleles delayed heading. All of these QTLs except the one detected at the *Vrn-D1* locus displayed QTL-by-environment interaction. At photoperiod loci, the magnitude of the additive effects differed among environments. At the *Vrn-A1* locus, QTLs were detected only in the MexObr10_D_CS and AusYan10_FI_LS experiments. For the mid-maturity subset, no QTLs were detected for time to heading.

Table 3.6 Summary of QTLs detected for the Zadoks score and days from sowing to heading (ear emergence time) for the random subset of Gladius/Drysdale mapping population. If the additive effect is positive, the Drysdale allele is associated with higher Zadoks score (accelerated development) and delayed heading (longer time to ear emergence).

	Linkage	Position	Closest		Additive effect[a]						
QTL	group	cM	marker[a]	LOD	AusRos09_SI_CS	AusYan09_FI_CS	MexObr10_D_CS	AusYan10_FI_CS	AusYan10_FI_LS	AusUrr10_SI_CS	AusUrr10_D_CS
Qzad.aww-2B	2B	100	*Ppd-B1*	33.5	-5.3	-2.0					
Qeet.aww-2B	2B	117	*gwm0429*	22.4			3.1	1.9	1.4	3.7	4.3
Qzad.aww-2D	2D	52	*Ppd-D1*	31.1	7.5	5.7					
Qeet.aww-2D	2D	52	*Ppd-D1*	65.0			-3.9	-3.1	-2.7	-6.9	-6.2
Qeet.aww-5A	5A	260	*Vrn-A1*	8.2			0.7	-	0.7	-	-
Qeet.aww-5D	5D	0	*Vrn-D1*	5.4			-1.1	-1.1	-1.1	-1.1	-1.1

[a] – QTL not detected in the environment; blank space when trait was not measured in the environment

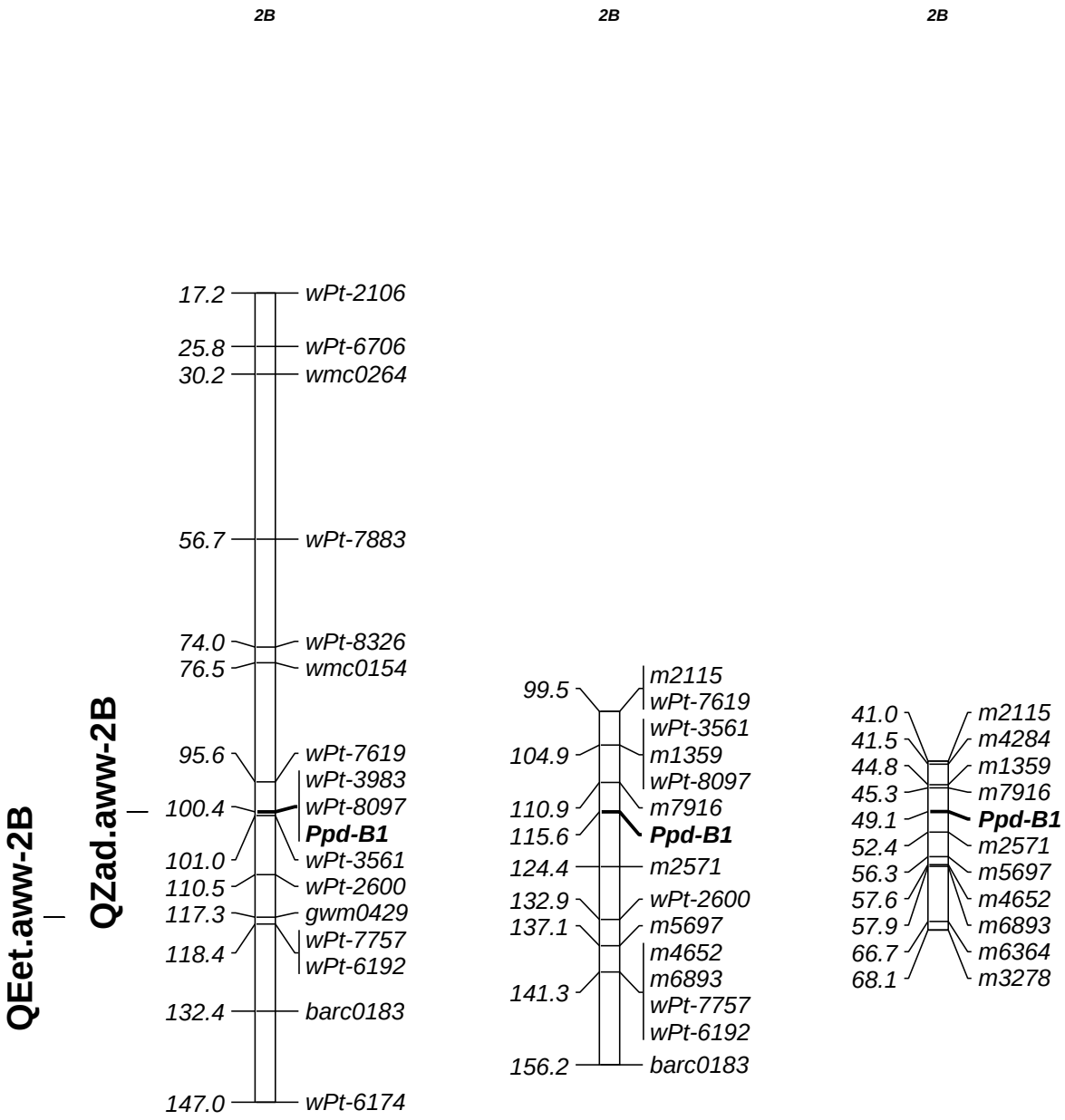

Figure 3.2 Zadoks score and heading date (ear emergence time) QTLs detected on chromosome 2B. The map on the left is the relevant portion of the linkage map for the random subset, the one in the middle is the relevant portion of the linkage map for the common subset (which serves as the link between the two maps) and the one on the right is the relevant portion of the linkage map for the mid-maturity subset.

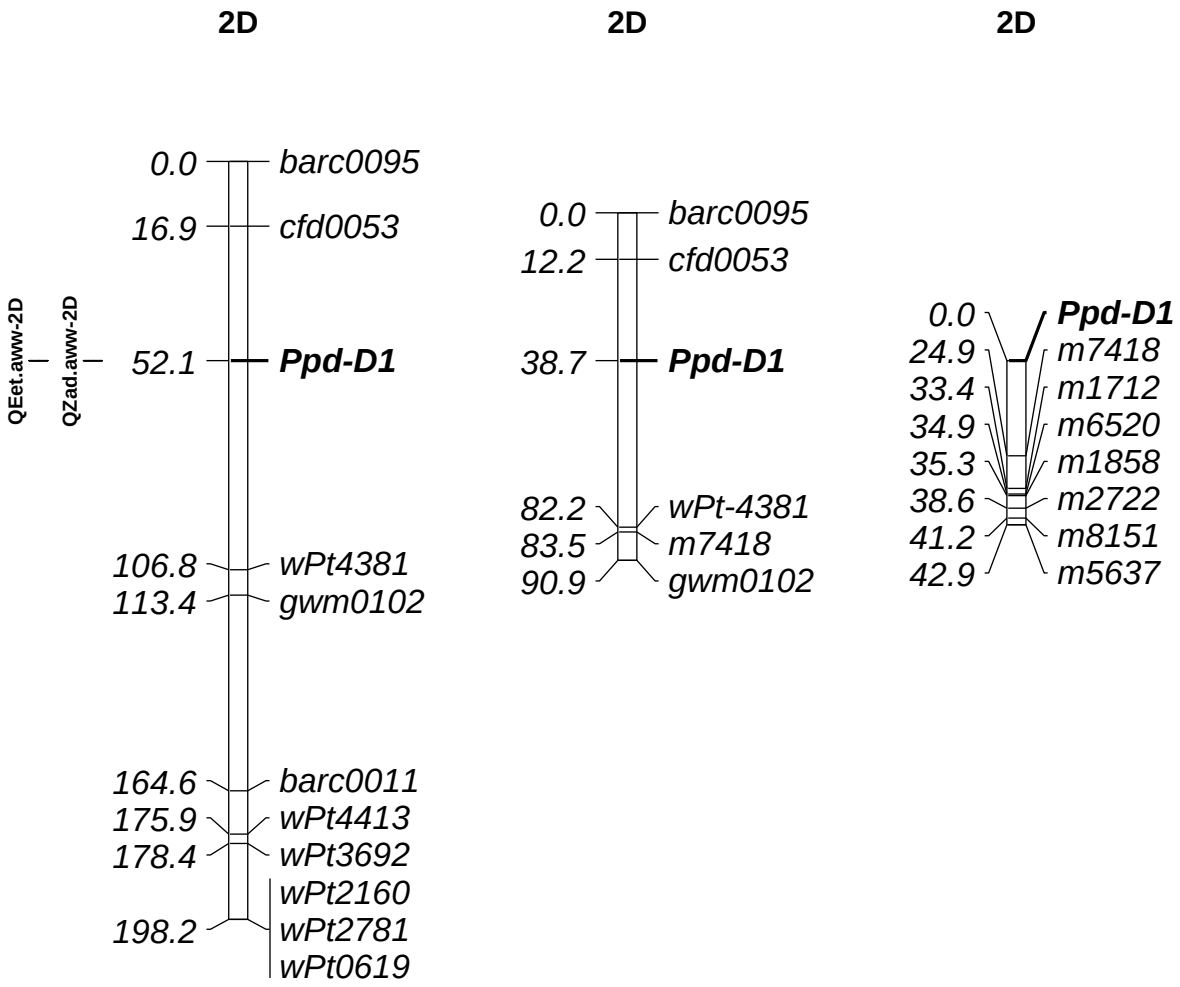

Figure 3.3 Zadoks score and heading date (ear emergence time) QTLs detected on chromosome 2D. The map on the left is for the random subset, the one in the middle is the relevant portion of the linkage map for the common subset (which serves as the link between the two maps) and the one on the right is the relevant portion of the linkage map for the mid-maturity subset.

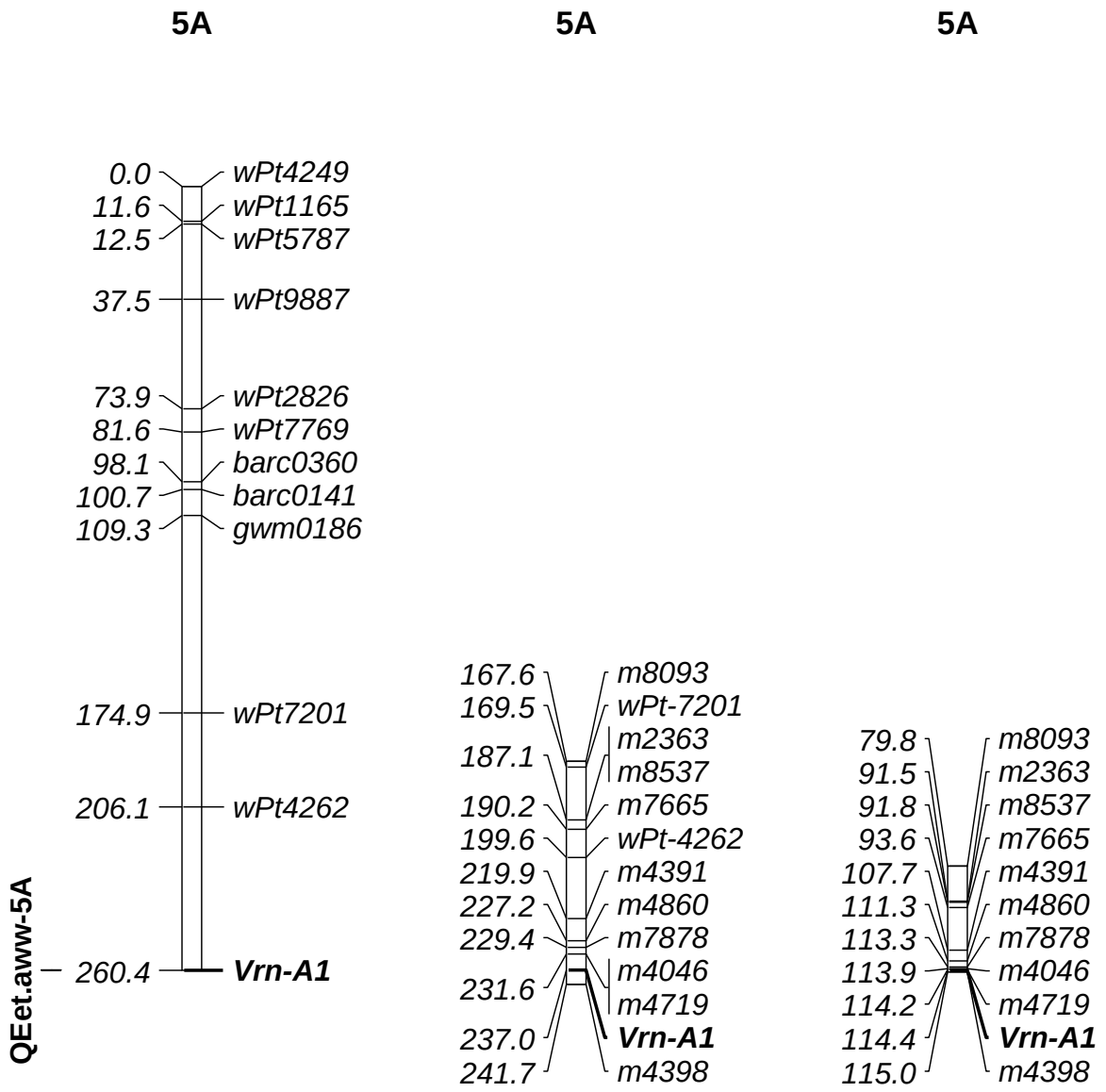

Figure 3.4 Heading date (ear emergence time) QTL detected on chromosome 5A. The map on the left is for the random subset, the one in the middle is the relevant portion of the linkage map for the common subset (which serves as the link between the two maps) and the one on the right is the relevant portion of the linkage map for the mid-maturity subset.

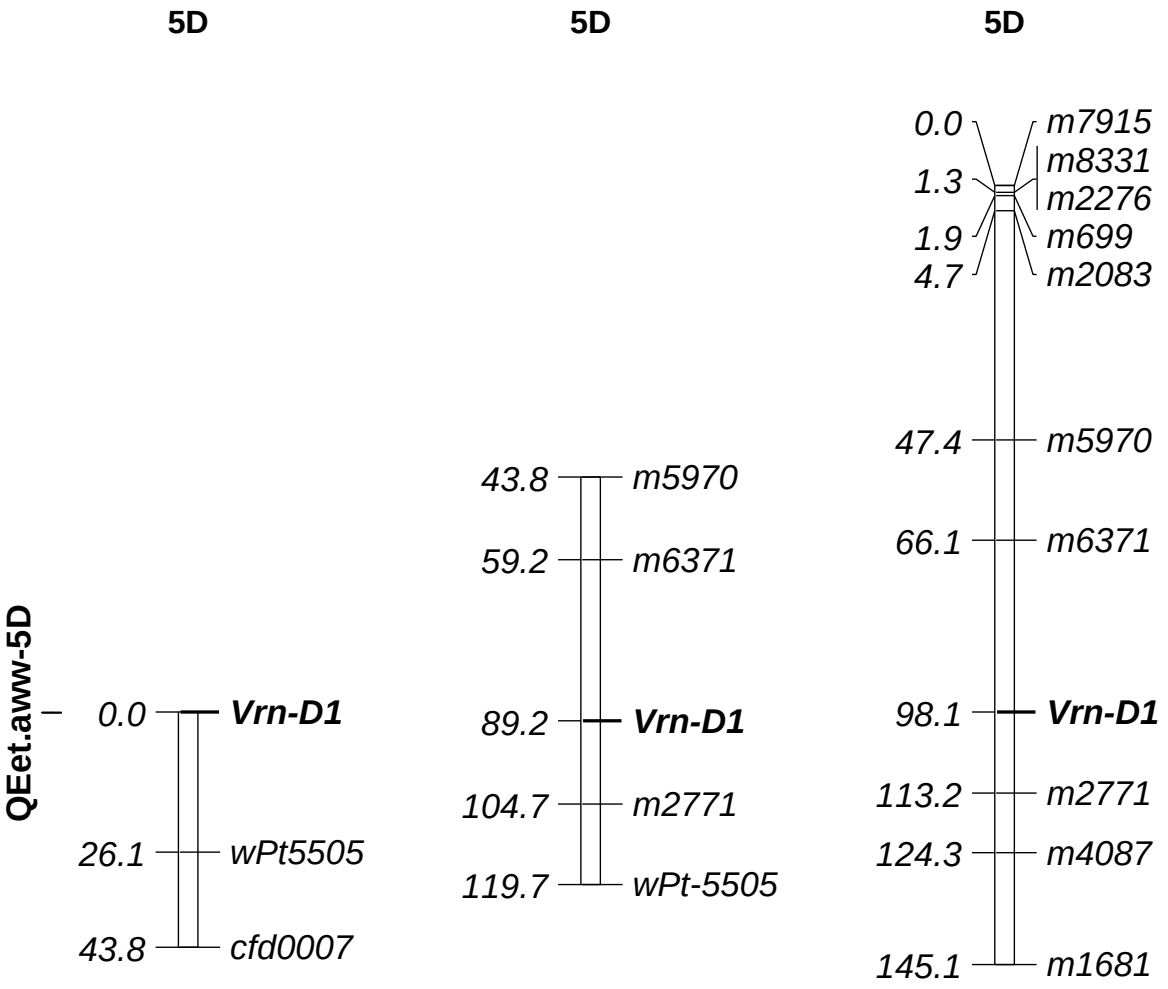

Figure 3.5 Heading date (ear emergence time) QTL detected on chromosome 5D. The map on the left is for the random subset, the one in the middle is the relevant portion of the linkage map for the common subset (which serves as the link between the two maps) and the one on the right is the relevant portion of the linkage map for the mid-maturity subset.

3.4 Discussion

The wheat cultivars Gladius and Drysdale have contrasting characteristics under heat and drought stress conditions (Fleury et al., 2010) making the Gladius/Drysdale mapping population suitable for genetic analysis of loci associated with response to drought and heat stress. Although the parents have similar time to heading, significantly differing in only one environment for which results are reported in this thesis Chapter, the progeny displayed large variation in time to heading and Zadoks score. Differences in time to heading can result in plants experiencing different stress conditions at different developmental stages. Variation in plant development can present problems for QTL analysis especially under drought and heat stress conditions. High temperatures often accelerate plant development and senescence and in the results reported in this thesis Chapter, plants in the experiments that had been sown late so that they would experience high temperatures mainly during grain filling had a shorter growing season.

Genotyping and phenotyping of random and mid-maturity subsets provided an opportunity to investigate the effects of selection based on heading date on linkage mapping. Selection based on heading date can lead to preferential selection of alleles from one parent thus causing segregation distortion. Although the selection significantly shifted allele frequency at two (*Ppd-B1* and *Vrn-A1*) of the four loci, all four loci were still segregating and could be mapped relative to molecular markers in the mid-maturity subset. Markers displaying segregation distortion are usually excluded from linkage map construction, but in the results reported in this thesis Chapter phenology genes were kept to test if they still had an effect on days to heading even after selection and they did not. Alleles from the same parent have the same effect on time to heading resulting in minimal differences in phenotypic data and leading to non-detection of QTLs as was observed here.

Time to heading was affected by the interaction of alleles in the four phenology loci. The allele combination at the photoperiod loci (sensitive *vs* insensitive) affected time to heading more than the allele combination at the vernalisation loci (winter *vs* spring) and QTL additive effects in Table 3.6 confirm this. This means that in these winter sown experiments, even if vernalisation requirements were satisfied, day length was still a major factor influencing time to heading. After selection, the combination involving alleles for photoperiod insensitivity resulted in late heading in the mid-maturity subset compared to the random subset. Selection excluded the early heading lines mostly carrying the alleles for photoperiod insensitivity, and

the remaining lines with alleles for photoperiod insensitivity were slightly late heading and in the 110-120 days to heading range. The combination involving alleles for photoperiod sensitivity resulted in earlier heading in the mid-maturity subset compared to the random subset. Similarly selection excluded most of the late heading lines carrying alleles for photoperiod sensitivity and this caused a shift towards the 110-120 days to heading range. Exclusion of early and late heading lines tended to be stabilising and might have minimised the expected phenotype of some allele combinations, such as photoperiod-insensitive and vernalisation-spring allele combinations (*aaaa* resulting in very early heading), and photoperiod-sensitive and vernalisation-winter allele combination (*bbvv* resulting in very late heading), leading to no differences in time to heading in the two subsets for these allele combinations.

The map distances between the phenology genes and flanking markers were different in the linkage maps of the two subsets except on chromosome 2B were the distances were similar. Selection and use of SNPs narrowed the gaps between flanking markers and phenology genes in the other three loci but on chromosome 2D, this led to loss of the portion of the chromosome. On chromosome 5D, selection and inclusion of SNPs led to addition of a portion of the chromosome. Changes in the types of markers might have affected the level of polymorphism detected in some chromosomal regions and thus affected map distances.

Both linkage maps have more than the desired 21 linkage groups for hexaploid wheat, with no DArT or SSR markers mapping to chromosomes 4D and 6D and no SNP markers assigned to chromosome 4D. This is similar to results in other studies, where no or few markers were mapped to chromosomes 3D, 4D and 6D (Akbari et al., 2006; Chalmers et al., 2001; Harker et al., 2001; Roder et al., 1998) due to low or no polymorphism in these chromosomes. Other studies also report poor map coverage for the D-genome (Chalmers et al., 2001; Chao et al., 2009; McIntyre et al., 2010). Chromosome 5D was too sparsely mapped to allow localisation of *Pina-D1* and *Pinb-D1* located on the short arm or *Vrn-D1* located on the long arm.

Zadoks score and time to heading were influenced by loci on chromosomes 2B, 2D, 5A and 5D at photoperiod and vernalisation genes that are well known to influence plant development, heading and flowering time (Eagles et al., 2009; Eagles et al., 2010; Trevaskis et al., 2007). Some of the detected QTL showed QTL-by-environment interactions. For the random subset, no association between the *Vrn-A1* locus and time to heading was found in

the experiments that were planted early when the temperatures were still relatively low and thus satisfied vernalisation needs. However, the vernalisation requiring winter allele delayed heading in late sown experiments in which the temperatures were higher during vegetative growth. For the random subset, the Gladius winter allele delayed time to heading at the *Vrn-D1* locus, but planting time did not influence vernalisation requirements as the effect was consistent in all environments. The *Vrn-D1* winter allele delays time to heading more than the *Vrn-A1* (Eagles et al., 2010) and here its additive effects were higher than for *Vrn-A1*. The *Vrn-D1* winter allele was still sensitive to temperature in late sown experiments though the temperatures had slightly increased. It has been reported that *Vrn-D1* has some residual vernalisation response that requires more days to be satisfied (Yoshida et al., 2010).

At the photoperiod loci, alleles for photoperiod-sensitivity delayed time to heading. The *Ppd-D1* locus had a larger effect than the *Ppd-B1* locus in all the environments. The plants were sown in winter when day length was short and thus affecting plants with the photoperiod sensitivity allele. The magnitudes of the QTL effects differed with environments with AusYan10_FI_LS which was sown late, when day length was becoming less limiting, being the least affected at both photoperiod loci.

The DArT, SSR, SNP and known genes comprising map for the common subset (middle map in Figures 3.3, 3.4, 3.5 and 3.6) serves as the link between the maps for the random subset and the mid-maturity subset. Although the genetic maps constructed in this thesis Chapter still have gaps and more than the desired 21 linkage groups, they provide an opportunity for QTL mapping of important physiological, agronomic and quality traits. Selection of mid-maturity lines did not result in severe segregation distortion in some of the phenology loci tested. Also, selection did not fix the phenology loci as they were still polymorphic and were added to the mid-maturity subset map. Use of similar maturing lines would result in a more uniform population and allow for the potential minimalisation of time to heading as a confounding factor through ensuring that plants are exposed to similar conditions at similar growth stages and thus offer more reliable phenotypic data.

Chapter 4

Genetic control of grain yield and grain characteristics in a wheat population grown under a range of environmental conditions

4.1 Introduction

The grain harvested from field experiments described in Chapter 3 of this thesis was analysed for grain characteristics and yield was calculated. The bread wheat industry aims to produce large quantities of high quality grain. Grain weight and number, which are often negatively correlated with each other (Miralles and Slafer, 2007) are important components of grain yield. Grain weight, which is commonly expressed as thousand grain weight, is also a grain quality factor, with varieties and grain lots required to meet minimum standards for the intended market. Screenings (small grains that can pass through a sieve of defined size) are undesirable and can compromise milling. Test weight (grain density) is a measure of the weight of grain of known volume. Low test weight increases the space required for (and thus the cost of) grain transportation and storage. Grain shape can affect grain classification, endosperm content and flour extraction (Berman et al., 1996; Symons and Fulcher, 1988). Grain uniformity allows even absorption of water during conditioning prior to milling and use of constant roller gaps during milling, ensuring steady flow of grain and thus potentially maximizing flour extraction, which is important in the milling industry (Evers and Millar, 2002; Marshall et al., 1986).

Other important grain characteristics are protein content and hardness. Grain with high protein content and hard texture is usually favoured for production of bread and noodles, while grain with low protein content and soft texture is preferred for cookies and biscuits. Harder grain requires more energy to mill and suffers more starch damage during milling than softer grain. Within the broad categories of ‘hard’ and ‘soft’ wheats, there is small within class variations that can still slightly influence flour extraction, processing and end use quality (Martin et al., 2001; Wang et al., 2012a).

Grain yield and grain characteristics are complex traits which are under the influence of the genotype, the environment and genotype-by-environment interactions. Genetic studies allow for dissection of the genetic control and detection of quantitative trait loci (QTLs) affecting these complex traits. Many QTLs, with both minor and major effects, have been detected for grain yield (Groos et al., 2003; Kirigwi et al., 2007), thousand grain weight (Groos et al.,

2003; Tsilo et al., 2010), percentage screenings (McIntyre et al., 2010), test weight (Sun et al., 2009; Tsilo et al., 2010), grain shape dimensions (length, width, thickness, aspect ratio, roundness and area) (Breseghello and Sorrells, 2007; Campbell et al., 1999; Dholakia et al., 2003; Gegas et al., 2010; Giura and Saulescu, 1996; Ramya et al., 2010; Sun et al., 2009; Tsilo et al., 2010), grain protein content (Groos et al., 2003; Prasad et al., 1999; Tsilo et al., 2010), grain hardness (Groos et al., 2004) and flour extraction (Tsilo et al., 2011).

Functional markers have been identified for some of the traits, for example grain hardness has been extensively studied and is largely controlled by the *Ha* (hardness) locus (Symes, 1965) located on the short arm of chromosome 5D (Morris, 2002; Sourdille et al., 1996). The *Ha* locus contains puroindoline a (*Pina-D1a*), puroindoline b (*Pina-D1b*), and grain softness protein (*GSP-1*) genes (Bhave and Morris, 2008; Morris, 2002). While the role of *GSP-1* in grain hardness is not clear, puroindoline allele combinations at this locus largely determine the phenotype of the wheat grain, with *Pina-D1a/Pinb-D1a* producing soft grain, *Pina-D1a/Pinb-D1b* producing moderately hard grain and *Pina-D1b/Pinb-D1a* producing very hard grain (Cane et al., 2004; Giroux and Morris, 1997; Giroux and Morris, 1998; Lillemo et al., 2006). The *Ha* locus can sometimes affect grain protein content (Turner et al., 2004).

A marker (*TaGW2*) associated with grain shape and weight in wheat has been developed (Su et al., 2011). The marker was developed through comparative studies with rice (Song et al., 2007). Regions close to this marker have been shown to be associated with grain yield in other studies (Snape et al., 2007; Sun et al., 2009). Some other markers such as *TaCwi-A1* on chromosome 2A (Ma et al., 2012) and *TaSus2-2B* on chromosome 2B (Jiang et al., 2011), have been detected in other genomic regions and are associated with grain shape and weight.

Some of the genetic studies listed above have measured quality traits in grain produced under favourable temperatures and with sufficient soil moisture. The genetic control of these traits under high temperatures and moisture deficit is not clearly understood. The yield, shape and characteristics of wheat grain are severely affected by environmental conditions such as moisture deficit and high temperatures (Gooding et al., 2003; Guttieri et al., 2001; Labuschagne et al., 2009; Weightman et al., 2008). These environmental conditions are common occurrences in many wheat growing regions and usually coincide with the sensitive grain filling phase.

The genetic approach to understanding the control of yield, grain shape and characteristics offers opportunities to predict and improve the performance of wheat varieties through

identifying potentially favourable alleles from both parents that can be combined in the progeny. It is hypothesised that QTL for some traits are environment or stress specific. Therefore, the aim of this study was to understand the genetic control of yield, grain shape and characteristics through identifying marker-trait associations in grain produced under a range of conditions including drought and heat conditions.

4.2 Materials and Methods

4.2.1 Plant material and field experiments

A random set of 205 Gladius/ Drysdale recombinant inbred lines (RILs) was grown in seven environments and a subset of 60 of these RILs was grown in two other environments under a range of conditions (Table 4.1). The characteristics of the parents and the experimental conditions were described in Chapter 3 of this thesis. Briefly, heat experiments were conducted at Yanco, New South Wales, Australia during the 2009 and 2010 seasons. These involved well watered and early sown experiments designed to escape heat stress, and well watered and late sown experiments designed to be exposed to heat stress during grain filling. At Urrbrae, South Australia in the 2010 season, the experiments were grown under heat, drought and well watered conditions. A drought experiment was conducted at Ciudad Obregon, Mexico in the 2010 season.

Table 4.1 Description of the environments in which the experiments were conducted, the lines tested and traits measured in each environment.

Environment[a]	Location[b]	Year	Latitude	Longitude	Altitude (M)	Lines tested	Traits measured[c,d]
MexObr09_D_LS	Ciudad de Obregon	2009	27° N	109° W	38	Random set of 205 RILs	Yield, GN, TGW
AusYan09_FI_CS	Yanco (NSW)	2009	34° S	146° E	136	Random set of 205 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC, % screenings
AusYan09_FI_LS	Yanco (NSW)	2009	34° S	146° E	136	Random set of 205 RILs	FE, Yield, GN, TGW, TW, PSI, GPC, % screenings
AusYan10_FI_CS	Yanco (NSW)	2010	34° S	146° E	136	Random set of 205 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC, % screenings
AusYan10_FI_LS	Yanco (NSW)	2010	34° S	146° E	136	Random set of 205 RILs	Weather damaged
AusUrr10_SI_CS	Urrbrae (SA)	2010	34° S	138° E	225	Random set of 205 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC
AusUrr10_D_CS	Urrbrae (SA)	2010	34° S	138° E	225	Random set of 205 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC
AusUrr10_H_SI_CS	Urrbrae (SA)	2010	34° S	138° E	225	Subset of 60 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC
AusUrr10_HD_CS	Urrbrae (SA)	2010	34° S	138° E	225	Subset of 60 RILs	FE, length, width, thickness, area, aspect ratio, roundness, yield, GN, TGW, TW, PSI, GPC

[a]Aus for Australia, Mex for Mexico, D for drought, H for heat, DH for drought and heat combined, CS for conventional sowing, LS for late sowing, FI for flood irrigation, SI for sprinkler irrigation

[b]NSW for New South Wales, SA for South Australia

[c]FE for flour extraction**,** GN for grain number, TGW for thousand grain weight, TW for test weight, PSI for particle size index, GPC for grain protein content

[d]Weather damaged and no analysis done

4.2.2 Harvesting and grain cleaning

For the experiments conducted at Urrbrae, grain was hand harvested and threshed with Kimseed Multi-Thresher CW08 (Kimseed Engineering, Australia). For the experiments conducted at Yanco, grain was mechanical harvested using a Kingaroy plot harvester fitted with a grain thresher (Kingaroy Engineering, Australia). Prior to quality analysis, harvested grain from experiments conducted at Yanco, was thoroughly cleaned using a grain cleaner (Kimseed Engineering, Australia). The grain cleaner has two round holed sieves, one of size 4.7 mm and the other of size 2.3 mm and the grain that passes through the 4.7 mm sieve but not the 2.3 mm sieve is collected and subsequently used in quality analysis.

4.2.3 Trait measurements

Different traits were measured in the different experiments (Table 4.1). Test weight, grain protein content, particle size index and flour extraction were measured for each experiment conducted in Australia. Percentage screenings was measured for each of the field experiments conducted at Yanco. Grain shape characteristics (length, width, thickness, area, aspect ratio and roundness) were measured for each of the experiments conducted in Australia except for AusYan09_FI_LS, the late-sown experiment at Yanco in 2009, from which all the grain had been milled prior to grain shape measurement. No analysis was done for AusYan10_FI_LS, the late-sown experiment at Yanco in 2010, from which the grain was severely weather damaged.

Yield was calculated as:

Yield (kg/ha) = clean grain weight (g) × (dry weight (g)/fresh weight (g))/ plot area (m^2) × 10.

Thousand grain weight (g) was estimated by filling a vial with clean grain and using a Contador grain counter (Pfueffer GmBH, Germany) to count out 250 grains. Each sample of 250 grains was weighed and its weight multiplied by four to provide the estimated weight of 1000 grains.

Grain number was calculated as:

Grain number = (clean grain weight (g)/thousand grain weight (g)) × 1000

A Graintech^{TM} grain screener (Engineering Service Providers, Australia) was used to measure percentage screenings. For each sample, a 300 g subsample was placed on a 2 mm slotted agitator screen and shaken 40 times to separate small grain (screenings) from plump grain. The weight of the sample passing through the screen was reported as a percentage of the initial sample weight.

A sliding test weight instrument (Wagga Wagga Agricultural Institute, in-house design) was used to measure test weight. Grain was steadily poured into a space of set volume, then weighed and the result was converted to kilograms per hectolitre (kg/hl) based on calibration to a full scale Franklin chrondrometer.

The conversion formula used was: kg/hl = (weight (g) × 1.9995) + 5.5635

A SeedCount digital imaging analysis scanner version 2.0 (Weiss Enterprises) was used to measure the grain shape dimensions length, width, thickness and calculate area, aspect ratio and roundness (Figure 4.1 and Table 4.2). The method requires about 30 g of grain sample and is non-destructive.

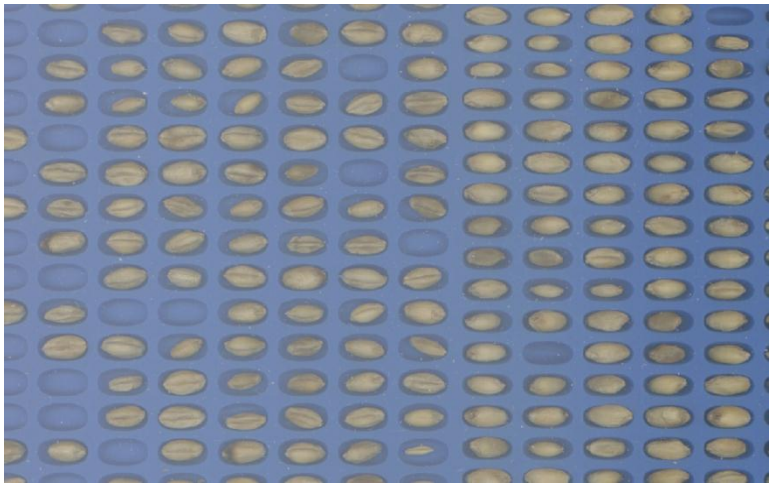

Figure 4.1 Indented tray holding grains for measuring grain dimensions using a SeedCount digital image system.

Table 4.2 Definition of the parameters estimated using the SeedCount digital imaging analysis system (Dell'Aquila, 2004).

Parameter	unit	definition
grain length	mm	The longest line through the grain almost always running from the embryo to the distal end
grain width	mm	The longest line through the grain at a right angle to the length
grain thickness	mm	The smallest dimension of the grain, it is the vertical measurement through the grain perpendicular to both the length and width
grain area	mm^2	The area of the polygon that defines the grain's outline
aspect ratio		Grain aspect ratio is a measure of grain shape and is calculated as the ratio of grain length to width. Circular grain will have an aspect ratio of one, while long thin grains will have an aspect ratio of five.
roundness		Roundness, a measure of average grain sphericity, is a three dimensional version of the aspect ratio. It is calculated as follows: Roundness = (length/width + length/thickness + width/thickness)/3 A perfect sphere has a value of one and wheat grain has roundness values on average of 1.93 and standard deviation of 0.25.

Percentage moisture content, grain protein content and particle size index (PSI) were estimated by NIR (RACI-CCD, 2010) using a Foss 6500 NIR instrument (FOSS NIR Systems, Inc., Laurel, MD). For grain moisture content, the NIR prediction was based on calibration to air oven moisture determination (AACC, 1999). For grain protein content, the NIR prediction was based on calibration to Leco protein determination (RACI-CCD 2010), and reported on an 'as is' basis. For PSI, the calibration was based on the particle size index method (AACC 1999). High PSI units correspond to low hardness (soft grain).

For the grain harvested from each experimental unit at Yanco, approximately 1 kg of grain was conditioned overnight to 15% moisture content (AACC, 1999) and milled on a Bühler MLU-202 laboratory test mill (Bühler AG, Uzwil, Switzerland). Recovered flour was weighed and flour extraction was expressed as a percentage of the initial grain weight. For grain harvested from each experimental unit of the polytunnel and netting experiments at Urrbrae, samples were allowed to equilibrate for three weeks in a conditioning cabinet and an equal amount of water was added to each sample before milling on a Quadrumat Junior test

mill (Brabender, Germany). Room temperature, humidity and mill temperature were kept as constant as is possible by using an air conditioned room and allowing the mill to cool down during the course of milling. Percentage flour extraction was calculated as a fraction of total products (flour and bran).

4.2.4 Statistical and genetic analysis

A two-step statistical and genetic analysis was done with GenStat (Payne et al., 2009) as described in Chapter 3 of this thesis. Briefly, a spatial analysis (Gilmour et al., 1997) was done to generate best linear unbiased estimates (BLUEs) taking into account the experimental designs. Multi-environment QTL analysis was done using composite interval mapping as described in Chapter 3 of this thesis. The linkage map used was described in Chapter 3 of this thesis, and consists of 720 DArT, SSR and gene-based markers distributed over 28 linkage groups (Appendix 1). Phenotypic correlations for the 60 subset of lines were calculated among the multi-environment means and for individual environment means with GenStat. Map diagrams were drawn using MapChart software (Voorrips, 2002).

4.3 Results

4.3.1 Performance of RILs and parents

The range of values for the RILs exceeded the value for each parent and heritability for all traits was low in the presence of heat (Tables 4.3 and 4.4). Heritability for the traits ranged from as low as 12% (grain number under both heat and drought stress in the polytunnel (AusUrr10_HD_CS) and grain width under heat stress in the polytunnel (AusUrr10_H_SI_CS)) to as high as 96% (grain length in the early sown control experiment at Yanco (AusYan09_FI_CS)). The thickness, width and area of grain from the 2010 experiment conducted at Yanco (AusYan10_FI_CS) were all greater than those for grain from other experiments for both parents and RILs, probably due to favourable weather conditions experienced that year, with higher rainfall and lower temperatures than at the same location in 2009. At Urrbrae in 2010, the grain harvested from the experiment that was managed to impose drought and heat conditions (AusUrr10_HD_CS) was thinner, narrower and longer and had higher aspect ratio and roundness values than that harvested from the control experiment (AusUrr10_SI_CS). Under most experimental conditions, grains of Gladius were wider, thicker and shorter than those of Drysdale (Table 4.3).

In the experiment conducted in Mexico, thousand grain weight was lower than in the experiments conducted in Australia. Gladius had higher thousand grain weight than Drysdale in the experiments conducted in Australia but lower mean weight in the experiment conducted in Mexico (Table 4.4).

There was a decrease in grain number and an increase in mean protein content due to heat or drought conditions compared to the controls (AusYan09_FI_CS and AusUrr10_SI_CS) in the experiments conducted in Australia.

Table 4.3 Mean grain dimensions for Gladius and Drysdale and the mean, minima, maxima and standard deviations for the same characteristics for 205 Gladius/Drysdale recombinant inbred lines (RILs) and (in parentheses) for a subset of 60 RILs for grain harvested from experiments in six environments.

		Parents		Population				Broad sense heritability (%)
Trait	Experiment	Gladius	Drysdale	RIL mean	minimum	maximum	Standard deviation	
Length	AusYan09_FI_CS	6.58	6.58	6.54 (6.54)	6.08 (6.16)	7.16 (6.92)	0.20 (0.18)	96
	AusYan10_FI_CS	6.83	6.63	6.62 (6.66)	6.12 (6.23)	7.23 (6.98)	0.22 (0.19)	93
	AusUrr10_SI_CS	6.50	6.52	6.49 (6.42)	6.05 (6.12)	7.12 (6.96)	0.20 (0.17)	87
	AusUrr10_D_CS	6.37	6.61	6.43 (6.43)	5.78 (6.07)	7.07 (6.84)	0.25 (0.17)	85
	AusUrr10_H_SI_CS	6.59	6.61	6.56	6.10	7.13	0.19	26
	AusUrr10_HD_CS	6.48	6.54	6.65	6.20	7.11	0.20	25
Width	AusYan09_FI_CS	3.36	3.28	3.27 (3.26)	3.01 (3.01)	3.56 (3.46)	0.12 (0.09)	91
	AusYan10_FI_CS	3.75	3.51	3.56 (3.58)	3.17 (3.33)	3.77 (3.76)	0.11 (0.08)	85
	AusUrr10_SI_CS	3.67	3.49	3.53 (3.50)	3.16 (3.19)	3.88 (3.73)	0.12 (0.11)	73
	AusUrr10_D_CS	3.56	3.45	3.48 (3.47)	2.96 (3.14)	3.90 (3.77)	0.20 (0.15)	79
	AusUrr10_H_SI_CS	3.72	3.56	3.53	3.27	3.84	0.11	12
	AusUrr10_HD_CS	3.32	3.13	3.44	3.00	3.68	0.13	16
Thickness	AusYan09_FI_CS	3.00	2.98	2.93 (2.93)	2.74 (2.76)	3.18 (3.07)	0.08 (0.07)	81
	AusYan10_FI_CS	3.21	3.01	3.07 (3.07)	2.86 (2.89)	3.33 (3.22)	0.09 (0.08)	85
	AusUrr10_SI_CS	3.20	3.00	3.03 (2.98)	2.69 (2.76)	3.33 (3.19)	0.11 (0.09)	72
	AusUrr10_D_CS	3.13	3.05	3.02 (3.00)	2.63 (2.69)	3.35 (3.27)	0.16 (0.13)	89
	AusUrr10_H_SI_CS	3.18	3.06	3.05	2.88	3.28	0.09	17
	AusUrr10_HD_CS	2.92	2.80	2.97	2.70	3.16	0.10	17
Area	AusYan09_FI_CS	15.63	15.31	15.18 (15.15)	13.13 (13.69)	17.72 (16.69)	0.85 (0.70)	93
	AusYan10_FI_CS	18.28	16.68	16.81 (17.06)	13.61 (15.11)	19.56 (18.76)	1.01 (0.78)	89
	AusUrr10_SI_CS	16.92	16.30	16.39 (16.04)	14.07 (14.07)	19.25 (17.77)	0.91 (0.79)	76
	AusUrr10_D_CS	16.10	16.35	16.04 (15.98)	12.08 (13.87)	19.38 (18.02)	1.45 (0.98)	81
	AusUrr10_H_SI_CS	17.37	16.90	16.54	14.88	18.84	0.82	18
	AusUrr10_HD_CS	15.17	14.45	16.20	13.59	18.24	0.89	19

Table 4.3 (continued)

Trait	Experiment	Parents		Population				Broad sense heritability (%)
		Gladius	Drysdale	RIL mean	minimum	maximum	Standard deviation	
Roundness	AusYan09_FI_CS	1.77	1.78	1.79 (1.80)	1.67 (1.71)	1.94 (1.91)	0.05 (0.04)	88
	AusYan10_FI_CS	1.71	1.76	1.74 (1.74)	1.63 (1.63)	2.00 (1.83)	0.05 (0.04)	83
	AusUrr10_SI_CS	1.66	1.74	1.73 (1.74)	1.60 (1.61)	1.95(1.83)	0.06 (0.05)	82
	AusUrr10_D_CS	1.66	1.74	1.72 (1.73)	1.57 (1.62)	1.91 (1.86)	0.06 (0.05)	84
	AusUrr10_H_SI_CS	1.68	1.74	1.73	1.60	1.85	0.05	22
	AusUrr10_HD_CS	1.78	1.87	1.79	1.66	1.92	0.06	28
Aspect	AusYan09_FI_CS	1.91	1.94	1.95 (1.95)	1.79 (1.84)	2.16 (2.11)	0.08 (0.07)	92
ratio	AusYan10_FI_CS	1.77	1.84	1.82 (1.81)	1.66 (1.70)	1.96 (1.90)	0.06 (0.05)	86
	AusUrr10_SI_CS	1.73	1.83	1.80 (1.80)	1.63 (1.67)	2.11 (1.96)	0.08 (0.07)	83
	AusUrr10_D_CS	1.74	1.88	1.81 (1.82)	1.63 (1.67)	2.13 (1.98)	0.09 (0.08)	81
	AusUrr10_H_SI_CS	1.73	1.82	1.82	1.64	1.98	0.07	20
	AusUrr10_HD_CS	1.92	2.07	1.89	1.70	2.08	0.09	28

Table 4.4 Mean grain yield, characteristics and % flour extraction for Gladius and Drysdale and the mean, minima, maxima and standard deviations for the same characteristics for 205 Gladius/Drysdale recombinant inbred lines (RILs) and (in parentheses) for a subset of 60 RILs for grain harvested from experiments in different environments.

				Population				
		Parents						Broad sense heritability (%)
Trait	Experiment	Gladius	Drysdale	mean	minimum	maximum	standard deviation	
Yield	AusYan09_FI_CS	5058	4473	4346 (4416)	2688 (2984)	5986 (5396)	661 (570)	75
(kg/ha)	AusYan09_FI_LS	1797	1499	1526 (1609)	554 (659)	2967 (2967)	461 (449)	45
	AusYan10_FI_CS	5512	5470	5387 (5500)	4084 (4287)	7557 (6730)	466 (506)	66
	AusUrr10_SI_CS	12490	6793	6656 (6645)	2271 (3020)	15350 (10408)	1810 (1916)	73
	AusUrr10_D_CS	3265	4718	5201 (5275)	1672 (2878)	8270 (7695)	1361 (1211)	45
	AusUrr10_H_SI_CS	4767	6265	5807	3101	9947	1453	16
	AusUrr10_HD_CS	2432	2318	4282	2461	6502	963	16
	MexObr10_D_LS	2900	3500	2850 (2752)	1329 (1329)	4155 (3981)	502 (589)	51
Grain	AusYan09_FI_CS	13267	12293	12256 (12604)	7500 (9207)	15559 (14839)	1451 (1337)	70
number	AusYan09_FI_LS	4844	3480	4183 (4501)	684 (1797)	9721 (7918)	1567 (1626)	39
	AusYan10_FI_CS	11246	12414	12478 (12518)	9040 (9895)	18019 (15155)	1398 (1253)	69
	AusUrr10_SI_CS	27415	15898	14346(14007)	5613 (5916)	36772 (24398)	4094 (4158)	69
	AusUrr10_D_CS	7149	10300	11705 (11859)	4232 (6650)	19241 (16062)	2591 (2291)	44
	AusUrr10_H_SI_CS	9613	13441	12980	7658	20925	2969	13
	AusUrr10_HD_CS	6223	6751	10135	6170	15902	2048	12
	MexObr10_D_LS	8651	9437	8633 (8406)	4280 (4280)	12454 (11152)	1352 (1528)	49
Thousand	AusYan09_FI_CS	38	36	35 (35)	27 (30)	47 (42)	3 (3)	84
grain weight (g)	AusYan09_FI_LS	36	35	35 (35)	24 (25)	45 (41)	4 (4)	81
	AusYan10_FI_CS	49	44	44 (44)	33 (39)	54 (51)	3 (3)	84
	AusUrr10_SI_CS	48	43	44 (42)	33 (34)	54 (50)	4 (4)	73
	AusUrr10_D_CS	46	46	44 (44)	29 (34)	60 (54)	7 (5)	82
	AusUrr10_H_SI_CS	50	47	45	37	56	4	15
	AusUrr10_HD_CS	39	34	42	31	49	4	19
	MexObr10_D_LS	34	37	33 (33)	24 (27)	42 (38)	3 (2)	85

Table 4.4 (continued)

		Parents		Population				
Trait	Experiment	Gladius	Drysdale	mean	minimum	maximum	standard deviation	Broad sense heritability (%)
Test weight	AusYan09_FI_CS	77.1	79.8	76.9 (76.9)	70.4 (71.3)	81.3 (80.9)	2.0 (2.0)	78
(kg/hl)	AusYan09_FI_LS	73.0	73.8	72.3 (72.1)	64.9 (65.2)	77.0 (76.6)	2.1 (2.4)	80
	AusYan10_FI_CS	75.2	79.4	76.6 (76.8)	71.0 (73.9)	79.8 (79.8)	1.6 (1.5)	84
Screenings	AusYan09_FI_CS	5.2	8.7	7.6 (8.6)	3.0 (3.0)	17.9 (17.9)	2.8 (3.5)	87
(%)	AusYan09_FI_LS	4.8	7.8	7.3 (7.7)	2.9 (3.3)	22.5 (17.6)	3.2 (3.6)	79
	AusYan10_FI_CS	3.6	4.6	4.6 (4.6)	1.8 (2.0)	9.2 (8.9)	1.4 (1.4)	38
Grain	AusYan09_FI_CS	14.2	13.6	14.1 (14.0)	12.0 (12.7)	16.6 (15.2)	0.8 (0.5)	89
protein	AusYan09_FI_LS	15.4	15.2	15.2 (15.3)	13.8 (13.8)	18.1 (18.1)	0.8 (0.8)	51
content (%)	AusYan10_FI_CS	11.2	11.9	11.6 (11.4)	10.3 (10.3)	13.5 (12.5)	0.6 (0.5)	48
	AusUrr10_SI_CS	12.7	12.8	13.1 (12.8)	10.7 (11.3)	15.9 (15.0)	1.0 (0.8)	50
	AusUrr10_D_CS	13.3	12.1	13.5 (13.0)	11.4 (11.4)	17.9 (14.8)	1.1 (0.8)	75
	AusUrr10_H_SI_CS	13.4	12.8	13.0	11.2	14.8	0.7	17
	AusUrr10_HD_CS	14.7	14.2	13.0	11.3	15.2	0.9	14
Particle	AusYan09_FI_CS	14.7	13.5	14.4 (14.7)	7.5 (8.0)	20.5 (19.0)	2.3 (2.4)	77
size	AusYan09_FI_LS	18.4	18.1	17.8 (18.2)	11.0 (12.0)	25.0 (23.0)	2.6 (2.5)	52
index	AusYan10_FI_CS	16.8	13.3	16.2 (16.4)	8.90 (12.9)	21.8 (20.0)	2.3 (1.9)	60
	AusUrr10_SI_CS	13.0	14.3	12.9 (12.9)	5.2 (7.2)	20.5 (18.6)	3.1 (2.8)	58
	AusUrr10_D_CS	11.5	9.5	11.1 (10.6)	3.1 (4.7)	23.1 (18.7)	3.3 (2.9)	60
	AusUrr10_H_SI_CS	12.0	8.0	12.7	6.2	18.0	2.4	20
	AusUrr10_HD_CS	19.6	14.8	8.8	0.9	12.6	2.5	16
Flour	AusYan09_FI_CS	76.2	74.9	75.4 (75.1)	71.3 (71.8)	78.7 (78.1)	1.4 (1.4)	65
extraction	AusYan09_FI_LS	76.1	75.7	75.3	66.10	77.8	1.5	56
	AusYan10_FI_CS	78.2	77.8	78.0 (77.8)	75.5 (76.1)	81 (79.5)	0.9 (0.9)	54
	AusUrr10_SI_CS	65.7	65.0	64.8 (65.0)	57.4 (59.4)	68.9 (68.9)	2.2 (2.0)	85
	AusUrr10_D_CS	66.5	65.2	65 (65.4)	58.4 (59.7)	70 (68.7)	2.3 (2.3)	91
	AusUrr10_H_SI_CS	65.5	64.5	64.9	58.7	68.9	2.3	22
	AusUrr10_HD_CS	67.1	62.9	64.9	58.9	68.9	2.4	22

4.3.1.1 Correlation among traits

There were some significant phenotypic correlations between some grain traits for the 60 similar maturing lines (Table 4.5). The direction and magnitude of the correlations were similar for both the 60 similar maturing lines and the whole population of 205 lines. Among the multi-environment means for traits measured, the strongest correlations (r = 0.90) were between grain number and yield and between grain area and thousand grain weight. Strong correlations were also observed between grain width and grain thickness, and between thousand grain weight with both grain width and thickness. Grain length was correlated with grain width but not with grain thickness. As expected, grain area, aspect ratio and roundness were each correlated with their component traits and aspect ratio and roundness were correlated with each other. Flour extraction was most correlated with particle size index. Grain yield exhibited very strong positive correlation with grain number, moderate positive correlation with test weight and thousand grain weight and moderate negative correlation with grain protein content. Thousand grain weight and test weight were positively correlated with each other and negatively correlated with percentage screenings.

Some specific within-environment correlations were also observed (Appendix 3). Grain width and grain number were positively correlated (r = 0.32) in AusUrr10_SI_CS but negatively (r = -0.31) in AusYan10_FI_CS and area and roundness were positively correlated in AusYan10_FI_CS (r = 0.35) and negatively (r = -0.33) in AusUrr10_D_CS. Grain protein content was negatively correlated with thousand grain weight and test weight in experiments conducted at Urrbrae but positively correlated with these traits in an experiment conducted at Yanco. Under heat conditions (AusYan09_FI_LS) particle size index and percentage screenings were positively correlated (r = 0.32) but negatively (r = -0.32) in the early sown experiment (AusYan09_FI_CS). Percentage screenings were negatively correlated (r = -0.46) with yield only in the 2009 experiments conducted at Yanco (AusYan09_FI_LS).

Table 4.5 Significant ($p < 0.001$) phenotypic correlation coefficients among the multi-environment means for grain morphology, yield, grain characteristic traits and flour extraction for a set of 60 Gladius/Drysdale recombinant inbred lines.

	Length	Width	Thickness	AR	Roundness	Grain area	GN	Yield	TKW	TW	PSI
Length	-	-	-	-	-	-	-	-	-	-	-
Width	0.28	-	-	-	-	-	-	-	-	-	-
Thickness	-	0.89	-	-	-	-	-	-	-	-	-
Aspect ratio (AR)	0.53	-0.67	-0.66	-	-	-	-	-	-	-	-
Roundness	0.61	-0.54	-0.67	0.95	-	-	-	-	-	-	-
Grain area	0.80	0.78	0.63	-	-	-	-	-	-	-	-
Grain number (GN)	-	-	-	-	-	-	-	-	-	-	-
Yield	-	0.41	-	-	-	0.38	0.90		-	-	-
Thousand grain weight (TGW)	0.61	0.84	0.81	-	-	0.90		0.43	-	-	-
Test weight (TW)	-	0.33	0.37	-0.32	-0.33		0.36	0.47	0.43		-
Percentage screenings	-	-0.42	-0.59		0.29	-0.35	-	-	-0.58	-0.31	-
Grain protein content (GPC)	-	-	-	-	-	-0.29	-0.32	-0.35	-	-	-
Particle size index (PSI)	-	-	-	-	-	-	-	-	-	-	-
Flour extraction (FE)	-	-	-	-	-	-	-	-	-	-	0.45

4.3.2 QTL detected

Quantitative trait loci for yield, flour extraction, grain shape and characteristics were detected in each experiment, with some chromosome regions affecting several traits (Table 4.6; Figures 4.2, 4.3 and 4.4). Some QTLs exhibited QTL-by-environment interaction.

The only QTL detected on a group 1 chromosome was on chromosome 1D. At that locus (position Q1 in Figure 4.2), the Drysdale allele was associated with higher test weight.

On group 2 chromosomes, QTLs were detected at or near photoperiod-sensitivity loci *Ppd-B1* (position Q5 in Figure 4.2) on chromosome 2B and *Ppd-D1* (position Q8 in Figure 4.2) on chromosome 2D. At the *Ppd-B1* locus, the Drysdale allele for photoperiod-sensitivity was associated with lower grain length, width, thickness, area, roundness, percentage screenings and thousand grain weight and with higher aspect ratio, test weight and grain protein content. That locus also affected grain yield and grain number, with the directions of the effects varying among environments. At the *Ppd-D1* locus, the Gladius allele for photoperiod-sensitivity was associated with lower grain width, thickness, area and higher aspect ratio and roundness. At that locus, the directions of effects on grain protein content, particle size index, percentage screenings, test weight, thousand grain weight and yield varied among environments.

At other loci on group 2 chromosomes, there were effects on particle size index (position Q2 in Figure 4.2) on chromosome 2A, grain number and aspect ratio (position Q3 in Figure 4.2), roundness (position Q4 in Figure 4.2), flour extraction (position Q6 in Figure 4.2) and percentage screenings (position Q7 in Figure 4.2) on chromosome 2B. There were also effects on length and flour extraction (position Q9 in Figure 4.2), thickness, aspect ratio and thousand grain weight (position Q10 in Figure 4.2) and width (position Q11 in Figure 4.2) on chromosome 2D.

On group 3 chromosomes, QTLs were detected on chromosomes 3A and 3B. On chromosome 3A, QTLs for grain number and yield co-located (position Q14 in Figure 4.3) with Drysdale alleles associated with higher grain number and yield, except at Ciudad Obregon where the Drysdale allele was were associated with lower grain yield. There were also QTLs on chromosome 3A at which the Drysdale allele was associated with increased roundness (position Q12 in Figure 4.3) or lower flour extraction (position Q13 in Figure 4.3). Drysdale alleles were associated with increased percentage screenings (position Q15 in

Figure 4.3) on chromosome 3A and (position Q16 in Figure 4.3) on chromosome 3B. At other loci on chromosome 3B, Drysdale alleles were associated with lower thousand grain weight and protein content (position Q17 in Figure 4.3) or test weight (position Q19 in Figure 4.3) and higher flour extraction (position Q18 in Figure 4.3). Also on chromosome 3B, Drysdale alleles were associated with lower percentage screenings (position Q21 in Figure 4.3), higher grain thickness, thousand grain weight and grain yield (position Q22 in Figure 4.3) and grain width (position Q23 in Figure 4.3). At another QTL on chromosome 3B (position Q20 in Figure 4.3) the Drysdale allele was associated with higher particle size index at Urrbrae but decreased this trait at Yanco.

On group 4 chromosomes, QTLs were detected on chromosomes 4A and 4B. At one position on chromosome 4A (position Q24 in Figure 4.3), the Drysdale allele was associated with higher grain thickness. At another position on chromosome 4A (position Q25 in Figure 4.3), the Drysdale allele was associated with higher grain number at Ciudad Obregon and Urrbrae but with lower grain number at Yanco. Drysdale alleles at other positions on this chromosome were associated with higher thousand grain weight and yield (position Q27 in Figure 4.3) and percentage screenings (position Q26 in Figure 4.3) at Yanco in 2010 and had a decreasing effect at Yanco in 2009. On chromosome 4B, only one QTL was detected (position Q28 in Figure 4.3). At that QTL, the Drysdale allele was associated with higher grain thickness.

On group 5 chromosomes, QTLs were detected at or near the *Vrn-A1* locus on chromosome 5A and at the *Ha* locus on chromosome 5D. The Drysdale allele was associated with lower grain protein content (position Q29 in Figure 4.4) on chromosome 5A. The Drysdale winter allele (*v*) (vernalisation requiring) was associated with lower grain number at Yanco but higher grain number at Urrbrae (position Q30 in Figure 4.4) at a region close to *Vrn-A1* on chromosome 5A. Drysdale allele combination (*Pina-D1b/Pinb-D1a*) that confers a harder phenotype was associated with lower particle size index and flour extraction (position Q31 in Figure 4.4) at the *Ha* locus on chromosome 5D.

On chromosome 6A, QTLs were detected at (position Q32 in Figure 4.4) or near (position Q33 in Figure 4.4) the *TaGW2* marker. At these QTLs, Drysdale alleles were associated with lower grain width, grain area and thousand grain weight but with higher grain roundness. The Drysdale allele at (position Q33 in Figure 4.4) was associated with higher grain number and grain yield in some environments but decreased both of these traits in other environments. A

QTL was detected on chromosome 6B (position Q34 in Figure 4.4) at which the Drysdale allele was associated with shorter grain.

On chromosome 7A, a QTL (position Q35 in Figure 4.4) was detected at which the Drysdale allele was associated with higher flour extraction. On chromosome 7B, a QTL (position Q36 in Figure 4.4) was detected at which the allele from Drysdale was associated with higher test weight.

Table 4.6 QTLs detected for yield, flour extraction, grain shape and characteristics in the Gladius/Drysdale mapping population grown in field experiments. Positive and negative additive effects indicate that the Drysdale allele increases or decreases the trait value, respectively. For QTLs for which the position estimate coincided exactly with the position of a marker locus, the name of that marker is shown. For QTLs for which the position estimate fell within intervals between marker loci, the names of the flanking markers are shown. QTL position numbers correspond to numbers shown in Figures 4.2, 4.3 and 4.4.

Chromosome	Position cM	closest/ flanking marker	QTL position	QTL	Trait	LOD	Additive effect[a]					
							MexObr10_D_LS	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS
1D	132.2	*wPt-0077*	Q1	*QTwg.aww-1D*	Test weight	4.4		-	0.43	0.27		
2A	46.8	*gwm0275*	Q2	*QPsi.aww-2A*	Particle size index	7.6		-0.51	-0.51	-0.51	-0.51	-0.51
2B	17.2	*wPt-2106*	Q3	*QGar .aww-2B-1*	Aspect ratio	4.7		-0.02		-0.02	-0.02	-0.02
2B	17.2	*wPt-2106*	Q3	*QGrn.aww-2B-1*	Grain number	4.4	202.00	306.00	171.00	-	1140.00	716.46
2B	25.8	*wPt-6706*	Q4	*QGro.aww-2B-1*	Roundness	5.9		-0.02		-0.02	-0.02	-0.02
2B	100.4	*Ppd-B1*	Q5	*QGar.aww-2B-2*	Aspect ratio	5.4		0.02		-	-	0.02
2B	100.4	*Ppd-B1*	Q5	*QGar.aww-2B*	Grain area	9.5		-0.22		-0.31	-0.14	-0.59
2B	100.4	*Ppd-B1*	Q5	*QGle.aww-2B*	Length	13.7		-0.02		-0.06	-	-0.09
2B	100.4	*Ppd-B1*	Q5	*QGro.aww-2B-2*	Roundness	7.9		-		-0.01	-	-
2B	100.4	*Ppd-B1*	Q5	*QGth.aww-2B*	Thickness	6.3		-		-	-	-0.04
2B	100.4	*Ppd-B1*	Q5	*QGwi.aww-2B*	Width	6.9		-0.03		-0.02	-	-0.07
2B	100.4	*Ppd-B1*	Q5	*QPsc.aww-2B-1*	percentage screenings	9.6		-	-0.28	-		
2B	100.4	*Ppd-B1*	Q5	*QTgw.aww-2B*	Thousand grain weight	7.5	-	-0.59	-	-0.53	-	-2.12
2B	100.4	*Ppd-B1*	Q5	*QGrn.aww-2B-2*	Grain number	3.0	-278.00	-299.00	142.00	-	415.00	-
2B	100.4	*Ppd-B1*	Q5	*QYld.aww-2B*	Yield	7.5	-99.59	-178.40	-	-79.24	242.46	-139.9
2B	101.0	*wPt-3561*	Q5	*QGpc.aww-2B*	Grain protein content	15.9		0.36	-	0.13	-	0.33
2B	101.0	*wPt-3561*	Q5	*QTwg.aww-2B*	Test weight	15.0		-	0.36	0.47		
2B	125.4	*wPt-6192/barc0183*	Q6	QFex.aww-2B	Flour extraction	5.9		-0.40		-	-0.71	-0.56
2B	193.2	*gwm0388*	Q7	*QPsc.aww-2B-2*	percentage screenings	5.1		-0.98	-0.42	-		

Table 4.6 (continued)

Chromosome	Position cM	closest/ flanking marker	QTL position	QTL	Trait	LOD	Additive effect[a]					
							MexObr10_D_LS	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS
2D	52.1	*Ppd-D1*	Q8	*QGar.aww-2D-1*	Aspect ratio	11.6		-0.03		-	-0.02	-0.03
2D	52.1	*Ppd-D1*	Q8	*QGar.aww-2D*	Grain area	12.3		0.28		0.28	0.20	0.69
2D	52.1	*Ppd-D1*	Q8	*QGro.aww-2D*	Roundness	17.1		-0.01		-	-0.01	-0.02
2D	52.1	*Ppd-D1*	Q8	*QGth.aww-2D-1*	Thickness	17.6		0.03		-	0.03	0.08
2D	52.1	*Ppd-D1*	Q8	*QGwi.aww-2D-1*	Width	15.3		0.05		0.03	0.03	0.09
2D	52.1	*Ppd-D1*	Q8	*QGpc.aww-2D*	Grain protein content	24.2		-0.38	-0.23	-0.06	0.12	-0.12
2D	52.1	*Ppd-D1*	Q8	*QPsi.aww-2D*	Particle size index	9.4		0.30	0.58	-0.69	1.66	-
2D	52.1	*Ppd-D1*	Q8	*QPsc.aww-2D*	percentage screenings	8.8		-0.61	0.60	-		
2D	52.1	*Ppd-D1*	Q8	*QTwg.aww-2D*	Test weight	32.0		0.39	-0.70	-0.59		
2D	52.1	*Ppd-D1*	Q8	*QTgw.aww-2D-1*	Thousand grain weight	21.4	-	1.22	-0.44	0.39	0.77	3.11
2D	52.1	*Ppd-D1*	Q8	*QYld.aww-2D*	Yield	15.3	65.02	288.60	-	136.42	-245.13	147.68
2D	61.2	*Ppd-D1/wPt-4381*	Q9	QFex.aww-2D	Flour extraction	10.5		0.60		0.23	1.10	0.71
2D	61.2	*Ppd-D1/wPt-4381*	Q9	*QGle.aww-2D*	Length	13.7		0.03		0.05	-	0.12
2D	139.0	*gwm0102/barc0011*	Q10	*QGar.aww-2D-2*	Aspect ratio	4.8		-		-0.02	-0.03	-0.03
2D	139.0	*gwm0102/barc0011*	Q10	*QGth.aww-2D-2*	Thickness	4.5		0.01		0.04	0.05	0.06
2D	139.0	*gwm0102/barc0011*	Q10	*QTgw.aww-2D-2*	Thousand grain weight	7.2	0.37	0.38	-	1.28	1.96	2.59
2D	147.5	*gwm0102/barc0011*	Q11	*QGwi.aww-2D-2*	Width	6.2		0.02		0.05	0.06	0.08
3A	173.3	*gwm0155/wPt-3278*	Q12	*QGro.aww-3A*	Roundness	3.7		0.02		0.02	0.02	0.02
3A	218.8	wPt-8658/barc0067	Q13	Q*Fex*.aww-3A	Flour extraction	6.2		-0.57		-0.26	-0.49	-0.79
3A	229.3	*barc0324*	Q14	*QYld.aww-3A*	Yield	3.3	-74.21	-	-	-	247.08	278.15
3A	231.5	*barc0306*	Q14	*QGrn.aww-3A*	Grain number	6.3	-	-	-	301.60	686.27	901.28
3A	246.7	*wPt-2639*	Q15	*QPsc.aww-3A*	percentage screenings	10.2		1.07	0.72	-		

Table 4.6 (continued)

Chromosome	Position	closest/ flanking marker	QTL	QTL	Trait	LOD	Additive effect[a]					
	cM		position				MexObr10_D_LS	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS
3B	6.6	*wPt-7984*	Q16	*QPsc.aww-3B-1*	percentage screenings	3.1		0.35	0.90	-		
3B	24.0	*wPt-7961/wPt-9066*	Q17	*QGpc.aww-3B*	Grain protein content	6.7		-	-0.34	-0.11	-	-
3B	24.0	*wPt-7961/wPt-9066*	Q17	*QTgw.aww-3B*	Thousand grain weight	4.5	-	-0.39	-1.35	-1.04	-0.48	-1.02
3B	136.0	wPt-6047	Q18	QFex.aww-3B	Flour extraction	5.2		0.49	0.35	-	-	-
3B	155.2	*barc0164*	Q19	*QTwg.aww-3B*	Test weight	4.1		-	-	-0.34		
3B	164.0	*wmc0418/gwm0383*	Q20	*QPsi.aww-3B*	Particle size index	4.4		-0.51	-0.43	-	-	0.47
3B	273.3	*wPt-0021*	Q21	*QPsc.aww-3B-2*	percentage screenings	4.3		-0.49	-0.51	-0.26		
3B	289.2	*wPt-2391*	Q22	*QTgw.aww-3B*	Thousand grain weight	5.7	0.67	0.67	0.67	0.67	0.67	0.67
3B	289.2	*wPt-2391*	Q22	*QYld.aww-3B*	Yield	7.0	122.72	175.47	73.39	-	137.51	-
3B	293.4	*wPt-1870*	Q22	*QGth.aww-3B*	Thickness	6.0		0.02		0.02	0.02	0.02
3B	301.7	*wPt-1870/wPt-2685*	Q23	*QGwi.aww-3B*	Width	4.2		0.03		0.03	0.03	0.03
4A	30.0	*wPt-0162/wPt-7939*	Q24	*QGth.aww-4A*	Thickness	5.9		0.03		0.03	0.03	0.03
4A	86.8	*wPt-9860/wPt-2909*	Q25	*QGrn.aww-4A*	Grain number	4.4	231	-399.63	-	-696.42	799.94	850.21
4A	119.2	*wPt-3150*	Q26	*QPsc.aww-4A*	percentage screenings	4.0		-0.44	-0.84	0.14		
4A	144.8	*wPt-0023*	Q27	*QTgw.aww-4A*	Thousand grain weight	3.6	-	0.67	1.02	-	0.64	1.09
4A	146.0	*wPt-8657*	Q27	*QYld.aww-4A*	Yield	4.1	-	-	-	-	460.26	462.84
4B	151.1	*wmc0349/wPt-5996*	Q28	*QGth.aww-4B*	Thickness	6.6		-0.04		-0.04	-0.04	-0.04
5A	206.0	*wPt-4262*	Q29	*QGpc.aww-5A*	Grain protein content	6.4		-0.20	-0.20	-0.20	-0.20	-0.20
5A	242.3	*wPt-4262/Vrn-A1*	Q30	*QGrn.aww-5A*	Grain number	7.3	-	752.7	-	-	-955.38	-991.61

Table 4.6 (continued)

Chromosome	Position	closest/ flanking marker	QTL	QTL	Trait	LOD	Additive effect[a]					
	cM		position				MexObr10_D_LS	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS
5D	0.0	*PinA*	Q31	*QPsi.aww-5D*	Particle size index	13.1		-1.15	-0.37	-	-0.41	-0.81
5D	0.0	*PinA*	Q31	QFex.aww-5D	Flour extraction	31.9		-0.34		-	-1.13	-1.41
6A	0.0	*TaGW2*	Q32	*QGth.aww-6A*	Thickness	11.1		-0.03		-0.03	-0.03	-0.03
6A	0.0	*TaGW2*	Q32	*QGwi.aww-6A*	Width	8.0		-0.03		-0.03	-0.03	-0.03
6A	0.0	*TaGW2*	Q32	*QGro.aww-6A*	Roundness	4.1		0.01		0.01	0.01	0.01
6A	1.7	*wPt-0902*	Q32	*QTgw.aww-6A*	Thousand grain weight	4.3	-0.58	-0.58	-0.58	-0.58	-0.58	-0.58
6A	8.0	*TaGW2/wPt-5834*	Q33	*QGar.aww-6A*	Grain area	3.8		-0.22		-0.26	-0.15	-
6A	10.1	*wPt-0902/wPt-5834*	Q33	*QGrn.aww-6A*	Grain number	3.2	-137.44	-146.48	-256.36	181.32	1185.64	278.20
6A	10.1	*wPt-0902/wPt-5834*	Q33	*QYld.aww-6A*	Yield	6.1	-88.25	-177.71	-111.82	66.75	469.88	186.83
6B	4.0	*wPt-0171*	Q34	*QGle.aww-6B*	Length	4.0		-0.06		-0.06	-0.06	-0.06
7A	168.0	wPt-0961	Q35	QFex.aww-7A	Flour extraction	4.5		-		-	-0.51	-0.33
7B	8.0	*wPt-7318/wPt-8283*	Q36	*QTwg.aww-7B*	Test weight	6.6		-	0.24	0.62		

[a] – QTL not detected in the environment; blank space when trait was not measured in the environment

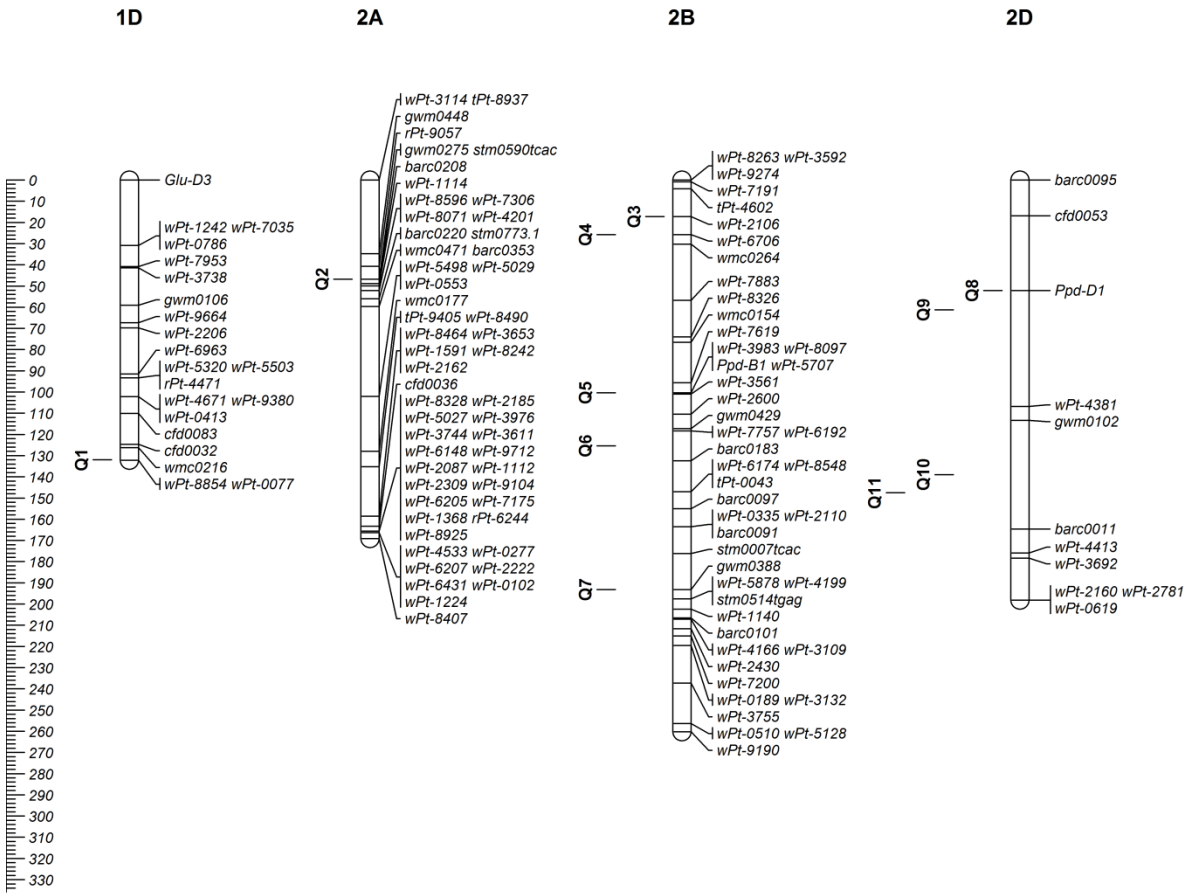

Figure 4.2 Locations (Q1 to Q11) on chromosomes of homeologous groups 1 and 2 of wheat at which QTLs were detected for one or more traits, as listed in Table 4.6. The scale on the left indicates genetic distances in cM.

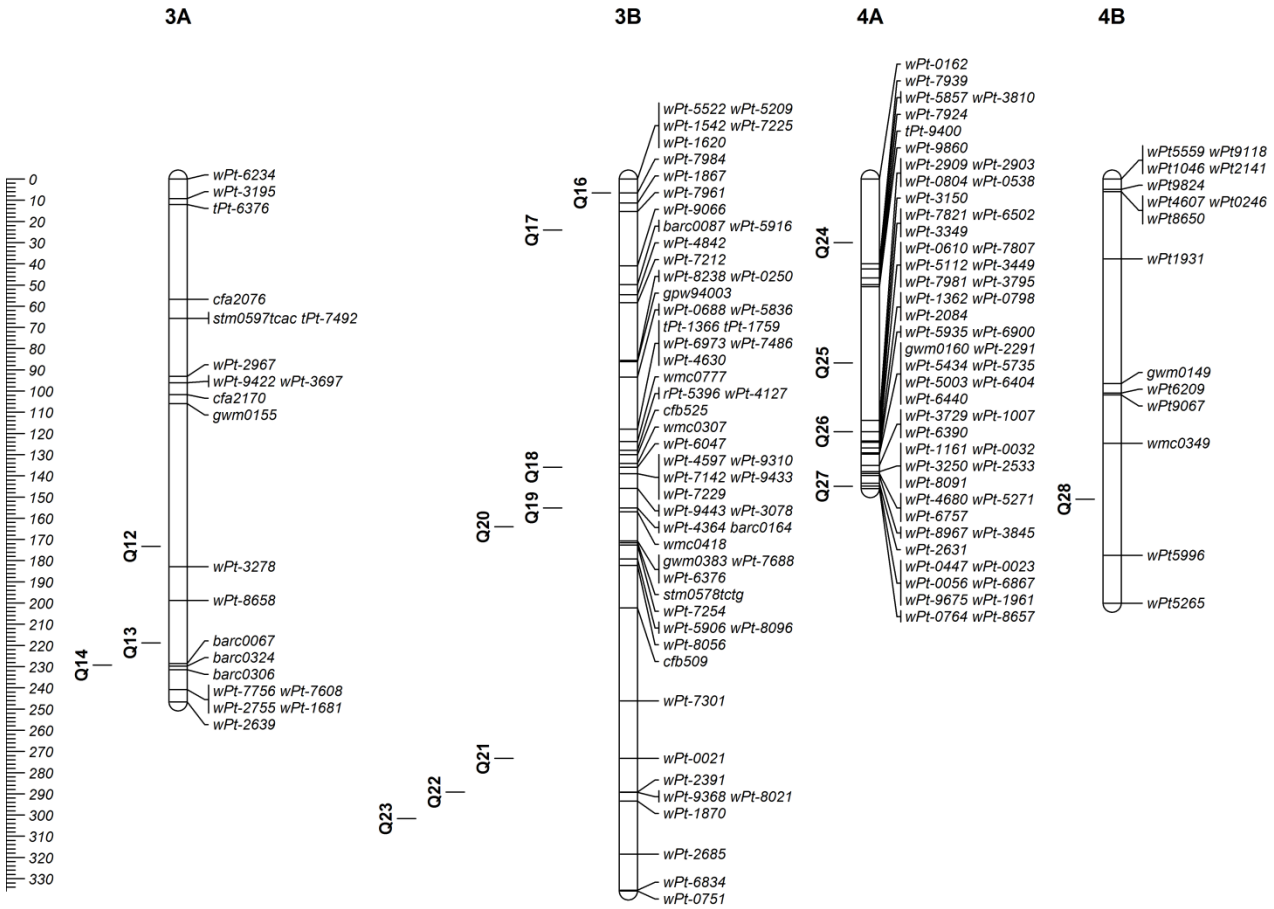

Figure 4.3 Locations (Q12 to Q28) on chromosomes of homeologous groups 3 and 4 of wheat at which QTLs were detected for one or more traits, as listed in Table 4.6. The scale on the left indicates genetic distances in cM.

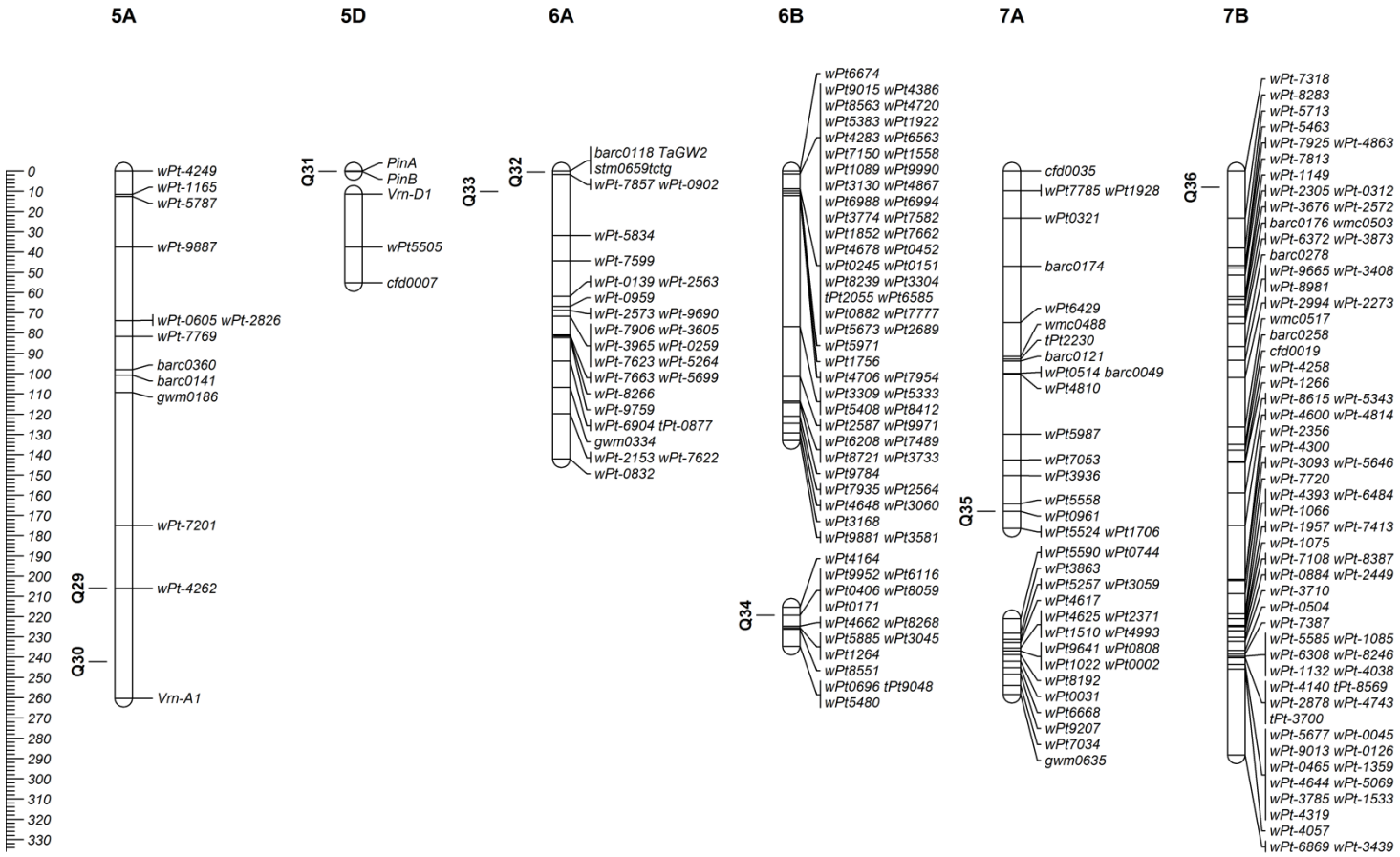

Figure 4.4 Locations (Q29 to Q36) on chromosomes of homeologous groups 5, 6 and 7 of wheat at which QTLs were detected for one or more traits, as listed in Table 4.6. The scale on the left indicates genetic distances in cM.

4.4 Discussion

As reported in Chapter 3, the alleles (*b*) for photoperiod-sensitivity at the *Ppd-B1* and *Ppd-D1* loci delayed flowering relative to the alleles (*a*) for photoperiod-insensitivity. Under most conditions, the alleles that confer photoperiod insensitivity, carried by Gladius at the *Ppd-B1* locus and by Drysdale at the *Ppd-D1* locus, were both associated with increased grain width, thickness, length, thousand grain weight and grain yield. Early flowering allows the plants to escape later season abiotic stresses and to have a longer grain filling duration thus allowing the grain dimensions to develop to maximum capacity. This could explain why lines carrying the insensitive allele have larger and heavier grains than those carrying the sensitive allele.

The alleles for photoperiod-sensitivity were usually associated with high percentage screenings, probably because late flowering shortened the grain filling duration, leaving insufficient time to fill the grain thus resulting in small grains. Under heat conditions, however, the opposite was observed, with the photoperiod-insensitivity alleles associated with higher percentage screenings and decreased thousand grain weight. This would be because early flowering plants, which had a longer grain filling duration, had their grain exposed to heat conditions for longer periods during grain filling resulting in smaller grains. High temperatures (above 30°C) in the field experiments were experienced at around heading and persisted until harvest maturity. The effect of photoperiod-sensitivity loci on grain characteristics observed here, have previously been reported in other studies, for example genomic regions close to the *Ppd-B1* locus have been shown to be associated with thousand grain weight (Groos et al., 2003; Huang et al., 2006) and grain protein content (Turner et al., 2004) and regions close to the *Ppd-D1* locus have been shown to affect protein content (Nelson et al., 2006), test weight and thousand grain weight (Huang et al., 2006).

Some QTL detected in this study were consistent over environments, while others showed QTL-by-environment interactions. No effects on grain yield were detected at the *Ppd-B1* or *Ppd-D1* loci under heat conditions. Under heat conditions, the growing season was shorter than in the control experiment (AusYan09_FI_CS) with shorter time to heading, and the additive effects for days to heading QTL were the lowest compared to other environments at both photoperiod loci (Chapter 3 of this thesis). The non-detection of QTL for yield at the photoperiod loci and low additive effects for time to heading under heat stress shows that photoperiod genes had no impact on the overall yield and little effect on time to heading. This is because the experiments were sown at different times, with the late sown heat exposed

experiment planted towards spring (August) when day length was slightly longer than in early winter (June) when the early sown experiment was planted. As a result, day length was no longer limiting in the late sown experiment and the differences between the sensitive and insensitive lines were minimised, leading to small differences in time to heading and on yield.

Although yield was negatively correlated with grain protein content as is often the case, there are some loci that affected these traits independently. Many grain protein content QTLs were detected indicating that many genes are responsible for its control. Polygenic control of grain protein content has also been observed in other studies (Groos et al., 2004; Groos et al., 2003; Turner et al., 2004). The detection of many QTLs for protein content makes it possible to circumvent the negative relationship with yield observed in this study by identifying independent loci (some affecting protein and others affecting grain yield) and combining them to increase both traits. At a locus on chromosome 3B (position Q17 in Figure 4.3) the Gladius allele has an increasing effect for both grain protein content and thousand grain weight (a yield component), providing an opportunity to simultaneously increase both traits. Another potentially favourable QTL, where many traits can be improved simultaneously, is on chromosome 3B (position Q22 in Figure 4.3), where grain thickness, thousand grain weight and yield QTLs co-locate and are all increased by Drysdale alleles. This genomic region has also been shown to be associated with yield in the RAC875/Kukri mapping population (Bennett et al., 2012c). Grain thickness QTL also co-located with thousand grain weight QTL on chromosome 2D (position Q10 in Figure 4.2), in a region not associated with *Ppd-D1* thus presenting opportunities to increase grain dimension and weight independent of the photoperiod-sensitivity alleles.

Grain hardness was expectedly associated with the *Ha* locus in this study, as it is known that hardness is largely influenced by the puroindoline genes at the *Ha* locus on chromosome 5D, which also contains *GSP-1*, whose exact role in grain hardness is not clearly understood (Giroux and Morris, 1997; Giroux and Morris, 1998; Morris et al., 2001; Morris, 2002). As expected, Gladius which has the allele combination *Pina-D1a/Pinb-D1b* at the *Ha* locus that confers softer phenotype, has alleles that increased particle size index and flour extraction. Drysdale is harder and has the allele combination *Pina-D1b/Pinb-D1a* at this locus. At *Pina-D1*, the *a* allele confers softness and the *b* (null) allele results in slightly increased hardness. At *Pinb-D1*, various mutations cause variation in puroindoline protein content and grain hardness (Bhave and Morris, 2008).

The relationship between grain hardness and flour extraction and the influence of the *Ha* locus on flour extraction observed here has been reported (Tsilo et al., 2011; Wang et al., 2012a). Softer grain yields more break flour and slightly more overall flour yield, though flour from softer grain is difficult to sieve and flows slowly through the mill. The flour extraction QTL (position Q35 in Figure 4.4) on chromosome 7A mostly likely coincides with the hardness QTL detected on a Spark/Rialto population (*wPt-0961*common marker is close to *Pinb-A2* gene) (Wilkinson et al., 2008). This region on chromosome 7A associated with flour extraction in this study coincides with a region where a new family of puroindoline genes, *Pinb-2* that have been found and shown to be associated with grain hardness (Chen et al., 2010; Geng et al., 2012a; Wilkinson et al., 2008).

The association between a region close to marker *TaGW2* (position Q32 in Figure 4.4) with grain dimensions and weight is consistent with the suggestion of Su et al. (2011) that *TaGW2-6A* can be used as a diagnostic marker for grain weight. This region was also associated with thickness, roundness and thousand grain weight and therefore this shows the contribution of grain dimensions on grain weight. Roundness is calculated from grain length, width and thickness, thus explaining the co-location of roundness QTL with width and thickness QTLs. The *TaGW2* marker was designed based on the rice *OsGW2* marker, which is also associated with grain width and weight (Song et al., 2007). Yield QTL were detected close to this marker in this study (position Q33 in Figure 4.4) and in other studies (Snape et al., 2007; Sun et al., 2009). The co-location of width and thickness QTLs in this region and on chromosome 2D (position Q8 in Figure 4.2) or in closely linked positions on chromosome 3B (positions Q22 and Q23 in Figure 4.3) indicates that the lateral dimensions of the grain can be under the same genetic control and an increase in these dimensions results in an increase in grain weight.

Grain length and width were shown to be largely under different genetic control mechanisms. The independence of length and width makes it possible to select allele combinations that increase both traits. Grain width and thickness were shown to be under the same or similar genetic control and they largely influenced thousand grain weight and ultimately yield. The correlations between grain dimensions and yield shows that an increase in any of them will result in improved yield. Though phenology genes were associated with yield, grain dimensions and characteristics, many other regions independent of phenology genes were shown to be associated with these traits.

Chapter 5

Genetic control of processing and bread making quality in a recombinant inbred line population grown under drought and heat conditions

5.1 Introduction

The grain harvested from field experiments described in Chapter 3 of this thesis was analysed for processing and baking quality. Bread wheat (*Triticum aestivum* L.) is a major agricultural cereal crop worldwide and is mainly consumed in the form of baked products. Dough properties and baking quality vary among wheat cultivars and are influenced by abiotic stresses such as high temperatures and moisture deficit especially during grain filling. Plants have evolved mechanisms to survive and reproduce under stressful conditions either through escaping or tolerance. Plant breeders also continuously breed for cultivars that are better able to withstand stressful conditions.

Crossing results in new genetic combinations in progeny providing a basis for selection to improve important traits, including those that affect processing and baking quality. Some specific genes or gene products have been found to be associated with flour and dough quality parameters. The *Psy1* (phytoene synthase) genes on group 7 chromosomes affect flour colour resulting in flour ranging from white, through pale yellow to extreme yellow (Howitt et al., 2009; Ravel et al., 2012). Puroindolines, though primarily associated with grain texture (Morris, 2002), can also influence flour extraction, water absorption and baking output (Hrušková et al., 2006; Pomeranz et al., 1984). Serpins, which function as defence proteins, also affect flour extraction (Cane et al., 2008). Gluten proteins (glutenins and gliadins) largely determine the viscoelasticity of wheat dough. Viscoelasticity allows the trapping of carbon dioxide during fermentation and the production of leavened bread. No specific genes have been identified as being directly associated with baking output. Numerous QTLs for flour colour, dough properties and baking output have been detected in many regions of the wheat genome (Elangovan et al., 2008; Groos et al., 2004; Groos et al., 2007; Kuchel et al., 2006; Law et al., 2005; Mann et al., 2009; Nelson et al., 2006).

Dough properties can be estimated using either large scale equipment such as the Brabender farinograph or small scale equipment such as the mixograph. Dough scoring systems and models have been devised to predict dough quality based on allele combinations (Békés et al., 2006; Eagles et al., 2002; Eagles et al., 2004; Payne et al., 1987). Measured and/or predicted

dough properties are in turn often used as predictors of baking quality. Various baking methods suited for different flour types and baking processes are used in industry and research. All of these methods aim to measure loaf parameters such as volume, structure and texture. Soft bread texture is generally preferred by consumers.

Environmental conditions including high temperature and water deficit can potentially affect wheat quality. The impact of high temperature and moisture deficit on quality is not properly understood, and it can be expected to vary with the developmental stage in which the stress occurs. High temperatures during grain filling have been reported to decrease dough strength (Randall and Moss, 1990). High temperatures above 30°C during late grain filling (at or around 25 days after anthesis) have been reported to decrease % UPP, a trait related to dough strength (Cavanagh et al., 2010; Irmak et al., 2008; Naeem and MacRitchie, 2005). Moisture deficit also affects wheat grain and processing quality. Moisture deficit increases protein content, slightly increases grain hardness and vitreosity (Weightman et al., 2008) and increases peak time (Guttieri et al., 2001). At later grain filling, drought stress decreases SDS sedimentation volume, a measure of dough quality (Gooding et al., 2003). The aim of the research reported in this thesis Chapter was to investigate the genetic control of flour, dough and baking output in grain produced under a range of conditions involving drought and heat stress, through mapping QTLs associated with these quality traits and investigating whether any of the detected QTLs are environment specific.

5.2 Materials and Methods

5.2.1 Plant Material

The wheat cultivars Gladius and Drysdale, which were the parents of the RIL population used in this thesis Chapter, were described in detail in Chapter 3 of this thesis. Briefly, these parents were chosen on the basis of their contrasting physiological and agronomic performance under drought and heat stress conditions, with Gladius being the drought and heat tolerant parent. Drysdale is more water use efficient, producing more biomass per unit area per unit of water taken (Fleury et al., 2010). As shown in Table 5.1, these cultivars differ for major phenology genes (Eagles et al. 2009), yet they head within five days of each other (Chapter 3 of this thesis). In environments in which they head at different times, Drysdale heads before Gladius. Gladius and Drysdale also differ for major quality-related genes (Eagles et al., 2009) (Table 5.1). They differ at the glutenin loci *Glu-B1*, *Glu-A3* and *Glu-D3* but not at *Glu-A1*, *Glu-D1* or *Glu-B3*.

Table 5.1 Alleles carried by Gladius and Drysdale at high molecular weight glutenin, low molecular weight glutenin, puroindoline, serpin and phenology loci.

		Parent	
Locus type	Locus	Gladius	Drysdale
High molecular weight glutenin	*Glu-A1*	*a*	*a*
		(Ax1)	*(Ax1)*
	Glu-B1	*b*	*i*
		(Bx7 + By8)	*(Bx17 + By18)*
	Glu-D1	*d*	*d*
		(Dx5 + Dy10)	*(Dx5 + Dy10)*
Low molecular weight glutenin	*Glu-A3*	*c*	*b*
	Glu-B3	*b*	*b*
	Glu-D3	*a*	*g*
Puroindoline	*Pina-D1*	*a*	*b*
	Pinb-D1	*b*	*a*
Serpin	*Srp*	*a*	*a*
Phenology	*Ppd-B1*	*a*	*b*
	Ppd-D1	*b*	*a*
	Vrn-A1	*a*	*v*
	Vrn-D1	*v*	*a*

5.2.2 Field trials

A set of 205 Gladius/Drysdale RILs was grown in the field under conditions involving drought and heat stress (Table 5.2). A more detailed description of the population, experimental designs and conditions is described in Chapter 3 of this thesis. Briefly, the field experiments were conducted at Yanco, New South Wales, Australia during the 2009 and 2010 seasons. These involved well watered and early sown experiments designed to escape heat stress, and well watered and late sown experiments designed to be exposed to heat stress during grain filling. At Urrbrae, South Australia in the 2010 season, the experiments were grown under drought and well watered conditions.

Table 5.2 Description of the environments in which the experiments were conducted showing location, year in which experiment was conducted and the traits measured.

Environment[a]	Location	Year	Latitude	Longitude	Altitude (M)	Traits measured		
						flour colour	dough properties	baking quality
AusYan09_FI_CS	Yanco (NSW)	2009	34° S	146° E	136	yes	yes (using farinograph)	yes
AusYan09_FI_LS	Yanco (NSW)	2009	34° S	146° E	136	yes	yes (using farinograph)	yes
AusYan10_FI_CS	Yanco (NSW)	2010	34° S	146° E	136	yes	yes (using farinograph)	yes
AusUrr10_SI_CS	Urrbrae (SA)	2010	34° S	138° E	225	yes	yes (using mixograph)	no
AusUrr10_D_CS	Urrbrae (SA)	2010	34° S	138° E	225	yes	yes (using mixograph)	no

[a] Aus Australia, CS for conventional sowing, LS for late sowing, D for drought, FI for flooding irrigation, SI for sprinkler irrigation

5.2.3 Quality analysis

From the experiments grown at Yanco, grain samples of 185 RILs of similar maturity were analysed for processing and bread making quality. From the experiment grown at Urrbrae, all 205 lines were analysed for quality traits. Milling was conducted as described in Chapter 4 of this thesis. Only flour from experiments conducted at Yanco was enough to do baking tests.

Flour colour properties were measured using a Minolta Chroma Meter CR-410 instrument (Minolta Co. Ltd., Osaka, Japan) utilising a D65 (daylight) light source, with colour expressed in CIE units (RACI-CCD, 2010). The CIE colour space measures brightness (L*) with higher values for brighter colour and blueness-yellowness (b*) with positive values indicating yellowness.

For the experiments conducted at Yanco, dough properties were assessed using the Perten-Newport Scientific DoughLAB (Newport Scientific Pty Ltd., NSW, Australia), which is an electronic alternative for the Brabender farinograph. The baseline is set by mixing flour only, for one minute before water is added. Dough stability (seconds), development time (seconds), softening (FU units) and percentage water absorption (RACI-CCD, 2010) are calculated.

For the drought and irrigated experiments grown at Urrbrae, a 10 g mixograph (National Manufacturing Co, Lincoln, Nebraska, USA) was used to assess dough properties and data were analysed using MixSmart software (National Manufacturing Co, Lincoln, Nebraska, USA) (AACC, 1999). Flour was allowed to equilibrate at room temperature for 24 hours. At the start of each day the mixograph was run for 20 minutes without sample to allow it to warm up. The traits measured are peak time (seconds), peak height and bandwidth (% full scale torque value), height at 8 minutes (% full scale torque value), slopes to the left and right of the peak (change in % height divided by time) and area or work input (expressed as an integral of % torque multiplied by time).

Baking performance was only investigated in the experiments conducted at Yanco. The baking test method used was the long fermentation method (RACI-CCD, 2010) which is based on AACC approved methods 10:09 and 10:10b (AACC, 1999) with bake mixing time taken as time to full dough development. The baking ingredients were flour, water, sugar, α-amylase, ammonium chloride, sodium chloride, fat, and ascorbic acid. Baked products were evaluated for loaf volume (cc units) using rapeseed displacement method (AACC, 1999) and loaf colour using the Minolta Chroma Meter CR-410 instrument. To permit evaluation of

crumb texture (feel) and crumb structure (appearance) loaves were cut diagonally from corner to corner to allow maximum view. For crumb texture a scale of 1-10 was used with 1 being "very tight under thumb pressure, crumb tears, rough to the feel" and 10 being "very soft feel under the thumb, very smooth, recovers with no indentation". For crumb structure a score of 1-10 was also used with 1 being "round cells with thick cell walls, crumpet like appearance" and 10 being "very fine cell walls, elongated cells around the edges of the loaf". Loaf volume score was obtained by dividing loaf volume by 48 and loaf volume score, loaf texture and structure scores were summed to provide loaf score.

5.2.4 Statistical and genetic analysis

A two-step statistical and genetic analysis was done with GenStat (Payne et al., 2009) as described in Chapter 3 of this thesis. A spatial analysis (Gilmour et al., 1997) was done to generate best linear unbiased estimates (BLUEs) taking into account the experimental designs. Multi-environment QTL analysis was done using composite interval mapping as described in Chapter 3 of this thesis. The linkage map used was described in Chapter 3 of this thesis, and consists of 720 DArT, SSR and gene-based markers distributed over 28 linkage groups (Appendix 1). Map diagrams were drawn using MapChart software (Voorrips, 2002).

5.3 Results

5.3.1 Summary of results

Recombinant inbred lines from the experiment that experienced high temperature generally had higher dough development time, stability, bake mixing time and decreased dough softening than those from experiments that did not experience high temperature (Table 5.3). Recombinant inbred lines from the higher temperature experiment also had a slightly higher loaf volume and score. Recombinant inbred lines from the experiment that experienced water deficit had higher time to peak (mixing time), peak width (dough strength) and decreased height at both peak and at 8 minutes (dough strength) than those from the experiment that received adequate moisture (Table 5.4).

Table 5.3 Mean values for flour, dough and loaf characteristics for Gladius and Drysdale and means, minima, maxima and standard deviations for the same characteristics in a set of Gladius/Drysdale population of recombinant inbred lines, all grown in heat experiments in New South Wales.

		Flour characteristics			Dough characteristics				Loaf characteristics						
		colour		Water	Dough	Dough	Bake	Dough	Loaf	Loaf	Loaf	Loaf	Loaf	colour	
Experiment	Parent or population	L* (CIE)	b* (CIE)	absorption (%)	development time (seconds)	stability (seconds)	mixing time (seconds)	softening (FU)	volume (cc)	volume score (out of 20)	texture (out of 10)	structure (out of 10)	score (out of 40)	L* (CIE)	b* (CIE)
AusYan09_FI_CS	Gladius	90.63	10.45	61.5	182	218	248	98	700	15	7	7	29	80.30	18.00
	Drysdale	90.53	10.61	61.8	161	187	225	108	805	17	8	6	31	81.50	17.00
	Population mean	90.63	10.36	62.5	169	195	236	109	757	16	7	6	29	81.04	16.65
	Population standard deviation	0.37	0.62	2.0	23	62	37	27	50	1	1	1	2	0.68	0.73
	Population minimum	89.47	8.93	58.2	123	67	154	49	639	13	4	5	22	78.90	14.35
	Population maximum	91.44	11.85	68.5	243	432	331	197	909	19	9	8	36	82.60	19.30
AusYan09_FI_LS	Gladius	90.43	8.20	60.4	188	206	285	109	648	14	6	6	26	80.85	14.55
	Drysdale	90.22	8.13	61.2	169	242	265	86	789	16	7	7	30	80.75	14.53
	Population mean	90.44	8.12	62.6	186	256	276	101	770	16	6	6	28	80.70	14.50
	Population standard deviation	0.40	0.46	3.0	31	90	56	34	88	2	1	1	3	0.76	0.53
	Population minimum	88.61	7.19	54.5	127	75	163	42	600	13	4	4	21	77.57	13.15
	Population maximum	91.47	9.23	70.4	373	546	375	251	960	20	8	9	37	82.52	15.95
AusYan10_FI_CS	Gladius	91.19	8.94	59.2	139	132	210	204	655	14	6	6	26	80.58	15.55
	Drysdale	91.17	8.82	61.4	147	146	188	147	744	15	8	7	30	81.48	15.03
	Population mean	91.14	9.01	58.9	140	138	207	182	677	14	6	6	26	80.85	15.47
	Population standard deviation	0.32	0.51	1.9	14	25	30	29	42	1	1	1	2	0.79	0.59
	Population minimum	90.23	8.07	54.5	101	62	121	125	559	12	3	3	18	79.02	14.13
	Population maximum	91.90	10.44	66.4	178	204	299	293	779	16	9	9	34	82.62	17.14

Table 5.4 Mean values for flour and dough characteristics for Gladius and Drysdale and means, minima, maxima and standard deviations for the same characteristics in a set of Gladius/Drysdale population of recombinant inbred lines, all grown in drought experiments in South Australia.

		Flour characteristics		Dough characteristics					
		colour		Dough	Right	Left	Peak	Peak	Peak height @
Experiment	Parent or population	L* (CIE)	b* (CIE)	mixing time (seconds)	slope (%/minute)	slope (%/minute)	width (%)	height (%)	8 minutes (%)
AusUrr10_SI_CS	Gladius	89.80	9.70	217	-7	9	29	75	60
	Drysdale	89.70	9.50	174	-7	9	30	77	59
	Population mean	89.24	9.80	188	-8	8	32	77	58
	Population standard deviation	0.50	0.69	24	2	5	5	6	4
	Population minimum	87.81	8.30	135	-15	1	21	64	47
	Population maximum	90.31	11.64	278	-4	19	43	92	69
AusUrr10_D_CS	Gladius	89.50	10.30	228	-8	12	35	74	57
	Drysdale	89.50	9.80	212	-7	11	35	71	57
	Population mean	89.33	10.11	199	-8	9	34	74	56
	Population standard deviation	0.54	0.83	26	2	5	5	5	4
	Population minimum	87.75	8.40	137	-13	1	24	61	49
	Population maximum	90.60	12.20	283	-3	34	52	90	68

5.3.2 QTLs detected

Flour, dough and baking output QTLs were detected on 11 chromosomes (Table 5.5; Figures 5.1 and 5.2). On group 1 chromosomes, QTLs were detected for envelope height at 8 minutes on chromosomes 1A (position Q1 in Figure 5.1), and for peak height and width co-locating on chromosome 1D (position Q2 in Figure 5.1), with Drysdale alleles associated with higher values for all the traits. On group 2 chromosomes, a region on chromosome 2A (position Q3 in Figure 5.1) was associated with dough development time and loaf texture, with the Gladius allele associated with higher values for both traits. Under heat conditions, this region affected loaf texture but not dough development time. On chromosomes 2B and 2D the phenology loci, *Ppd-B1* and *Ppd-D1* were associated with flour yellowness (positions Q4 and Q7 in Figure 5.1), with Gladius alleles associated with intense yellow colour at position Q4 but associated with less yellowness at position Q7. Bake mixing time and dough development time were associated with loci on chromosome 2B (positions Q5 and Q6 respectively in Figure 5.1), with Gladius alleles associated with higher values for both traits. A bake mixing time QTL (position Q8 in Figure 5.1) was detected on chromosome 2D, with the Drysdale allele extending the mixing time.

On group 3 chromosomes the only QTL detected was on chromosome 3A (position Q9 in Figure 5.2) with the Dysdale allele associated with intense flour yellowness. On group 4 chromosomes, a QTL for loaf score (position Q10 in Figure 5.2) was detected on chromosome 4A under heat conditions and a QTL for flour yellowness was detected on chromosome 4B (position Q12 in Figure 5.2) with Gladius alleles associated with higher values for both traits. Also on chromosome 4B, a QTL for peak width was detected (position Q11 in Figure 5.2) with the Gladius allele widening the peak under drought conditions but narrowing it under well watered conditions.

On group 5 chromosomes, Gladius alleles were associated with increased envelope height at 8 minutes (position Q13 in Figure 5.2) and peak height (position Q14 in Figure 5.2) on chromosome 5A. At the *Ha* locus on chromosome 5D, Drysdale alleles were associated with increased envelope height at 8 minutes, flour yellowness, loaf texture and score. The loaf texture and score QTLs were detected under heat conditions. Two flour colour QTLs were detected on chromosome 7B (positions Q16 and Q17 in Figure 5.2) with Drysdale alleles associated with intense yellowness at position Q17 and less yellowness at position Q16.

Table 5.5 QTLs detected for flour, dough and loaf traits in the Gladius/Drysdale mapping population grown in field experiments. Positive additive effects indicate that the Drysdale allele increases the trait value, while negative effects indicate that the Gladius allele increases the trait value. For QTLs for which the position estimate coincided exactly with the position of a marker locus, the name of that marker is shown. For QTLs for which the position estimate fell within intervals between marker loci, the names of the flanking markers are shown. QTL position numbers correspond to numbers shown in Figures 5.1 and 5.2.

				QTL position number			Additive effect[a]				
Chromosome	QTL	Trait	Position cM	(Figures 5.1&5.2)	Closest/ flanking markers	LOD	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS
1A	*QEh8.aww-1A*	Envelope height@ 8 min	12.4	Q1	*wPt-0164*	4.0				1.24	0.63
1D	*QPkh.aww-1D*	Peak height	126.2	Q2	*wmc0216*	4.2				1.72	0.81
1D	*QPkw.aww-1D*	Peak width	126.2	Q2	*wmc0216*	4.8				1.55	0.58
2A	*QDdt.aww-2A*	Dough development time	168.0	Q3	*wPt-1224/wPt-8407*	4.4	-4.45	-4.45	-4.45		
2A	*QLtx.aww-2A*	Loaf texture	168.0	Q3	*wPt-1224/wPt-8407*	3.7	-	-	-0.35		
2B	*QFcb.aww-2B*	Flour colour b*	100.4	Q4	*Ppd-B1*	12.0				0.30	0.30
2B	*QBmt.aww-2B*	Bake mixing time	147.0	Q5	*wPt-6174*	12.6	-14.24	-28.75	-10.06		
2B	*QDdt.aww-2B*	Dough development time	169.9	Q6	*barc0091/stm0007tcac*	7.7	-10.27	-12.62	-2.63		
2D	*QFcb.aww-2D*	Flour colour b*	61.22	Q7	*Ppd-D1/wPt-4381*	13.8				-0.35	-0.47
2D	*QBmt.aww-2D*	Bake mixing time	79.5	Q8	*Ppd-D1/wPt-4381*	4.2	-	33.34	-		
3A	*QFcb.aww-3A*	Flour colour b*	231.5	Q9	*barc0306*	4.9				0.18	0.18
4A	*QLsc.aww-4A*	Loaf score	129.2	Q10	*wPt-5935*	4.7	-	-0.73	-		
4B	*QPkw.aww-4B*	Peak width	4.8	Q11	*wPt-9824*	4.2				1.02	-0.64
4B	*QFcb.aww-4B*	Flour colour b*	76.9	Q12	*wPt-1931/gwm0149*	5.5	-0.26	-0.26	-0.26		
5A	*QEh8.aww-5A*	Envelope height@ 8 min	128	Q13	*gwm0186/wPt7201*	4.6				-1.09	-1.09
5A	*QPkh.aww-5A*	Peak height	190.5	Q14	*wPt-7201/wPt4262*	3.8				-2.35	-1.12
5D	*QEh8.aww-5D*	Envelope height@ 8 min	0.0	Q15	*PinA*	4.4				0.44	1.02
5D	*QFcb.aww-5D*	Flour colour b*	0.0	Q15	*PinA*	6.7				0.22	0.22
5D	*QLtx.aww-5D*	Loaf texture	0.0	Q15	*PinA*	13.4	-	0.49	-		
5D	*QLsc.aww-5D*	Loaf score	0.0	Q15	*PinA*	14.5	-	1.38	-		
7B	*QFcb.aww-7B*	Flour colour b*	110.0	Q16	*wPt2273/wmc0517*	4.4	-	-	0.16		
7B	*QFcb.aww-7B*	Flour colour b*	221.0	Q17	*wPt1066*	9.0	-0.24	-0.07	-0.21		

[a] – QTL not detected in the environment; blank space when trait was not measured in the environment

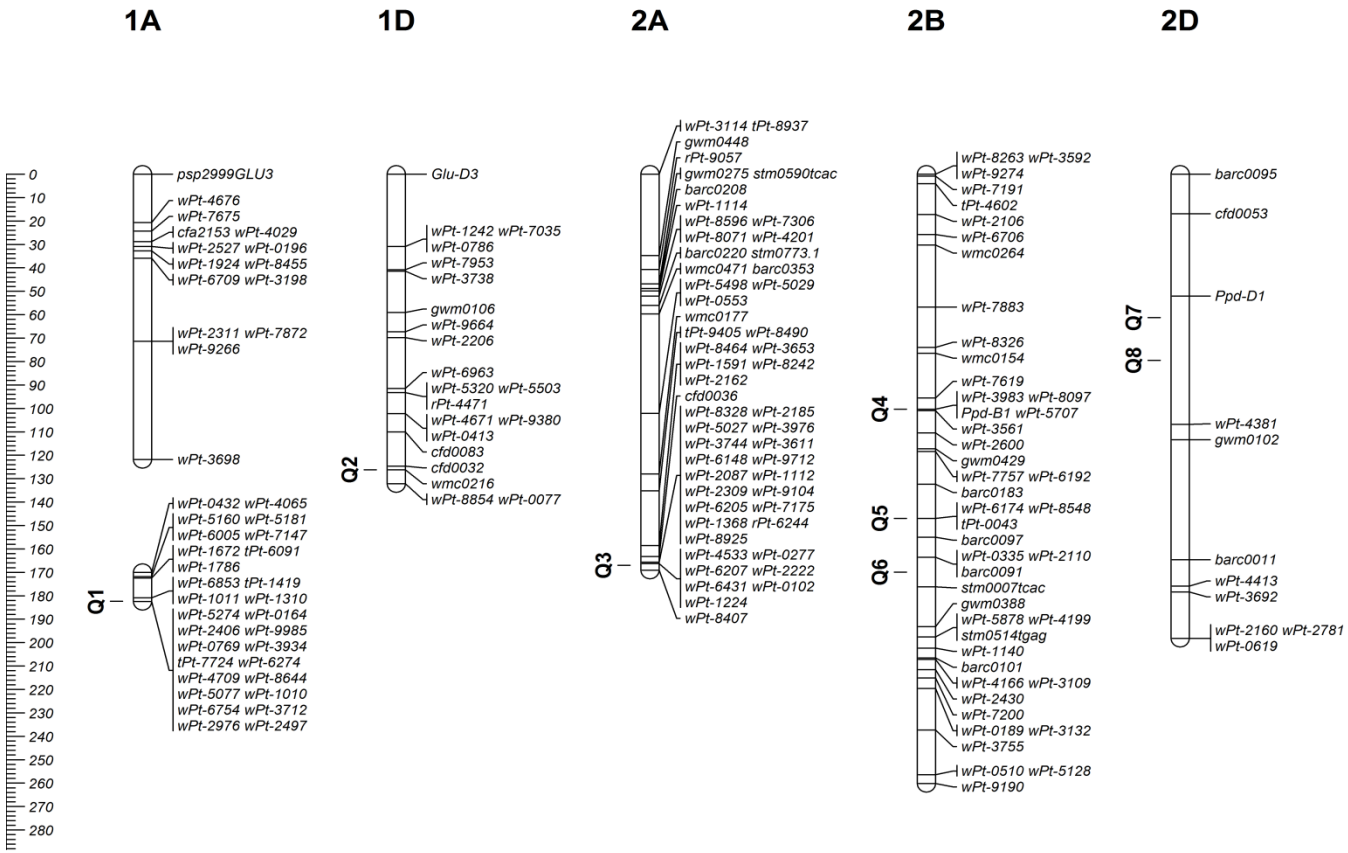

Figure 5.1 Locations (Q1-Q8) on chromosomes of homeologous groups 1 and 2 of wheat at which QTLs were detected for one or more traits, as listed in Table 5.5. The scale on the left indicates genetic distances in cM.

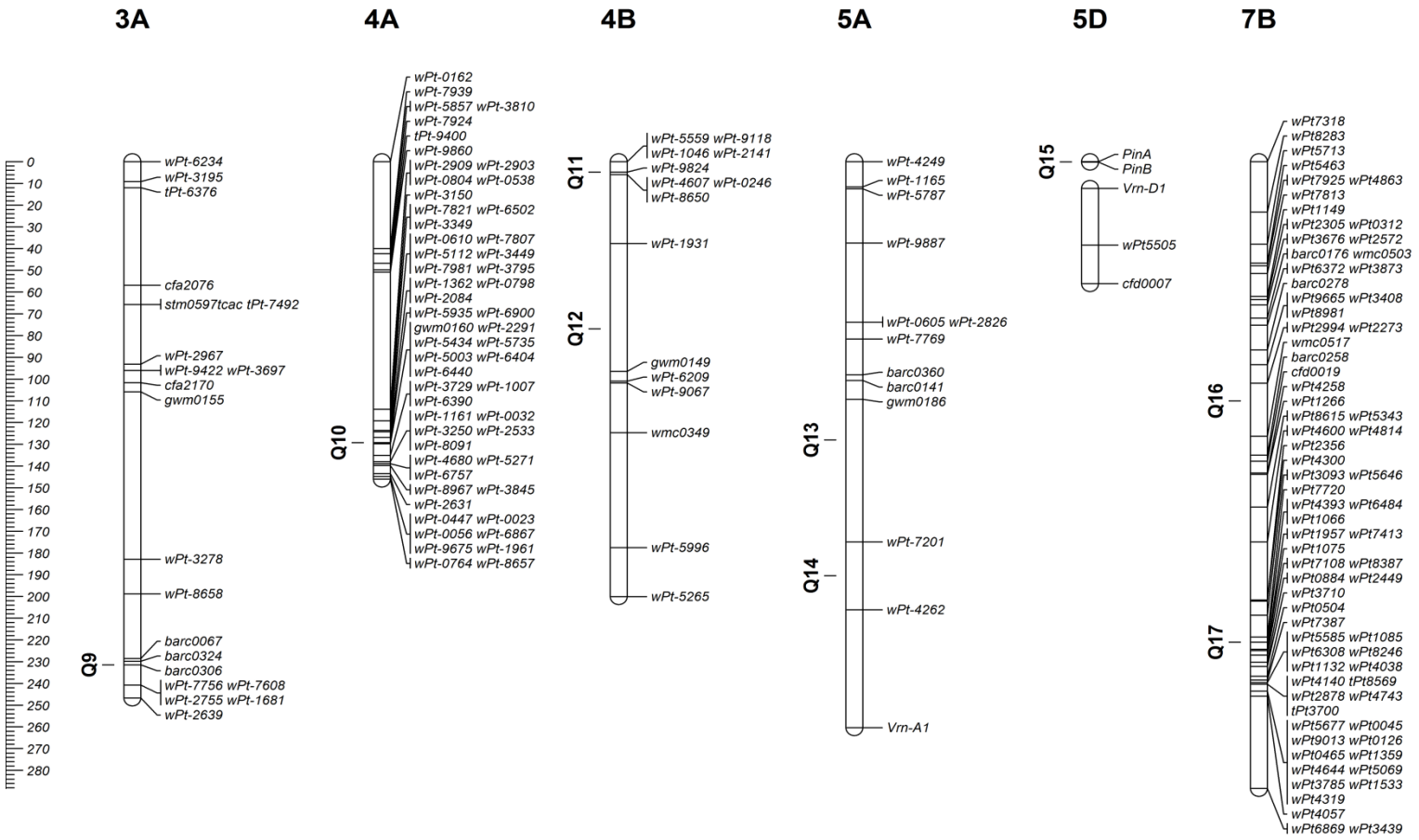

Figure 5.2 Locations (Q9-Q17) on chromosomes of homeologous groups 3, 4, 5 and 7 of wheat at which QTLs were detected for one or more traits, as listed in Table 5.5. The scale on the left indicates genetic distances in cM.

5.4 Discussion

The high loaf volume and score observed in the heat exposed experiment compared to the early sown one are consistent with the high grain protein content achieved in that experiment (Chapter 4 of this thesis), as high protein content has been shown to be highly correlated with loaf volume and score (Barak et al., 2012; Groos et al., 2007; Stojceska and Butler, 2012). High protein content may also have led to very long dough development time and greater dough stability observed under heat conditions, similar to findings in another study (Blumenthal et al., 1991). The observed increase in loaf volume due to heat stress is consistent with findings in another study (Li et al., 2012). The higher loaf score in the experiment grown under heat conditions is partly because of high loaf volume, as loaf score is a summation of loaf volume, texture and structure scores.

In terms of baking output, Drysdale performed better than Gladius under all experimental conditions, and both parents performed better in the early sown trial than in the heat exposed experiment in the 2009 season. In contrast, the population of RILs had higher mean loaf volume in the heat-exposed experiment than in the early sown one. This shows that some lines performed worse than the unfavourable parent, while others performed better than the favourable parent, under control and heat conditions, an indication of transgressive segregation in the population. The better performance of some of the RILs shows that some of the lines might have superior allele combinations and these better performing lines can potentially be selected for future use in quality improvement and cultivar development.

Potentially novel QTLs for dough properties were detected on group 1 chromosomes in genomic regions other than those encoding gluten proteins, which are well known to be associated with dough properties. The QTL on chromosome 1A that affected envelope height at 8 minutes (position Q1 in Figure 5.1) is not associated with the *Glu-A3* locus (or the closely linked/collocating *Gli-A1* locus) on the short arm and this population is also fixed at the *Glu-A1* locus on the long arm. Peak height and width QTLs (position Q2 in Table 5.5 and Figure 5.1) co-locating on the long arm of chromosome 1D are not associated with *Glu-D3* locus (or the closely linked/collocating *Gli-D1* locus) on the short arm and *Glu-D1* on the long arm is not segregating in this population.

On chromosomes 2A and 2B, the QTLs (positions Q3 and Q6 in Figure 5.1) for dough development time appear to be at homoeologous positions, and on chromosome 2A the QTL co-locates with QTL for loaf texture in a region previously shown to be associated with

dough properties and loaf volume in another population (Kuchel et al., 2006). Bake mixing time QTLs were also detected on chromosomes 2B and 2D (positions Q5 and Q8 in Figure 5.1) in genomic regions that have not been previously shown to be associated with dough properties. The role of group 2 chromosomes in dough and baking quality has not been extensively demonstrated and therefore detection of these QTLs offers opportunities for further understanding the role of these chromosomes in processing quality. If these loci can prove to be consistent in additional studies, they can provide more information on the relationship between dough properties and baking quality and possibly allow use of dough properties to more accurately predict baking quality.

Flour colour is important in determining the end use of wheat flour and in this thesis Chapter, as in previous research involving three wheat populations (Mares and Campbell, 2001), QTLs for flour colour were detected on numerous chromosomes. The QTL for flour yellowness (b*) that was detected on chromosome 7B (position Q17 in Figure 5.2) may coincide with previously detected QTLs (Kuchel et al., 2006; Mares and Campbell, 2001) and with the *Psy-B1* locus (He et al., 2009; Ravel et al., 2012). This QTL seems to be in a homeologous position to the QTL detected on chromosome 7A in other studies (He et al., 2008; Mares and Campbell, 2001; Parker et al., 1998; Zhang et al., 2006) and probably to the *Psy-A1* locus (He et al., 2008). The *Ppd-B1* region and a region close to *Ppd-D1* were also associated with flour colour, with the alleles for photoperiod-sensitivity associated with intense flour yellowness. These alleles also delayed heading date (Chapter 3 of this thesis) and decreased grain weight (Chapter 4 of this thesis). The effects of the alleles on grain weight and flour colour is similar to a reported negative correlation between total yellow pigment and grain weight in durum wheat (Digesù et al., 2009). Decrease in grain weight is due to reduced starch accumulation in late flowering lines, and might result in less dilution effect and thus a higher concentration of yellow pigment.

The *Pina-D1b/Pinb-D1a* allele combination that Drysdale carries at the *Ha* locus cause slightly harder grain than the *Pina-D1a/Pinb-D1b* allele combination carried by Gladius (Chapter 4 of this thesis), and the allele combination associated with harder phenotype increased envelope height (a measure that can be used to infer dough extensibility), improved loaf texture and loaf score and increased flour yellowness. Association of the hardness locus with dough and baking output observed in this Chapter is due to the differences in starch damage between the hardness classes during milling which influences water absorption and ultimately dough and baking quality, and it has been reported that harder grain suffers more

starch damage than softer grain (Barak et al., 2012). Damage to starch particles also increases the access to starch of α -amylase enzymes thus providing more sugars for the fermentation process resulting in production of more carbon dioxide thus potentially increasing loaf volume. However, severe starch damage which is mostly likely to occur in extremely hard grain can result in poor loaf properties (Békés, 2012). Selection for the favourable Drysdale alleles at this locus could be readily implemented using puroindoline molecular markers, but it is worth noting that these alleles were also associated with greater flour yellowness in this study, since yellow flour is undesirable for bread making. Similarly, others have observed greater yellowness and less brightness to be associated with harder haplotypes (Peterson et al., 2001; Tsilo et al., 2011).

Some novel and potentially valuable QTLs were detected. The QTL on chromosome 4A (position Q10 in Figure 5.2) at which the allele from the heat tolerant parent Gladius was associated with better loaf score under heat stress conditions could be useful for improving baking output under heat stress conditions. The QTLs (positions Q9 and Q12 in Figure 5.2) associated with flour colour on chromosomes 3A and 4B, QTL (position Q11 in Figure 5.2) associated with mixograph traits on chromosomes 4B and QTLs (positions Q13 and Q14 in Figure 5.2) associated with mixograph traits on chromosomes 5A have not been reported and are potentially novel.

The effect of the environment on quality traits was demonstrated in this thesis Chapter. While heat conditions seemed to improve loaf properties, it is important to view all the steps in the wheat production process, from grain to bread. Low yields obtained from heat stress environments are not profitable for grain growers. Further, very long dough development time can be undesirable, due to high energy requirements, so bakers prefer intermediate dough development time. Some of the QTLs detected in this study showed QTL-by-environment interaction, and some were environment specific. No QTL was detected under drought conditions only. The QTLs at positions Q8, Q10 and Q15 (Figures 5.1 and 5.2) were detected only under heat conditions thus offering opportunities to select allele combinations and lines that perform and/or maintain quality even under high temperatures.

Chapter 6

Genetic control of processing quality in a bread wheat mapping population grown in water-limited environments

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The text, figures and tables presented in this thesis Chapter are exactly the same as published except minor formatting changes such as renumbering to make them consistent with the thesis format. The statement of acknowledgements and the reference list that were included in the published article have been incorporated into the acknowledgements and bibliography of the thesis.

Statement of Authorship

Maphosa L. (Candidate)

Assessed dough properties, measured flour colour, genotyping the mapping population with gene-based markers, interpreted the results and prepared the manuscript.

I hereby certify that the statement of contribution is accurate.

Signed..... Date.....

Langridge P.

Initiated the research and collaboration with the New South Wales Department of Primary Industries, provided valuable advice and research supervision to the candidate and contributed to revision of the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Taylor H.

Measured grain characteristics, milled the grain samples, conducted baking tests, trained the candidate in the quality analysis methods and contributed in the interpretation of quality results.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

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Chalmers K.J.

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Bennett D.

Conducted the field experiments, collected climatic data and contributed to revision of the manuscript.

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Kuchel H.

Developed the doubled haploid population and contributed to revision of the manuscript.

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Mather D.E.

Supervised the research, worked closely with the candidate in revising the manuscript including formatting tables and figures prior to publication and acted as the corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Abstract

End-use quality of bread wheat (*Triticum aestivum* L.) is affected by both genetic and environmental variation. Current understanding of the genetic control of wheat quality traits is mainly based on genetic experiments conducted using grain produced in favourable conditions. The objective of this research was to extend the genetic analysis of these traits by using grain produced in water-limited environments. Grain samples harvested from a mapping population grown in field experiments at two locations in Australia were used to assess characteristics of the grain, flour, dough and bread. Quantitative trait loci were mapped. The parents of the population, RAC875 and Kukri, differ at several loci that are known to affect grain quality or plant phenology. Of these, a high-molecular-weight glutenin locus (*Glu-B1*) affected dough properties, the puroindoline-encoding *Ha* locus affected grain hardness, flour and loaf properties and a photoperiod response locus (*Ppd-D1*) affected flour extraction and protein content. Similarly, several previously reported quantitative trait loci (not associated with specific genes) also had effects in the stress environments used here. In addition, novel QTL were detected for bread wheat quality traits; these may be specific to materials grown under drought and heat stress.

Key words: Wheat quality; drought; heat; QTL

6.1 Introduction

Bread wheat (*Triticum aestivum* L.) is an important crop worldwide and end-use quality is key to its marketability. To consistently satisfy market demands, growers need to produce wheat of reliably good quality regardless of growing conditions. In many wheat production regions, including those in Australia, overall wheat production is severely constrained by biotic and abiotic factors, some of which affect end-use quality. Amongst abiotic factors, drought and heat stress have been found to influence wheat grain development, yield and quality as reviewed by Altenbach (2012).

The classes of wheat that are suitable for particular end uses differ from each other for a number of characteristics, including grain colour and hardness. White flour is generally preferred for baking, while yellow or cream-coloured flour may be suitable for production of yellow alkaline noodles. Hard wheats are preferred for the production of bread and noodles, while soft wheats are preferred for the production of cookies and pastry. Evaluation of wheat quality for bread making involves rigorous testing of wheat grain, flour, dough and bread in sequential stages. The grain characteristics measured include grain size, weight, hardness, protein content and milling yield (the amount of flour obtained from a given quantity of grain). The principal flour characteristic that is measured is colour. Dough rheology tests estimate parameters such as stability, mixing time, extensibility, resistance to extension, tolerance to breakdown, strength, and water absorption. Baking performance is assessed by measuring traits such as loaf volume, texture and structure.

A number of genetic loci are known to affect quality traits in bread wheat. As detailed in a review by Gale (2005), these include the *Glu-1* loci on the long arms of homeologous group 1 chromosomes (encoding high-molecular-weight glutenin subunits (HMW-GS)), the *Glu-3* loci on the short arms of group 1 chromosomes (encoding low-molecular-weight glutenin subunits (LMW-GS)), and gliadin loci on the short arms of group 1 (closely linked with the LMW-GS loci) and 6 chromosomes. Genotypes in these protein coding regions have been used as predictors of dough rheology (Payne et al., 1987). Puroindoline genes at the *Ha* locus on the short arm of chromosome 5D are the main determinants of grain texture (Morris, 2002). Serpin proteins encoded at a locus on the long arm of chromosome 5B, have been shown to affect milling yield (Cane et al., 2008).

Quantitative trait loci (QTLs) for wheat quality traits have been mapped (e.g.,Groos et al., 2007; Kuchel et al., 2006; Mann et al., 2009; Radanovic et al., 2002; Raman et al., 2009),

based on the analysis of grain harvested from trials that had been conducted under favourable conditions. Given that QTLs can interact with environments, this study was carried out to investigate the genetic control of quality traits when plants are exposed to the hot and dry conditions under which wheat must often be grown in regions with Mediterranean climates. Accordingly, QTLs for quality traits were mapped using data from grain grown in low-yielding experiments conducted in water-limited environments in Australia.

6.2 Material and methods

6.2.1 Plant Material

The wheat breeding line RAC875 (RAC655/3/Sr21/4*Lance//4*Bayonet) and the wheat cultivar Kukri (76ECN44/76ECN36//Madden/6*RAC177), which are the parents of the doubled haploid (DH) population used in this study, were chosen on the basis of their contrasting performance under drought and heat stress conditions, with RAC875 being more tolerant to drought and heat than Kukri (Izanloo et al., 2008). The two parents carry the same alleles at the phenology loci *Vrn-A1* and *Vrn-D1* (Table 6.1) and they head within 2 to 5 days of each other (Bennett et al., 2012a). They differ at photoperiod-sensitivity loci, for puroindoline genes, at two high-molecular-weight glutenin loci and three low-molecular weight glutenin loci (Table 6.1).

Table 6.1 Alleles carried by RAC875 and Kukri at high molecular weight glutenin, low molecular weight glutenin, puroindoline, serpin and phenology loci.

		Parent	
Locus type	Locus	RAC875	Kukri
High molecular weight glutenin	*Glu-A1*	*b*	*a*
		(Ax2)*	*(Ax1)*
	Glu-B1	*i*	*al*
		(Bx17 + By18)	*($Bx7^{OE}$* + By8*)*
	Glu-D1	*d*	*d*
		(Dx5 + Dy10)	*(Dx5 + Dy10)*
Low molecular weight glutenin	*Glu-A3*	*c*	*d*
	Glu-B3	*b*	*h*
	Glu-D3	*k*	*b*
Puroindoline	*Pina-D1*	*a*	*b*
	Pinb-D1	*b*	*a*
Serpin	*Srp*	*a*	*a*
Phenology	*Ppd-B1*	*a*	*b*
	Ppd-D1	*b*	*a*
	Vrn-A1	*a*	*a*
	Vrn-D1	*v*	*v*

From among 368 available RAC875/Kukri DH lines, 192 early to mid-maturing lines were selected for use in the experiments described here. Selection of these lines was based on the number of calendar days from sowing to ear emergence in a preliminary evaluation of the population that was conducted at Roseworthy, South Australia in 2007. The distribution of these DH lines according to the time to ear emergence has been described by Bennett et al. (2012a).

6.2.2 Field experiments

The field experiments used for analysis of quality traits were selected from among many in which this population had been evaluated for agronomic traits (Bennett et al., 2012b). The selected experiments were those conducted at Piednippie and Streaky Bay in South Australia in 2008. These locations are about 60 km apart (33°S latitude, 134°E longitude) and have

similar grey calcareous sandy loam soils types. The experiments were sown and harvested within one week of each other. In the 2008 growing season, rainfall was only 212 mm (188 mm prior to anthesis and 24 mm during grain filling) at Piednippie and even less (95 mm total, 85 mm prior to anthesis and 10 mm during grain filling) at Streaky Bay. Throughout the growing season, maximum temperatures were higher at Piednippie than at Streaky Bay. The number of days with temperatures above 35°C was also higher at Piednippie than at Streaky Bay.

Each of the experiments consisted of two randomised complete blocks, each of which included 192 RAC875/Kukri DH lines, the two parents and six controls (the cultivars Gladius, Drysdale, Excalibur, Krichauff, Wyalkatchem and Yitpi), all sown at about 200 seeds m^{-2}. Local management practices for fertiliser and chemical application were applied to minimise potential confounding factors such as non-uniform soil fertility and disease incidence. From each plot, the area harvested was 7 m^2.

From each experiment, 208 grain samples were prepared for evaluation of quality characteristics. These included one sample of each of 192 RAC875/Kukri DH lines, 10 samples of control cultivars (three Gladius, one Drysdale, three Excalibur, one Krichauff, one Wyalkatchem and one Yitpi) and three samples of each parent. Each of these samples was a composite of grain from across two blocks. After examination of the grain samples, 15 samples from Piednippie (all DH lines) had to be excluded due to the presence of common bunt disease caused by a *Tilletia* spp. fungal pathogen.

6.2.3 Grain characteristics

Percentage moisture content, grain protein content and particle size index (PSI) were estimated by near-infrared reflectance (NIR) (RACI-CCD, 2010) using a Foss 6500 NIR instrument (FOSS NIR Systems, Inc., Laurel, MD). For grain moisture content, the NIR prediction is based on calibration to air oven moisture determination (AACC, 1999). For grain protein content, the NIR prediction is based on calibration to Leco protein determination (RACI-CCD, 2010) and reported on an "as is" basis. For PSI, the calibration was based on the particle size index method (AACC, 1999). Hard grain is expected to have lower particle size index values than soft grain.

6.2.4 Flour extraction

Approximately 1 kg of grain from each bulked sample was conditioned overnight to 15% moisture content (AACC, 1999) and milled on a Bühler MLU-202 laboratory test mill (Bühler AG, Uzwil, Switzerland). Recovered flour was weighed and flour extraction (milling yield) was expressed as a percentage of the initial grain weight.

6.2.5 Colour parameters

Flour and loaf colour were measured using a Minolta Chroma Meter CR-410 instrument (Minolta Co. Ltd., Osaka, Japan) utilising a D65 (daylight) light source, with colour expressed in CIE units (RACI-CCD, 2010). The CIE colour space measures brightness (L*) with higher values for brighter colour and blueness-yellowness (b*) with positive values indicating yellowness.

6.2.6 Dough properties

Dough properties were assessed using the Newport Scientific DoughLAB (Newport Scientific Pty Ltd., NSW, Australia), which is an electronic alternative for the Brabender farinograph. It records dough stability (seconds), development time (seconds), softening (FU units) and percentage water absorption (RACI-CCD, 2010). The baseline is set by mixing flour for one minute before water is added.

6.2.7 Baking quality

The baking test method used in this study was the long fermentation method (RACI-CCD, 2010) which is based on AACC approved methods 10:09 and 10:10b (AACC, 1999) with bake mixing time taken as time to full dough development. The baking ingredients were flour, water, sugar, α-amylase, ammonium chloride, sodium chloride, fat, and ascorbic acid. Baked products were evaluated for loaf volume using the rapeseed displacement method (AACC, 1999) and loaf colour using the Minolta Chroma Meter CR-410 instrument. To permit evaluation of crumb texture (feel) and crumb structure (appearance), loaves were cut diagonally from corner to corner to allow maximum view. For crumb texture a scale of 1 to 10 was used with 1 being “very tight under thumb pressure, crumb tears, rough to the feel”

and 10 being “very soft feel under the thumb, very smooth, recovers with no indentation”. For crumb structure a score of 1 to 10 was also used with 1 being “round cells with thick cell walls, crumpet like appearance” and 10 being “very fine cell walls, elongated cells around the edges of the loaf”. Loaf volume score was obtained by dividing loaf volume by 48 and loaf volume score, loaf texture and loaf structure scores were summed to provide loaf score.

6.2.8 Marker genotyping

Each DH line was genotyped with gene-based markers for the loci *Pina-D1*, *Pinb-D1*, *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-D3* and *Ppd-B1*. For *Pina-D1* and *Pinb-D1*, the assays were as described by Cane et al. (2004). The *Glu-A1b* allele (subunit Ax2*) carried by RAC875 was detected using the assay described by Ma et al. (2003). The *Glu-B1al* over-expression allele (subunit $Bx7^{OE}$*)* was detected using assays described by Ragupathy et al. (2008). For *Glu-A3* the method described by Zhang et al. (2004) was used to differentiate between the *c* and *d* alleles. For *Ppd-B1* the method described by Díaz et al. (2012) was used to detect the *a* allele. All of these markers are dominant, so reactions were performed at least twice to ensure that absence of products was not due to failed PCR reactions. For *Glu-D3*, a multiplex PCR assay (Appelbee et al., 2009) involving primer pairs M2F12/M2R12 and S13F2/S13R1 was used to detect both the *b* allele (from Kukri) and the *k* allele (from RAC875).

6.2.9 Genetic analysis

The linkage map used for genetic analysis was based on the one used by Bennett et al. (2012a) for analysis of agronomic traits in the same population. That map, which was constructed using genotypic data from 368 RAC875/Kukri DH lines, includes 457 marker loci (246 DArT markers and 210 SSR markers) and the *Ppd-D1* gene. The gene-based markers described in section 6.2.8 were added to the map to bring the total number of markers to 464. The map consists of 21 linkage groups with a total map length of 3452 cM. A multiple environment QTL analysis using a genome-wide significance threshold ($p = 0.05$) was done using composite interval mapping with GenStat 14 (http://www.vsni.co.uk/software/genstat).

6.3 Results

6.3.1 Phenotypic variation

Samples from the Streaky Bay experiment had softer grain, slightly higher grain protein content, lower flour extraction, slightly longer dough development time, longer bake mixing time, more stable dough, less dough softening and larger loaf volume than those from the Piednippie experiment. For each quality trait, the range of values observed among the DH lines exceeded that of the two parents (Table 6.2). For pairs of traits that exhibited significant phenotypic correlations at both sites, the directions of the associations were the same at both sites (Table 6.3). Dough development time, dough stability and mixing time were positively correlated with each other and negatively correlated with dough softening. The baking output parameters loaf texture, structure and volume were positively correlated with each other.

Table 6.2 Mean values for grain, flour and loaf characteristics for RAC875 and Kukri and means, minima, maxima and standard deviations (s.d.) for the same characteristics in a set of RAC875/Kukri population of doubled haploid lines, all grown at two locations (Piednippie and Streaky Bay) in South Australia.

	Grain characteristics			Flour characteristics				Dough characteristics				Loaf characteristics						
	Protein	Particle	Moisture	Flour	Colour		Water	Dough	Dough	Bake	Dough	Loaf	Loaf	Loaf	Loaf		Colour	
Parent or population	content (%)	size index	content (%)	extraction (%)	L* (CIE)	b* (CIE)	absorption (%)	development time (s)	stability (s)	mixing time (s)	softening (FU)	volume (cc)	volume score[a]	texture score[b]	structure score[b]	Loaf score[c]	L* (CIE)	b* (CIE)
Piednippie																		
RAC875	11.6	13	9.8	75.5	90.9	9.4	61.3	155	148	228	132	752	16	7	6	29	81.1	16.3
Kukri	12.0	12	9.7	75.3	91.4	10.1	59.7	202	305	288	82	767	15	7	7	29	82.4	17.3
Population																		
Mean	12.0	12	9.7	75.3	91.2	9.8	61.6	197	247	283	99	763	16	7	6	29	81.9	16.8
s.d.	0.6	3	0.2	1.2	0.4	0.6	2.4	44	94	48	29	48	2	1	1	2	1.0	0.8
Minimum	10.6	4	9.3	70.8	89.9	8.1	55.4	117	86	150	39	615	14	5	4	23	78.5	14.6
Maximum	13.7	21	10.4	78.7	92.3	11.1	68.6	417	480	360	183	890	17	9	9	35	84.0	19.6
Streaky Bay																		
RAC875	13.0	15	9.0	72.1	91.3	10.0	59.6	185	204	303	100	813	17	8	6	31	82.2	17.4
Kukri	13.9	17	8.9	72.0	91.4	10.5	59.5	211	304	352	77	802	17	6	7	30	82.3	18.0
Population																		
mean	13.6	15	9.0	72.0	91.3	10.4	60.7	210	301	314	80	828	18	7	6	31	81.9	17.7
s.d	0.6	3	0.2	1.8	0.4	0.7	2.3	47	108	44	23	74	2	1	1	2	0.6	0.8
Minimum	12.1	9	8.4	63.3	90.4	8.5	55.1	139	119	195	77	645	15	4	5	24	78.4	15.8
Maximum	15.5	24	9.5	75.8	92.2	12.1	67.0	444	644	360	137	1040	19	9	9	37	83.7	19.9

[a]On a scale from 1 to 20; [b]On a scale from 1 to 10; [c]Sum of loaf volume score, loaf texture score and loaf structure score.

Table 6.3 Significant ($p < 0.001$) phenotypic correlation coefficients between traits for the RAC875/Kukri doubled haploid population in the experimental sites Streaky Bay (below the diagonal) and Piednippie (above the diagonal).

	GPC	**PSI**	**FEX**	**FWA**	**FCB**	**FCL**	**DDT**	**DSO**	**DST**	**BMT**	**LVO**	**LST**	**LTX**	**LCB**	**LCL**
Grain protein content (GPC)		-	-	-	-	-	-	-	-	-	0.37	-	-	-	-
Particle size index (PSI)	0.30		-	-0.51	-0.41	0.55	-	-	-	-	-	0.39	-	-	0.45
Flour extraction (FEX)	-0.36	-0.30		-0.31	-0.34	-	-	0.27	-0.27	-	-	-	-	-0.31	-
Flour water absorption (FWA)	-	-	-		0.31	-0.48	-	-	-	-	-	-0.41	0.29	-	-0.37
Flour colour b* (FCB)	-	-0.36	-	-		-0.48	-	-	-	-	-	-	-	0.50	-
Flour colour L* (FCL)	-	0.51	-	-	-0.68		-	-	-	-	-	0.36	-	-	0.58
Dough development time (DDT)	-	-	-	-	-	-		-0.77	0.86	0.59	-0.26	-	-	-	-
Dough softening (DSO)	-	-	0.36	-	-	-	-0.73		-0.90	-0.55	-	-	-	-	-
Dough stability (DST)	-	-	-0.40	-	-	-	0.87	-0.93		0.64	-0.31	-	-	-	-
Bake mixing time (BMT)	-	-	-0.26	-	-	-	0.73	-0.79	0.82		-0.47	-	-	-	-
Loaf volume (LVO)	-	-	-	-	0.30	-0.39	-	-	-	-0.28		-	0.46	-	-
Loaf structure (LST)	-	-	-	-	-	0.36	-	-	-	-	-		-	-	0.48
Loaf texture (LTX)	-	-0.46	-	-	0.27	-	-	-	-	-	0.61	-		-	-
Loaf colour b* (LCB)	-	-	-	-	0.46	-	-	-	-	-	-	-	-		-
Loaf colour L* (LCL)	-	-	-	-	-0.32	0.41	-	-	-	-	-	0.36	-	-0.34	

6.3.2 Quantitative trait loci

Quantitative trait loci were detected for all traits except for blueness-yellowness (b*) of loaf colour and on 14 of the 21 chromosomes of the wheat genome (Table 6.4, Figures 6.1 and 6.2). The *Ha* locus on chromosome 5D (detected using markers for puroindoline genes) was associated with several grain, flour and loaf traits. The RAC875 haplotype decreased flour water absorption and flour yellowness, increased flour brightness and loaf brightness, and decreased overall loaf score, with negative effects on loaf texture score, and loaf volume but positive effects on loaf structure score. The *Ha* locus exhibited QTL-by-environment interaction for some traits, affecting flour water absorption only at Piednippie and loaf texture and loaf score only at Streaky Bay.

Within 8.5 cM of the *Glu-A3* locus on chromosome 1A (positions Q1 and Q2 in Figure 6.1), QTLs were detected for particle size index, flour yellowness, flour brightness, flour water absorption and loaf texture (Table 6.4). The Kukri alleles increased all the traits except particle size index and flour brightness. All of these QTLs were detected in both environments, except for the water absorption QTL, which was detected only at Piednippie.

At the *Glu-B1* locus on chromosome 1B, Kukri carries the *Glu-B1al* allele, which includes two copies of the gene encoding the $Bx7^{OE}$ HMW-GS (Ragupathy et al., 2008) and confers over-expression of that subunit relative to other HMW-GS. In the RAC875/Kukri population, this chromosome region (positions Q4 and Q5 in Figure 6.1) affected rheology traits (dough development time, stability and softening) and another region on chromosome 1B (position Q3 in Figure 6.1) affected dough stability and softening. In both regions, the Kukri alleles increased dough development time and stability and reduced dough softening. The *Ppd-D1* locus on chromosome 2D (position Q7 in Figure 6.1) was associated with grain protein content at both locations and with flour extraction at Streaky Bay, with the Kukri allele increasing protein content and the RAC875 allele increasing flour extraction. Quantitative trait loci for wheat quality traits were also detected in genomic regions in which the RAC875/Kukri population is not known to segregate for any specific genes already known to affect grain quality or plant phenology (Table 6.4, Figures 6.1 and 6.2).

Table 6.4 Summary of QTLs detected for grain, flour and loaf traits in the RAC875/Kukri mapping population, with estimated additive effects of the RAC875 allele (within or across locations depending on whether or not statistically significant QTL-by-environment interaction was detected).

Chromosome	Position (cM)	Closest marker(s)	QTL position number in figures	QTL	Trait	LOD	Estimated additive effect of RAC875 allele		
							Significant QTL×E		No significant QTL×E
							Piednippie	Streaky Bay	
1A	5.3	*wPt-6564*	*Q1*	*QPsi.aww-1A*	Particle size index	7.6			0.9
	5.7	*cfa2153*	*Q1*	*QFcb.aww-1A*	Flour colour b* (CIE)	6.8			-0.2
	20.9	*gdm0033a*	*Q2*	*QFcl.aww-1A*	Flour colour L* (CIE)	17.7			0.2
				QFwa.aww-1A	Flour water absorption (%)	4.1	-0.7	-	
	21.6	*wPt-8770*	*Q2*	*QLtx.aww-1A*	Loaf texture	4.2			-0.2
1B	72.0	*gwm0413 barc0137a*	*Q3*	*QDso.aww-1B-1*	Dough softening (FU)	4.7	11.2	5.8	
				QDst.aww-1B-1	Dough stability (s)	5.2			-38.5
	103.9	*wPt-0202*	*Q4*	*QDso.aww-1B-2*	Dough softening (FU)	2.0			5.4
				QDst.aww-1B-2	Dough stability (s)	2.1			-22.7
	109.2	*Glu-B1*	*Q5*	*QDdt.aww-1B*	Dough development time (s)	22.3			-25.4
2B	151.4	*wPt-7360*	*Q6*	*QGpc.aww-2B*	Grain protein content (%)	4.6	0.2	-	
2D	35.0	*Ppd-D1*	*Q7*	*QGpc.aww-2D*	Grain protein content (%)	5.3			-0.2
				QFex.aww-2D	Flour extraction (%)	6.1	-	0.7	
3A	26.1	*wPt-0714*	*Q8*	*QFcl.aww-3A*	Flour colour L* (CIE)	3.8	-0.1	-	
	66.1	*wPt-4077*	*Q9*	*QGpc.aww-3A*	Grain protein content (%)	3.4	-	-0.2	
	83.9	*wPt-8658*	*Q10*	*QFex.aww-3A*	Flour extraction (%)	5.6			0.4
3B	96.2	*gwm0285 gwm0131a*	*Q11*	*QDso.aww-3B*	Dough softening (FU)	4.8			6.4
				QDst.aww-3B	Dough stability (s)	4.9			-26.4
				QBmt.aww-3B	Bake mixing time (s)	2.0			-8.8
	102.1	*gwm0131a*	*Q12*	*QDdt.aww-3B*	Dough development time (s)	4.3			-10.4
3D	189.5	*wPt-0485*	*Q13*	*QFex.aww-3D*	Flour extraction (%)	3.2			-0.3
4A	108.7	*wPt-5694*	*Q14*	*QFex.aww-4A*	Flour extraction (%)	5.2			-0.4

Table 6.4 (continued)

Chromosome	Position (cM)	Closest marker(s)	QTL position number in figures	QTL	Trait	LOD	Estimated additive effect of RAC875 allele		
							Significant QTL×E		No significant QTL×E
							Piednippie	Streaky Bay	
		Pina-D1		*QPsi.aww-5D*	Particle size index	25.0			1.6
		Pinb-D1		*QFcb.aww-5D*	Flour colour b* (CIE)	19.9			-0.4
				QFcl.aww-5D	Flour colour L* (CIE)	42.1			0.2
				QFwa.aww-5D	Flour water absorption (%)	12.6	-1.2	-	
5D	0.0		*Q16*	*QLst.aww-5D*	Loaf structure	6.4			0.3
				QLtx.aww-5D	Loaf texture	12.4	-	-0.6	
				QLcl.aww-5D	Loaf colour L* (CIE)	5.7	0.3	0.3	
				QLsc.aww-5D	Loaf score	5.7	-	-0.7	
				QLvo.aww-5D	Loaf volume (cc)	5.1	-6.6	-25.4	
				QLsc.aww-6A	Loaf score	5.0	0.5	0.5	
6A	21.2	*wPt-8266*	*Q17*	*QLtx.aww-6A*	Loaf texture	6.0	0.2	0.2	
				QLvo.aww-6A	Loaf volume (cc)	4.1	14.1	14.1	
	80.8	*barc0118*	*Q18*	*QGpc.aww-6A*	Grain protein content (%)	7.3	-0.2	-0.2	
6B	93.1	*gwm0626*	*Q19*	*QDso.aww-6B*	Dough softening (s)	4.6	5.9	5.9	
	115.6	*barc0108*	*Q20*	*QGpc.aww-7A*	Grain protein content (%)	5.9	-	-0.2	
7A	117.8	*wmc0603b*		*QFcb.aww-7A-1*	Flour colour b* (CIE)	7.1	-0.2	-0.2	
	145.2	*gwm0276*	*Q21*	*QPsi.aww-7A*	Particle size index	2.7	0.6	-	
	236.2	*wPt-7763*	*Q22*	*QFcb.aww-7A-2*	Flour colour b* (CIE)	4.9	0.2	0.2	
7B	32.6	*gwm0297*	*Q23*	*QBmt.aww-7B*	Bake mixing time (s)	4.7	11.2	-	
7D	101.8	*stm0001tcac*	*Q24*	*QPsi.aww-7D*	Particle size index	8.0	0.9	-0.3	

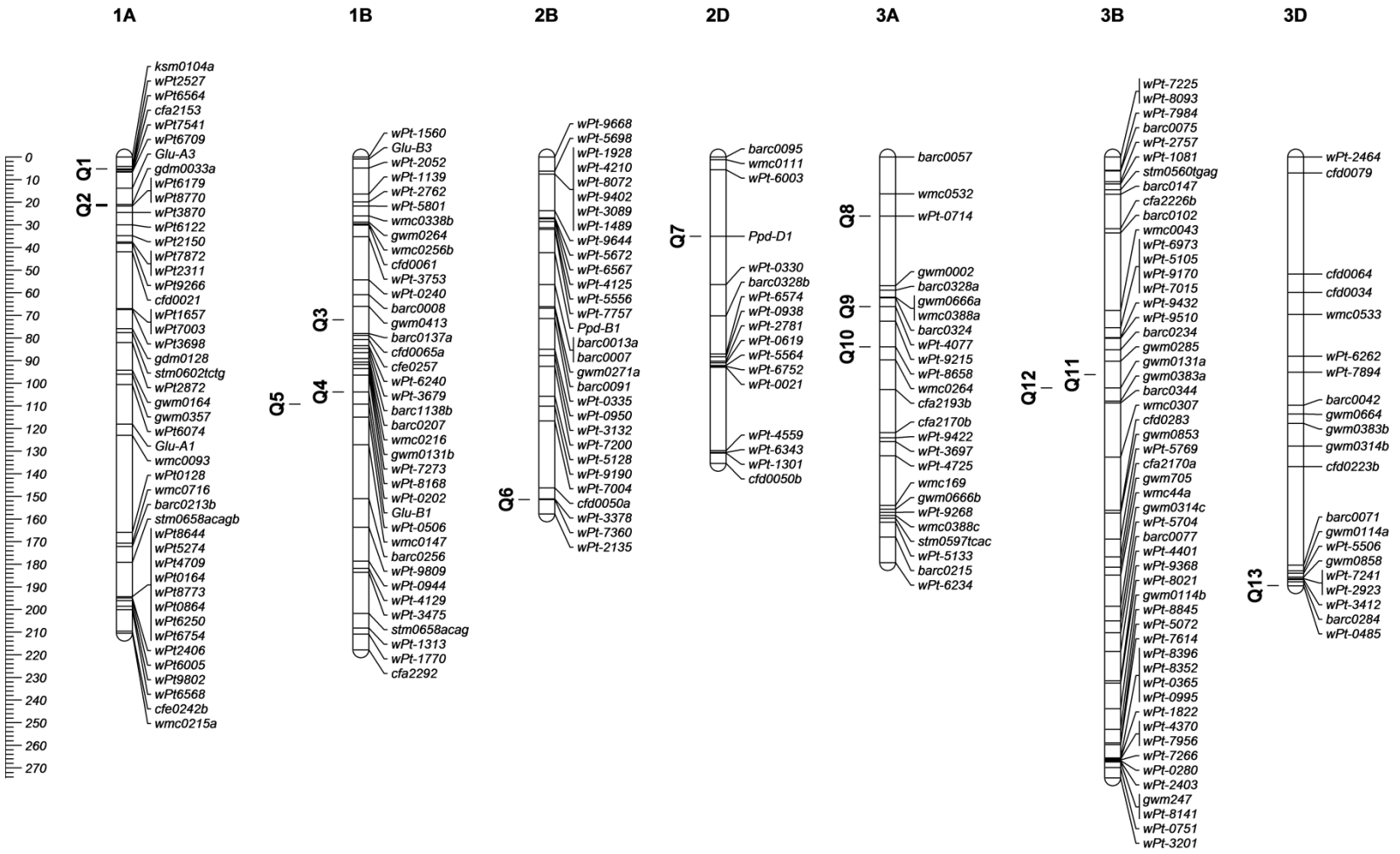

Figure 6.1 Locations (Q1 to Q13) on chromosomes of homeologous groups 1, 2 and 3 of wheat at which QTLs were detected for one or more traits, as listed in Table 6.4. The scale on the left indicates genetic distances in cM.

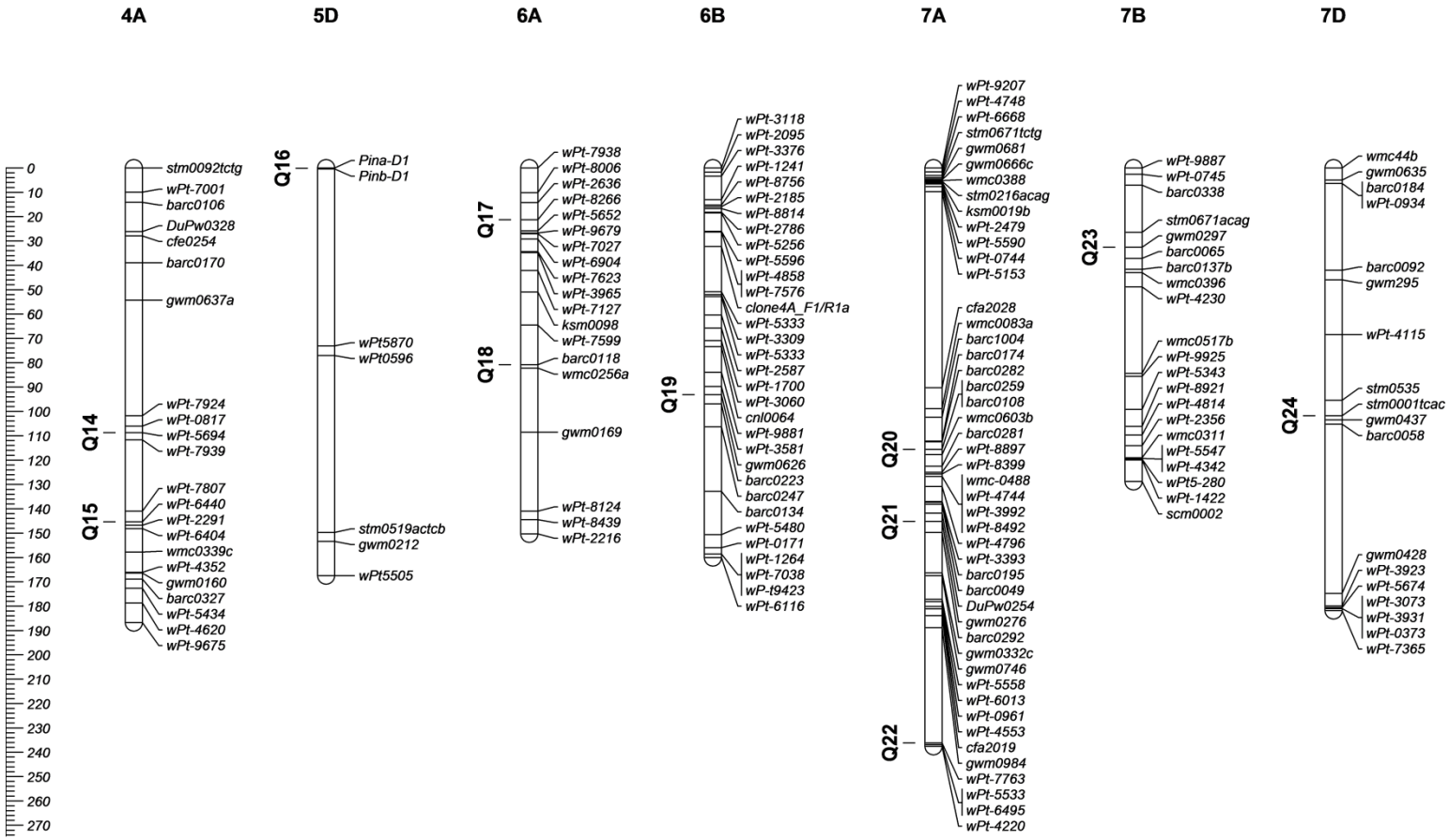

Figure 6.2 Locations (Q14 to Q24) on chromosomes of homeologous groups 4, 5, 6 and 7 of wheat at which QTLs were detected for one or more traits, as listed in Table 6.4. The scale on the left indicates genetic distances in cM.

6.4 Discussion

It is well known that bread wheat quality is affected by genetic and environmental factors and by their interaction. The parents of the mapping population used in this study were chosen on the basis of their contrasting agronomic and physiological responses to drought and heat stress and they were known to differ for some quality traits. The grain samples evaluated for quality traits were produced under conditions of water deficit and high temperature at two locations, making it possible to map QTLs that affect quality characteristics of grain grown under abiotic stress conditions.

The co-location of QTLs for particle size index (grain hardness), flour and loaf colour, flour water absorption and loaf structure and texture on chromosome 5D (position Q16 in Figure 6.2) and on chromosome 1A (positions Q1 and Q2 in Figure 6.1) can be interpreted with respect to the relationships among those traits and the segregation of the *Ha* and *Glu-A3* loci in the mapping population. During milling, starch particles suffer damage to varying degrees, particularly if the grain is hard. Damage to starch particles can influence the amount of water absorbed by flour (Cane et al., 2004). In addition, starch damage may also increase the access of yeast to starch during fermentation and thereby influence loaf properties. On both chromosomes, the parent with alleles that increased grain hardness (Kukri) also has alleles that increase flour yellowness, water absorption, loaf texture and decrease flour brightness. This is consistent with a previous observation that an increase in grain hardness result in darker grain with decreased brightness and increased yellowness (Peterson et al., 2001). At the *Ha* locus the RAC875 haplotype (*Pina-D1a* and *Pinb-D1b*) is associated with moderately hard grain and the Kukri haplotype (*Pina-D1b* and *Pinb-D1a*) is associated with very hard grain (Cane et al., 2004). The QTL for particle size index on chromosome 7A (position Q21 in Figure 6.2), which was detected only in Piednippie, might correspond to the one previously detected and near *Pinb-A2* (Wilkinson et al., 2008), which is part of the recently discovered *Pinb-2* gene family, which has members on all three group 7 chromosomes (Geng et al., 2012a)

Here, the *Glu-B1al* allele increased dough development time and stability and decreased dough softening relative to the *Glu-B1i* allele from RAC875 confirming that the *Glu-B1al* over-expression allele affects dough properties even under stress conditions. The increased proportion of the over-expressed $Bx7^{OE}$ subunit results in strong dough that requires more energy and time to mix, which remains stable for a longer time and does not break down

(soften) easily. The *Glu-B1al* over-expression allele, which Kukri carries, is known to improve dough strength, significantly increasing extensibility, dough development time and maximum dough resistance (Rmax) relative to other *Glu-B1* alleles (Butow et al., 2003; Eagles et al., 2004; Mann et al., 2009). Quantitative trait loci for dough characteristics have previously been mapped at *Glu-B1* in wheat populations that segregate for the *Glu-B1al* (Mann et al., 2009; Radovanovic et al., 2002; Raman et al., 2009).

Other QTLs affecting dough properties were detected (at positions Q3, Q11, Q12, Q19 and Q23 in Table 6.4 and Figures 6.1 and 6.2), but none of them are closely linked with any loci previously known to affect grain quality. One of these QTLs (at position Q3 in Figure 6.1) is on chromosome 1B but is not closely linked with either *Glu-B1* or *Glu-B3*. Another one is on chromosome 6B (at position Q19 in Figure 6.2) but is not near the gliadin loci on the short arm of that chromosome. The alleles associated with weak dough (high dough softening values) all came from RAC875, except at position Q23, at which the RAC875 allele was associated with stronger dough and increased bake mixing time.

Based on comparisons with published maps available on Graingenes (http://wheat.pw.usda.gov/) and Triticarte (http://www.triticarte.com.au/) web sites, a QTL affecting loaf properties (position Q17 in Figure 6.2) seems to be at or near the *Gli-A2* gliadin locus on the short arm of chromosome 6A, with the RAC875 allele favourable for all traits. Gliadin proteins mainly affect dough viscosity but their effect on loaf properties is not clearly defined in literature. Heat stress has been reported to increase gliadin synthesis, mainly because of the close proximity of gliadins to heat shock proteins, thus increasing the gliadin:glutenin ratio and modifying overall protein composition (Blumenthal et al., 1993). This increased gliadin synthesis is often cited as a possible cause of reduced dough strength in heat stressed wheat.

Grain protein content was found to be under polygenic control in RAC875/Kukri. One locus (position Q9 on chromosome 3A in Figure 6.1) that affected protein content seems to coincide with a locus detected in a Kukri/Janz population by Mann et al. (2009). In both studies, the Kukri alleles increased protein content. This locus may also coincide with loci that have been shown to be associated with baking quality in other populations (Groos et al., 2007; Kuchel et al., 2006). At the *Ppd-D1* locus on chromosome 2D (position Q7 in Figure 6.1), effects on protein content and flour extraction were detected. At this locus, increased protein content and reduced flour extraction were associated with the *Ppd-D1a* allele from

Kukri, an allele that confers photoperiod insensitivity and results in early flowering (Bennett et al., 2012a). This region was not associated with yield; the closest yield QTL was about 40 cM away (Bennett et al., 2012b).

Differences in flour colour can be due to inherent differences in pigment concentration in wheat endosperm, while loaf colour can be influenced both by initial flour colour and by biochemical reactions during baking. In some populations, flour yellowness QTLs have been detected on the long arm of chromosome 7A (Mares and Campbell, 2001; Parker et al., 1998), at or near the phytoene synthase gene *Psy-A1* (Howitt et al., 2009). In this study, a flour colour QTL was detected at a similar location (position Q22 in Figure 6.2), but this QTL cannot be attributed to *Psy-A1,* because both RAC875 and Kukri carry the same allele (*p,* for pale yellow flour colour) at *Psy-A1*. Accordingly, it seems that *Psy-A1* is not the only gene in this region that affects flour colour. No QTL were detected for loaf yellowness, which may mean that pigments influencing yellow colour in flour are degraded during baking or that other pigments overshadow them in the baked product. In contrast, Mann et al. (2009) detected co-locating QTLs for flour and loaf colour on the long arm of chromosome 7A in a Kukri/Janz mapping population. In that case, the flour colour effect could be due *Psy-A1* locus, as Janz carries the *PsyY-A1e* allele for white colour (Howitt et al., 2009). In contrast to the differences in the genetic control between flour and loaf yellowness, flour and loaf brightness were under common genetic control, with greater brightness associated with the RAC875 allele at the *Ha* locus.

This population has been extensively investigated for agronomic performance in many environments including the two in this study (Bennett et al., 2012a; Bennett et al., 2012b). Some of the QTLs detected in this study co-located with QTLs for agronomic performance and physiological attributes. In a region on chromosome 7A (position Q20 in Figure 6.2), early flowering due to the RAC875 allele was accompanied by increased yield and thousand grain weight but decreased grain number and protein content. The inverse relationship between protein content and yield and yield components is not surprising and is probably due to a dilution effect in well-filled grain. On chromosome 3A, a QTL for flour extraction (position Q10 in Figure 6.1) co-located with a QTL for leaf glaucousness, with RAC875 alleles increasing both traits. Though glaucousness is thought to be a stress survival mechanism, it is not clear how it aids plants to survive unfavourable conditions. If glaucousness plays a role in stress survival, it might lead to well filled grain and better flour extraction. Some other co-locations are difficult to explain: for example, time to ear

emergence and water soluble carbohydrates with bake mixing time on chromosome 7B (position Q23 in Figure 6.2) and canopy temperature with flour brightness (L*) on chromosome 3A (position Q8 in Figure 6.1).

Some of the QTLs detected here displayed QTL-by-environment interaction (Table 6.4) with effects only at Piednippie (positions Q2, Q6, Q8, Q16, Q21 and Q23 in Table 6.4) or only at Streaky Bay (positions Q7, Q9, Q15, Q16 and Q20 in Table 6.4). Four of the QTLs displaying QTL-by-environment interaction (positions Q6, Q9, Q15 and Q20) were for protein content, a trait known to be highly dependent on environmental conditions. Consistent with the general expectation that water deficit increases protein content, grain from the Streaky Bay experiment was higher in protein than grain from the Piednippie experiment. The inter-related traits grain hardness, flour extraction, water absorption, brightness, bake mixing time, loaf score and texture were also environmentally dependent.

Some of the QTLs detected for quality traits in this study correspond with known genes for grain quality traits (positions Q5 and Q16 in Figures 6.1 and 6.2) or with previously detected QTLs for grain quality traits (position Q9 in Figure 6.1) while others have not been detected in previous studies. Some of these novel QTLs may be useful for developing wheat cultivars that can maintain their end-use quality under drought and heat stress conditions. For example, QTLs at positions Q11 and Q12 on chromosome 3B (with favourable alleles coming from Kukri) offer opportunities to improve several dough traits and the QTL at position Q17 on chromosome 6A (with favourable alleles coming from RAC875) could allow for the improvement of loaf properties. The presence of favourable alleles at different genomic regions in the two parents presents prospects for combining favourable alleles from both parents to improve quality of wheat grain grown in water-limited environments.

Chapter 7

Effect of post-anthesis heat on bread wheat lines with and without the *Gpc-B1* introgression segment from wild emmer wheat

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To be submitted for publication:

Statement of authorship

Maphosa L. (Candidate)

Designed the experiment, conducted the glasshouse and laboratory experiments, interpreted results and prepared the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Collins N.

Devised experimental methods for evaluation of the effects of heat stress and worked closely with the candidate in conducting the glasshouse experiments and contributed to revision of the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Taylor J.

Did the statistical analysis, interpretation of the results and contributed to revision of the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Mather D.E.

Initiated the experiment, oversaw the molecular marker-assisted selection for the backcrossing, suggested the experimental design, assisted in the interpretation of the results, contributed to revision of the manuscript and is acting as the corresponding author

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the article in the thesis.

Signed..... Date.....

Abstract

Heat stress compromises bread wheat production and quality. The main aim of this study was to investigate the effects of high temperature in two pairs of backcross derivatives, with and without a chromosomal introgression segment from wild emmer wheat that contains the *Gpc-B1* gene, in their corresponding recurrent parents (RAC1262A and Wyalkatchem) and in Australian cultivars; Gladius and Drysdale. The experiment was conducted in a glasshouse under normal growing conditions (24/18°C, 14/10 h day/night) and half of the plants were put into a heat chamber (37/27°C, 14/10 h day/night) at 15 d after anthesis for three days. The presence of the *Gpc-B1* introgression segment significantly increased protein content and unextractable polymeric protein in the Wyalkatchem background and accelerated senescence compared to backcross derivatives with the alternate alleles in both backgrounds. The *Gpc-B1* segment had no effect on grain number or weight and therefore its presence might not lead to any yield penalty. The non-*Gpc-B1* lines were more similar genetically and phenotypically to their respective recurrent parents than the *Gpc-B1* lines. The non-*Gpc-B1* and *Gpc-B1* lines differed mainly on chromosomes 6B were the segment was introgressed. Wyalkatchem and RAC1262A, and Gladius and Drysdale pairs differed for a number of traits. Grain weight and senescence were the traits mostly affected by heat stress in all the lines. Heat treatment increased unextractable polymeric protein and protein content in the Wyalkatchem background.

Key words: Gpc-B1, grain filling, grain weight, heat stress, percentage unextractable polymeric protein, senescence

7.1 Introduction

In both hexaploid bread wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*T. turgidum* L. ssp. *durum* (Desf.) Husn., a chromosome segment introgressed from wild emmer wheat (*T. turgidum* L. subsp. *dicoccoides* (Körn. Ex. Asch. & Graebn.) Thell.) is known to accelerate senescence (as indicated by degradation of chlorophyll in the flag leaf), shorten the grain filling period, and increase the concentration of protein, iron and zinc in the grain (Distelfeld et al., 2007; Joppa et al., 1997; Olmos et al., 2003; Uauy et al., 2006a; Uauy et al., 2006b). These effects have been attributed to a NAC transcriptional factor encoded by the *NAM-B1* gene at the *Gpc-B1* locus (Uauy et al., 2006b). The increase in grain protein concentration is thought to be due to enhanced remobilisation of nitrogen from other plant organs to the developing grain (Brevis and Dubcovsky, 2010; Kade et al., 2005; Waters et al., 2009). Traits associated with high protein concentration, including water absorption, mixing time and loaf volume have been reported to be increased by *Gpc-B1* (Brevis et al. 2010).

The introgression segment that carries *Gpc-B1* has been found to reduce grain weight, and test weight (Brevis and Dubcovsky, 2010; Brevis et al., 2010). Grain yield was not affected as much as grain weight, indicating that other yield components may partially compensate for the decrease in grain weight (Brevis and Dubcovsky, 2010). The decrease in grain weight was greater in tetraploid than in hexaploid wheats. This may be due to a dosage effect: tetraploids have two other functional *Gpc* genes (on chromosomes 6A and 2B) while hexaploids have four (on chromosomes 6A, 2B, 2D and 6D).

The effects of *Gpc-B1* are similar in some respects to those that have been reported for heat stress during grain filling. Heat stress has been reported to shorten the grain filling period (accelerate senescence), increase grain protein concentration, reduce grain weight and reduce grain yield (Altenbach et al., 2003; Dias and Lidon, 2009; Gooding et al., 2003; Hurkman et al., 2009; Labuschagne et al., 2009; Stone and Nicolas, 1995a; 1995b). Changes in grain size and grain protein concentration have been attributed to reduced starch deposition, possibly due to the effect of heat on starch synthesising enzymes. Heat stress, like *Gpc-B1*, has also been reported to increase mixing time and loaf volume probably due to increased protein concentration (Blumenthal et al., 1991; Li et al., 2012). Increases in temperature up to 30°C have been shown to increase dough strength but further increases can decrease dough strength (Randall and Moss, 1990). Heat-induced reductions in dough strength have been attributed to lower glutenin:gliadin ratios under heat stress (Blumenthal et al., 1993;

Blumenthal et al., 1995a). Heat has been reported to decrease percentage unextractable polymeric protein (% UPP, which is used as an indicator of dough strength) when applied at later grain filling stages (Irmak et al., 2008) but to increase % UPP when applied in early stages of grain filling (Balla et al., 2011). Effects of *Gpc-B1* on protein quality traits such as dough strength, glutenin:gliadin ratios and % UPP have not been reported.

Here, to directly compare the effects of heat stress with those of *Gpc-B1* and to investigate how the effects of post-anthesis heat stress would affect *Gpc-B1*-carrying wheat lines, pairs of backcross-derived wheat lines with and without *Gpc-B1* were grown with and without exposure to a high temperature treatment early in grain filling.

7.2. Materials and Methods

7.2.1 Plant materials

The lines used in this research included two pairs of backcross-derived lines of bread wheat with contrasting genotypes at *Gpc-B1*. The lines RS4-11-10 and RS4-11-16, both have the Australian breeding line RAC1262A (a selection from the breeding line RAC1262, which was later released as the cultivar Gladius) as their recurrent parent and the *Gpc-B1*-carrying Canadian wheat cultivar Somerset (Fox et al., 2006) as their donor parent. Each of these lines was derived by selfing from a single progeny plant of a BC_4 plant (RS4-11), which was heterozygous for *Gpc-B1*. RS4-11-10 is homozygous for the *Gpc-B1*-containing introgression segment while RS4-11-16 lacks that introgression segment. Similarly, WB4-1-6 and WB4-1-8 have the Australian cultivar Wyalkatchem as their recurrent parent and the *Gpc-B1*-carrying Canadian cultivar Burnside (Humphreys et al., 2010) as their donor parent. WB4-1-6 carries the *Gpc-B1* segment and WB4-1-8 lacks that introgression. In addition, the experiment included the recurrent parents RAC1262A and Wyalkatchem, and the Australian cultivars Gladius and Drysdale. Gladius, which is considered to be a heat-tolerant cultivar (Fleury et al., 2010) was derived from a complex cross involving derivatives of RAC875, Krichauff, Excalibur and Kukri (http://pbr.ipaustralia.plantbreeders.gov.au/). The pedigree of Drysdale is Hartog*3/Quarrion (http://pbr.ipaustralia.plantbreeders.gov.au/) and that of Wyalkatchem is Machete///Gutha//Jacup*2/11thISEPTON-135 (http://wheatpedigree.net/).

7.2.2 DNA extraction and SNP genotyping

DNA was extracted from approximately 2.0 g of leaf tissue from one four-week-old plant of each of the recurrent parents RAC1262A and Wyalkatchem and each of the backcross

derivatives RS4-11-10, RS4-11-16, WB4-1-6 and WB4-1-8 using a mini prep ball bearing extraction method (Rogowsky et al., 1991) with some modifications (Pallotta et al., 2000). DNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies). For each of these lines, approximately 100ng/µl DNA (30 µl) was sent to Department of Primary Industries, Victoria to be assayed on an Infinium 9K SNP assay as described by Akhunov et al. (2009). Gladius and Drysdale had previously been genotyped on the same array.

7.2.3 Glasshouse experiment

Plants were grown in a naturally-lit glasshouse in the Plant Accelerator, Urrbrae, Australia, in which photoperiod was 14 h at flowering and the minimum and maximum temperatures were set at 18 and 24°C. The experimental design consisted of 30 complete blocks where each block consisted of 16 pots (arranged in two rows of eight) and contained four sub-blocks, each of which consisted of four pots (arranged two-by-two). Each of four pairs of lines (RS4-11-10 and RS4-11-16; WB4-1-6 and WB4-1-8; RAC1262A and Wyalkatchem; Gladius and Drysdale) was assigned at random to a sub-block within each block. Within each sub-block, each of the two lines was assigned at random to two of the four pots. One plant of each line was allocated at random to be subjected to a heat treatment, while the other one was used as a control.

Seeds were sown on 24 May 2011 in pots containing Waikerie sand and coco peat soil mixture (pH 6-6.5) which also contained 202 mg/l dolomite lime, 561 mg/l agricultural lime, 131 mg/l hydrated lime, 202 mg/l gypsum, 202 mg/l super phosphate, 505 mg/l iron phosphate, 33.7 mg/l iron chelate, 202 mg/l micronutrients (Scotts Micromax), 505 mg/l calcium nitrate and 2022 mg/l slow-release fertilizer (Osmocote). Aquasol multi-nutrient fertiliser (nitrogen, phosphorus, potassium, sulfur, magnesium, manganese, copper, iron, zinc, boron and molybdenum) was dissolved in water and applied to the soil at two-week intervals after anthesis at the recommended rate.

For each plant, the date on which the first spike reached anthesis (the day in which extruded anthers first became visible) was recorded. That spike was labelled for further use, and other tillers were removed. At 15 d after anthesis, plants allocated for heat treatment were transferred to a growth chamber (BDW120, Conviron) with 37/27°C day/night temperatures, a 14 h day cycle, and an average 80/60% day/night relative humidity. The temperature was

gradually raised from 27 ºC to 37 ºC (within 3 hours at a rate of 3.3 ºC/hour) after which it was maintained at 37 ºC for 8 hours, then gradually cooled down from 37ºC to 27 ºC (within 3 hours at a rate 3.3 ºC/hour). In the growth chamber, pots stood in tubs containing around 3 cm of water to minimize drought stress. After 3 d in the growth chamber, the plants were returned to their original positions in the glasshouse.

For each plant, the date of senescence (complete peduncle yellowing) was recorded. Grain was harvested on 28 October 2011 after all plants had reached physiological maturity (complete peduncle and spike yellowing). The grains harvested from each spike were counted (grain number) and weighed. Mean grain weight was obtained by dividing grain weight by grain number.

7.2.4 Size-exclusion high-performance liquid chromatography (SE-HPLC)

Grain from each hand threshed spike was crushed with a hammer. Flour that passed through a 280 µm sieve was retained. The flour was allowed to rest for at least 7 d in a refrigerator prior to protein extraction. Protein was extracted from flour using an established method (Appelbee, 2007). It involved weighing 25 mg of flour into a 1.5 ml tube and adding 1 ml of 0.05 M phosphate extraction buffer (pH 6.9). Phosphate extraction buffer was made by preparing two solutions, solution A (3.55 g Na_2HPO_4; 2.5 g SDS; 500 ml water) and solution B (3 g NaH_2PO_4; 2.5 g SDS; 500 ml water) then gradually adding solution B to solution A until the pH reached 6.9. The protein extraction procedure was a two-step process. In the first step, the flour was mixed with 1 ml of phosphate extraction buffer, and centrifuged for 10 minutes at 13000 rpm. The supernatant was aspirated from the pellet to a new tube and represented the SDS-soluble protein (‘extractable polymeric protein’). In the second step, 1 ml of phosphate extraction buffer was added to the pellet and the tube sonicated at room temperature using a sonifier (Branson model B-12 cell disrupter, Danbury, CT) set at 10 W (output four) for 30 seconds, then spun again and supernatant collected. This represented the SDS-insoluble protein fraction (‘unextractable polymeric protein’). Both soluble and insoluble extracts were filtered through 0.45 µm PVDF filters into 1 ml glass HPLC vials. Proteases were inactivated by incubating the vials at 80°C for two minutes.

The protein extracts were run on a Waters HPLC system (Milford, MA) Protein-Pak 300TM column (C18, 300 Å pore size, 3.5 µm particle size, 150 mm x 4.6 mm) using a Waters 717 plus auto sampler, a Waters 600 system controller and a Waters 486 detector. The samples

were run in 50% (v/v) acetonitrile containing 0.1% trifluoroacetic acid (TFA), for 50 minutes (soluble fraction) or 40 minutes (insoluble fraction) (Batey et al., 1991) at a flow rate of 0.5 ml/minute. The software Millennium32 (version 3.2) was used for data acquisition and processing. Protein was detected by UV absorbance at 214 nm (Stone and Nicolas, 1996a) and the areas under the peaks were used to estimate amounts of soluble and insoluble protein. The first major peak eluting at about 14.5 minutes in the soluble fraction was defined as total soluble polymeric protein (Appendix 4) and the first major peak in the insoluble fraction eluting at about 15.5 minutes was defined as total insoluble polymeric protein (Appendix 5). Two aliquots of each sample were each run separately through the HPLC and the results of the two averaged to get a value for the sample. Total polymeric protein and percentage of SDS-unextractable polymeric protein (% UPP) were calculated as follows:

Total polymeric protein = Area of soluble polymeric protein peak + Area of insoluble polymeric protein peak

% UPP = (Area of insoluble polymeric protein peak/Total polymeric protein) × 100

7.2.5 Total protein measurement

For each line, total grain protein concentration was measured for blocks 1, 15 and 30. Total nitrogen content was measured using the Dumas total combustion method (Buckee, 1994) using the Elementar rapid N III Nitrogen analyser, Version J **(**Elementar, Germany). Total protein concentration expressed on an "as is" was obtained by multiplying nitrogen concentration by 5.7 (conversion factor for wheat).

7.2.6 Statistical analysis

For each of the measured traits a spatial analysis was conducted using a linear mixed model (Gilmour et al., 1997) that accounted for possible variation due to position of the pot in glasshouse as well as variation arising from the design of the experiment such as blocks and sub-blocks. The model also contained a fixed treatment-by-line interaction term which provided adjusted means for the measured traits of each factorial combination of the treatment levels. All models were analysed using ASReml-R (Butler et al., 2009) in the R statistical computing environment (R Development Core Team, 2012). With each trait model, post-analysis multiple comparisons were performed to test the significance of the effects of

the heat treatment on each of the lines and to directly compare the lines within pairs (Gladius and Drysdale, RAC1262A and Wyalkatchem, RS4-11-10 and RS4-11-16, and WB4-1-6 and WB4-1-8) under heat treatment and control conditions. Correction for the family wise error rate (FWER) for the number of comparisons performed was done using the Bonferroni correction and calculated as 0.05 divided by number of comparisons (eight for anthesis and 16 for the other traits).

7.3. Results

7.3.1 SNP genotypes of the backcross derivatives

In the SNP analysis, RS4-11-10 differed from RAC1262A for 113 of 297 markers (38%) that had previously been mapped in a 134-cM region between markers wsnp_Ex_rep_c67887_66598188 and wsnp_Ex_c12674_20142989 on chromosome 6B (Appendix 6), whereas RS-4-11-16 differed from RAC1262A at only two of those loci (<0.5%). Across the remaining 5817 SNP markers that had previously been mapped elsewhere in the genome, RS4-11-10 and RS4-11-16 each differed from the recurrent parent at less than 0.5% of loci (Appendix 7). Similarly, WB4-1-6 differed from Wyalkatchem at 41.8% of the markers in a 93-cM region between wsnp_Ex_c8011_13584847 and wsnp_BE591931B_Ta_2_2 on chromosome 6B (Appendix 6), while WB-4-1-8 was identical to Wyalkatchem at all of those markers. Elsewhere in the genome, WB4-1-6 and WB-4-1-8 differed from Wyalkatchem at less than 0.5% of markers (Appendix 7).

7.3.2 Comparison of the paired lines

In overall comparisons made between the lines within pairs (under control or heat treatments) (Table 7.1), significant differences were detected for timing of anthesis (WB4-1-6 earlier than WB4-1-8 under control conditions), grain number under both control and heat treatments (lower in Gladius than in Drysdale; lower in RAC1262A than in Wyalkatchem), timing of senescence (Drysdale earlier than Gladius and RS4-11-10 earlier than RS4-11-16 after heat treatment; Wyalkatchem earlier than RAC1262A and WB4-1-6 earlier than WB4-1-8 under control conditions), grain weight (lower in Gladius than in Drysdale under control conditions; lower in RAC1262A than in Wyalkatchem under both control and heat treatments), grain protein concentration (higher in Gladius than in Drysdale under both control and heat treatments; higher in RAC1262A than in Wyalkatchem under both control and heat

treatments; higher in WB4-1-6 than in WB4-1-8 under control conditions) and % UPP (higher in Gladius than in Drysdale under both control and heat treatments; higher in RAC1262A than in Wyalkatchem after heat treatment; higher in WB4-1-6 than in WB4-1-8 under control conditions).

7.3.3 Effects of the heat treatment

In each of the eight lines evaluated, senescence was significantly accelerated by exposure to heat (Table 7.1). The heat treatment reduced grain weight in Drysdale, Wyalkatchem, RS4-11-10, WB4-1-6 and WB4-1-8, increased grain protein concentration in WB4-1-8 and increased % UPP in WB4-1-6.

Table 7.1 Adjusted mean values for days to anthesis, grain number per spike, senescence (days from anthesis to complete peduncle yellowing), grain weight, percentage flour protein and percentage unextractable polymeric protein (% UPP) in four pairs of wheat lines in control and heat treatments, and *p*-values for comparisons of lines within pairs and the effect of post-anthesis exposure to heat for three days for each wheat line. *P*-values displayed in bold font are less than the Bonferroni-corrected significance levels of 0.006 (0.05/8) for days to anthesis and 0.0031 (0.05/16) for other traits, indicating statistical significance of the corresponding comparisons.

	Days to anthesis		**Grain number**		**Days from anthesis to senescence**		**Grain weight (mg)**		**% Protein**		**% UPP**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Wheat line						**Mean**						
Gladius	68	68	32	32	61	55	38	35	20.0	20.8	46.8	48.3
Drysdale	70	71	51	50	60	49	49	38	17.3	18.0	34.9	36.1
Wyalkatchem	75	76	37	36	62	54	47	43	17.7	17.7	39.7	41.5
RAC1262A	63	63	28	28	66	59	34	33	20.0	20.3	46.9	48.0
WB4-1-6	74	75	41	39	56	51	46	40	20.0	19.5	39.9	42.9
WB4-1-8	76	75	38	38	63	55	48	40	16.8	19.9	39.0	39.9
RS4-11-10	65	66	29	28	60	52	36	33	22.2	21.5	48.4	49.1
RS4-11-16	66	65	31	29	62	57	38	34	20.6	22.2	46.9	48.0
Comparison						***p*-value**						
Gladius *vs.* Drysdale	0.123		**0.000**	**0.000**	0.158	**0.000**	**0.000**	0.036	**0.000**	**0.000**	**0.002**	**0.001**
Wyalkatchem *vs.* RAC1262A	0.277		**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.005	**0.002**
WB4-1-6 *vs.* WB4-1-8	**0.002**		0.132	0.337	**0.000**	**0.001**	0.080	0.702	**0.000**	0.295	**0.000**	0.583
RS4-11-10 *vs.* RS4-11-16	0.856		0.215	0.464	0.022	**0.000**	0.264	0.091	0.006	0.204	0.006	0.410
Heat *vs.* Control												
Gladius			0.906		**0.000**		0.071		0.070		0.410	
Drysdale			0.612		**0.000**		**0.000**		0.147		0.347	
Wyalkatchem			0.710		**0.000**		**0.001**		0.033		0.969	
RAC1262A			0.734		**0.000**		0.369		0.194		0.724	
WB4-1-6			0.723		**0.000**		**0.000**		0.316		**0.000**	
WB4-1-8			0.369		**0.000**		**0.000**		**0.000**		0.530	
RS4-11-10			0.263		**0.000**		**0.003**		0.167		0.060	
RS4-11-16			0.541		**0.000**		0.015		0.408		0.410	

7.4. Discussion

The two pairs of backcross derivatives used here were developed independently, using different donors and different recurrent parents. Based on the results of SNP genotyping, each of the non-*Gpc-B1* backcross derivatives is nearly identical to its recurrent parent, and the members of each pair of backcross derivatives are nearly identical to each other, except on chromosome 6B. On chromosome 6B, the non-*Gpc-B1* lines were nearly identical to their recurrent parents, while the *Gpc-B1* lines differed from them at many marker loci that had previously been mapped within regions of 134 cM (RS4-11-10) or 93 cM (WB4-1-6). These introgressions would include both the actual *Gpc-B1* containing segment from wild emmer, which has been estimated to be approximately 15-30 cM long (Khan et al., 2000; Mesfin et al., 1999), and flanking regions derived from the donor wheat cultivars Somerset (in RS4-11-10) and Burnside (in WB4-1-6). Considering that there would have been six opportunities for recombination in the development of these BC_4-F_1 derived lines, the observed introgression segments are surprisingly long, possibly indicating suppression of recombination around the *Gpc-B1* locus. With continued backcrossing there should be further opportunities for recombination, and it might be possible to reduce the introgression segment size, especially if marker-based selection is used to favour recombinant progeny.

Phenotypically, the non-*Gpc-B1* lines were similar to their recurrent parents. Compared with RAC1262A and RS4-11-16, WB4-1-8 and Wyalkatchem both reached anthesis later, set more grains per spike, had shorter intervals between anthesis and senescence and had larger grains with lower protein concentration and lower % UPP. The segments introgressed from Burnside into a Wyalkatchem background and from Somerset into an RAC1262A background had both accelerated senescence, as was expected based on previously reported effects for *Gpc-B1* backcrossed into other wheat backgrounds (Brevis and Dubcovsky, 2010; Uauy et al., 2006b). Under control conditions, the acceleration was greater in the Wyalkatchem background than in the RAC1262A background, which already had a long period from anthesis to senescence. With the heat treatment, the delay in senescence in WB4-1-6 was similar to that in RS4-11-10.

In the Wyalkatchem background, the *Gpc-B1* segment significantly increased grain protein content (20.0% for WB4-1-6 compared to 16.8% for WB4-1-8). In the higher-protein RAC1262A background, the difference was numerically smaller (22.2% for RS4-11-10 compared to 20.6% in RS4-11-16) and not quite significant with the stringent (Bonferroni-

corrected) Type I error control employed here. This lack of significance is probably due to the relatively low number of replicates used for this trait. With more replicates, the statistical test would likely have had sufficient power to detect an increase in grain protein content due to *Gpc-B1* in both backgrounds.

In the experiment described here, post-anthesis heat treatment had the expected effects of accelerating senescence (in all lines) and reducing grain weight (in some lines). The cultivars Gladius and Drysdale did not differ in time to anthesis, but they differed from each other in grain number, senescence, grain weight and grain protein content. In Drysdale, heat treatment accelerated senescence more than in Gladius (by 11 days compared to 7 days) and significantly reduced grain weight. Gladius, which is considered to be a heat tolerant cultivar (Fleury et al., 2010) did not suffer significant reduction in grain weight after exposure to heat. Similarly, RAC1262A (Gladius or a sister line of Gladius) did not suffer significant reduction in grain weight under heat stress. The decrease in grain weight observed in Drysdale under heat stress might be due to its early senescence shortening the grain filling duration and interfering with the supply of substrates to the developing grain. Reduction of grain growth duration has previously been reported to decrease grain weight (Viswanathan and Khanna-Chopra, 2001). In contrast to these observations for the effect of the heat treatment, the *Gpc-B1* introgression segment accelerated senescence without significantly decreasing grain weight, indicating that factors that reduce grain-filling duration do not necessarily limit grain size.

Given that the whole plant was exposed to the heat treatment, the heat-induced reduction in grain weight may have involved interference with important physiological processes in vegetative tissues and/or in the developing grain. These could include nutrient uptake, substrate translocation and starch synthesis. Soluble starch synthase, which is involved in starch biosynthesis, has been shown to be affected by high temperatures, leading to reduction of starch deposition in the developing grain (Jenner, 1994; Labuschagne et al., 2009). Since a large proportion of the wheat grain is starch, suppression of starch synthesis would be expected to reduce grain weight. Reduction of starch synthesis might be expected to be accompanied by increased grain protein concentration due to dilution effects. In this experiment, a significant heat-induced reduction in grain weight was accompanied by significant increase in grain protein content only in the non-*Gpc-B1* line WB4-1-8. Interestingly, although the heat treatment resulted in a significant increase in grain protein content of WB4-1-8, and the *Gpc-B1* introgression segment significantly increased grain

protein content in the same background (WB4-1-6 *vs.* WB4-1-8), there was no further increase due to the combination of *Gpc-B1* and heat. Grain protein concentration may have already reached an upper limit.

Estimation of % UPP made it possible to investigate the effects of *Gpc-B1* and/or heat on a functional property of grain protein. Measurement of % UPP requires only a small sample of flour, yet gives an indication of the extent of glutenin polymerisation and is a predictor of dough strength. These characteristics are well known to be affected by allelic variation at the *Glu-1* loci, which encode high-molecular-weight glutenins. Gladius, RAC1262A, RS4-11-10 and RS411-16 all carry the *Glu-A1a*, *Glu-B1b* and *Glu-D1d* alleles, which encode the HMW-GS *Ax1*, *Bx7*, *By8*, *Dx5* and *Dy10*. Wyalkatchem, WB4-1-6 and WB4-1-8 all carry the *Glu-A1a*, *Glu-B1b* and *Glu-D1a* alleles, which encode the HMW-GS *Ax1*, *Bx7*, *By8*, *Dx2* and *Dy12*. Drysdale carries *Glu-A1a*, *Glu-B1i* and *Glu-D1d* alleles, which encode the HWM-GS *Ax1*, *Bx17*, *By18*, *Dx5* and *Dy10*. According to effect estimates presented by Eagles et al. (2002), the allelic combination carried by Gladius should lead to stronger dough (higher resistance to extension, Rmax) than the combinations carried by Wyalkatchem and Drysdale. Consistent with these expectations, the lines carrying the Gladius allelic combination had consistently higher % UPP than Drysdale or those carrying the Wyalkatchem combinations, under control conditions and after post-anthesis heat treatment. Such differences in dough strength are partly due to the extent of glutenin polymerisation.

Accumulation of SDS-insoluble polymers has been shown to occur at about 31 days after anthesis and to be accompanied by reductions in monomers and SDS-soluble polymers (Carceller and Aussenac, 1999; Gupta et al., 1996). Consistent with this, exposure to heat late in grain filling (in field experiments or applied at 25 days after anthesis) has been reported to reduce % UPP and dough mixing time and strength (Cavanagh et al., 2010; Irmak et al. 2008). In contrast, earlier heat treatments (applied at 10 or 12 days after anthesis) have been reported to increase % UPP (Balla et al., 2011) and increase sodium dodecyl sulphate sedimentation height/volume (another indicator of glutenin polymerisation and dough strength) (Beecher et al., 2012). In the study by Balla et al. 2012, exposure to heat led to moderate increases in protein concentration (smaller than those observed for exposure to drought or to both drought and heat) as was observed in this thesis Chapter for some of the lines following exposure to heat treatment.

In the experiment conducted here, plants were exposed to heat early in grain filling (15 days after anthesis) and for only 3 days. In the non-*Gpc-B1* lines, this heat treatment had no significant effects on % UPP. In the *Gpc-B1* line WB4-1-6, however, the heat treatment increased % UPP from 39.9% to 42.9%. Despite high grain protein concentration achieved in that line under control conditions (20.0%), the % UPP was relatively low (39.9% compared to 48.4% for RS4-11-10, probably due to the Wyalkatchem background). Perhaps in the high-protein condition conferred by *Gpc-B1* (20.0% compared to 16.8% for WB4-1-8) the heat treatment triggered a change in the glutenin composition, favouring subunits that enhance polymerisation. In the other *Gpc-B1* line, RS4-11-10, the corresponding % UPP values were 48.4% (heat) and 49.1% (control); the smaller difference between these was in the same direction, but not statistically significant ($p = 0.056$). Compared with WB4-1-6, RS4-11-10 may have had been less capacity for heat to increase % UPP, given that the control level of % UPP was already high.

In the experiment reported here, the use of controlled environment facilities made it possible to expose wheat plants to high temperatures without the limitation in water supply that usually accompanies heat events in the field. Under experimental conditions used here, it is not possible to realistically measure bread making quality or grain yield, but it was possible to obtain data on indicator variables including grain protein concentration, % UPP, grain number and grain weight. In the experimental conditions used here, *Gpc-B1* increased not only grain protein concentration but also protein polymerisation. It accelerated senescence yet did not reduce grain number or grain weight. A heat treatment imposed between 15 and 18 days had similar effects on senescence, but reduced grain size. This early exposure to heat did not affect % UPP in most lines, and it increased grain protein concentration in only one of the eight lines investigated. Although it would not be appropriate to extrapolate directly from the results of a glasshouse experiment to production conditions in the field, it is worth noting that no deleterious effects of *Gpc-B1* were observed here in either genetic background, even after post-anthesis exposure to heat.

Chapter 8

General Discussion

Genetic linkage maps are useful for QTL analysis. In this thesis research, two genetic linkage maps were generated for the Gladius/Drysdale population, one using a random subset of lines (genotyped with DArT, SSR and gene-based markers) and the other using a selection of mid-maturing lines (genotyped with SNP and gene-based markers) (Chapter 3). In the Gladius/Drysdale population, selection against very early and very late heading did not fix any of the four known phenology loci for which this population segregates as it was possible to genetically map them, and relative to molecular marker loci. In both linkage maps the number of linkage groups was more than the 21 wheat chromosomes. The D genome which is known to exhibit low polymorphism was poorly mapped compared to the A and B genomes in both linkage maps. The SNP markers used to genotype the mid-maturity subset lines increased marker density.

Creation of a mapping population composed of similar maturing lines (uniform population) can be a useful strategy of minimising the role of phenology loci in mapping studies. This ensures that plants are exposed to the same conditions at the same developmental stage. Based on the results of this thesis research it is recommended that the number of lines in the mid-maturity subset be increased from the current 134 and the new lines be genotyped with SNPs as well. Lines used in the two subsets are part of a large population of 5000 RILs which provide adequate resources for fine mapping. Fine mapping increases marker density and results in better marker order because a large population allows identification of more recombinants and thus separate markers, which would otherwise co-locate in a smaller population. Fine mapping further allows clearer identification of markers flanking the candidate gene.

In this thesis research, two mapping populations, the RAC875/Kukri (DH) and Gladius/Drysdale (RIL) were used to evaluate wheat quality. The parents of the mapping populations were chosen on the basis of their contrasting field performance under drought and heat conditions, with RAC875 and Gladius being drought and heat tolerant and Kukri and Drysdale being more sensitive to these abiotic stresses (Fleury et al., 2010; Izanloo et al., 2008). The quality of wheat grain produced under a range of environmental conditions was investigated, through investigating grain yield, shape, characteristics, flour colour, dough properties and baking performance, and investigating whether the different quality traits are

associated with the same QTLs. The RAC875/Kukri DH population was used for the research reported in one experimental Chapter (Chapter 6) and the Gladius/Drysdale RIL population was used for the bulk of the thesis research (Chapters 3 to 5). Prior to this research, the RAC875/Kukri population had already been thoroughly studied for yield and agronomic traits in a range of environments (Bennett et al., 2012a; Bennett et al., 2012b; Bennett et al., 2012c), and grain samples were already available for quality analysis. On the other hand, the Gladius/Drysdale population was a fairly new population and had not been extensively characterised. The Gladius/Drysdale population was analysed for traits ranging from yield up to bread making over two seasons in many locations. Measuring all traits ranging from grain characteristics to baking quality permitted investigation of the genetic control of grain and processing quality and how these can influence end product quality.

The populations were grown under a range of conditions including drought and/or heat stress in Australia and Mexico and climatic data was collected in all experiments. Both populations were evaluated for quality using identical testing and statistical methods to make it easier and more valid to compare between the populations although it was not possible to grow the two populations in exactly the same environments. In the RAC875/Kukri population there was no control experiment and results obtained under water limiting conditions were compared with published results of experiments conducted under optimal conditions. The testing methods were also validated and instruments were serviced accordingly to minimise errors and to get accurate and precise results as QTL detection heavily depends on accurate phenotypic data.

Field experiments were conducted at Urrbrae (South Australia), Yanco (New South Wales, Australia) and Ciudad Obregon (Mexico) for Gladius/Drysdale under a range of conditions involving drought and heat stress. Drought and heat stress often coincide in the field and separating their individual effects is very difficult. At Urrbrae and Ciudad Obregon, drought stress was imposed by withholding water and the experiments were conventional sown so that they will escape heat stress. At Yanco, the effect of heat stress was investigated through conventional planting so that plants can escape heat stress and late planting so that plants are exposed to heat stress during grain filling, with both sets of experiments well watered to avoid drought stress. In the glasshouse and heat chamber experiment (Chapter 7) the plants were kept in tubs filled with water in an attempt to minimise moisture deficit and thus study the effects of heat treatment.

Unlike RAC875 and Kukri, Gladius and Drysdale had not been previously characterised in depth under various abiotic stress regimes. Gladius and Drysdale were therefore evaluated in glasshouse conditions for their response to heat treatment at 15 days after anthesis, with Gladius showing heat tolerance while Drysdale was sensitive to heat treatment (Chapter 7). Though the two parents did not differ in time to anthesis, Drysdale had higher grain number, reached senescence earlier, had larger grains with lower protein concentration and lower % UPP than Gladius.

Quality evaluation and QTL analysis for the Gladius/Drysdale population was based on the random subset. Phenology genes are known to influence overall plant development and maturity and their effects vary with environmental growth conditions. Vernalisation (cold temperature requirement) and photoperiod (day length) genes determine when a plant switches from the vegetative phase to the reproductive phase and were associated with time to ear emergence and Zadoks score in the Gladius/Drysdale mapping population (Chapter 3) and in the RAC875/Kukri mapping population (Bennett et al., 2012a). In the random subset, the alleles that confer photoperiod insensitivity, carried by Gladius at the *Ppd-B1* locus and by Drysdale at the *Ppd-D1* locus, were associated with early flowering/accelerated plant development. The winter alleles carried by Drysdale at the *Vrn-A1* locus and Gladius at the *Vrn-D1* locus were associated with delayed time to heading but the vernalisation loci had minimal effects on other traits.

The alleles that confer photoperiod insensitivity generally led to increased grain dimensions, grain weight, yield, and decreased percentage screenings and flour yellowness. The alleles that confer photoperiod insensitivity were also associated with a higher proportion of screenings under heat stress and this might be because the developing grain was exposed to heat stress at a more critical grain setting or early in grain filling. It has been shown that grain is very sensitive to heat stress and prone to abortion in early grain filling (Tashiro and Wardlaw, 1990). In the absence of heat stress, alleles that confer photoperiod sensitivity were associated with higher screenings at the *Ppd-D1* locus and there was no effect at the *Ppd-B1* locus. In the mid-maturity subset, no QTL for days to heading was detected. It is speculated based on the lack of association between phenology loci and plant development that had the mid-maturity subset been used, the effects of phenology loci on quality traits would have been minimised. This would allow identification of other major QTLs associated with the quality traits without the confounding effect of phenology.

For Gladius/Drysdale, the field experiment, AusYan09_FI_CS (early sown at Yanco in the 2009 season under well watered conditions) had moderate yield and tended to have QTLs (for grain number, percentage screenings, flour extraction, particle size index and yield) detected in the heat stressed experiment, AusYan09_FI_LS (late sown at Yanco in the 2009 season under well watered conditions) but not in the higher yielding AusYan10_FI_CS (early sown at Yanco in the 2010 season under well watered conditions) experiment. The year 2009 was characterised by very high temperatures in early spring and it is therefore possible that the AusYan09_FI_CS experiment might have been slightly exposed to heat stress. In contrast, year 2010 was cooler and had received more rainfall than 2009. This might have led to more water availability in 2010 than in the irrigated 2009 experiment. As a result, AusYan09_FI_CS experiment might be moderately stressed compared to AusYan10_FI_CS experiment.

In AusYan10_FI_CS experiment the alleles for photoperiod sensitivity were associated with higher yield in contrast to other environments where alleles for photoperiod insensitivity were associated with higher yield. In the absence of later season stresses, which was most likely the case in 2010, late flowering might allow plants to accumulate enough biomass and storage reserves, which can then be translocated to the developing grain resulting in higher yield. The early sown experiment, (AusYan10_FI_CS) was characterised by very high yield and low protein content levels (Chapter 4) while the late sown trial (AusYan10_FI_LS) was severely weather damaged and was not used for quality analysis making same year comparison only possible for the 2009 season.

High temperature and water deficit affected overall yield and quality in the Gladius/Drysdale population in this thesis research. Yield is a function of grain number and weight and there were positive correlations between these traits and yield, and co-location of QTLs for yield with either QTLs for grain number or thousand grain weight. Onset of stress before anthesis in the field decreased yield mainly through grain number reduction (Chapter 4) while stress after anthesis decreased grain weight (Chapter 7). Grain number reduction due to stress after anthesis may also occur if the stress is intense enough to induce grain abortion but this was not observed in the experiment reported in Chapter 7. Under heat stress the growth period was shortened (Chapter 3; Chapter 7). Usually the rate of grain filling increases under stress to try and compensate for the shortened duration. This was not ascertained in the research reported here because the rate of grain filling was not measured.

In the Gladius/Drysdale population, experiments exposed to drought had lower grain weight at both Urrbrae and Ciudad Obregon and longer mixing time at Urrbrae (Chapter 4; Chapter 5). Exposure to heat also resulted in lower yields, higher protein content and slightly better baking outcome (Chapter 4; Chapter 5). The high protein content most likely accounted for longer dough development time, mixing time, high loaf volume and score in material from the stressed experiments as the correlation between protein content and these traits is well known. Although high loaf volume is desirable, long dough development time requires more energy to be expended in preparing the dough prior to baking and may be undesirable. In the heat chamber, heat treatment decreased grain weight and accelerated senescence in all the lines (Chapter 7). The combination of drought and heat stress at Urrbrae had very substantial effects on yield and grain characteristics than either stress alone (Chapter 4). This thesis research acknowledges that late and early flowering lines experienced stresses at different developmental stages and for different durations, hence the inclusions of a small subset of 60 similar maturing lines for the Gladius/Drysdale mapping population under drought and heat conditions at Urrbrae.

For the RAC875/Kukri population, field experiments were conducted in Streaky Bay and Piednippie in South Australia. The RAC875/Kukri lines used for quality evaluation were a selection of early to mid-maturing lines to minimise the confounding effects of maturity. High temperature and water deficit affected yield and agronomic traits in the RAC875/Kukri population (Bennett et al., 2012b). Samples from the Streaky Bay experiment, which received less rainfall but had slightly lower temperatures during the growing season, had softer grain, slightly higher grain protein content, lower flour extraction, slightly longer dough development time, longer bake mixing time, more stable dough, less dough softening and larger loaf volume than those from the Piednippie experiment that received more rainfall and had slightly higher temperatures during the growing season (Chapter 6).

Concern over the potentially confounding effects of maturity in grain produced in stress environments was expressed when a wide range of phenology was noted during the initial grain increase of the random subset of 205 Gladius/Drysdale RILs in 2009 in the experiment conducted at Roseworthy. Although genotyping and phenotyping continued to be carried out on the random subset of 205 RILs, 360 additional lines were evaluated for heading date in 2010, and 134 mid-maturing lines (some from the random subset and some from the additional lines) were selected for future agronomic, physiological and quality research (Chapter 3). No association between days to heading and phenology was observed in the mid-

maturity subset and future agronomic, physiological and quality research with the mid-maturity subset will ascertain if the remaining phenotypic variation is sufficient to confound the results. From among 205+360=565 lines evaluated for heading date, 134 were selected, which is rather a small population but according to the results in Chapter 3 there were 137 other lines in the same range of heading dates that could have been selected. This would give 271 lines which are sufficient for mapping agronomic, quality and physiological traits.

It has been suggested that grain shape can influence milling yield (Berman et al., 1996; Marshall et al., 1984; Marshall et al., 1986). An increase of a percent in flour yield can translate to millions of dollars for the industry per year. In this thesis research (Chapter 4), QTLs for grain shape and milling yield did not co-locate indicating that these traits are under different genetic control mechanisms. This indicates that in the Gladius/Drysdale population, selection to improve grain shape would not directly increase milling yield. Grain shape was not measured in the RAC875/Kukri population making it uncertain if the QTLs associated with grain shape in Gladius/Drysdale would also be detected in the RAC875/Kukri population.

In the Gladius/Drysdale population, grain width and thickness were largely under the same or similar genetic control and they largely influenced thousand grain weight and ultimately yield, but grain length and width were largely under different genetic control mechanisms. Regions on chromosomes 3B (position Q22 in Figure 4.3) and 6A (position Q32 in Figure 4.4) were associated with grain width and/or thickness, thousand grain weight and yield. This shows that increase in the lateral dimension of the grain leads to increase in grain weight probably through increase in cell numbers. The QTL on chromosome 3B in the Gladius/Drysdale population coincides with one for thousand grain weight detected in the RAC875/Kukri population (Bennett et al., 2012b). This region has been fine mapped in the RAC875/Kukri population with the aim of identifying candidate genes (Bonneau et al., 2012). On chromosome 6A, grain dimensions and weight were associated with a polymorphism in the *TaGW2-6A* gene that has previously been shown to be associated with grain width and weight (Su et al., 2011). The *TaGW2* genes of wheat were isolated based on sequence of the rice gene *OsGW2*, which is also associated with grain weight in rice (Song et al., 2007).

Wheat grain is broadly classified into hard or soft types with hard wheat preferred for bread making and soft wheat preferred for pastry products. Grain hardness has a bearing on flour

extraction, flour properties, dough properties, processing quality and baking outcome. All the four parents used here are hard types suitable for bread making, with Drysdale and Kukri carrying the (*Pina-D1b* and *Pinb-D1a*) and Gladius and RAC875 carrying (*Pina-D1a* and *Pinb-D1b*) allele combination at the *Ha* locus on chromosome 5D. The *Ha* locus is a major determinant of grain hardness and in the research reported here, hardness was as expected largely controlled by the *Ha* locus in both populations. The *Ha* locus contains the puroindoline genes and grain softness protein, whose role is not well defined.

The *Ha* locus influenced flour colour, dough properties and baking output in both populations. Increase in hardness due to the allelic combination in both Drysdale and Kukri (*Pina-D1b* and *Pinb-D1a*) was generally accompanied by decreased particle size index (increased hardness), flour extraction, brightness, increased flour yellowness, flour water absorption, dough strength, loaf texture and score in both populations. Similar effects of grain hardness on processing and baking quality have been observed in other studies involving both hard and soft wheat grain (Carter et al., 2012; Peterson et al., 2001; Tsilo et al., 2011). During milling, the endosperm is crushed and the degree of crushing varies between soft and hard wheat types. Hard wheat usually has more damaged starch particles than soft wheat allowing more water absorption that can potentially influence loaf properties.

Flour colour is important for flour end use, with yellow coloured flour preferred for yellow alkaline noodles and white flour favoured for bread making. In the research reported here, various genomic regions influenced flour colour. Homoeologous copies of *Psy-1* (*Psy-A1*, *Psy-B1* and *Psy-D1*) are present in all the group 7 chromosomes (Ravel et al., 2012) and the group 7 chromosomes affected variation in flour yellowness in this thesis research. In the RAC875/Kukri population, a region on chromosome 7A previously shown to affect flour yellowness (Mares and Campbell, 2001; Parker et al., 1998) was associated with variation in flour yellowness. The *Psy-A1* gene, located on chromosome 7A is known to be involved in yellow pigment synthesis (He et al., 2008; Howitt et al., 2009). However, both RAC875 and Kukri are monomorphic for *Psy-A1* indicating that this gene was not the only gene on chromosome 7AL that affected variation in flour yellowness and that part of the variation is due to some other gene very close to *Psy-A1*. A similar observation where flour yellowness was associated with a locus close to *Psy-A1* has been reported (Crawford et al., 2011). A region seemingly homoeologous to this locus on chromosome 7B, and which might be close to or corresponding to *Psy-B1* was associated with flour yellowness in the Gladius/Drysdale

population. The QTL on chromosome 7B might correspond to a previously detected QTL associated with flour colour (Mares and Campbell, 2001).

Gliadins and glutenins, which together form the gluten complex, are major determinants of dough properties (Gras et al., 2001). The gliadins were not assayed in both populations. In the RAC875/Kukri population, *Glu-A1* and *Glu-B1* (HMW-GS), and *Glu-A3*, *Glu-B3* and *Glu-D3* (LMW-GS) were polymorphic and were assayed. The LMW-GS and gliadins are closely linked on the short arms of group 1 chromosomes. A region within 8.5 cM of the *Glu-A3* locus on chromosome 1A, was associated with particle size index, flour yellowness, flour brightness, flour water absorption and loaf texture. The relationship between water absorption and baking output can facilitate the use the former to predict the latter. In the RAC875/Kukri population, the *Glu-B1al* over-expression allele was associated with dough properties even under water limiting conditions. This allele has been shown to increase dough strength in other studies (Butow et al., 2003; Eagles et al., 2004; Mann et al., 2009). The other glutenin loci were not associated with any trait in this thesis research. Based on map comparisons, a region that seems to be at or near the *Gli-A2* gliadin locus on the short arm of chromosome 6A was associated with loaf properties. Other genomic regions, other than the gluten loci also affected dough properties with chromosomes 1B and 3B mainly associated with rheology traits.

In the Gladius/Drysdale population, *Glu-B1* (HMW-GS), and *Glu-A3* and *Glu-D3* (LMW-GS) were polymorphic and were assayed but none were associated with rheology traits. For the Gladius/Drysdale population, both groups 1 and 2 chromosomes were largely associated with rheology traits. The loci on chromosomes 2A and 2B associated with rheology traits might be in homoeologous positions. The region on chromosome 2A was also associated with dough properties and loaf volume in another population (Kuchel et al., 2006). Chromosomes 2B and 3B have not been widely demonstrated before to affect rheology, so the QTLs detected on these chromosomes might be novel. If consistently detected across populations and environments, these QTLs may be useful in selecting for dough rheology.

In both populations, some of the QTLs showed QTL-by-environment interactions, while some were stable and were detected regardless of growth conditions. This thesis research shows the importance of comparing QTLs for bread making quality traits across diverse environments. The range of environments used in this research enabled the detection of responsive QTLs, that are present under certain environmental conditions, and constitutive

QTLs that are stable across environments. Future fine mapping and candidate gene identification studies can target loci such as positions Q17 and Q22 (Chapter 4) on chromosome 3B, which is now fully sequenced. At position Q17, the Gladius allele is associated with higher grain protein content and thousand grain weight, providing an opportunity to simultaneously increase both traits, as more often these traits are negatively correlated. At position Q22, Drysdale alleles are associated with higher grain thickness, thousand grain weight and yield thus offering an opportunity to simultaneously improve all these traits. Though in these loci the favourable alleles came from opposite parents, these loci are not closely linked and are 265 cM apart making selection of desired allele combinations possible. The QTL on chromosome 4A (position Q10) (Chapter 5) at which the allele from the heat tolerant parent (Gladius) was associated with better loaf score under heat stress conditions could be useful for improving baking output under heat stress conditions. The QTLs at positions Q8 and Q15 (Chapter 5) were also detected only under heat conditions and thus offer opportunities to select lines with allele combinations that allow them to perform and/or maintain quality even under high temperatures.

Two pairs of backcross derivatives, one having a grain protein content gene (*Gpc-B1*) introgression segment and the other without the segment and their recurrent parents were grown in the glasshouse in a heat treatment experiment (Chapter 7). The recurrent parents were Wyalkatchem and RAC1262A. Heat stress expectedly accelerated senescence (in all the lines) and reduced grain weight (in some lines) in the glasshouse experiment. Wyalkatchem and its backcross derivatives suffered grain weight losses due to heat treatment. RAC1262A a sister line of Gladius was heat tolerant. Exposure to heat treatment in early grain filling might lead to increased % UPP. Early exposure to heat treatment (15 days after anthesis) resulted in increased protein polymerisation as measured by percentage unextractable polymeric protein (% UPP) in WB4-1-6 and had no effect on others. The increase in % UPP is consistent with a study which showed increased % UPP when heat stress was initiated at 12 days after anthesis (Balla et al., 2011) and one that observed an increase in sodium dodecyl sulphate sedimentation height/volume when plants were exposed to heat stress at 10 days after pollination (Beecher et al., 2012). However, late exposure at about 25 days after anthesis or in field trials has been shown to be accompanied by decreased % UPP (Cavanagh et al., 2010; Irmak et al., 2008).

Heat stress and *Gpc-B1* had similar effects on some traits, and they both increased protein content and reduced the grain filling duration. The increase in protein content due to the high

grain protein (*Gpc-B1*) locus observed under normal conditions was expected and has been reported in other studies as well (Brevis and Dubcovsky, 2010; Uauy et al., 2006b). The *Gpc-B1* locus is also known to accelerate senescence and this was observed in this thesis research. There were no negative effects of the *Gpc-B1* segment observed in both backgrounds in this thesis research.

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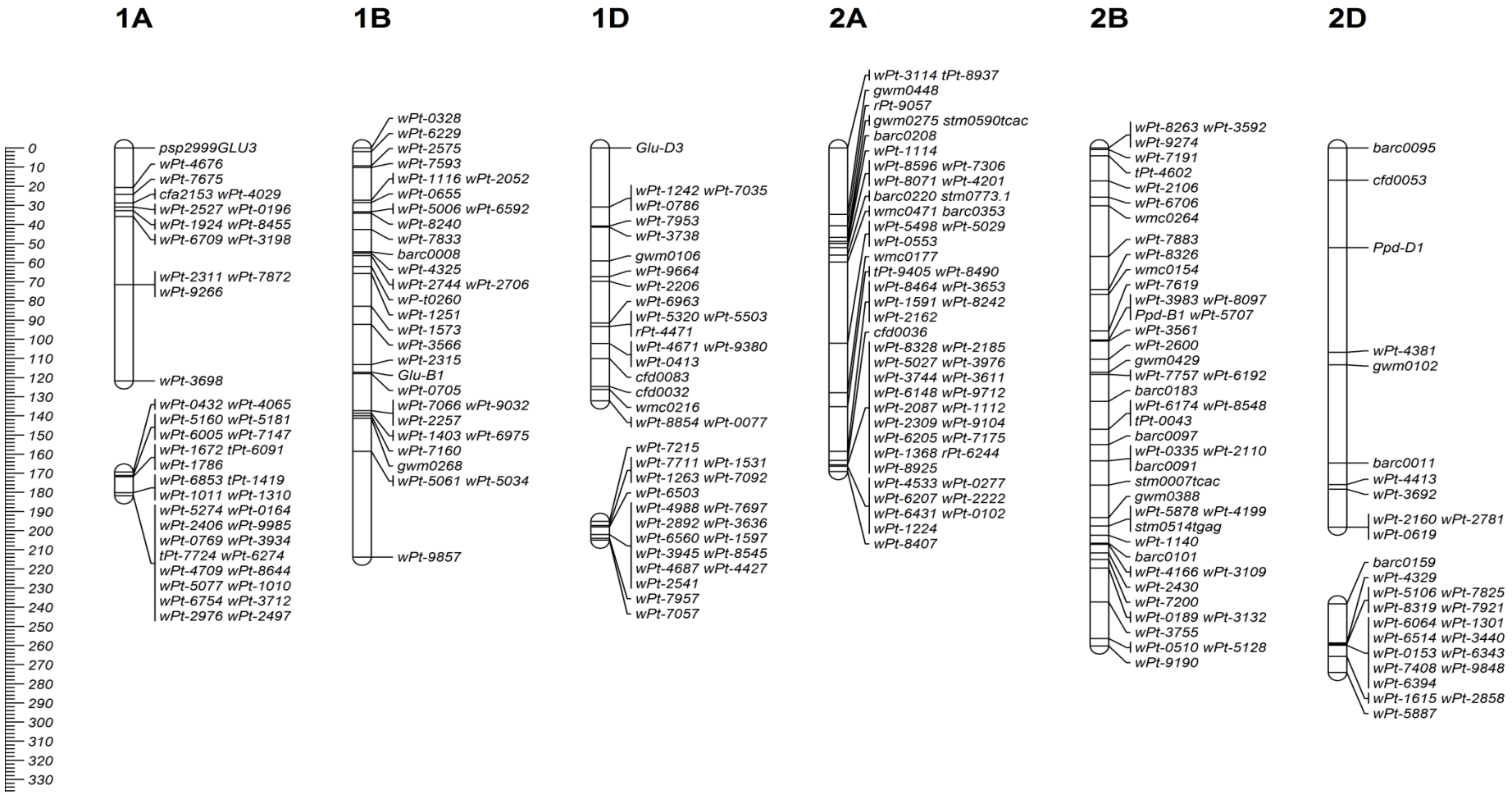
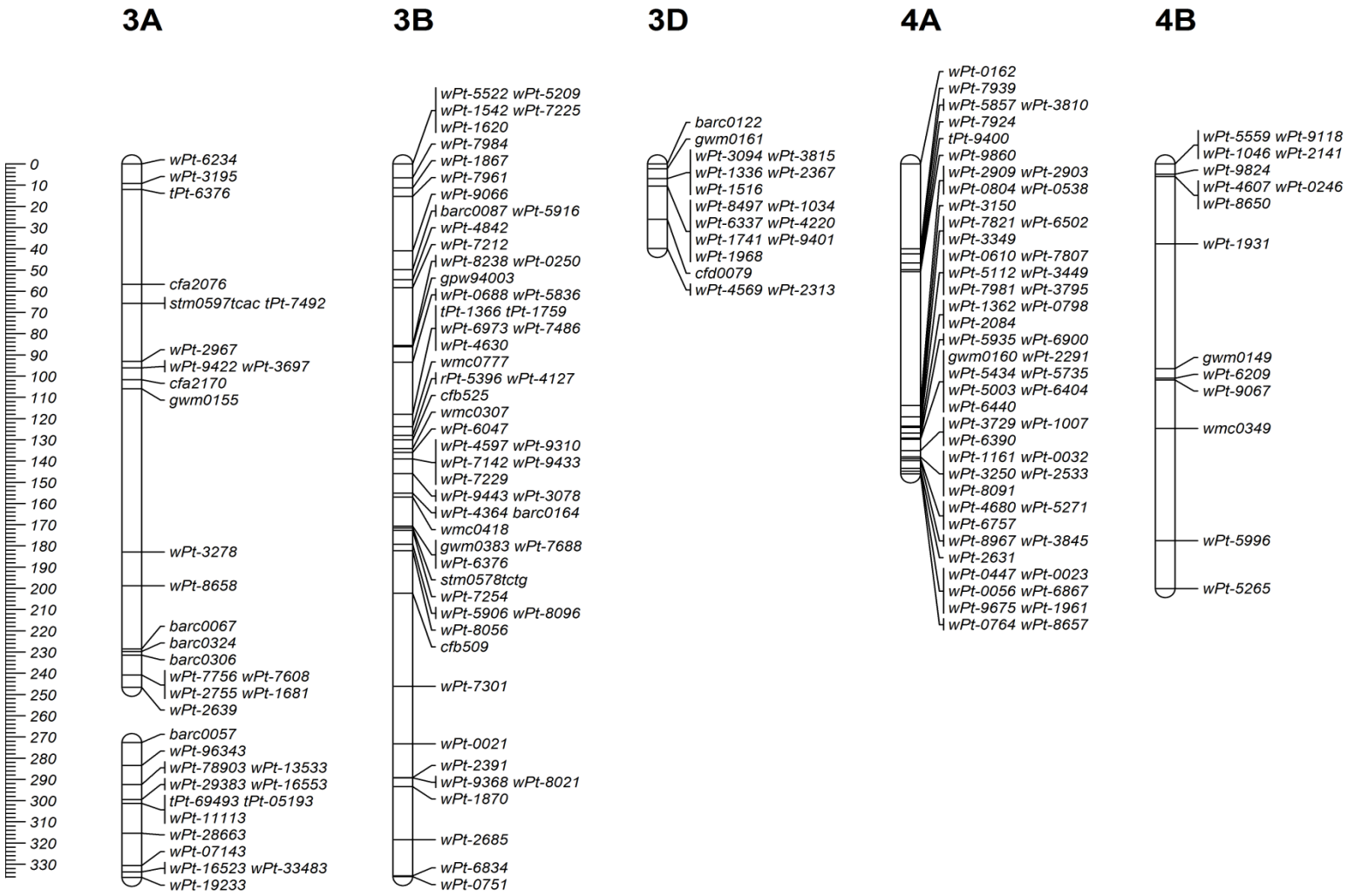
Appendix 1. Genetic linkage map generated for the random subset. The scale on the left indicates genetic distances in cM.



Appendix 1. (continued)



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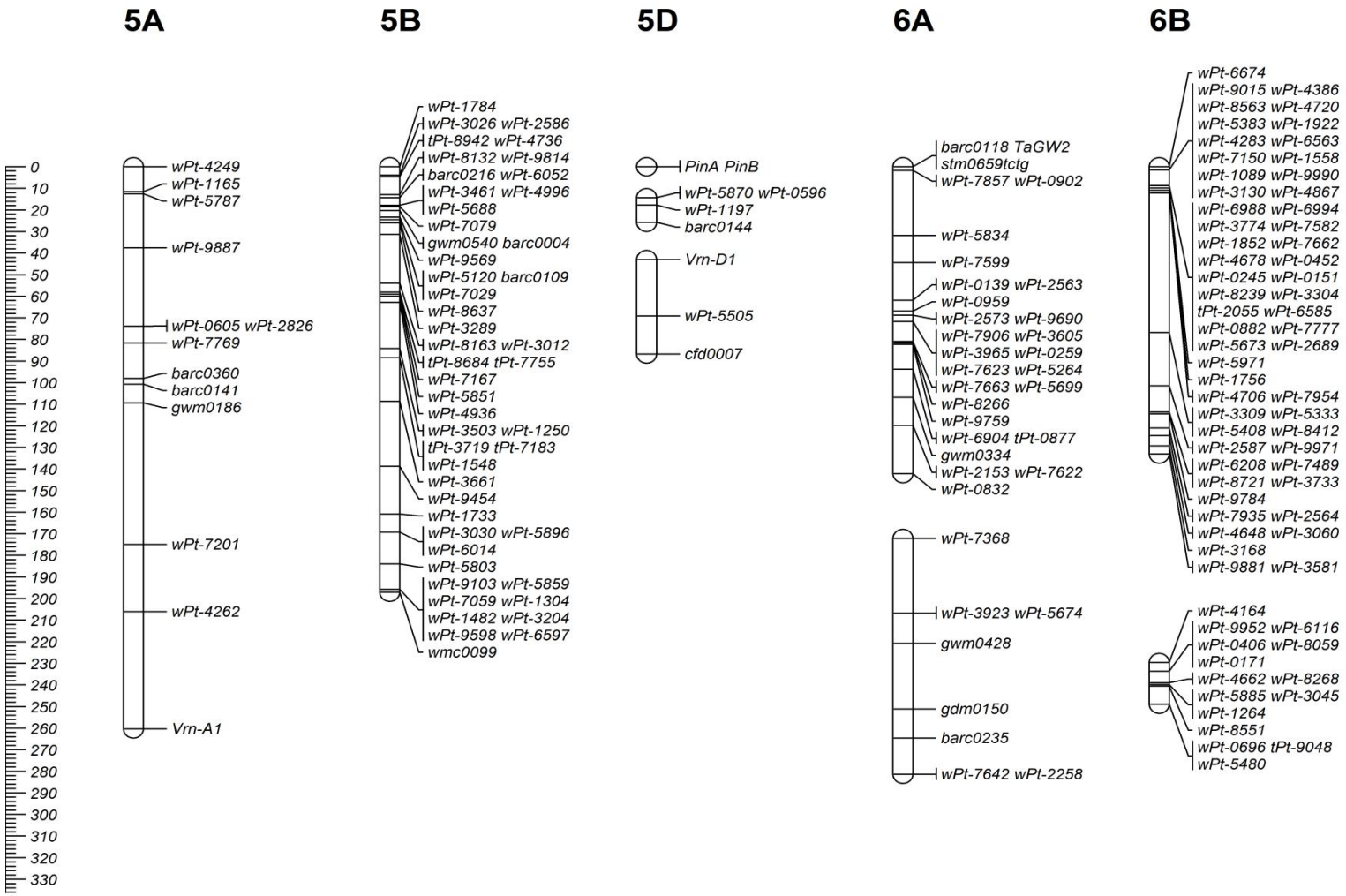
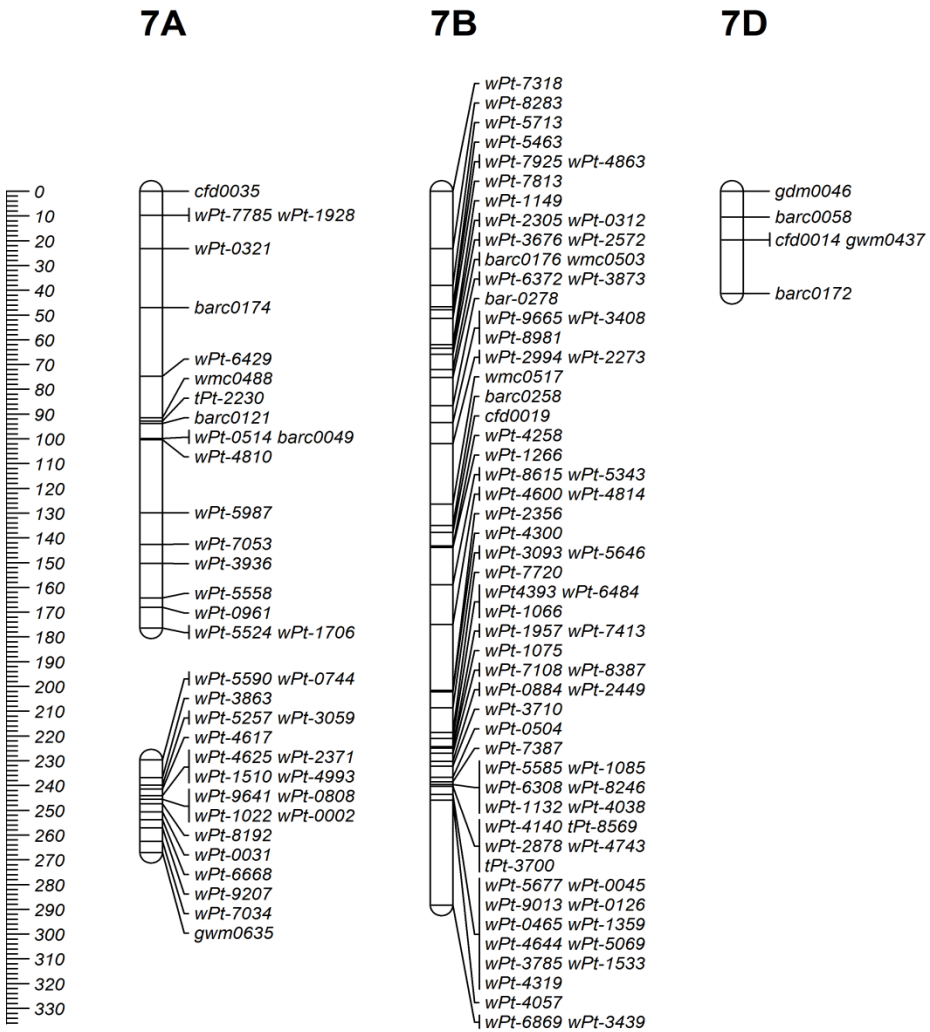
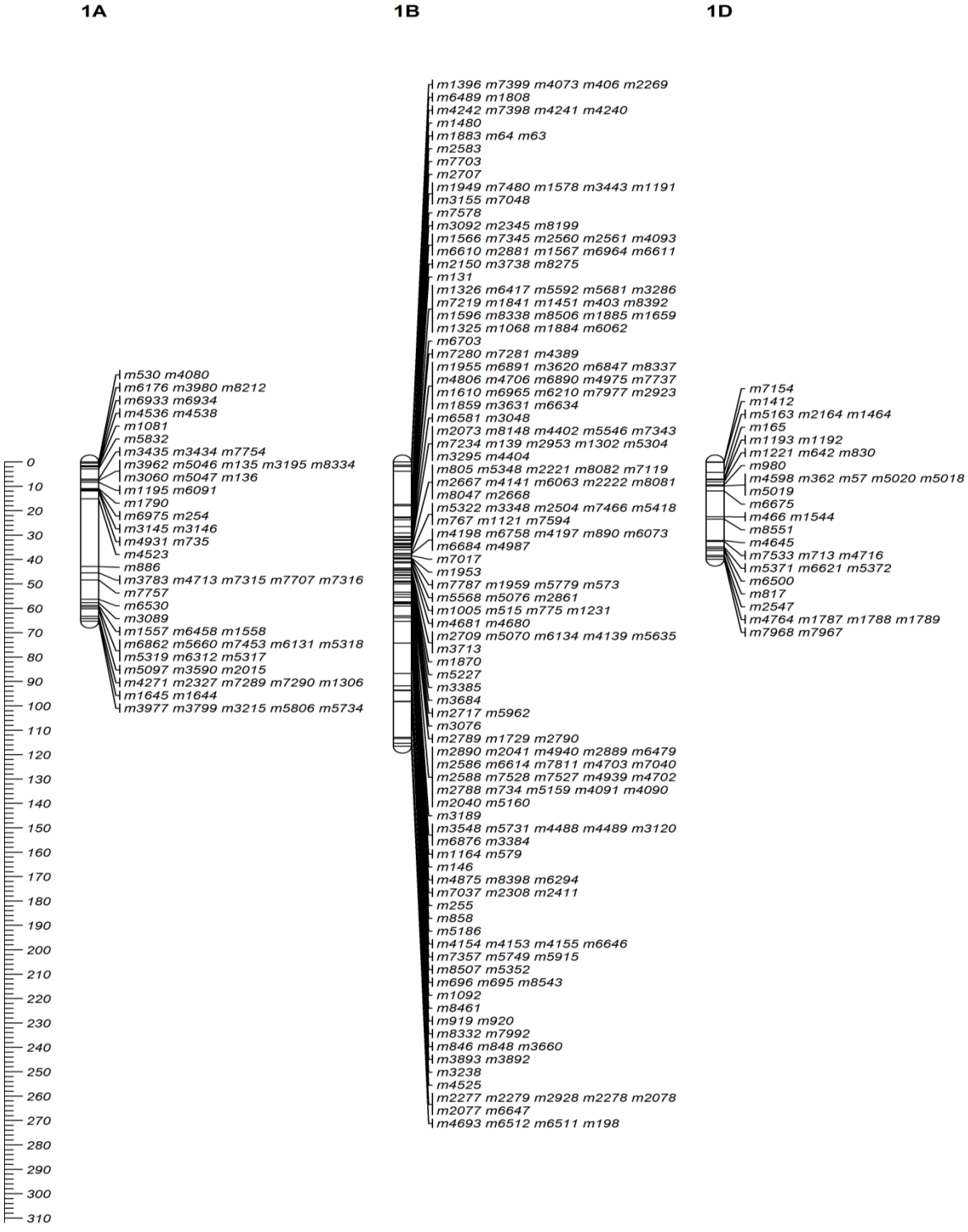


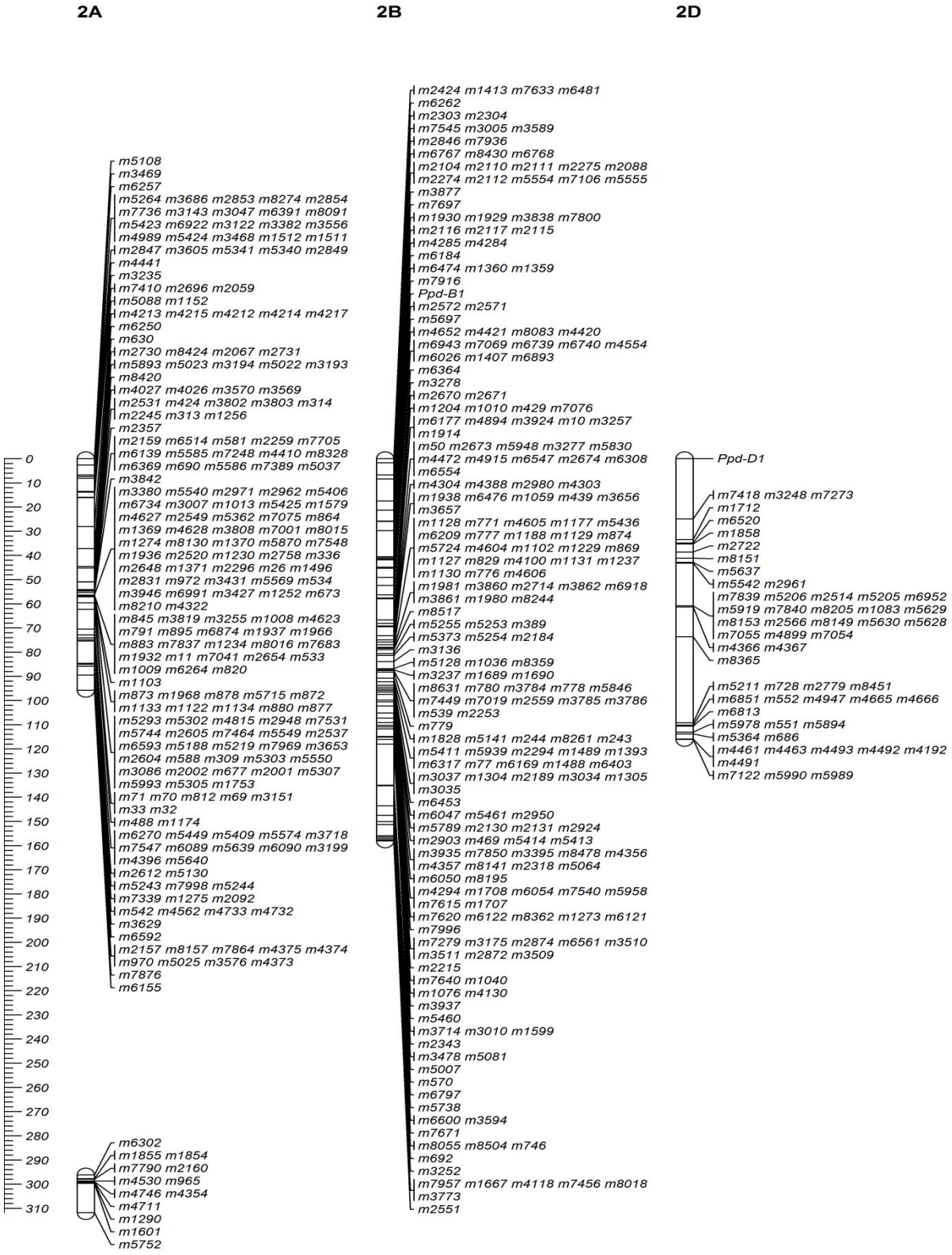
Appendix 1. (continued)



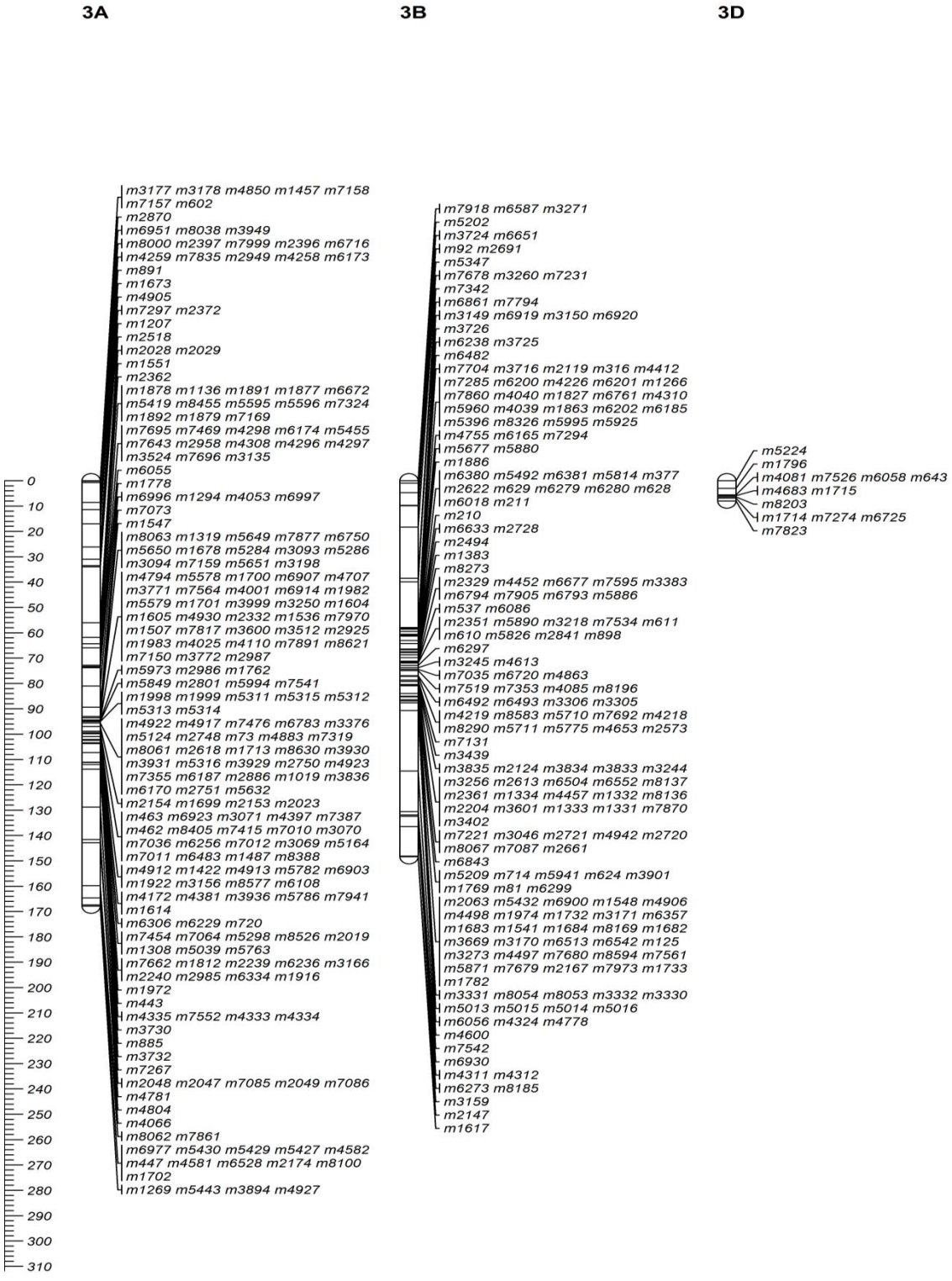
Appendix 2. Genetic linkage map generated for the mid-maturity subset. The scale on the left indicates genetic distances in cM.



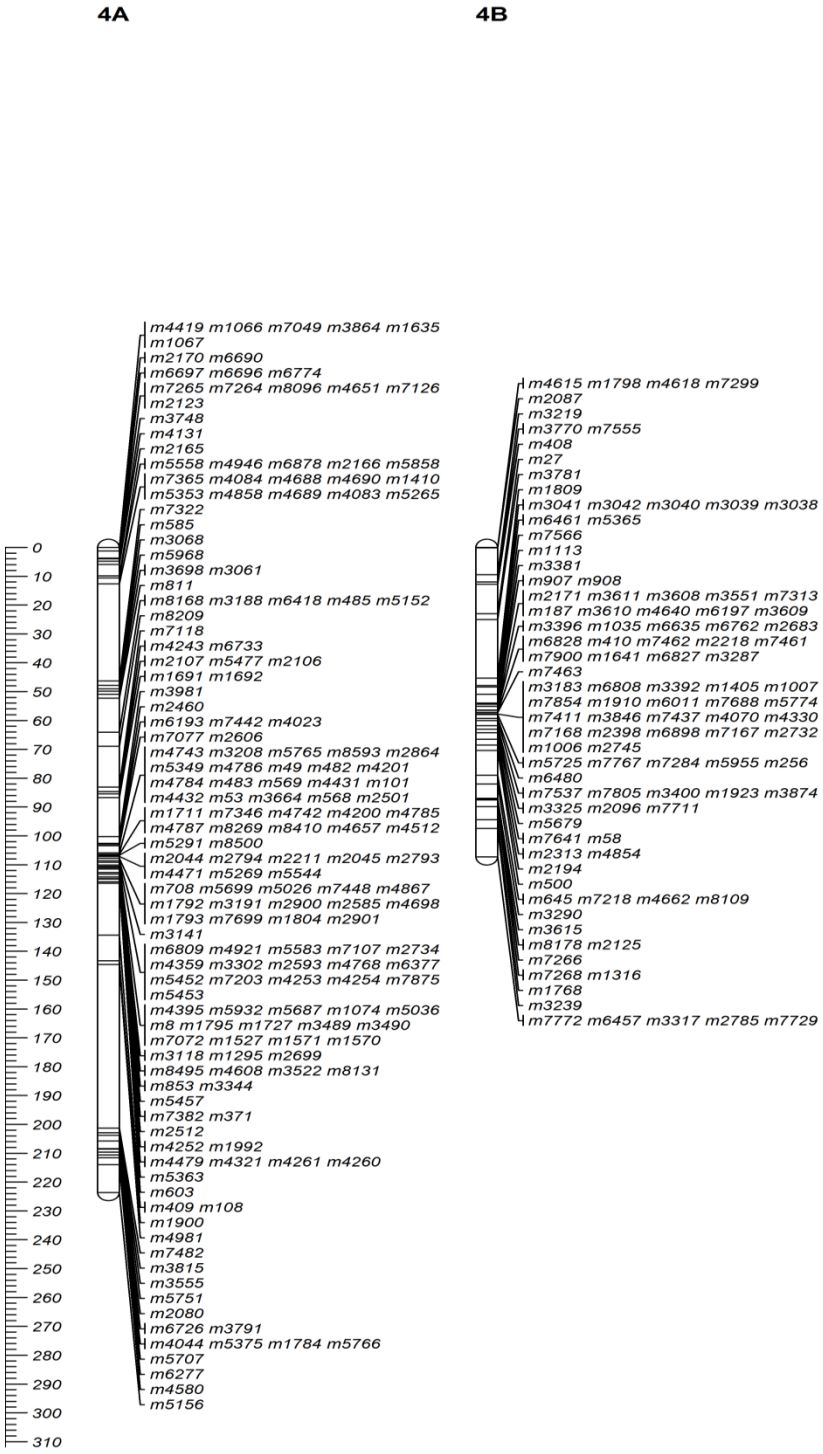
Appendix 2. (continued)



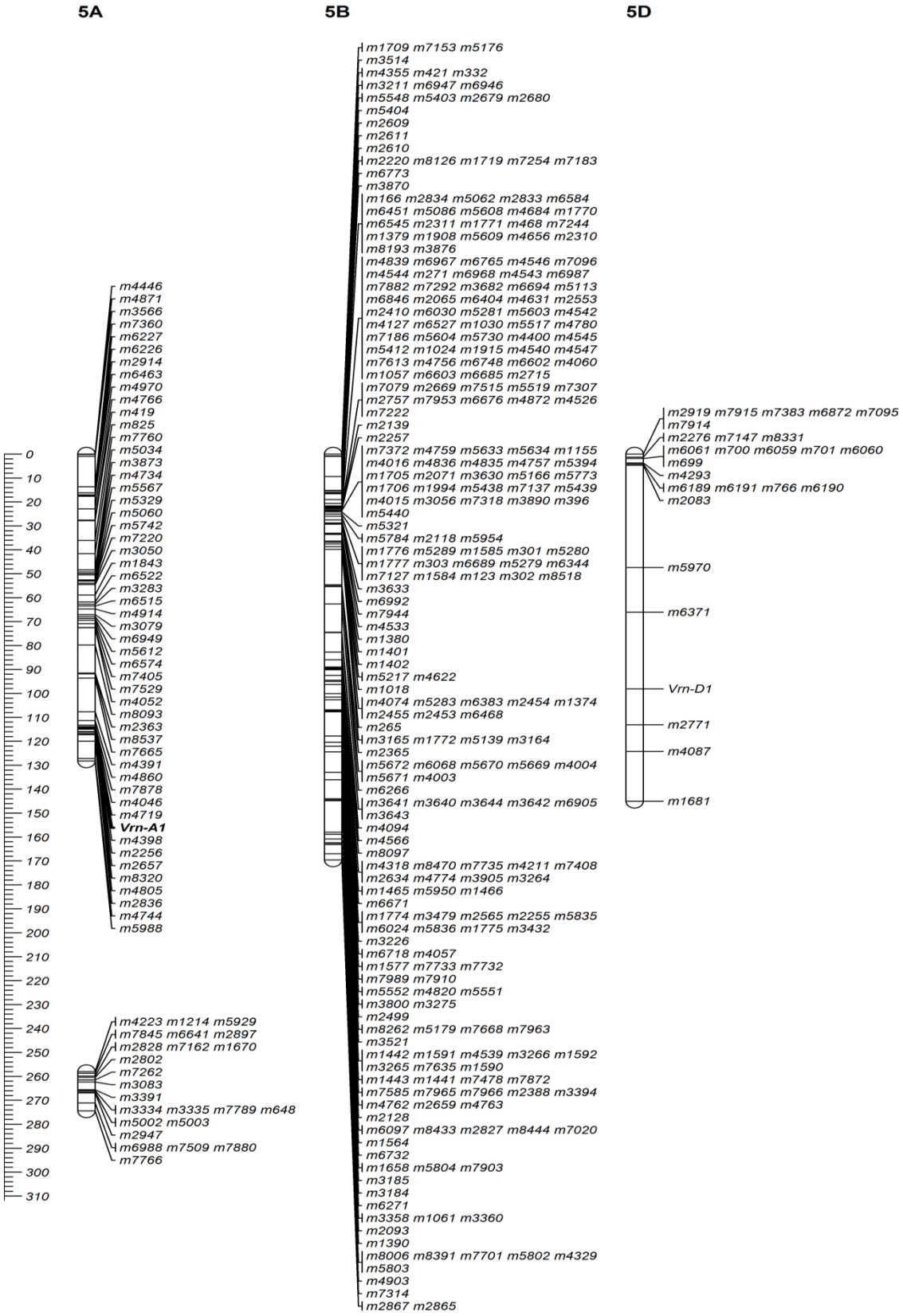
Appendix 2. (continued)



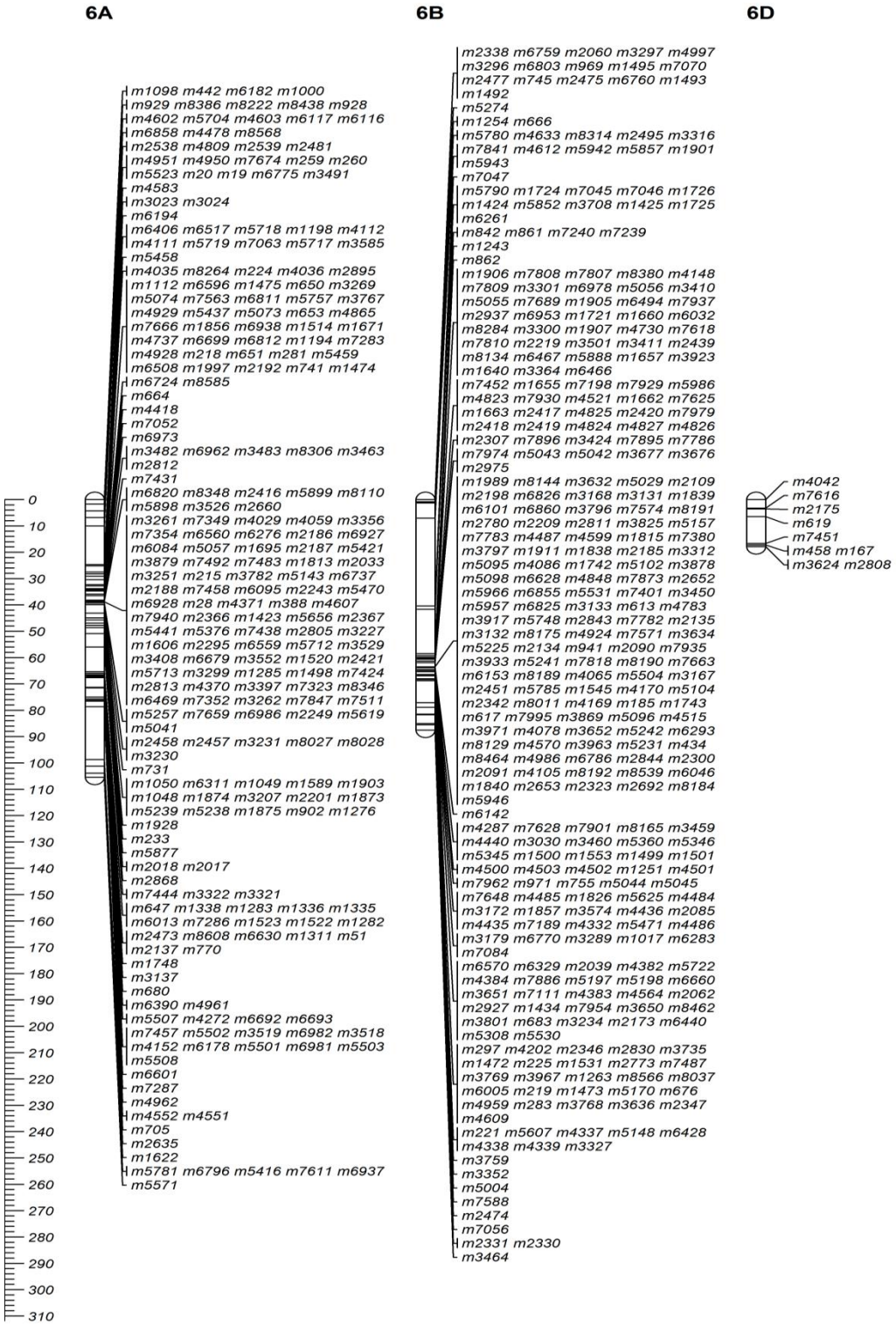
Appendix 2. (continued)



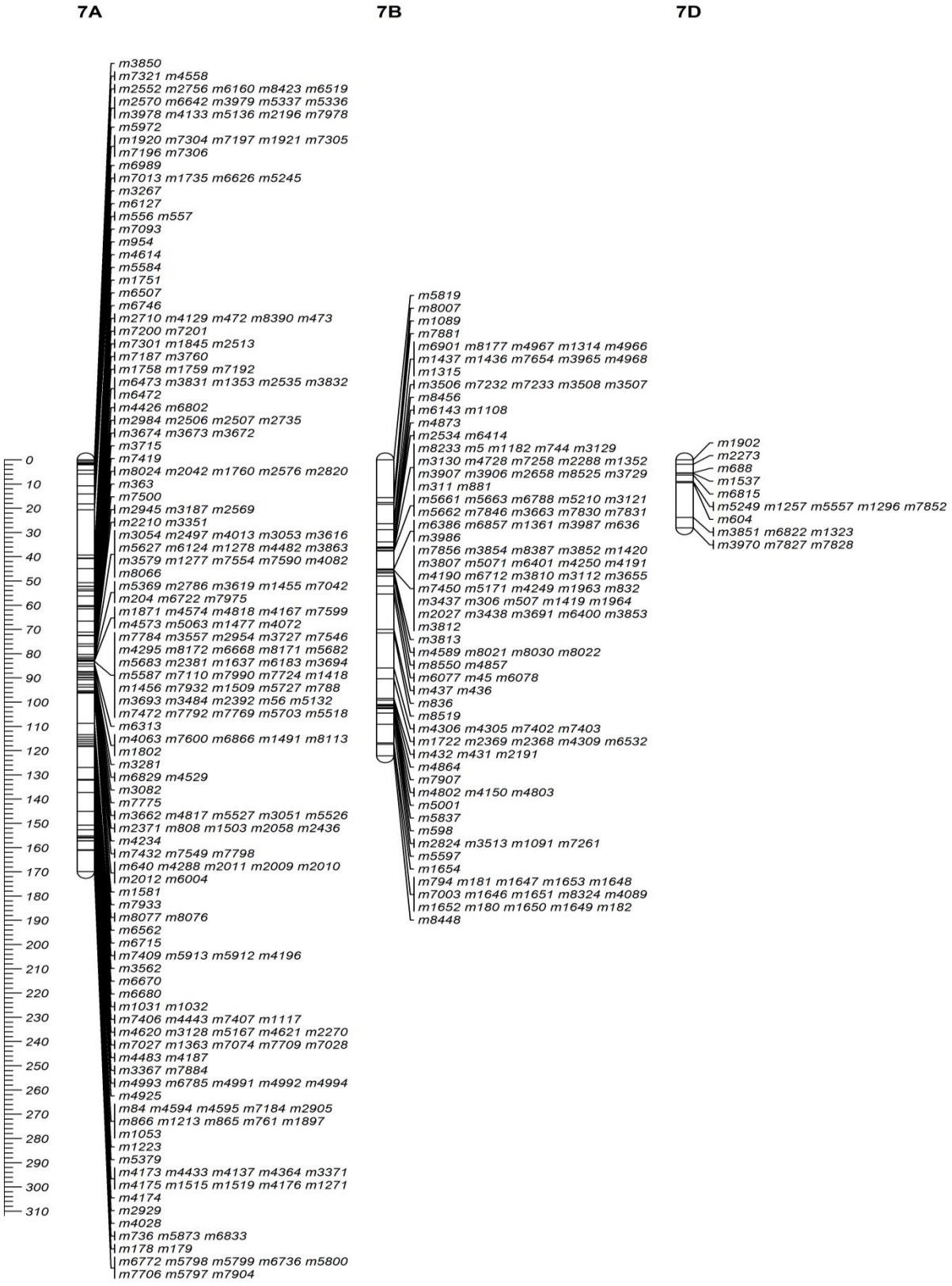
Appendix 2. (continued)



Appendix 2. (continued)



Appendix 2. (continued)



Appendix 3. Significant ($p < 0.001$) within environment phenotypic correlation coefficients between traits for the 60 subset of the Gladius/Drysdale recombinant inbred line population.

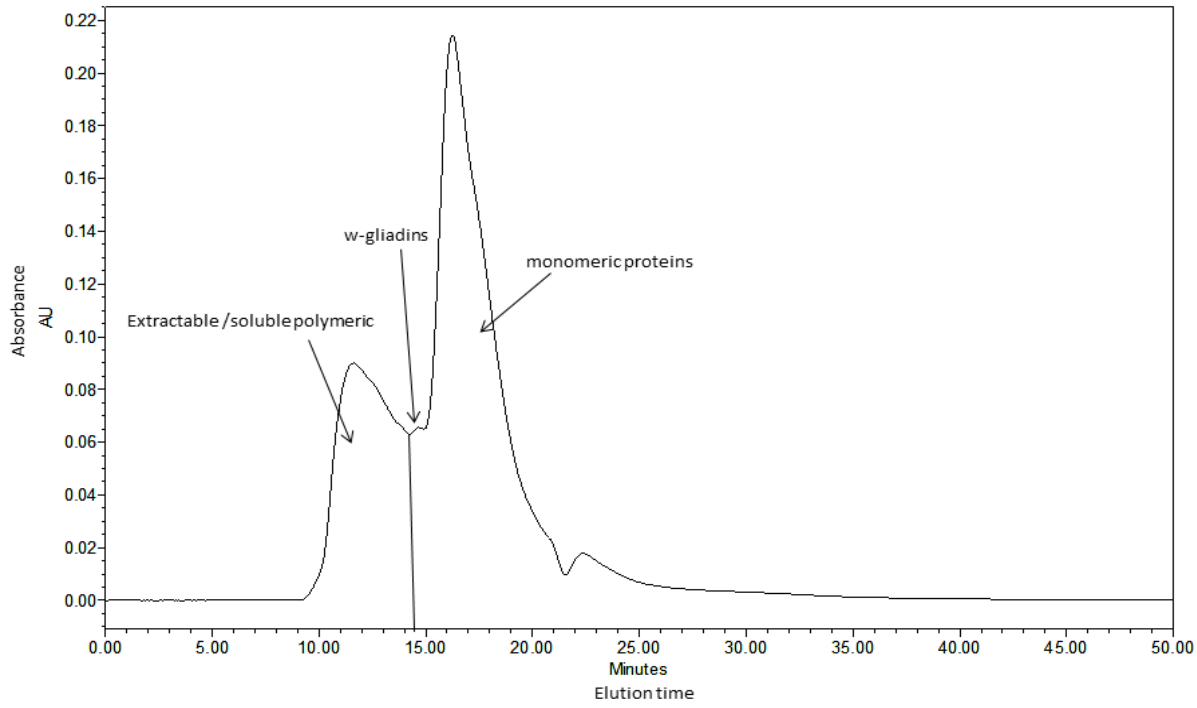
	Environment							
Correlated traits[a]	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS	AusUrr10_H_SI_CS	AusUrr10_HD_CS	MexObr10_D_LS
Length and TW					0.36			
Length and yield					0.45			
Length and FE					0.32			
Length and width	0.35		0.51	0.37	0.50			
Length and GPC				-0.29	-0.43			
Length and thickness			0.36		0.42			
Width and PSI	-0.29		-0.35					
Width and FE					0.33			
Width and TW			-0.29					
Width and GPC				-0.40	-0.49		-0.55	
Width and GN			-0.31	0.32				
Thickness and FE					0.29			
Thickness and GPC					-0.29		-0.37	
Thickness and GN			-0.35					
Thickness and PSI			-0.34					
Thickness and yield	0.41			0.35	0.37		0.31	
AR and area					-0.48			
AR and GN	-0.30			-0.29				
AR and screenings	-0.39							
AR and TGW	-0.35			-0.45	-0.62	-0.45	-0.54	
AR and yield	-0.40			-0.35	-0.32			
AR and GPC							0.45	

Appendix 3. (continued)

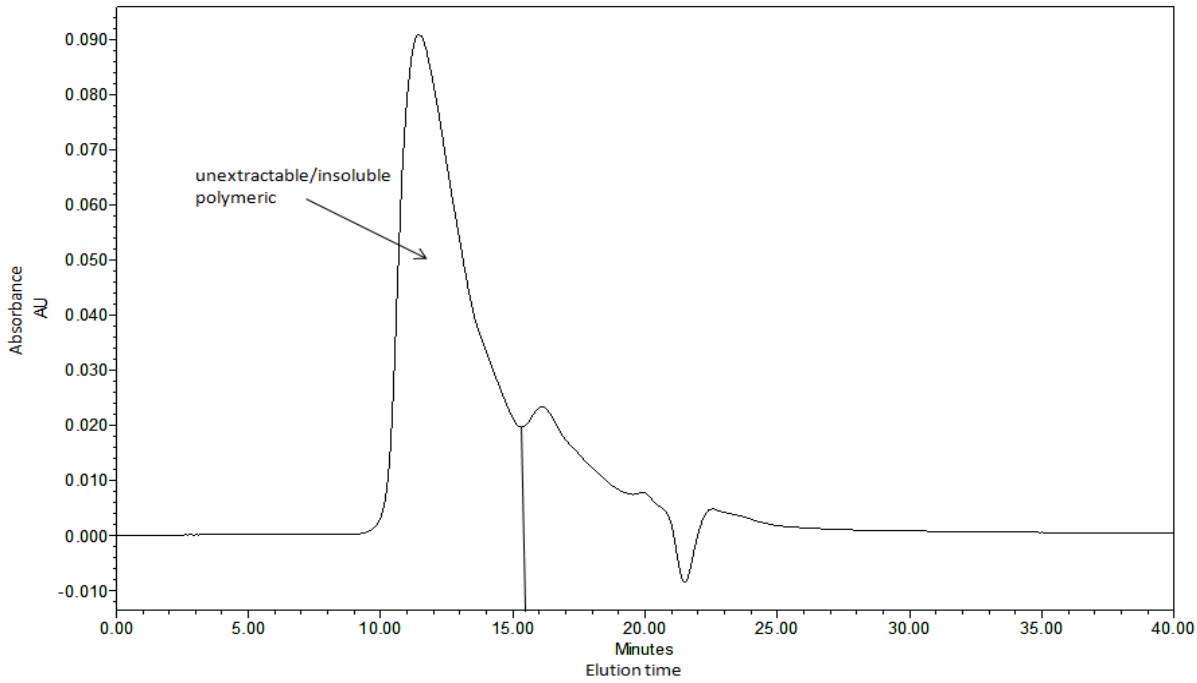
	Environment							
Correlated traits[a]	AusYan09_FI_CS	AusYan09_FI_LS	AusYan10_FI_CS	AusUrr10_SI_CS	AusUrr10_D_CS	AusUrr10_H_SI_CS	AusUrr10_HD_CS	MexObr10_D_LS
Roundness and TGW				-0.33	-0.54	-0.36	-0.45	
Area and GN			-0.31					
Area and PSI			-0.34					
Area and roundness			0.35		-0.33			
Area and FE					0.38			
Area and TW					0.66	0.31	0.46	
GN and TGW			-0.40	0.32				0.35
GN and PSI					-0.44			
GN and screenings	-0.44							
Yield and PSI	-0.37				-0.36		-0.36	
Yield and screenings		-0.46						
TGW and PSI			-0.46				-0.31	
TGW and GPC		0.39		-0.42	-0.50		-0.54	
TGW and FE		0.33			0.36			
TW and GPC		0.29			-0.51	-0.31	-0.67	
TW and PSI							-0.4	
TW and FE		0.30	0.39		0.29	-0.31		
Screenings and GPC		-0.42						
Screenings and PSI	-0.32	0.32						
GPC and PSI			-0.54	-0.29			0.38	
PSI and FE		-0.30						

[a] AR for aspect ratio, GN for grain number, TGW for thousand grain weight, PSI for particle size index, GPC for grain protein content, TW for test weight, screenings for percentage screenings, FE for flour extraction

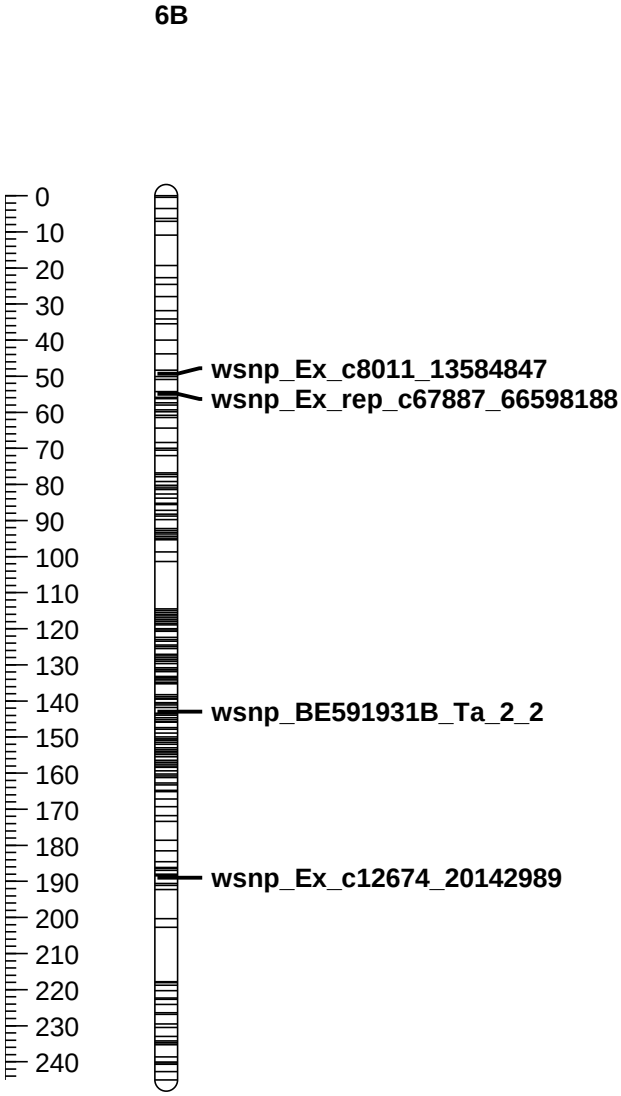
Appendix 4. Size-exclusion-HPLC separation of SDS-extractable polymeric proteins. The area to the left of the vertical line (14.5 minutes) defined the amount of polymeric SDS-extractable protein.



Appendix 5. Size-exclusion HPLC separation of SDS-unextractable polymeric proteins. The area to the left of the vertical line (15.5 minutes) defined the amount of SDS-unextractable polymeric protein.



Appendix 6. Chromosome 6B showing the introgressed *Gpc-B1* segment in RS4-11-10 (between wsnp_Ex_rep_c67887_66598188 and wsnp_Ex_c12674_20142989) and WB4-1-6 (between wsnp_Ex_c8011_13584847 and wsnp_BE591931B_Ta_2_2) which are RAC1262A and Wyalkatchem backcross derivatives respectively. The regions between the shown markers contain polymorphic markers. The horizontal lines on the chromosome represent positions of the markers and marker names were excluded for clearer presentation. The scale on the left indicates genetic distances in cM.



Appendix 7. Polymorphic loci in the SNP consensus map. B for Burnside (non-Wyalkatchem) and S for Somerset (non-RAC1262A) show the regions donated by these donor parents in the rest of the genome (excluding 6B). The horizontal lines on the chromosomes represent positions of the markers and marker names were excluded for clearer presentation. The scale on the left indicates genetic distances in cM.