The Genetic Analysis and Manipulation of Economically Important Traits in Bread Wheat

By

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Abstract

The aims of this thesis were to firstly gain an improved understanding of the genetic basis to economically important complex traits in bread wheat, and secondly, to investigate marker assisted selection (MAS) methodologies that may lead to improved rates of genetic gain. An elite Australian breeder's line, 'Stylet', and its parents 'Trident' and 'Molineux' were used as the basis of this study.

A doubled-haploid (DH) population previously produced from a cross between 'Trident' and 'Molineux' (T/M DH) was used to dissect the genetic basis to end-use quality and agronomic performance. The study of end-use quality confirmed the widely published relationship between the glutenin loci and dough rheology. However this study also identified a quantitative trait locus (QTL) on chromosome 2A that was shown to be associated with dough resistance and baking quality, and another QTL on 3A that was associated with baking quality.

QTL were identified in the T/M DH population that were involved in the control of time to ear-emergence through their effects on vernalisation sensitivity, photoperiod sensitivity and earliness *per se*. In addition to the well characterised *Vrn-A1* and *Ppd-B1* genes, six other QTL were identified. Three of these, *QPpd.agt-1A*, *QPpd.agt-7A* and *QPpd.agt-7B* are putative new loci involved in the control of photoperiod sensitivity in wheat. *QPpd.agt-1A* appears homoeologous to the photoperiod response gene *Ppd-H2* in barley. *QPpd.agt-7A* and *QPpd.agt-7B* are located in homoeologous regions, and may represent a new phenology gene series in wheat.

The T/M DH population was also used to dissect the genetic basis to grain yield and grain yield components, and to examine the influence of QTL-by-environmental covariable interaction on genotype-by-environment interaction. The association of plant height genes, rust resistance genes and phenology

genes with grain yield were determined. Overall, semi-dwarf rust resistant DH lines, carrying alleles conferring a short time to ear-emergence, showed the highest and most stable grain yield. Nine genetic associations with grain yield, without effects on plant height, time to ear-emergence and rust resistance, were identified. Two QTL, *QGyld.agt-1B* and *QGyld.agt-4D* were shown to have large and frequent associations with grain yield. *QGyld.agt-1B* showed only low levels of interaction with environmental covariables and therefore constitutes a prime candidate for MAS for grain yield.

The second part of this study investigated the potential role of MAS through a practical breeding strategy and by computer simulation. An 'Anneullo/2*Stylet' cross aimed at producing a rust resistant 'Stylet' derivative with improved end-use quality was used as the model for this analysis. MAS was shown to be highly effective at improving the rate of genetic gain for rust resistance and end-use quality. This was most evident when undertaken on the BC_1F_1 population, although MAS also improved the efficiency of the breeding programme when performed on fixed lines. Practical implementation of the MAS breeding strategy validated the results from the simulation study and produced elite lines approaching the grain yield level of 'Stylet', with resistance to leaf, stem and stripe rust, and with improved end-use quality.

While the results from this study highlight the complex nature of the major economically important traits being manipulated by wheat breeders, this study also concluded that improvements in rate of genetic gain are possible through the application of MAS.

Statement

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Chapter 1

Review of the Relevant Literature and an Introduction to the Research

1.0 Review of the Relevant Literature and an Introduction to the Research

1.1 Introduction

Wheat production holds a dominant position in Australia's agricultural industries. From 1998-2002, wheat comprised around 68% of Australia's area sown to cereal crops, 65% of total cereal production and 69% of its gross cereal crop value. Across all Australian agricultural production, wheat is consistently placed second in value, making up 15% of the total agricultural revenue (ABS 2001; ABS 2004). Beyond expanding the area sown to wheat, the ability to increase the profitability of the Australian wheat industry relies on improving two broad factors; productivity, tonnes per hectare, and price, dollars per tonne. Both the productivity, and value of a wheat crop, are to some degree a function of the genetic potential of the varieties being sown and the characteristics of the environment in which the crop is being grown.

Changes in agronomic practice; namely the introduction of macro- and micro-nutrient application, herbicide based weed management, fungicide mediated control of cereal diseases, optimisation of crop rotation and improvements in tillage and seeding technology have improved the grain yields achieved by Australian growers and allowed the expansion of the cereal zone into otherwise unprofitable geographical regions. Likewise, careful harvest of the wheat crop, appropriate nitrogen based fertiliser application, and environmental factors such as hot dry conditions during harvest, have helped to improve the value of the Australian wheat crop. These factors can ensure a grain sample possessing low moisture content, having a high test weight and achieving an appropriate grain protein level (Simmonds 1989).

Genetic improvement of wheat through the recombination and selection of superior genotypes is capable of improving both the productivity and value of the Australian wheat crop. This dual aim of wheat improvement was recognised by the pioneering Australian wheat breeder, William Farrer (Wenholz 1937). As early as the 1880's, Farrer started crossing wheat cultivars and selecting their progeny for improved grain yield and disease resistance (Wrigley et al. 1981). Although farming systems have changed dramatically over the ensuing century, and the extent of genetic knowledge concerning wheat quality and grain yield has deepened, the aims of current wheat breeding programmes remain substantially the same. The science of wheat breeding has now progressed to the extent that DNA sequence variation can be used to identify the genetic basis of complex traits such as grain yield and end-use quality, and thereby allow breeders to begin using genotypic rather than phenotypic selection (Koebner et al. 2003).

Genotypic selection, using molecular markers (Thoday 1961) designed to assay DNA polymorphisms linked to genes controlling economically important traits, has been suggested as a method to improve the rate of genetic gain in plant breeding programmes (Young 1999). Unlike phenotypic based selection, marker assisted selection (MAS) has the advantage of not being influenced by environmental variation. DNA based assays can also be performed at any growth stage, for any number of genes, and are relatively inexpensive. These factors make the use of molecular markers in a breeding programme an attractive option where the traditional trait based selection can be expensive, not possible (such as for exotic diseases, or end-use quality very early in a breeding programme), or is subject to substantial extraneous error (Koebner et al. 2003). Currently, there are few published reports detailing successful MAS in wheat breeding. It appears likely that two basic

requirements must be met before we see the routine and successful application of MAS in wheat breeding; 1) there must be genetic analysis of relevant and economically important traits, and 2) systems must be in place to effectively apply genotypic selection for these economically important traits. Consequently, the aim of this literature review is to summarise the current status of genetic knowledge regarding some traits of economic importance to southern Australia and outline the ways in which genetic knowledge in wheat has been, and may be, used to improve the rate of genetic gain within wheat breeding.

1.2 Genetic Analysis of Economically Important Traits in Bread Wheat

1.2.1 Genetic Analysis of End-Use Quality

Australian wheat has traditionally been used for baking, and although some rudimentary improvements in baking quality were achieved by Farrer in the earliest breeding efforts (Wrigley et al. 1981), much scope for improvement remained. During the next century the grain quality characteristics of Australian wheat changed from soft to hard texture and from weak to strong dough. This shifted Australia's critical export commodity from a product used in blends with better quality wheat, to a product capable of attracting a premium in export markets (Whitwell et al. 1991). Australian wheat has also expanded from a product aimed almost exclusively at leavened and unleavened bread and biscuit products, to a flour of choice for Asian noodles and steam breads (Simmonds 1989). However, with this widening of the market for Australian wheat, comes a need for wheat breeders to gain an improved understanding of the genetic basis of the traits required for each end-product.

The attributes affecting wheat quality can be separated into two categories: 1) physical and 2) chemical. Physical properties include moisture content, grain size, test weight, and cleanliness (absence of foreign particulate matter). Both moisture content and cleanliness are controlled largely by environmental factors and are therefore not considered further in this review. Grain size and test weight both form critical receival and marketing standards and are under substantial genetic control (Bhatt et al. 1975; Pearson et al. 1981). Grain size, as one of the components of grain yield, will be considered as part of the review of genetic loci affecting grain yield.

The chemical properties affecting wheat quality are particularly complex, but can be dissected into three groups. Firstly, those factors influencing milling quality, otherwise described as the quantity and quality of flour production. Secondly those factors affecting the performance of the flour as it is being mixed into a dough and the dough itself, and finally the unique characters of the dough that dictate the quality of the final end-product (Whitwell et al. 1991).

1.2.1.1 Genetic Factors Controlling Milling Quality

The genetic basis of milling quality can be dissected into the following traits; grain protein content, flour yield, grain texture, and flour colour.

Grain Protein Content

As grain protein is responsible for much of the functionality of flour, the concentration of protein within each grain forms a key quality criterion (Stoddard et al. 1990). The quantity of protein in a wheat grain, expressed as a proportion of grain weight, is heavily influenced by both the nitrogen and carbon supply to the developing grain. Given that final grain weight is also influenced by the movement of

carbon assimilate into the developing grain, a strong inverse relationship exists between grain yield and protein concentration (Fischer et al. 1990; Stoddard et al. 1990; Fabrizius et al. 1997; Cooper et al. 2001). It would therefore be expected that many of the genes responsible for the grain yield of wheat (Section 1.2.2) would also influence grain protein content. However, genes that confer high grain protein independent of grain yield would be of more interest to wheat breeders attempting to improve both grain yield and protein concentration simultaneously.

A gene influencing protein concentration, apparently independent of grain yield, was identified in *Triticum turgidum* on chromosome 6BS (Joppa et al. 1997). This gene was transferred to bread wheat, resulting in the variety 'GluPro' (Khan et al. 2000). Since then, the gene has successfully been incorporated into commercial varieties such as 'Lillian' (De Pauw et al. 2005), and 'Somerset' (Fox et al. 2006), and molecular markers have been developed to aid its selection (Khan et al. 2000). Uauy et al. (2006a) showed that *Gpc-B1* has pleiotropic effects on the rate of senescence, grain size and grain protein concentration. Since then, this gene has been cloned, and has also been shown to increase the remobilisation of iron and zinc to the grain (Uauy et al. 2006b). Numerous studies have reported QTL associated with protein content (Prasad et al. 1999; Groos et al. 2003; Prasad et al. 2003; Turner et al. 2004; Breseghello et al. 2005; Huang et al. 2006), and these can be shown to be distributed across most of the genome (Table 1). Unfortunately, many of these studies were performed without accounting for grain yield, so it is difficult to determine if selection for these high protein alleles within a breeding programme would result in an increase *per se* in protein concentration without a corresponding drop in grain yield. However it is interesting to note the large number of grain and flour protein content QTL that are coincident with grain yield and grain weight QTL identified in a range of mapping

populations (Figure 1). This tends to confirm the strong genetic basis to the relationship between protein content and grain yield.

Table 1. Genetic loci associated with the control of economically important agronomic and end-use quality characters in bread wheat. Not all genetic loci associated with the traits are included. Instead, where possible, the significance of the genetic associations was extracted from the relevant publication and QTL with a LOD>3, or those identified in multiple environments, have been included in the table. Where more than one environment was used for analysis, and an association with the mean of those environments was not listed, the highest association is quoted. Genetic loci have only been included in the table if detected, or actively used, in *Triticum aestivum*. Some genes (eg *Glu-1* and *Pin* series) have been studied widely and consequently the referencing of papers citing their impact has focussed on the most thorough publications. The seminal reference for a locus is not necessarily cited in the table if later research more thoroughly explains the function of the locus (see text for seminal references). The position of makers, QTL and genes was determined using the CMap resource (http://rye.pw.usda.gov/cmap) and the combination of the references cited in the table. With CMap, the composite map produced by R. Appels (http://rye.pw.usda.gov/cmap), the map of Gale (1995) and the consensus map of Somers et al. (2004) were used.

Trait	Abbreviation	Gene[a]	Chromosome[b]	Position[b]	Closest Marker(s)	Significance[c]	Reference(s)[c]
Grain Protein	GPC						
			1A	54	Xgwm135	r^2 4.6	(Groos et al. 2003)
			1B	41	Xcdo1188	r^2 6.5	(Perretant et al. 2000)
			2A	15	Xgwm400	r^2 8.9	(Groos et al. 2003)
			2A	15	Xgwm830	LOD 3.9	(Prasad et al. 2003)
			2B	76	Xgwm1249	LOD 3.5	(Prasad et al. 2003)
			2D	90	Xgwm1264	LOD 4.1	(Prasad et al. 2003)
			3A	60	Xgwm666	r^2 8.3, r^2 8.2	(Groos et al. 2003; Groos et al. 2004)
			3B	17	Xcfd79	r^2 5.3	(Groos et al. 2003)
			3D	64	Xgwm456	LOD 4.0	(Prasad et al. 2003)
			4A	69	Xgwm397	r^2 5.5	(Groos et al. 2003)
		Rht-D1	4D	28	Xwmc52	LOD 8.3	(Huang et al. 2006)
		Rht-D1	4D	43	Xcfd71	r^2 10.3, r^2 6.2	(Groos et al. 2003; Groos et al. 2004)
		B1	5A	107		r^2 19.0	(Ma et al. 1999)
		Vrn-B1	5B	101	Xgwm271	r^2 4.6	(Groos et al. 2003)
			6A	54	$Xe38m60_{200}$	r^2 17.1	(Perretant et al. 2000)
			6A	118	Xgwm570	r^2 4.2	(Groos et al. 2003)
		Gpc-6B1	6B	70	Xucw67	LOD 7.7	(Joppa et al. 1997; Olmos et al. 2003; Distelfeld et al. 2004)
			6B	150	Xgwm889	LOD 3.3	(Prasad et al. 2003)
			7A	53	Xcfa2049	r^2 4.5	(Groos et al. 2003)
			7A	75	Xgwm1171	LOD 6.5	(Prasad et al. 2003)
			7B	40	Xwmc662	LOD 4.5	(Huang et al. 2006)
			7D	25	Xgdm86	LOD 4.0	(Prasad et al. 2003)
			7D	235	Xcfd69	r^2 10.4, r^2 9.6	(Groos et al. 2003; Groos et al. 2004)
Flour Protein	FPC						
		Gli-A1	1A	12		P<0.01	(Igrejas et al. 2002)
		Glu-A3	1A	12		P<0.05	(Igrejas et al. 2002)
		Glu-A1	1A	68		P<0.01	(Igrejas et al. 2002)
			1A	28	Xabc156b	LOD 3	(Campbell et al. 2001)
		Glu-B1	1B	72	XksuG34	LOD~6	(Rousset et al. 2001)
			2A	31	Xbcd855	LOD 7.2	(Breseghello et al. 2005)
			2B		Xcdo1445b	LOD 5.2	(Campbell et al. 2001)
			2B	100	Xbcd1688a	LOD4.5	(Campbell et al. 2001)
			2B	109?	Xggat12	LOD 4.3	(Breseghello et al. 2005)
			2D	33	Xwmc453	LOD 3.1	(Huang et al. 2006)
			4B	0	Xggat27	LOD 3.6	(Breseghello et al. 2005)
		Rht-B1	4B	30	Xwmc48c	LOD 3.8	(Breseghello et al. 2005)
		Rht-D1	4D	28	Xwmc52	LOD 8.3	(Huang et al. 2006)
			6B	107	Xcdo524	LOD 6.5	(Breseghello et al. 2005)
			7B	40	Xwmc662	LOD 4.5	(Huang et al. 2006)
Flour yield	FY						
		Glu-A1	1A	68		P<0.01	(Igrejas et al. 2002)
			1D	13	Xwmc432	LRS 9.9	(Smith et al. 2001)
			2B	35	Xwmc154	LRS 10.4	(Smith et al. 2001)
			2B	94?	Xccat8	LOD 3.5	(Breseghello et al. 2005)
			2D	25	Xccac3	LOD 3.8	(Breseghello et al. 2005)
			2D	26	Xwmc025.1	LRS 17.7	(Smith et al. 2001)
			2D	111	Xbcd410C	LRS 10.0	(Smith et al. 2001)
			3A (3B?)	0	Xccag4	LOD 5.4	(Breseghello et al. 2005)
			3A	90	Xbcd115	LRS 16.2	(Parker et al. 1999)
			3B	63	Xpaat.mcac5	LRS 10.2	(Smith et al. 2001)
			3B	65?	Xbcd706	r^2 5.0	(Campbell et al. 2001)
			4B	26	Xggta12	LOD 5.4	(Breseghello et al. 2005)
			5A	16	Xabg397	LRS 14.9	(Smith et al. 2001)

		Vrn-A1	5A	70	Xwua56	LRS 12.1	(Parker et al. 1999)
			5B	80	Xp34.p519	LRS 9.2	(Smith et al. 2001)
			5B	87	Xpact.mcca1	LRS 16.4	(Smith et al. 2001)
			5B	113	Xwmc235	LOD 4.0	(Breseghello et al. 2005)
			6B	>200?	Xp42.m501	LRS 9.9	(Smith et al. 2001)
		PinA-D1	5D	0		P<0.01, P<0.01	(Martin et al. 2001; Cane et al. 2004)
		PinB-D1	5D	0		LOD 10.9 P<0.01, P<0.01	(Campbell et al. 2001; Martin et al. 2001; Cane et al. 2004)
			6B	129	Xgwm626	LRS 10.4	(Smith et al. 2001)
			7B	90	Xpaca.mcaa1	LRS 10.0	(Smith et al. 2001)
			7D	22	Xcdo1400	LRS 14.3	(Smith et al. 2001)
			7D	82	Xgwm111	LRS 16.1	(Parker et al. 1999)
Flour purity	FP						
		PinA-D1	5D	0		P<0.01	(Martin et al. 2001)
		PinB-D1	5D	0		P<0.01	(Martin et al. 2001)
Grain texture	GT						
		Glu-A3	1A	12	Gli-A1	r^2 17.0	(Groos et al. 2004)
		Glu-A3	1A	15	Xcfa2153	P<0.001	(Arbelbide et al. 2006)
		Glu-A1	1A	68		P<0.05	(Igrejas et al. 2002)
		Glu-A1	1A	68	Xbcd808	LOD 3.8	(Breseghello et al. 2005)
			1A	85	Xfba92	r^2 3.1	(Perretant et al. 2000)
			1A/1D?		Xgcat7	LOD 3.1	(Breseghello et al. 2005)
		Glu-B1	1B	61	Xgwm403a	LOD 3.3	(Breseghello et al. 2005)
		Glu-D1	1D	80		P<0.05	(Igrejas et al. 2002)
			2A	98	XksuF11	r^2 5.7	(Sourdille et al. 1996)
			2D	54	Xbcd120	r^2 4.0	(Sourdille et al. 1996)
			3A		XksuG53	r^2 8.4	(Sourdille et al. 1996)
			3B	83	Xksum29	LOD 4.1	(Narasimhamoorthy et al. 2006)
			4A			r^2 8.1	(Groos et al. 2004)
			5B	55	XksuA1	r^2 5.3	(Sourdille et al. 1996)
		Vrn-B1?	5B	101	Xgwm271a	r^2 6.3	(Groos et al. 2004)
		PinA/B-D1	5D	5	Xgwm190	P<0.001	(Arbelbide et al. 2006)
		PinA/B-D1	5D	0	Xmta9	r^2 63.2	(Sourdille et al. 1996)
		PinA/B-D1	5D	0	Xmta10	r^2 66.9	(Perretant et al. 2000)
		PinA/B-D1	5D	0	Xcfd18	LOD 14.6	(Narasimhamoorthy et al. 2006)
		PinA-D1	5D	0		P<0.01, P<0.01	(Martin et al. 2001; Cane et al. 2004)
		PinB-D1	5D	0		r^2 64, P<0.01, P<0.01	(Campbell et al. 1999; Martin et al. 2001; Cane et al. 2004)
			6D	36	XksuG48	r^2 4.8	(Sourdille et al. 1996)
			6D	59	Xcfd33	r^2 6.2	(Groos et al. 2004)
			6D	83	Xgwm55	r^2 5.5	(Perretant et al. 2000)
			7A/7B/2B?		Xgwm130	r^2 12.7	(Groos et al. 2004)
Flour Minolta b*	b*						
			2D	25	Xwmc025a	LOD 5.2	(Mares et al. 2001)
			3A	85	Xwmc428	LOD 5.5	(Mares et al. 2001)
			3A	112	Xbcd828	LRS 12	(Parker et al. 1998)
			3B	53	Xgwm285	LOD 4.8	(Mares et al. 2001)
		Rht-B1	4B	30	Xwmc048c	LOD 3.6	(Mares et al. 2001)
			5B	68	Xgwm499	LOD 4.5	(Mares et al. 2001)
		PinA-D1	5D	0		LOD 5.9	(Mares et al. 2001)
			6A	60	Xp37m92	LOD 3.8	(Mares et al. 2001)
			7A	190	XmurFC3	LOD 12.1	(Mares et al. 2001)
			7A	200	Xcdo347	LRS 45	(Parker et al. 1998)
			7B	120	Xpsr680a	LOD 4.4	(Mares et al. 2001)

Flour Minolta L	L						
			1A	78	Xbcd808	LOD 4.7	(Mares et al. 2001)
		Glu-B3	1B	13	Xbcd1434	LOD 5.4	(Mares et al. 2001)
			2D	35	Xmwg950	LOD 3.9	(Mares et al. 2001)
		Rht-B1	4B	30	Xwmc048c	LOD 6.6	(Mares et al. 2001)
			5B	64	Xbcd508	LOD 4.0	(Mares et al. 2001)
		PinA-D1	5D	0		LOD 5.4	(Mares et al. 2001)
Water Absorption	WA						
		B1	5A	107		r^2 12.0	(Ma et al. 1999)
		PinA-D1	5D	0		P<0.01	(Cane et al. 2004)
		PinB-D1	5D	0		LOD 10.9, P<0.01	(Campbell et al. 1999; Cane et al. 2004)
Viscosity	RVA						
		Gli-A1	1A	12		P<0.001	(Igrejas et al. 2002)
		Glu-A1	1A	68		P<0.01	(Igrejas et al. 2002)
		Glu-B1	1B	66		P<0.001	(Igrejas et al. 2002)
		Glu-D3	1D	3		P<0.001	(Igrejas et al. 2002)
			2A	84	Xbcd1307d	P<0.05	(Udall et al. 1999)
			2B	41	Xbcd18c	P<0.05	(Udall et al. 1999)
			2D	70	Xcdo678	P<0.05	(Udall et al. 1999)
			3B	58	Xcdo718	P<0.05	(Udall et al. 1999)
		Wx-B1	4A	113		P<0.001?, P<0.001	(Zhao et al. 1998; Araki et al. 2000)
		Wx-A1	7A	25?		P<0.01	(Araki et al. 2000)
		Wx-D1	7D	25?		P<0.001	(Araki et al. 2000)
Dough resistance/tenacity	R_{max}						
		Glu-A3	1A	12		P<0.05	(Gupta et al. 1989)
		Glu-A1	1A	68		P<0.05	(Eagles et al. 2002b)
		Glu-B3	1B	1		P<0.05	(Eagles et al. 2002b)
		Glu-B1	1B	66		P<0.05	(Eagles et al. 2002b)
		Glu-D3	1D	3			
		Glu-D1	1D	80		P<0.05	(Eagles et al. 2002b)
			2A		Xp12-330W	r^2 9	(Ma et al. 1999)
			2B			r^2 5.4	(Groos et al. 2004)
			3B	54	Xgwm131b	r^2 11.6	(Groos et al. 2004)
			5A		Xp35-82dW	r^2 18	(Ma et al. 1999)
			6D			r^2 7.2	(Groos et al. 2004)
			7A/7B/2B?		Xgwm130	r^2 13.7	(Groos et al. 2004)
Dough Extensibility	Ext						
		Glu-A3	1A	12		P<0.05	(Eagles et al. 2002b)
		Glu-A1	1A	68		P<0.05, P<0.001	(Eagles et al. 2002b; Igrejas et al. 2002)
		Glu-B3	1B	1		P<0.05	(Eagles et al. 2002b)
		Glu-B1	1B	66		P<0.05, P<0.01	(Eagles et al. 2002b; Igrejas et al. 2002)
		Glu-D3	1D	3		P<0.001, P<0.05	(Appels et al. 2001; Igrejas et al. 2002)
		Glu-D1	1D	80		P<0.05	(Eagles et al. 2002b)
			2B			r^2 5.3	(Groos et al. 2004)
			2D	22	Xgwm261	P<0.001	(Appels et al. 2001)
			3?		Xc19-510fM	r^2 15	(Ma et al. 1999)
			3B	54	Xgwm131b	r^2 17.1	(Groos et al. 2004)
			5B	50	Xgwm371	r^2 5.5	(Groos et al. 2004)
			7A/7B/2B?		Xgwm130	r^2 6.9	(Groos et al. 2004)
Dough strength	W						
		Gli-A1	1A	12		P<0.05	(Igrejas et al. 2002)
		Glu-A1	1A	68		P<0.001, P<0.001	(Igrejas et al. 2002; Arbelbide et al. 2006)

		Gli-B1/Glu-B3	1B	1		r^2 6.3	(Groos et al. 2004)
		Glu-B1	1B	66		P<0.001, r^2 10.2	(Igrejas et al. 2002; Groos et al. 2004)
		Glu-B1	1B	66		P<0.001	(Arbelbide et al. 2006)
		Glu-D3	1D	3		P<0.05	(Igrejas et al. 2002)
		Glu-D1	1D	80		P<0.001	(Arbelbide et al. 2006)
			3A	117	Xfbb250	r^2 10.7	(Groos et al. 2004)
			3B	27	XksuE3	r^2 9.4	(Perretant et al. 2000)
			5B	21	Xgwm234	P<0.001	(Arbelbide et al. 2006)
		PinA/B	5D	0	Xmta10	r^2 19.5	
Bread quality	BQ						
		Glu-A1	1A	68		P<0.001	(Payne et al. 1987)
		Glu-B1	1B	66		P<0.05	(Payne et al. 1987)
		Glu-D1	1D	80		P<0.001	(Payne et al. 1987)
Loaf volume	Vol						
		Glu-A1	1A	68		P<0.05, LOD>4	(Rousset et al. 1992; Rousset et al. 2001)
		Glu-B3	1B	12		LOD~4	(Rousset et al. 2001)
		Glu-B1	1B	66		P<0.05	(Rousset et al. 1992)
		Glu-D1	1D	80		P<0.05	(Rousset et al. 1992)
		Lvl 1	3A	85	Xgwm720	P<0.001	(Law et al. 2005)
		Wx-B1	4A	113		P<0.001	(Martin et al. 2004)
		PinA-D1	5D	0		P<0.05	(Martin et al. 2001)
		PinB-D1	5D	0		P<0.05	(Martin et al. 2001)
Crumb Score	CS						
		Wx-B1	4A	113		P<0.001	(Martin et al. 2004)
		PinA-D1	5D	0		P<0.05	(Martin et al. 2001)
		PinB-D1	5D	0		P<0.05	(Martin et al. 2001)
Cookie quality	CQ						
		Glu-A1	1A	68		P<0.001	(Igrejas et al. 2002)
		Glu-B1	1B	66		P<0.05	(Igrejas et al. 2002)
			5B?	56	Xcdo412	LOD 3.7	(Campbell et al. 2001)
		PinB-D1	5D	0		LOD 8.2	(Campbell et al. 2001)
Noodle texture	NT						
		Wx-B1	4A	113		P<0.05, P<0.001	(Epstein et al. 2002; Martin et al. 2004)
		PinB-D1	5D	0		P<0.05	(Storlie et al. 2006)
		Wx-A1	7A	25?		P<0.05	(Epstein et al. 2002)
		Wx-D1	7D	25?		P<0.05	(Epstein et al. 2002)
Stability/PPO	PPO						
			2A	53	Xgwm294a	LOD 6.9, r^2 84	(Mares et al. 2001; Raman et al. 2005)
			2D	80?	Xbcd266b	LOD 26.9	(Mares et al. 2001)
		Rht-B1	4B	30	Xwmc048c	LOD 3.7	(Mares et al. 2001)
Height	HT						
			1A	25	Xgwm1104	LOD 4.8	(Huang et al. 2004)
			1A	38	Xbcd96	LOD>3	(Borner et al. 2002)
			1D	60	Xgwm848	LOD 3.3	(Huang et al. 2004)
		Rht4	2B	125	Xwmc317	P<0.01	(Ellis et al. 2005)
		Ppd-D1/Rht8	2D	22	Xfba400	LOD>3	(Borner et al. 2002)
		Rht8	2D	23	Xwmc503	P<0.01	(Ellis et al. 2005)
			2D	42	BE497718–260	LOD 4.2	(McCartney et al. 2005)

	Rht5	3B	30	Xbarc102	P<0.01	(Ellis et al. 2005)
		3B	63	Xgwm108	LOD 3.8	(Huang et al. 2004)
		4A	31	Xbcd1738	r^2 26	(Araki et al. 1999)
	Wx-B1	4A	93	Xabg390	LOD>3	(Borner et al. 2002)
	Wx-B1	4A	113		r^2 29	(Araki et al. 1999)
	Rht-B1	4B	30		P<0.0001	(Butler et al. 2005)
	Rht-B1	4B	30	Xgwm1167a	LOD 4.0	(Huang et al. 2004)
	Rht-B1	4B	43	Xgwm513	LOD 7.7	(McCartney et al. 2005)
		4B	63	Xaac.ctg1	LOD 6.7	(Marza et al. 2006)
	Rht-D1	4D	23	Xwmc48	LOD 30.9	(McCartney et al. 2005)
	Rht-D1	4D	28		P<0.0001	(Butler et al. 2005)
	Rht-D1	4D	43	Xcfd71a	LOD 14	(Huang et al. 2006)
		5A	24	Xgwm304	LOD 5.8	(Huang et al. 2004)
		5A	51	Xgwm156	LOD 7.1	(Huang et al. 2004)
	Rht9	5A	54	Xbarc151	P<0.01	(Ellis et al. 2005)
	Rht12	5A	105	Xwmc410	P<0.01	(Ellis et al. 2005)
		5B	120	Xwmc640	LOD 6.1	(McCartney et al. 2005)
		5D	107	Xwmc640a	LOD 5.4	(Huang et al. 2006)
		6A	75	Xgwm786	LOD 4.1	(Huang et al. 2004)
		6A	78	Xcdo329	LOD>3	(Borner et al. 2002)
		6D	90	Xgwm1241	LOD 3.9	(Huang et al. 2004)
		7A	130	Xwmc139	LOD 3.3	(McCartney et al. 2005)
		7B	20	Xgwm537	LOD 3.8	(Huang et al. 2006)
		7B	46	Xgwm333	LOD 3.3	(McCartney et al. 2005)
	Rht13	7B	105	Xgwm577	P<0.01	(Ellis et al. 2005)
		7D	60	Xgwm1002	LOD 4.0	(Huang et al. 2004)
Phenology	Phen					
		1A				(Law et al. 1998)
		1A				(Halloran et al. 1967)
		1B				(Law et al. 1998)
		1D				(Law et al. 1998)
	Ppd-A1	2A				(Law et al. 1978b)
	Ppd-B1	2B	50			(Scarth et al. 1983; Mohler et al. 2004)
	Ppd-B1	2B	62	Xgwm148	LOD 7.9	(Hanocq et al. 2004)
		2B	62	Xgwm148	LOD 3.5	(Hanocq et al. 2004)
		2B				(Scarth et al. 1983)
	Ppd-D1	2D	56			(Welsh et al. 1973; Borner et al. 1998)
	Ppd-D1	2D	26	Xgwm484, Xfba400	LOD>3, LOD 7.7	(Borner et al. 2002; Hanocq et al. 2004)
	Ppd-D1	2D	22	Xgwm261	LOD 4.5	(Hanocq et al. 2004)
	Ppd-D1	2D	22	Xgwm261	LOD 4.0	(Narasimhamoorthy et al. 2006)
		2D	90	Xgdm6	LOD 3.4	(Huang et al. 2004)
		3A				(Hoogendoorn 1985)
		3A				(Miura et al. 1994)
		3B				(Halloran et al. 1967)
		3B				(Miura et al. 1994)
		3D	25	Xgwm161	LOD 5.0	(Narasimhamoorthy et al. 2006)
		4A	110	Xgwm1081	LOD 6.2	(Huang et al. 2004)
	Wx-B1	4A	113		r^2 37, LOD 6.1	(Araki et al. 1999; McCartney et al. 2005)
	Rht-B1	4B	30		P<0.001	(Butler et al. 2005)
		4B				(Hoogendoorn 1985)
		4B				(Halloran et al. 1967)
	Rht-D1	4D	28		P<0.001	(Butler et al. 2005)
		4D				(Hoogendoorn 1985)
		4D	30	Xwmc48	LOD 5.1	(McCartney et al. 2005)
		5A	50	Xglk407	r^2 6.9	(Sourdille et al. 2000)
	Vrn-A1	5A	66			(Law et al. 1976)

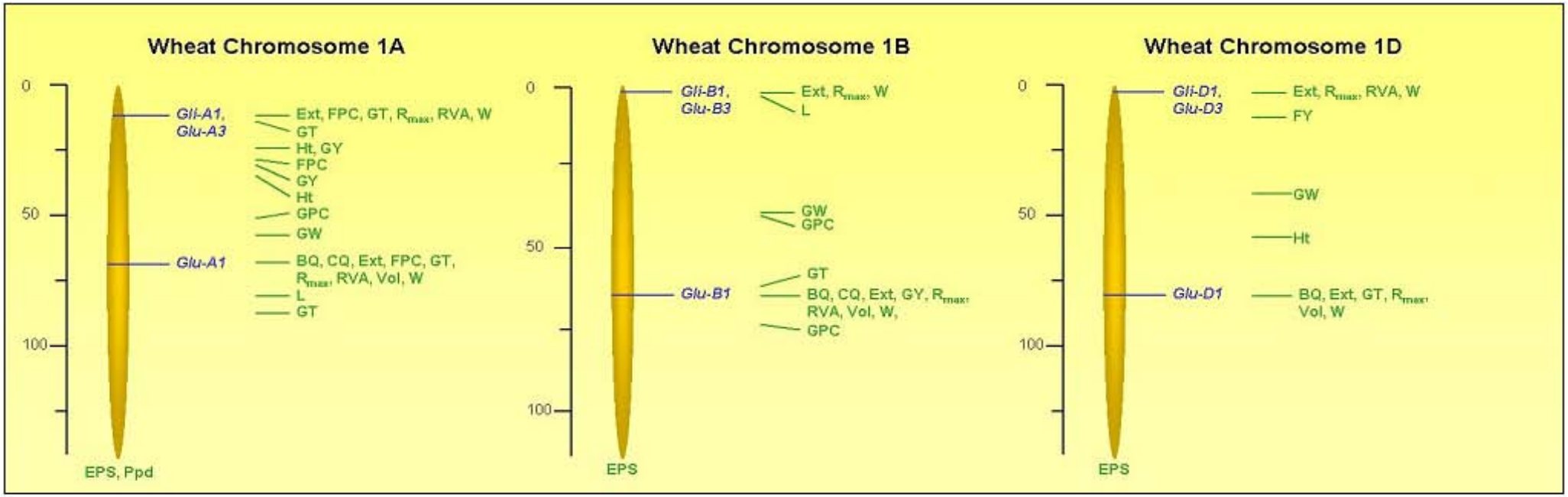
		Vrn-A1	5A	72	Xgwm271	LOD 18.8	(Hanocq et al. 2004)
			5B	0?	Xaca.cta13	LOD 4.7	(Marza et al. 2006)
			5B	50	Xgwm371	LOD 3.9	(Hanocq et al. 2004)
			5B	71	Xgwm639a	LOD 3.4	(Hanocq et al. 2004)
		Vrn-B1	5B	108	Xgwm408	LOD 34.7	(Leonova et al. 2003)
		Vrn-D1	5D	61?			(Law et al. 1976)
		Vrn-D1	5D	52	Xgwm174	r^2 9.2	(Sourdille et al. 2000)
			5D	64	Xbcd450	LOD>3	(Borner et al. 2002)
			5D	87	Xwmc640	LOD 3.2	(Huang et al. 2006)
			5D	97	Xbcd1421	LOD 3.0	(Hanocq et al. 2004)
			6B				(Islam-Faridi et al. 1996)
			6B				(Hoogendoorn 1985)
			6B				(Halloran et al. 1967)
		Vrn-B4	7B	20	XksuD18	9.7	(Sourdille et al. 2000)
			7D				(Halloran et al. 1967)
			7D	60	Xgwm1220	LOD 4.4	(Huang et al. 2004)
			7D	70	Xgwm130	LOD 17.5	(McCartney et al. 2005)
			7D	97	Xwmc405a	LOD 6.7	(Huang et al. 2006)
Grain weight	GW						
		Glu-A1	1A	59	Xcdo92	r^2 11.8	(Campbell et al. 1999)
			1B	40	Xgwm1050	LOD 3.3	(Huang et al. 2004)
			1B	40	XksuG9	r^2 11.1	(Campbell et al. 1999)
			1D	43	Xgwm337	r^2 8.7	(Groos et al. 2003)
			2A	61	Xcdo456B	r^2 5.8	(Campbell et al. 1999)
			2B	10	Xwmc661	LOD 3.1	(Huang et al. 2006)
			2B	74	Xgwm374	r^2 19.7	(Groos et al. 2003)
		Ppd-D1	2D	22	Xgwm261	r^2 6.8	(Groos et al. 2003)
		Ppd-D1	2D	27	Xwmc112	LOD 6.5	(Huang et al. 2006)
			2D	90	Xgdm6	LOD 4.1	(Huang et al. 2004)
			3A	60	Xgwm666	r^2 4.9	(Groos et al. 2003)
			3B	55	Xbarc164	LOD 3.1	(Huang et al. 2006)
			3B	58	Xcdo718	r^2 12.2	(Campbell et al. 1999)
			3D	65	Xgwm341	LOD 4.3	(McCartney et al. 2005)
			3?	105	Xbcd361	r^2 10.9	(Campbell et al. 1999)
			4A	31	Xbcd1738	r^2 17	(Araki et al. 1999)
			4A	100	Xgwm162	LOD 6.7	(McCartney et al. 2005)
		Rht-B1	4B	30		P<0.01	(Butler et al. 2005)
		Rht-B1	4B	31	Xgwm107	LOD 3.2	(Huang et al. 2004)
		Rht-B1	4B	31	Xwmc238	LOD 11.6	(McCartney et al. 2005)
		Rht-B1	4B	45	Xcfd39b	LOD 4.6	(Huang et al. 2006)
		Rht-D1	4D	23	Xwmc48	LOD 20.9	(McCartney et al. 2005)
		Rht-D1	4D	28		P<0.0001	(Butler et al. 2005)
		Rht-D1	4D	43	Xcfd71a	LOD 10.9	(Huang et al. 2006)
		Vrn-A1	5A	78	Xfba351	LOD>3	(Borner et al. 2002)
		Vrn-B1	5B	101	Xgwm271	r^2 10.4	(Groos et al. 2003)
			6A	28	Xgwm334a	LOD 3.9	(Huang et al. 2004)
			6A	80	Xgwm1150	LOD 6.2	(Huang et al. 2004)
			6A	90	Xbarc146	LOD 7.3	(Huang et al. 2006)
			6A	118	Xgwm570	r^2 6.7	(Groos et al. 2003)
			6B	119	Xbcd1495	LOD>3	(Borner et al. 2002)
			6D	59	Xcfd33	r^2 7.5	(Groos et al. 2003)
			6D	106	Xgwm55	LOD 3.9	(McCartney et al. 2005)
			7A	12	Xgwm834	LOD 3.3	(Huang et al. 2004)
			7A	53	Xcfa2049	r^2 10.3	(Groos et al. 2003)
			7A	170	Xgwm282	LOD 4.6	(Huang et al. 2004)
			7D	60	Xgwm1220	LOD 7.0	(Huang et al. 2004)

			7D	235	Xcfd69	r^2 7.5	(Groos et al. 2003)
Grain yield	GY						
			1A	20	Xgwm1104	LOD 4.1	(Huang et al. 2004)
			1A	31	Xm71p78.5	P<0.05	(Quarrie et al. 2005)
		Glu-B1	1B	66		P<0.05	(Quarrie et al. 2005)
			2A	56	Xgwm339	LOD 3.0	(McCartney et al. 2005)
			2B	45	Xm86p65.1	P<0.05	(Quarrie et al. 2005)
			2B	47	Xgwm257	LOD 9.4	(McCartney et al. 2005)
			2B	56	Xaag.cagt12	LOD 3.5	(Marza et al. 2006)
			2B	74	Xgwm374	r^2 5.6	(Groos et al. 2003)
			2B	114	Xgwm382	P<0.05	(Verma et al. 2004)
			2D	22	Xgwm261	LOD 6.0	(Narasimhamoorthy et al. 2006)
			2D	110	Xgwm382	P<0.05	(Verma et al. 2004)
			3A	72	Xbarc67	LRS~33	(Campbell et al. 2003)
			3B	17	Xcfd79	r^2 6.5	(Groos et al. 2003)
			3D	64	Xgwm456	LOD 6.2	(Huang et al. 2004)
			3D	75	Xbarc042	P<0.05	(Quarrie et al. 2005)
			4A	12	Xwmc179.3	P<0.05	(Quarrie et al. 2005)
			4A	31	Xbcd1738	r^2 27	(Araki et al. 1999)
			4A	69	Xgwm397	LOD 4.4	(McCartney et al. 2005)
			4A	122	Xpsr490.2Ss1	P<0.05	(Quarrie et al. 2005)
			4A	126	Xcdo545	r^2 5.4	(Groos et al. 2003)
		Rht-B1	4B	30		P<0.01	(Butler et al. 2005)
		Rht-B1	4B	31	Xgwm113	r^2 6.1	(Groos et al. 2003)
			4B	48	Xgwm165.1	P<0.05	(Quarrie et al. 2005)
			4B	70	Xact.cat11	LOD 4.0	(Marza et al. 2006)
			4B	92	Xdupw043	P<0.05	(Quarrie et al. 2005)
		Rht-D1	4D	28		P<0.0001	(Butler et al. 2005)
			4D	47	Xgwm165.2	P<0.05	(Quarrie et al. 2005)
			4D		Xgwm1163	LOD 5.2	(Huang et al. 2004)
			5A	19	Xacg.gac1.2	LOD 6.0	(Marza et al. 2006)
			5A	24	Xgwm304	LOD 3.7	(Huang et al. 2004)
			5A	51	Xgwm156	LOD 3.3	(Huang et al. 2004)
		Vrn-A1	5A	66		P<0.05	(Quarrie et al. 2005)
		Vrn-A1	5A	72	Xgwm271	r^2 5.2	(Groos et al. 2003)
		Vrn-A1	5A	83	Xcfd39a	LOD 4.7	(Huang et al. 2006)
			5B	45	Xwg232.2, Xbarc074	P<0.05	(Quarrie et al. 2005)
		Vrn-B1	5B	101	Xgwm271	r^2 6.8	(Groos et al. 2003)
			5D	20	Xbarc044	P<0.05	(Quarrie et al. 2005)
			5D	72	Xgwm212	P<0.05	(Quarrie et al. 2005)
			6B		Xgctg.ctt1	LOD 3.1	(Marza et al. 2006)
			6B	120	Xm87p78.5a	P<0.05	(Quarrie et al. 2005)
			6D	90	Xgwm1241	LOD 3.4	(Huang et al. 2004)
			7A	66	Xwmc83	LOD 3.1	(Huang et al. 2006)
			7A	105	Xbarc108	LOD 7.0	(Marza et al. 2006)
			7A	202	Xpsp3094.1, Xm68p78.6	P<0.05	(Quarrie et al. 2005)
			7B	10	Xm59p78.7	P<0.05	(Quarrie et al. 2005)
			7B	89	Xm43p78.14, Xm86p65.0	P<0.05	(Quarrie et al. 2005)
			7D	231	Xgwm37	LOD 4.0	(Narasimhamoorthy et al. 2006)
			7D	235	Xcfd69	r^2 15.7	(Groos et al. 2003)

[a]The putative gene responsible for the genetic association is listed in black if cited in the text of the reporting paper, or in blue when considered likely by this author.

[b]If the chromosomal location of a QTL has not been reported, or the exact location is unknown the 'Position' is left blank. In others, where the position has been difficult to determine, the figure is followed by a '?'.

[c]For some well characterised loci, more than one study is reported in the table. In these cases, the significances and references are presented in the same order.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50
100
Gli-A1, Glu-A3
Glu-A1
EPS, Ppd
Ext, FPC, GT, Rmax, RVA, W
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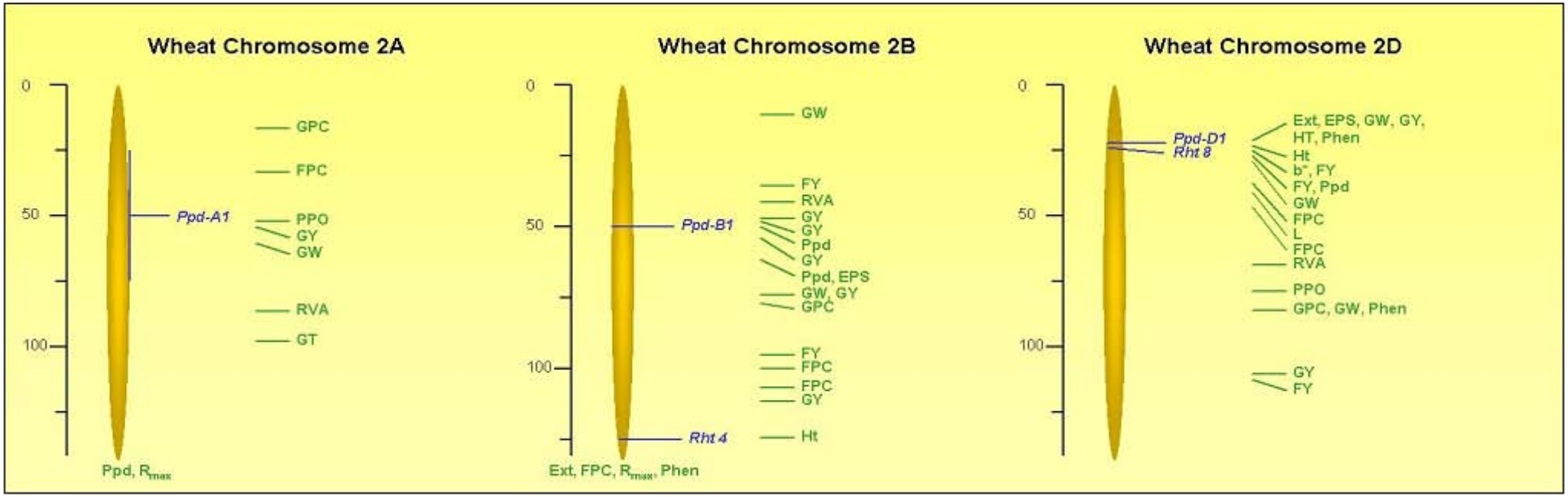
FPC
GY
Ht
GPC
GW
BQ, CQ, Ext, FPC, GT, Rmax, RVA, Vol, W
L
GT
Wheat Chromosome 1B
0
50
100
Gli-B1, Glu-B3
Glu-B1
EPS
Ext, Rmax, W
L
GW
GPC
GT
BQ, CQ, Ext, GY, Rmax, RVA, Vol, W,
GPC
Wheat Chromosome 1D
0
50
100
Gli-D1, Glu-D3
Glu-D1
EPS
Ext, Rmax, RVA, W
FY
GW
Ht
BQ, Ext, GT, Rmax, Vol, W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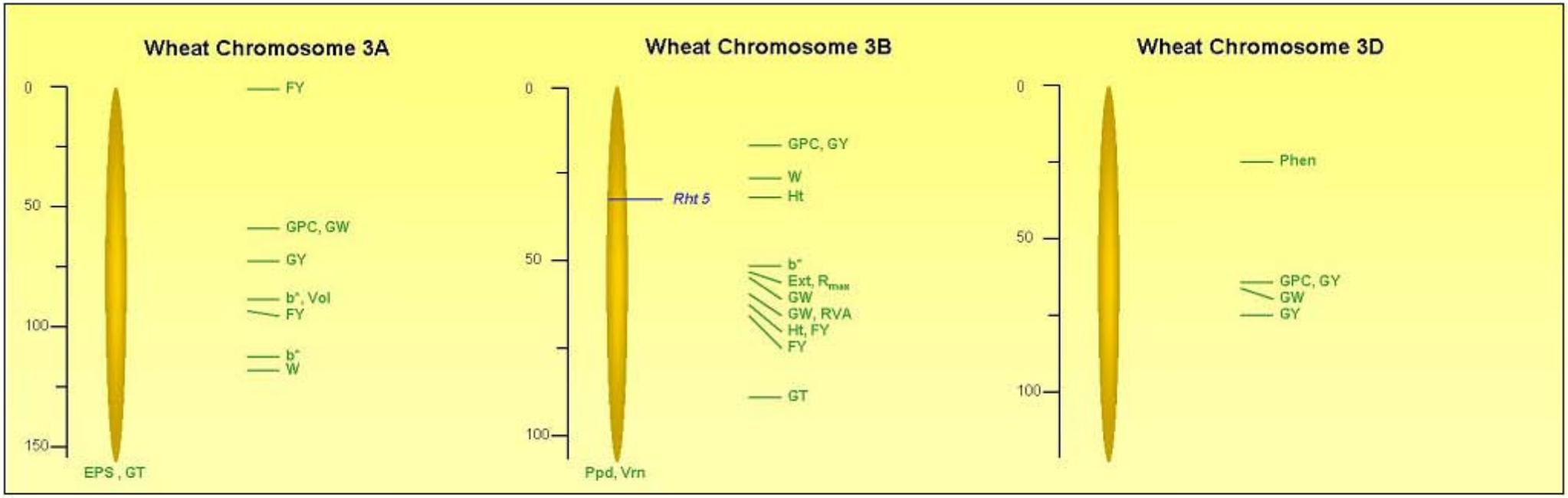
50
100
Ppd-A1
Ppd, Rmax
GPC
FPC
PPO
GY
GW
RVA
GT
Wheat Chromosome 2B
0
50
100
Ppd-B1
Rht 4
Ext, FPC, Rmax, Phen
GW
FY
RVA
GY
GY
Ppd
GY
Ppd, EPS
GW, GY
GPC
FY
FPC
FPC
GY
Ht
Wheat Chromosome 2D
0
50
100
Ppd-D1
Rht 8
Ext, EPS, GW, GY, HT, Phen
Ht
b*, FY
FY, Ppd
GW
FPC
L
FPC
RVA
PPO
GPC, GW, Phen
GY
FY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b*, Vol
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b*
W
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100
Rht 5
GPC, GY
W
Ht
b*
Ext, Rmax
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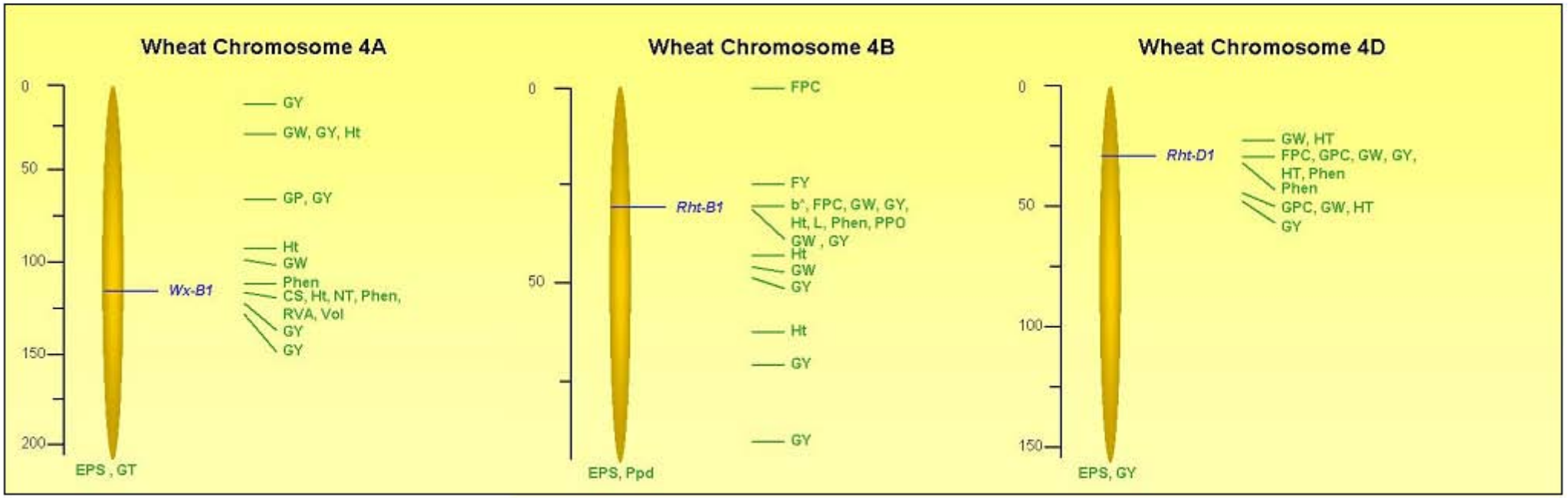
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GW
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RVA, Vol
GY
GY
Wx-B1
EPS , GT
Wheat Chromosome 4B
0
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FPC
FY
b*, FPC, GW, GY,
Ht, L, Phen, PPO
GW , GY
Ht
GW
GY
Ht
GY
GY
Rht-B1
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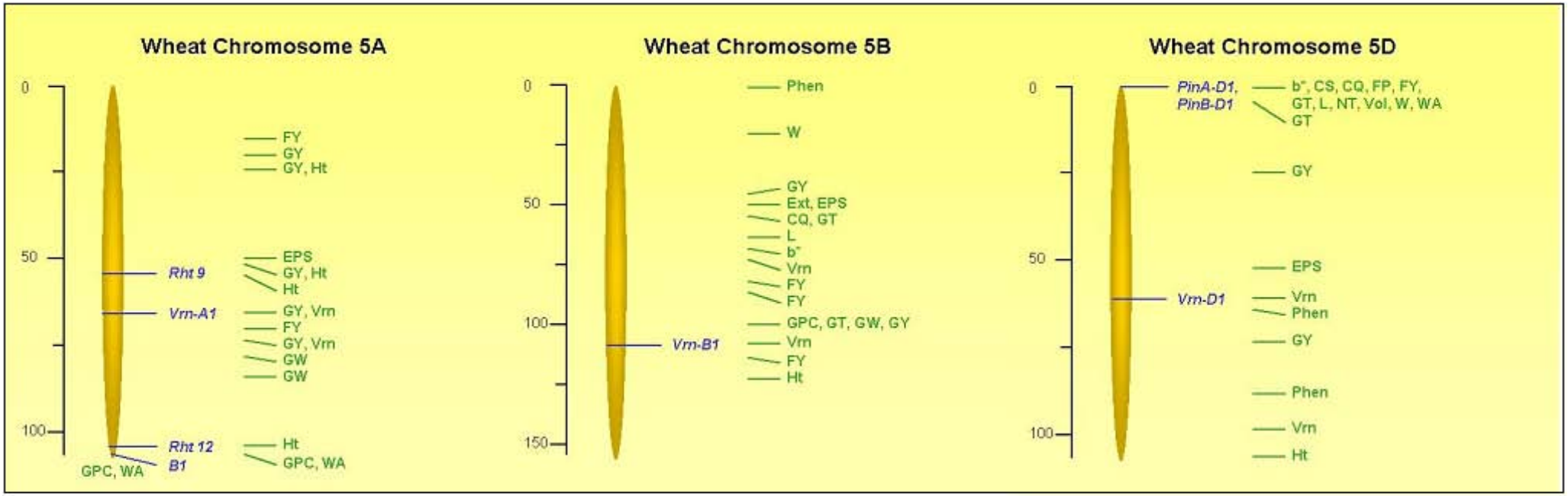
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Vrn-A1
Rht 12
B1
GPC, WA
FY
GY
GY, Ht
EPS
GY, Ht
Ht
GY, Vrn
FY
GY, Vrn
GW
GW
Ht
GPC, WA
Wheat Chromosome 5B
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50
100
150
Vrn-B1
Phen
W
GY
Ext, EPS
CQ, GT
L
b*
Vrn
FY
FY
GPC, GT, GW, GY
Vrn
FY
Ht
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PinB-D1
Vrn-D1
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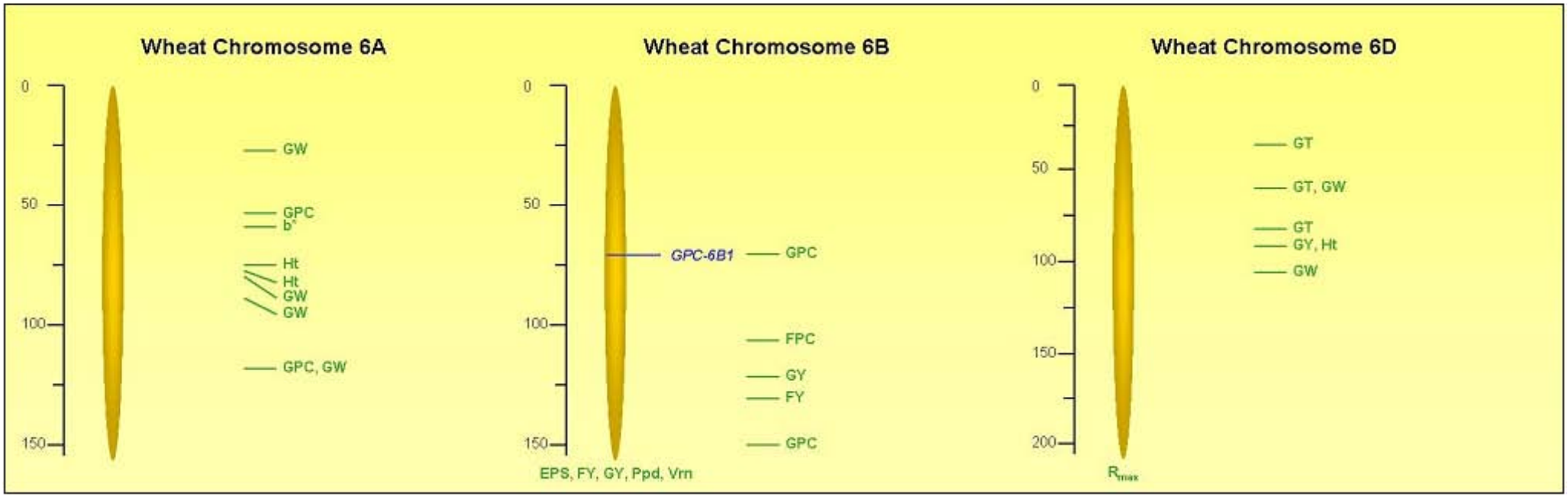
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GPC
b*
Ht
Ht
GW
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GPC, GW
Wheat Chromosome 6B
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100
150
GPC-6B1
GPC
FPC
GY
FY
GPC
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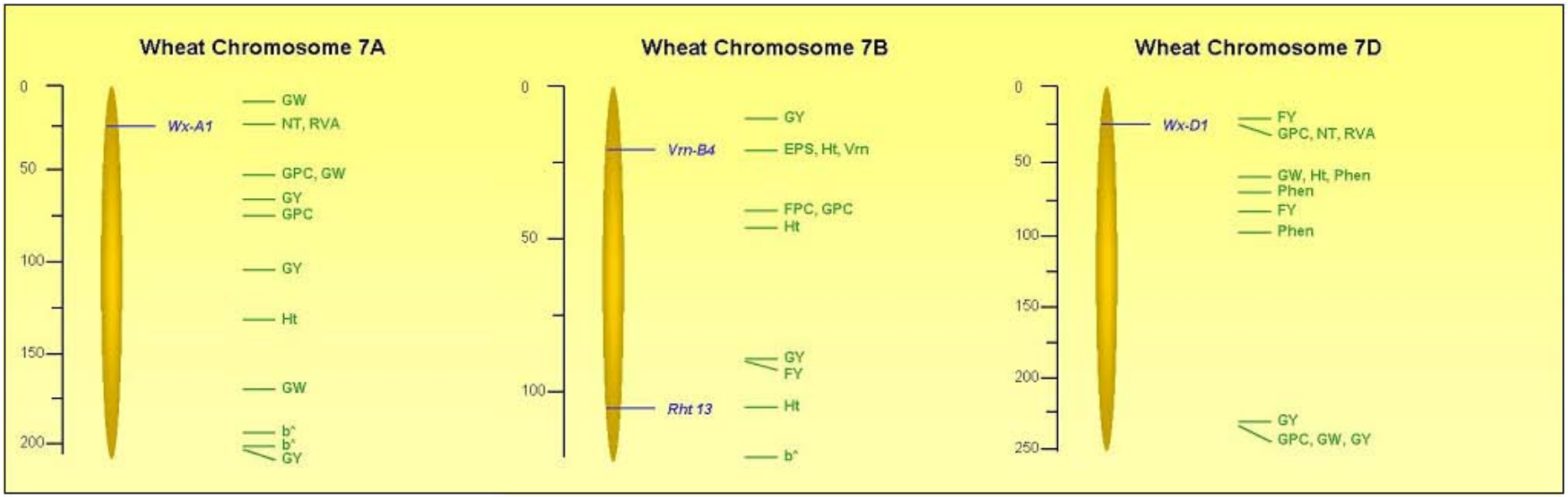

Figure 1. A composite map of QTL/genes identified in wheat influencing some economically important agronomic and quality traits. Characterised genes are listed in blue and QTL in green. Data is taken from Table 1, and the trait abbreviations are the same. QTL without reported intra-chromosomal positions are listed at the bottom of the relevant chromosomes.

Flour Yield

The quantity of flour that can be extracted from a wheat kernel (flour yield) forms one of the major milling quality traits to be considered by a wheat breeder. Flour is extracted primarily from the endosperm of a wheat grain, and is composed of starch granules encased in a protein matrix. Surrounding the endosperm, the bran, along with the embryo (germ) forms the non-flour fraction of the grain. The quantity of flour able to be extracted from a grain is consequently a function of the bran and embryo to endosperm ratio, which is dictated by grain size and morphology, as well as the ease with which the endosperm is released from the bran (Simmonds 1989). Grain size and morphology characters are under both environmental and genetic control (Bhatt et al. 1975; Pearson et al. 1981). A number of authors have reported genetic associations with milling yield (Table 1) and many have not been experimentally related to grain size and shape (Figure 1).

Grain Texture

Due to the strong relationship between the starch granules and protein matrix, hard textured varieties incur greater starch damage during milling than soft textured varieties. Soft textured varieties are better suited to biscuit, cookie and some noodle manufacture while hard grained varieties are generally used for bread and some noodle products (Simmonds 1989). While grain hardness has shown to be correlated with grain protein content (Giroux et al. 2000), genetic control of texture independent of protein content has been reported (Table 1).

The major locus involved in the control of grain texture, *Ha* (Symes 1965), was localised to the short arm of chromosome 5D (Mattern et al. 1973; Sourdille et al. 1996). Subsequently, two closely linked genes encoding puroindoline proteins

(Gautier et al. 1994) were identified in the same region and most likely encode the variation in grain hardness associated with the *Ha* locus (Giroux et al. 1997; Giroux et al. 1998). Giroux et al. (1997) suggested that a mutation in the puroindoline-b gene (*Pinb-D1*), leading to an amino acid change, results in altered protein structure and consequently the strength with which the puroindoline protein binds with membrane polar lipids. This in turn alters the strength of the bond between the starch granules and protein matrix. In a later report Giroux et al. (1998) identified a null allele at the other puroindoline gene, *Pina-D1*. Consequently, if a variety possesses either of the mutant alleles (*Pina-D1b* or *Pinb-D1b*) at these loci, the resultant grain has a hard texture (Cane et al. 2004). Although a number of other alleles have now been detected at these loci (Morris et al. 2001), three genotypes predominate in released cultivars; 'soft', *Pina-D1a/Pinb-D1a*, 'hard', *Pina-D1a/Pinb-D1b* and 'extra hard', *Pina-D1b/Pinb-D1a* (Cane et al. 2004). The water absorption of these three genotypic classes is positively correlated with grain hardness. In the work of Cane et al. (2004), varieties with the 'extra hard' genotype absorbed 3.5% more water than varieties with the 'hard' genotype and 8.3% more than those with the 'soft' genotype. However the distinction between the water absorption of the 'extra hard' and 'hard' classes was not observed by Martin et al. (2001). Both of these studies showed lower milling yield to be associated with the 'extra hard' class. Beyond this major gene for grain texture, numerous QTL associated with grain hardness have been reported (Table 1). However it is likely that many of these relationships are due to associations with grain protein content (Figure 1).

Flour Colour

The yellowness of flour and its end products, often recorded as b* using a Minolta meter, results largely from variation of xanthophyll levels in the grain (Mares et al. 1997). Yellow flour is generally regarded as undesirable for bread products, while flour for noodle production can vary from a creamy to yellow colour depending on the style of noodle being made (Simmonds 1989). A major gene series controlling the xanthophyll content and therefore yellowness of flour is situated on chromosome group seven of wheat (Parker et al. 1998; Ma et al. 1999). Smaller less significant associations with flour yellowness have also been detected on chromosome group three (Parker et al. 1998; Mares et al. 2001) and a number of other chromosomes (Table 1).

1.2.1.2 Genetic Factors Controlling Aspects of Dough Formation and Rheology

As water is mixed with flour a dough is formed which can then be manipulated to produce various end products. The quantity of water absorbed by the flour, the viscosity of the flour paste, and the rheological characteristics of the dough all influence flour's functionality.

Water Absorption

The quantity of water absorbed by flour during dough formation is largely influenced by factors under genetic control (O'Brien et al. 1987; Eagles et al. 2002a). For bread products, a relatively high water absorption is required, whereas for biscuits and noodle production a lower water absorption is desirable (Simmonds 1989). The

water absorption of flour is heavily influenced by grain texture, grain protein content and the concentration of non-starch polysaccharides. Although damage to starch granules can be altered by the conditions used during milling, this attribute is also related to grain texture and is therefore under significant genetic control (discussed previously under 'Grain Texture'). A thorough literature search revealed just two loci shown to be involved in the control of flour water absorption (Campbell et al. 1999; Ma et al. 1999; Cane et al. 2004). One of these loci was also shown to be associated with grain texture (Campbell et al. 1999; Cane et al. 2004) (discussed under 'Grain Texture') while the other was associated with protein concentration (Ma et al. 1999). Although non-starch polysaccharides have been shown to influence the water absorption properties of wheat flour (Shogren et al. 1987; Andersson et al. 1994), no reports of genetic associations between non-starch polysaccharides and water absorption were found in the literature.

Flour Paste Viscosity

The paste viscosity of flour is critical in determining the quality of Japanese white salted (Oda et al. 1980; Konik et al. 1992) and Chinese noodles (Miskelly et al. 1985). In a breeding programme, both the Rapid Viscoanalyser (Newport Scientific) and flour swelling volume test have been used to determine the viscosity and therefore quality of wheat for noodle manufacture (Crosbie 1991; Panozzo et al. 1993). Biochemically, the ratio of amylose to amylopectin present in starch is one of the major determinants of this viscosity (Sasaki et al. 2000). In 1992 Yamamori et al. showed a positive correlation between the quantity of granule bound starch synthase (GBSS) in wheat flour and flour amylose content. The location of a homoeologous gene series (*Wx-A1*, *-B1* and *-D1*) on chromosomes 7A, 4A (ancient translocation

from 7B) and 7D (Chao et al. 1989) which control the production of GBSS, and the subsequent development of molecular markers to aid in the selection of the null alleles at each of these loci (McLauchlan et al. 2001), has provided an important tool for the improvement of noodle quality. Very few studies (Udall et al. 1999; Igrejas et al. 2002) have identified genetic associations with flour viscosity that do not involve the *Wx* gene series (Table 1).

Dough Rheology

The physical properties of dough play a large role in determining its functionality. The extent to which a piece of dough can be stretched, and the force required to do so helps to determine the suitability of a variety for specific end-uses. For example, leavened bread is best produced from dough that possesses strong and balanced rheological properties. Whereas dough most suited to biscuit production is generally less resistant to extension but is able to be extended a large distance before rupturing. This allows the dough to flatten and spread into a large, flat, evenly shaped biscuit (Simmonds 1989). Dough rheology is often measured using either an Extensograph (Brabender, Germany) or an Alveograph (Chopin). In both cases, slow sample throughput, the requirement for large sample sizes and the impact of extraneous error hamper genetic gain for improved rheological properties in wheat. The search for the genetic basis of dough rheology has uncovered the important role of a major set of proteins, the glutenins (Payne et al. 1987).

Variation in the alleles of the wheat storage proteins, low molecular weight (LMW) and high molecular weight (HMW) glutenins, is responsible for much of the variation in dough rheological properties (Gupta et al. 1989). The glutenins, along with gliadins, form the gluten protein mass that holds dough together and provides its

characteristic elasticity (Gupta et al. 1989). Changes to the length and structure of these proteins alter the behaviour of the gluten and consequently the dough (Simmonds 1989). A high level of allelic variability for the high and low molecular weight glutenins has been demonstrated, and the functional effects of many combinations have been characterised. Loci on the long arms of chromosomes 1A, 1B, and 1D encode the HMW glutenins (*Glu-A1, -B1, -D1*), while alleles encoding the LMW forms are located on the short arms of the same chromosomes (*Glu-A3, -B3, -D3*) (McIntosh et al. 2003). Together, allelic variation at these six loci have been shown to control as much as 46% of the variation in dough resistance and 23% of variation in dough extensibility in Australian wheat germplasm (Eagles et al. 2002b). Although there are a very large number of possible allele combinations, leading to a range of phenotypes, four alleles in particular have been shown to impart substantial influences on dough rheology (Eagles et al. 2002b; Eagles et al. 2004). At the *Glu-A1* locus, a null allele leads to low dough resistance levels (Payne et al. 1987; Eagles et al. 2004), making this allele a key target when breeding varieties for biscuit production, but making these varieties less desirable for bread making. Likewise, the null allele at *Glu-A3* is associated with low dough resistance (Eagles et al. 2004), however it is also associated with low extensibility. This allele is therefore generally undesirable regardless of the end product targeted. At the *Glu-B1* locus, an over-expressed allele (*al*) is associated with high dough resistance (Butow et al. 2003). This allele has been shown to be associated with a rise in dough strength of 130 BU over the average of the alternative alleles at that locus (Eagles et al. 2004). At *Glu-D1*, the *d* allele is associated with dough resistance (Payne et al. 1987) 121 BU over the *a* allele (Eagles et al. 2004). In both cases, selection for these alleles may be a target when trying to improve the bread baking potential of wheat varieties.

Glutenin alleles can be identified by extracting the glutenin proteins from seed and separating them on gels (Singh et al. 1991). Although capable of detecting a wide range of alleles at the six glutenin loci, this method is slow and expensive, often limiting its use to advanced breeding material. In addition, protein based markers for seed expressed genes are difficult to select in segregating germplasm due to the triploid nature of wheat endosperm. Alternatively, DNA based molecular markers for the glutenin loci have been developed to aid in selection for improved dough rheology (D'Ovidio et al. 1994; Devos et al. 1995; Ahmad 2000; Juhasz et al. 2003; Radovanovic et al. 2003; Zhang et al. 2004; Gale 2005).

Given that the glutenin loci control such a large proportion of the variation in dough rheology, little attention has been paid to alternative loci that may also contribute to dough quality. Reports of identification of alternative loci are rare (Table 1). Most likely, the disappointing results gleaned from this work have arisen from; 1) small population sizes used in mapping, 2) epistatic effects between characterised and uncharacterised loci, and 3) large glutenin allele derived differences in dough rheology masking the effects of other loci. Ideally, future mapping would utilise populations with minimal glutenin allele segregation to identify, and tag with selectable markers, non-glutenin based variation.

1.2.1.3 Genetic Factors Controlling the Quality of End-Products

Ultimately, water absorption, grain texture, protein content, flour colour, and dough rheology, among others, are *predictors* of the likely end-product performance of a wheat variety. In order to characterise the actual quality of a wheat variety, flour samples must be used to produce the targeted end-products. For leavened loaves the principal quality characteristics are loaf volume, crumb colour and crumb structure.

While for both white salted noodles and yellow alkaline noodles the texture, brightness, brightness stability and yellowness/whiteness of the noodle are all important.

While many of the characters measured on end-products are either partly or entirely attributable to variation in milling quality and dough rheology traits described previously, genetic associations with end-product quality unrelated to these traits have also been reported (Table 1). In general however, as can be observed from Table 1, the majority of genetic associations with end-products are coincident with genes responsible for variation in milling and dough quality.

1.2.2 The Genetic Basis of Grain Yield

As the determinant of productivity, the grain yield achieved by a wheat crop is a primary driver of farm profitability. Consequently, it was one of the first traits targeted by the earliest wheat breeders, and remains high on the set of objectives for all Australian breeding programmes (Wrigley et al. 1981). The grain yield of a crop can be considered the result of a combination of the genetic potential of the variety being grown, and the environment that it is grown in. The genetic basis of grain yield can be further separated into grain yield potential and grain yield protection. Although the distinction between these terms can be blurred, grain yield potential can be classed as those genetic factors that lead to high grain yield in the absence of disease. Grain yield protection on the other hand can be used to group the genetic loci that contribute to resistance against disease and therefore protect the inherent grain yield potential of a variety in an environment. In southern Australia a number of diseases have the potential to reduce the grain yield of a wheat crop. Predominate diseases include: the foliar diseases; leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), stripe rust

(*Puccinia striiformis*), yellow leaf spot (*Pyrenophora tritici-repentis*), *Septoria tritici* blotch, *Stagonospora nodorum* blotch and the root diseases; cereal cyst nematode (*Heterodera avenae*), root lesion nematode (*Pratylencus neglectus* and *P. thornei*), crown rot (*Fusarium pseudograminearum*), take-all (*Gaeumannomyces graminis* var. *tritici*), and *Rhizoctonia solani* (Butler 1961; Murray et al. 1987; Eastwood et al. 1991; Klein et al. 1991; Vanstone et al. 1998). For all but take-all (Cook 2003) and rhizoctonia (Smith et al. 2003), useful genetic resistance has been characterised (Paull et al. 1998; Eastwood et al. 1991; Jahier et al. 2001; Williams et al. 2002; Adhikari et al. 2003; Schnurbusch et al. 2003; Adhikari et al. 2004a; Adhikari et al. 2004b; Haen et al. 2004; Wallwork et al. 2004; Zwart et al. 2004; Collard et al. 2005; Schmidt et al. 2005), and utilised in wheat breeding. Clearly, grain yield protection is a key route to farm profitability. However, for the purposes of this thesis, this literature review will be limited to those factors that influence grain yield potential. In the review of genetic factors involved in grain yield potential, the influences of genes/QTL related to plant height and phenology as well as grain yield genes/QTL with uncharacterised function will be discussed. Abiotic stresses such as aluminium (Fisher et al. 1993), boron (Moody et al. 1993) and salt (Colmer et al. 2006) toxicity, as well as manganese (Reuter et al. 1988), copper (Leach et al. 2006) and zinc deficiency are known to reduce the grain yield of wheat in southern Australia (Reuter et al. 1988) and genetic associations providing tolerance to some of these stresses have been identified (Dubcovsky et al. 1996; Jefferies et al. 2000; Sasaki et al. 2004; Leach et al. 2006). However these were not the focus of this research and will not be considered further in this review.

1.2.2.1 Genotype-by-Environment Interaction

Unfortunately variety performance for grain yield is not simply a function of the additive combination of the effects of the genotype and the environment. The performance of one variety may also differ relative to another across different environments. One of the simplest examples to illustrate this scenario is the interaction between disease severity and disease resistance on grain yield. In an environment devoid of a particular disease, two varieties, one susceptible to the disease, and the other resistant, may achieve the same grain yield. But in an environment where the disease is infecting crops, the resistant variety will outperform the susceptible variety for grain yield. This genotype-by-environment interaction (GEI) reduces the accuracy and therefore efficacy of selection decisions (Cooper et al. 1995). This is particularly evident where a cross-over interaction occurs (Bernardo 2002). Using the example discussed previously: if the disease resistance gene carried some metabolic cost, it may be possible that the susceptible variety would actually be higher yielding than the resistant variety in environments without disease pressure. In fact, GEI can be classified into four distinct patterns (Figure 2).

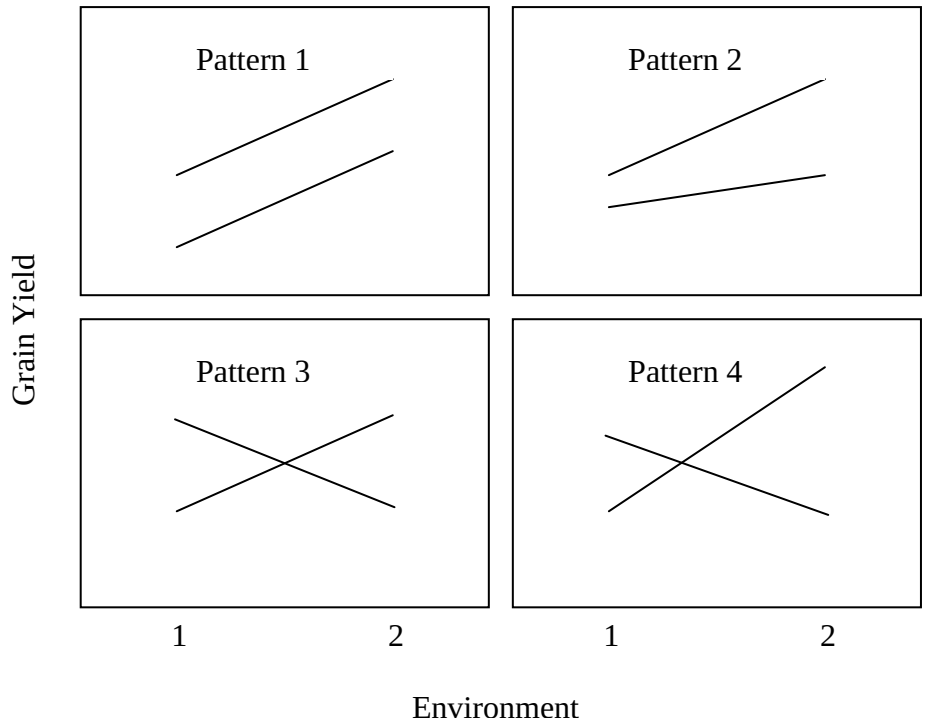

Figure 2. A schematic of genotype-by-environment interaction patterns (taken from (Ouyang et al. 1995) and (Bernardo 2002))

From a breeding perspective; Pattern 1, where no GEI is detected, is ideal. This allows selection to be performed in one environment and the results extrapolated across all other environments, only altering the mean grain yield of the population with changes in environment. However this is rarely seen, instead, Pattern 2 interactions are more common (Eberhart et al. 1966), and make sense biologically. One variety may be higher yielding than another in a low yielding environment, but in a higher yielding environment, the difference between them is amplified. Expressed another way; there are fewer limitations to achieving grain yield potential in a favourable environment and so it follows that the superiority of a variety will be more evident in a favourable environment. Although GEI is observed in such an example, its impacts on breeding are trivial, as the elite variety remains so regardless of

environment. In fact, it is observations such as this that have led some breeders to utilise high yielding environments to test the "yield potential" of breeding material (Rajaram et al. 1996). It is GEI Patterns 3 and 4 that pose the greatest complication to genetic improvement (Bernardo 2002). Cross-over GEI (Patterns 3 and 4) mean that results from one site can not be extrapolated to another. This has resulted in breeders adopting wide-scale testing of genotypes in an attempt to assess grain yield across the range of environments expected within the target population of environments (TPE). However, wide-scale testing for grain yield is not practical at the early stages in a breeding programme. Limited seed, but more importantly the sheer number of genotypes requiring testing, restrict the number of environments able to be sampled. In general, as genotypes pass through a breeding programme and confidence in their performance increases, they are tested more widely, ensuring that their superiority is maintained across the TPE. Numerous methods have been employed to sample the TPE as early as possible in the breeding programme and to increase the effectiveness of grain yield assessment through appropriate statistical treatments (Basford et al. 1998). However, ultimately, there can be no avoiding the fact that cross-over GEI reduces the accuracy of phenotypic based selection for grain yield improvement and wide adaptation. Selection for genes conferring high grain yield with molecular markers, not influenced by the environment, therefore provides a attractive alternative to phenotypic selection for grain yield. However, this raises an important point. Before MAS is used to select for genes/QTL involved in the control of traits that are substantially influenced by cross-over GEI, it is critical that the environmental interaction of those genes/QTL be characterised. One such GEI influenced trait is grain yield. Many studies have reported QTL associated with grain yield (Table 1), but it is unlikely that breeders have undertaken MAS targeting these loci unless

convinced of the stability of the QTL effects across environments. The concept of QTL (or gene)-by-environment interaction (Sari-Gorla et al. 1997; Crossa et al. 1999; Piepho 2000; Verbyla et al. 2003; Campbell et al. 2004; Malosetti et al. 2004; Piepho 2005; Yan et al. 2005) should therefore be considered as part of any genetic analysis in wheat involving traits influenced by environmental interactions.

1.2.2.2 Genetic Factors Influencing Grain Yield Through Plant Height

Bread wheat, in its wild-type form, is tall. Although varying with the fertility of the environment, tall varieties often reach heights greater than 130 cm (Law et al. 1978a; Fischer et al. 1990; Flintham et al. 1997). In populations not segregating for major genes controlling plant height, Law et al. (1978a) showed a positive correlation between grain yield and plant height, likely to be arising through pleiotropy. The relationship was so strong, they concluded that selection for plant height on a single plant basis would result in greater gains in grain yield than single plant based selection for grain yield itself. The genetic basis for this correlation between height and grain yield appeared to be due to minor genes located on most wheat chromosomes. However, the work of Fischer et al. (1990) disagreed with that of Law et al. (1978a). Fischer et al. (1990) concluded that regardless of whether reduced height was conferred by minor or major gene action, the most desirable plant height was 70cm under optimal conditions. They also questioned the value of breeding for 'tall-dwarfs' (lines with a major gene for dwarfism, but possessing minor genes that result in 'tall-dwarfs') as proposed by Law et al. (1978a). However Fischer et al. (1990) did admit that the results and conclusions of Law et al. (1978a) may have arisen through differences in the latitude of test locations (Australia vs the United Kingdom). The results of Richards (1992a) support the conclusions of Fischer et al.

(1990), where, like Fischer et al. (1990), experiments were undertaken in Australia. However at the lower yielding environments used by Richards (1992a), an optimum height of 70-100cm was suggested. Consequently, it can be concluded that on average, selection for cultivars with reduced plant height with respect to the wild type will improve grain yield potential in Australia, although some GEI is present for these genes.

The introduction of major height reducing genes through wheat breeding probably constitutes the single largest impact of genetic improvement on wheat production in history. In fact Dr Norman Borlaug received the Nobel peace prize for his contribution to the green revolution through the deployment of reduced stature wheats (Hedden 2003). Gibberellic acid insensitive height reducing genes (*Rht-B1b* and *Rht-D1b*) from the Japanese variety Norin-10 were introduced into the CIMMYT breeding programme and subsequently much of the world.

Sensitivity to the plant growth factor gibberellic acid is required for stem elongation. Where the *Rht-B1* and *Rht-D1* genes are present this elongation does not occur normally, leading to reduced height. With this height reduction comes an improvement in harvest index (the proportion of grain to above ground biomass) and kernel number, and consequently grain yield potential (Fischer et al. 1990). In high yielding environments the reduction in height also leads to lodging resistance (Rebetzke et al. 2000). Although unlikely to be a major benefit in many of the low yielding environments of Australia, resistance to lodging allowed substantial increases in the application of fertiliser, which was a major contributor to the green revolution (Hedden 2003). In dryer environments such as that in southern Australia, the improvements in grain yield are likely to be attributable to improved harvest index (Laing et al. 1977) and an increase in kernel number. However, some authors have

suggested that the height reduction resulting from the introgression of *Rht-B1b* and *Rht-D1b* may be too extreme for dry environments (Richards 1992a) such as those in Australia. In addition, the *Rht-B1* and *Rht-D1* semi-dwarf varieties have short coleoptiles, which are believed to be associated with slower establishment (Allan 1989). This in turn may lead to reduced water-use efficiency (Richards 1992b) and weed competitiveness (Rebetzke et al. 2000).

Efforts have been undertaken to characterise and introgress alternative height reducing genes that retain sensitivity to gibberellic acid and therefore maintain their coleoptile length. The most well known of these, *Rht8*, has been used in wheat breeding for many years, particularly in Eastern Europe (Worland et al. 1988). However, more recently, a number of additional gibberellic acid sensitive height reducing genes have been characterised (Loskutova 1998). Of the gibberellic acid sensitive height reducing genes, *Rht13* appears particularly promising, conferring similar reductions in height to *Rht-B1* and *Rht-D1*, but without the adverse effects on coleoptile length (Ellis et al. 2004). Molecular markers have been developed for many of these genes (Korzun et al. 1997; Korzun et al. 1998; Ellis et al. 2005) to aid in the efficient replacement of the existing semi-dwarf genes (Speilmeyer et al. 2001). As yet, there has been only a small amount of work (Loskutova 1998; Rebetzke et al. 2000; Ellis et al. 2004) investigating the potential yield improvements offered by these loci, or their effects on other economically important traits.

Additional loci influencing plant stature, not formally classified as height reducing genes, have also been identified in a number of QTL based analyses (Table 1). Some of the genes/QTL may be associated with phenological processes that have pleiotropic effects on plant height, or genes/QTL that contribute to the specific

adaptation of wheat varieties. In either case, it is likely that numerous minor genes, as yet uncharacterised, could influence plant height and therefore grain yield.

1.2.2.3 Genetic Control of Plant Phenology Traits Influencing Grain Yield

There are key stages within the life cycle of wheat that define its potential to produce optimal levels of grain in any given environment. The early stages of growth, as floral primordia are being developed, largely dictate the number of grains that may eventually be produced (Worland 1996). Stress, be it nutritional, moisture or temperature related, may severely reduce the potential grain yield of a crop. In some environments the vegetative phase can be excessively long and flowering and grain fill may occur under moisture and temperature stress. Ultimately, for each environment, a fine balance exists between the duration of the vegetative and reproductive phases (Cockram et al. 2007). Ideally, the grain yield potential, as determined by spike and spikelet number, will be large, but flowering needs to occur early enough to allow sufficient time for grain fill to achieve the grain yield potential. Should flowering occur too early in Australia, a wheat crop will experience a greater chance of being afflicted by reproductive frost damage. However, should flowering occur too late, the hot dry conditions of late spring and early summer could result in premature death and incompletely filled grain (Reinheimer et al. 2002). The timing of anthesis can be altered through changes in crop planting date. However the period of suitable planting dates is usually narrow and can change from year-to-year. Consequently, it is desirable to select varieties with a flowering date that is ideal for a specific environment and preferably does not display large year-to-year fluctuations: irrespective of planting date (Boyd et al. 2003).

The genetic basis of wheat development can be split into three components, those that control response to photoperiod (Halloran et al. 1967), those that control

responsiveness to cold (vernalisation) (Flood et al. 1984b) and those that control the rate of plant development (basic development rate or earliness *per se*) as it relates to temperature (Flood et al. 1984a). A series of major genes *Ppd-A1*, *Ppd-B1* and *Ppd-D1* on chromosomes 2A, 2B and 2D respectively have been shown to control photoperiod sensitivity, while a homoeologous gene series for vernalisation sensitivity are located on chromosomes 5A, 5B and 5D (Snape et al. 2001). No such gene series has been identified for the control of earliness *per se*. However, numerous reports from studies using either substitution or addition lines, or QTL mapping, have identified a large number of genetic associations with timing of flowering (Table 1).

Although plant phenology is known to have a large influence on grain yield in Australia (Fisher 1979), few attempts have been made to characterise the grain yield effects of specific phenological genes (Snape et al. 2001). Worland (1996) and Worland et al. (1998) reported on the influences of the *Ppd-B1* and *Ppd-D1* loci on grain yield in Europe. They showed that both genes had a significant influence, but that the magnitude and direction of the effects changed from year-to-year and from site-to-site. Consequently, it appears that the photoperiod sensitive loci may contribute to the cross-over GEI discussed earlier. However Worland (1996) showed that when averaged across years, the introduction of the *Ppd-D1* photoperiod insensitivity gene resulted in reasonably consistent effects on some grain yield components. Across England, Germany and Yugoslavia, photoperiod insensitivity was associated with a larger number of spikes and spikelets per spike, but fewer fertile florets per spikelet. The relationship between photoperiod sensitivity and grain weight, on the other hand, varied with environment (Worland 1996).

Work in North America, studying the impact of the *Ppd-D1* gene on grain yield across 21 site-year combinations, reported higher grain yields (4.9%) associated

with the photoperiod sensitivity allele (Dyck et al. 2004). However, in contrast to the work of Worland (1996), they found that photoperiod insensitivity was associated with fewer spikelets per spike but no significant relationship was established with seeds per spike or grain weight (Dyck et al. 2004). This GEI is not surprising given that both photoperiod and vernalisation genes are responsive to environmental signals. Consequently, it is important that the number and location of genes responsible for the variation in phenology observed in Australian wheat germplasm is determined, and their effects on grain yield over a range of environments characterised.

1.2.2.4 Genetic Factors Influencing Grain Yield *per se*

Both physiologically and morphologically, it is not difficult to imagine routes other than those associated with plant height and phenology that could be manipulated to improve the grain yield potential of wheat. Where light is limiting; leaf area and duration, and photosynthetic efficiency could be manipulated to increase carbohydrate production (Austin 1982; Simmons 1987). In environments where water is limiting, improved root growth and therefore water extraction, increased water use efficiency and improved osmotic adjustment could be used to improve drought tolerance (Gusta et al. 1987). In general however, the genetics of grain yield *per se* is poorly understood, perhaps mainly due to the effort that has been required to investigate the more readily observable grain yield related traits, such as height, phenology, disease resistance and stress tolerance. As the complexity of grain yield is dissected, it may become possible to characterise alternative routes to achieve high grain yield. Numerous QTL have been reported to be associated with grain yield independent of plant height, phenology and disease resistance (Table 1). Some studies have also reported genetic associations with grain weight, some of which coincide with grain

yield QTL (Table 1). However these studies have not determined the molecular or physiological basis of the genetic associations. This poses an obvious challenge to the wheat geneticist. A concerted effort is required to identify QTL for grain yield and characterise their pathways from 'gene-to-phenotype'. This information may provide breeders with the confidence to utilise genotypic based selection for the improvement of grain yield.

1.3 Application to Breeding, of Genetic Knowledge Concerning Economically Important Traits in Bread Wheat

In the early days of wheat breeding, parents were identified, crosses were made and segregants selected and multiplied based entirely on their macro-phenotype. As the physiological and biochemical basis of economically important traits has become better understood, selection decisions have also improved (Gusta et al. 1987; Simmons 1987).

A simple example of this is the shift from the use of complete baking tests to measure end-product quality to the use of correlated dough rheological properties to predict end-product quality. Not only does this improve the throughput and heritability of selection, it also provides a greater understanding to the basis of end-product quality. This may in turn lead to the selection of parents with complimentary rheological properties. Two varieties may produce similar leavened loaf volume, but examination of dough resistance and extensibility may expose a clearer picture of the end-product potential of a cross formed between them. In a case where both generate resistant but inextensible dough, little genetic gain in baking performance may be expected. However, where one of the varieties produces less resistant but more

extensible dough, transgressive segregation could be expected and consequently superior baking varieties could be achieved.

Understanding of the physiological and biochemical basis to complex traits has greatly aided selection and breeding strategy design. Improved genetic knowledge is also likely to lead to increased genetic gain through enhanced crossing and selection decisions.

In the following section, the outcomes of genetic analysis in wheat is discussed with reference to its impact on crossing strategies, phenotypic selection decisions, and MAS. These concepts are presented using some specific examples.

1.3.1 Genotypic Based Trait Dissection and its Impacts on Phenotypic Selection

Gibberellic acid-sensitive genes for height reduction are thought to provide a number of agronomic advantages over gibberellic acid-insensitive genes (see Section 1.2.2.2). The introgression of the gibberellic-acid insensitive height reducing genes *Rht-B1* and *Rht-D1* has been shown to result in shortened coleoptile length which in turn can lead to poor emergence of wheat when sown deep (Trethowan et al. 2001). However, genetic analysis has also shown that the effects of gibberellic-acid sensitive height reducing genes such as *Rht8, 9* and *12* have much smaller effects on coleoptile length. Lines carrying these dwarfing genes produce coleoptiles only seven to 13% shorter than the wild type and 47% longer than lines carrying the *Rht-B1b* and *Rht-D1b* genes (Rebetzke et al. 2004). The longer coleoptiles associated with gibberellic acid-sensitive height reduction genes has led to the introduction of phenotypic selection methods designed to exploit this characteristic. At CIMMYT (Mexico), populations known to be segregating for non *Rht-B1b* or *Rht-D1b* height reducing genes are sown at depth in order to select against individuals that have shortened

coleoptiles and are therefore likely to be carrying *Rht-B1b* or *Rht-D1b* (Trethowan et al. 2005). In this case, genetic and physiological analysis has provided the basis for the design of an effective phenotypic selection method.

In an example of trait dissection taken from barley, genetic analysis has helped to explain the poor genetic gain that had been made historically for tolerance to boron toxicity. Boron toxicity is known to reduce the grain yield of cereals in southern Australia (Cartwright et al. 1984), and in barley causes substantial leaf necrosis (Jefferies et al. 1999). Genetic variation for tolerance to boron (Cartwright et al. 1987) had been utilised by barley breeders in southern Australia for a number of years but with modest success (SP Jefferies, personal communication 2000). Breeding methodology relied predominantly on selection of genotypes with reduced leaf necrosis when grown under boron toxic conditions. However the genetic analysis of Jefferies et al. (1999) showed that the genetic regions largely responsible for the variation in leaf symptom (2H), were not the same as the major QTL (4H) responsible for whole shoot boron concentration and whole-shoot dry weight. The results of Jefferies et al. (1999) highlight the inadequacy of the previously used leaf symptom based selection methods and consequently, phenotypic selection using a combination of boron shoot content and leaf symptoms under the influence of boron toxicity was proposed as better selection method (SP Jefferies, personal communication 2000).

1.3.2 The Impact of Improved Understanding of Gene Effects, Linkage and Pleiotropy on Crossing and Selection Strategies

Both genetic linkage amongst genes, and the occurrence of pleiotropy, can be either beneficial or a hindrance to genetic improvement. The phase of linkage and pleiotropy often determines which is beneficial and which is a hindrance.

Genetic improvement in a breeding programme relies on the presence of genetic variation and the ability of the breeder to identify desirable genotypes. For a single trait, response to selection depends on additive genetic variance, heritability and selection intensity (Bernardo 2002). For two or more traits, genetic and environmental covariances become important drivers of genetic gain. As the number of traits targeted within a breeding programme is increased, selection intensity can be maintained through a corresponding increase in population size. However, ultimately population size will be constrained by the availability of resources. Consequently, genetic gain per trait is generally reduced as the number of traits and consequently the number of genes being targeted is increased. However, should two desirable genes be linked in repulsion, or if one gene has desirable effects on one trait and undesirable effects on another (pleiotropy), genetic gain will be reduced further. In the case of pleiotropy, the effects are unavoidable, and negative genetic gain for one of the traits will need to be compensated for by the positive effects of one or more alternative loci: thereby further decreasing the rate of genetic gain. Two genes linked in repulsion offer a slightly more favourable possibility over pleiotropy. The likelihood of identifying a recombinant carrying both favourable alleles is a direct function of the linkage distance between the loci. So although genetic gain is reduced, it is not impossible to achieve.

The opposite is also possible. Genetic gain is increased through linkage of two genes in coupling phase (desirable alleles inherited together) or a gene with favourable effects on more than one trait (positive pleiotropy). In these cases, genetic improvement can be made for more than one trait at the same time without necessarily increasing population size and therefore resource requirements.

For example, the root lesion nematode, *P. neglectus*, can cause substantial reductions in grain yield in southern Australia (Vanstone et al. 1998). Varieties such as 'Excalibur', 'Krichauff' and 'Worrakatta' have proven good sources of resistance (Vanstone et al. 1998). However, it has been noted that a very large number of root lesion nematode resistant derivatives of 'Krichauff' and 'Worrakatta' possess undesirable levels of yellow pigment (high flour Minolta b* value) in their flour for most of Australia's current export markets (SP Jefferies, personal communication 2000). Recent results from genetic analysis carried out by Williams et al. (2002) showed that the root lesion nematode resistance locus (*Rlnn1*) carried by 'Excalibur', 'Krichauff' and 'Worrakatta' is located on chromosome 7A, less than 10cM from the yellow flour colour QTL described by Parker et al. (1998 & 1999) which is also carried by 'Krichauff' and 'Worrakatta'. Consequently, it could be predicted that approximately 95% of progeny from a cross with 'Krichauff' or 'Worrakatta' would be either susceptible to root lesion nematode or possess an unacceptably high flour Minolta b* value. Beyond increasing population size, and consequently the probability of finding favourable recombinants, the simplest way to breed varieties resistant to root lesion nematode but producing acceptable flour colour may be through selection of an alternative donor parent. 'Excalibur', and more recently 'Wyalkatchem', have been identified as resistant to root lesion nematode but producing acceptable flour colour. Consequently, crossing and selection strategies have been developed that utilise these alternative varieties as donors of *P. neglectus* resistance rather than 'Krichauff' or 'Worrakatta' (H. Kuchel, unpublished data).

In another example of undesirable linkage (or perhaps pleiotropic effects), Gororo et al. (2001) showed that grain size co-segregated with the cereal cyst nematode (*H. avenae*) resistance locus *Cre1*. Unfortunately, the allele providing

resistance to the cereal cyst nematode (CCN), was associated with small grain. There has been some debate (R.F. Eastwood, personal communication 2001) as to whether the association is due to pleiotropy, or linkage with genes carried on the genomic segment introgressed from the donor landrace (Slootmaker et al. 1974). Regardless, the use of the *Cre1* gene in the Victorian wheat breeding programme seems to have resulted in a high proportion of progeny producing small grain (R.F. Eastwood, personal communication 2001). Unlike *Rlnn1*, alternative CCN resistance genes have been characterised. *Cre3* (Eastwood et al. 1991) provides a similar level of CCN resistance (using CCN strain Ha13) to *Cre1*, while the protection afforded by *Cre8* (Paull et al. 1998) is marginally lower, and other minor genes such as *Cre5* (Jahier et al. 2001) may 'enhance' CCN resistance. In addition, none of these other CCN resistance genes have shown the same detrimental effects on grain size. Consequently, genetic analysis has allowed breeders to select parents carrying alternative genes for CCN resistance, avoiding the detrimental effects conferred on grain size by the use of *Cre1*.

Recently, the work of numerous researchers, but particularly that of Cane et al. (2004) and Eagles et al. (2002b), has provided a thorough and robust estimation of the gene effects associated with the glutenin and puroindoline genes on wheat quality in Australia. The main and interaction effects of these loci have been used as the basis of a wheat quality 'cross-predictor' (Eagles et al. 2001; Cornish et al. 2006) using the QU-GENE computer simulator (Podlich et al. 1998). Taking the example used previously for the design of crosses based on rheological rather than baking quality: the wheat quality 'cross-predictor' enables breeders to simulate the outcomes of individual crosses and determine the likely success of each parental combination prior to investing valuable resources. Due particularly to epistatic effects between glutenin

loci (Eagles et al. 2002b), selection of parental material by phenotype only, may not provide a reliable prediction of their general, or more particularly, their specific combining ability. Consequently, genetic analysis of wheat quality, and its packaging into a tactical software tool for breeders, provides another example of improved crossing decisions facilitated through the outcomes of genetic analysis.

Australian wheat breeders have used the knowledge gained from the genetic analysis of traits including root lesion nematode resistance, flour colour, CCN resistance, and dough rheology to design and implement crossing and selection strategies that have resulted in enhanced rates of genetic gain (S. Jefferies and R. Eastwood, personal communication 2006). It would therefore be beneficial to gain a thorough understanding of the main and pleiotropic effects, as well as any linkage implications, of other economically important genes being manipulated within a breeding programme.

1.3.3 Marker-Assisted Selection

Although the benefits of an improved understanding of the genetic basis to economically important traits can be demonstrated across a wide range of breeding activities, selection for these traits using molecular markers has been touted as potentially having one of the largest impacts on the way wheat is bred (Koebner et al. 2003). Phenotypic markers, exploiting serendipitous linkages with morphological variation, has been utilised by breeders to target specific genes for a number of years. The stem rust gene *Sr2* is linked to the expression of pseudo-black chaff (Hare et al. 1979), the stripe rust gene *Yr10* is associated with brown chaff (Metzger et al. 1970), while leaf tip necrosis is observed on individuals carrying *Lr34/Yr18* (Singh 1992). In these cases, breeders have been able to achieve genetic gain for rust resistance where

no disease is present or where other major genes are masking the expression of the target locus. However, useful phenotypic linkages such as these are the exception rather than the rule, and like the genes of interest themselves, the expression of the marker traits may also be influenced by the environment as well as background effects from gene modifiers (H Bariana, personal communication 2006). Molecular markers, based on DNA sequence variation, do not suffer either of these disadvantages and unlike phenotypic markers, are ubiquitous.

1.3.3.1 Why Use Marker-Assisted Selection?

In a wheat breeding programme, genetic gain is often hampered by genotypic effects such as epistasis and recessiveness, as well as error sourced from environmental variation and experimental inaccuracies. In addition, selection for some traits requires large quantities of grain (end-use quality), is expensive (grain yield analysis) or not practical (resistance to exotic disease). Also, selection systems for different traits can at times be mutually exclusive; for example, determining resistance to multiple root diseases such as cereal cyst nematode and root lesion nematode as well as tolerance to boron and aluminium toxicity, in one assay, is not possible due to the confounding effects of one assay system on another. It is in these situations that DNA based MAS has been suggested as a more effective alternative to phenotypic based selection (Dudley 1993; Knapp 1998), potentially offering synergistic benefits to the overall breeding programme (Stuber et al. 1999). More specifically, MAS has been studied and proposed in inbred crops for donor gene (Ribaut et al. 2002) and recurrent parent selection in a backcrossing programme (Frisch et al. 1999), recurrent selection to accumulate QTL (Charmet et al. 1999; Charmet et al. 2001), and diversity analysis (Charcosset et al. 2004). A general consensus has emerged from the literature

that the relative efficiency of MAS will be higher when the phenotypic alternative has low to moderate heritability (Hospital et al. 1997; Knapp 1998). However, a caveat has been voiced regarding this conclusion. Where the heritability of a trait is too low, the inaccuracies associated with the original genetic analysis used to detect the QTL may reduce the benefits of MAS (Hospital et al. 1997). DNA based markers have also been suggested for variety identification and in turn assisting in the capture of variety royalties.

1.3.3.2 Limitations to the Application of Marker-Assisted Selection

A computer based search of literature (Biological Abstracts 1980-2006) using the term “marker assisted selection” (or similar) and the keyword “wheat”, revealed a total of 249 publications. The results from the classification of these into four broad subject areas is displayed in Table 2. Although there are a number of publications making reference to MAS in wheat, those describing practical examples of MAS in a breeding context was just 17 (7%). This may reflect the difficulty of publishing the results obtained from pragmatic breeding activities. However since some journals such as ‘Euphytica’, ‘Molecular Breeding’, and ‘Plant Breeding’ are dedicated to the publication of applied research in plant breeding, this seems unlikely. It could also be countered that pragmatic breeders, focussed on the release of improved cultivars, are not interested in publishing ‘MAS success stories’. However, based on the observations of this writer and others (S. Jefferies, P. Langridge and J. Reinheimer, personal communication 2006), it seems that worldwide, MAS in wheat breeding still has considerably more scope to develop. Consequently, it seems likely that this publication record is an accurate reflection of the rate with which the results of genetic analysis have been adopted within many wheat breeding programmes.

Table 2. Classification of the 249 references within Biological Abstracts 1980-2006 mentioning marker-assisted selection with reference to wheat.

	Number of papers	Percentage
Genetic analysis and new marker technologies	175	70
Reviews and simulations	13	5
Practical examples of MAS in wheat	17	7
Other (not related directly to wheat and/or molecular markers)	44	18

So the question is posed: Given the benefits to genetic improvement that may potentially be afforded by MAS, why has their application in wheat breeding not progressed further? Numerous reasons could be put forward, for example; 1) not enough economically important loci have been tagged with markers, 2) the markers linked to target loci are not user friendly or robust enough, 3) the linkage between loci and identified markers is too loose, 4) economically important traits are too complex for MAS, 5) it is too expensive to implement MAS in a practical breeding programme, and 6) current wheat breeders are not trained in molecular breeding or convinced of its benefits. Many of these reasons are interrelated, and perhaps a matter of opinion, but below is a discussion of the aforementioned six possible reasons for slow MAS adoption.

1) Not enough economically important loci have been tagged with markers

Breeders routinely manipulate a large number of loci within their germplasm pools, and although a number of these loci are common across most of the globe (such as rust resistance genes and glutenins for wheat quality), many are likely to be breeding programme (or at least region) specific. For example, boron tolerance, cereal cyst nematode resistance and root lesion nematode (*P. neglectus*) resistance have been key selection targets for southern Australia, and have consequently been tagged with molecular markers by co-located researchers (Paull et al. 1998; Jefferies et al. 2000;

Williams et al. 2002). In northern Australia however, these marker-trait associations are not particularly useful, and breeders in that region would prefer to use markers for traits such as crown rot tolerance and root lesion nematode (*P. thornei*) resistance. If this scenario is considered across the range of traits selected in each of the world's wheat breeding programmes, it is easy to imagine the vast number of genetic analyses required to supply their needs. When considering traits under complex control, the situation becomes even more daunting. Although the number of studies reporting trait-marker associations substantially outweigh the reports of practical marker-assisted selection, our knowledge concerning the genetic basis of economically important traits remains far from complete.

2) The markers linked to target loci are not user friendly or robust enough

Numerous molecular marker technologies are available for MAS in wheat, and are reviewed by Langridge et al. (2001). In the first few years of DNA based MAS, restriction fragment length polymorphisms (RFLPs) formed the greatest proportion of markers available for implementation within wheat breeding programmes. However, as they rely on hybridisation technology and require high grade DNA, throughput was slow, justifying the conclusions of some breeders that the MAS technology theoretically offered much, but in reality was unable to deliver at the level required by their breeding programmes.

The advent of polymerase chain reaction (PCR) based marker technologies such as simple sequence repeats (SSRs), randomly amplified polymorphic DNAs (RAPD), sequence characterised amplified regions (SCARs) and sequence tagged sites (STS) allowed the use of lower quality DNA and achieved a substantially higher throughput (Langridge et al. 2001). SSRs in particular, due to their high

polymorphism level, use in mapping experiments, technical robustness, and genomic ubiquitousness (Langridge et al. 2001), have proven to be the marker type of choice for many wheat breeding programmes. In a large part, this technology development has alleviated many of the problems that resulted in the criticism that MAS is not a user friendly technology. However, most SSRs have multiple alleles but are often used to select di-allelic genetic loci, thereby potentially making selection by the uninitiated confusing. In the simplest and most desirable scenario, each phenotypic allele would be entirely associated with a single marker allele. This will be discussed further in the following section.

3) The linkage between genetic loci and molecular markers is too loose

One of the major benefits of MAS over trait-based phenotypic selection is its improved accuracy. Whereas trait based selection is subject to extraneous error, MAS may theoretically approach a heritability of one. However, loose linkage between a marker and the locus being selected reduces the effectiveness of MAS. Within any particular cross, the impact of poor linkage on response to selection will be proportional to the frequency of recombination between the marker and locus. Where the linkage distance between the marker and locus is not large (eg <10cM), the reduction in selection efficacy may be quite small. However, after the completion of several breeding cycles, recombination distances even closer than 10cM can have a large effect on the effectiveness of MAS. Unless the linkage phase between the marker and locus is confirmed at the end of each crossing cycle, it is very possible for crosses to be made, and selected with markers, that do not even segregate for the locus of interest. Identification of linkage phase between the selectable marker and target locus before crossing will overcome this problem. This may be a relatively straight

forward process for loci with a discrete (or near discrete) phenotype; such as glutenins, puroindolines, or a well characterised rust gene, or for traits controlled by very few genes; for example boron tolerance or root lesion nematode (*P. neglectus*) resistance. In these cases, the association between a marker allele and gene allele can be easily identified. For complex traits, determination of linkage phase is not so simple. By their very definition, quantitative traits are either under the control of multiple genetic loci and/or are heavily influenced by the environment. Ideally, each locus under selection would be cloned and the mutations responsible for phenotypic variation would be subsequently tagged. This would remove the requirement to confirm linkage phase before each cycle of crossing and selection. Until such "diagnostic markers" are available for each locus of interest, it will be necessary for the breeding community to develop systems that cope with an imperfect selection system (such as tandem selection with flanking markers). Regardless, it would be useful to assess, and potentially demonstrate to breeders, the in/effectiveness of selection with loosely linked markers.

4) Economically important traits are too complex for MAS

Quantitative traits are characterised as being controlled by a large number of genes and/or are influenced by environmental and often genotype-by-environmental variation. Consequently, heritability for quantitative traits is generally low and genetic gain for these traits is slow. Therefore, they appear prime candidates for MAS. However, for the very reasons that they are difficult to manipulate by traditional selection techniques in a breeding programme, they are also difficult to dissect genetically (Hospital et al. 1997; Kruger et al. 2001).

Grain yield forms one of the most complex traits manipulated by wheat breeders. Although a number of publications cite QTL associated with grain yield in wheat (Borner et al. 2002; Campbell et al. 2003; Groos et al. 2003; McCartney et al. 2005; Quarrie et al. 2005; Marza et al. 2006), their physiological basis is rarely investigated, and their interaction with the environment poorly characterised. In barley, MAS for complex traits such as grain yield, has met with mixed success (Romagosa et al. 1999; Zhu et al. 1999; Kandemir et al. 2000; Schmierer et al. 2004). Before MAS for improved grain yield (and other complex traits) is realised, a thorough dissection of its genetic basis will be required.

5) It is too expensive to implement MAS in a practical breeding programme

The development of new marker systems has led to a dramatic increase in the capacity and throughput of molecular laboratories. However, many of these improvements have arisen through the use of expensive equipment that is perceived to be beyond the reach of many breeding programmes. In the USA, this has led to the centralisation of molecular resources and the establishment of four genotyping centres servicing each of the publicly funded USA wheat breeding programmes (G. Brown-Guedira, personal communication 2005). In Australia, centralisation has not occurred, with each wheat breeding company running their own molecular marker facility. However, these companies have also accessed the high-throughput capacity of commercial enterprises such as the federally funded Australian Genome Research Facility. Regardless of the system used to complete the assays, the cost to set up or contract out molecular analysis is seen by some as prohibitive. However, this should be viewed in perspective and within the context of the whole breeding strategy. An end-use quality laboratory is also a large monetary investment, but is seen as

obligatory. Likewise, the cost of field equipment for grain yield assessment is substantial but considered essential. Consequently, it is probably fair to conclude that when viewed as another selection technique, equal in merit to the established systems, the cost of molecular analysis should not be an impediment to its uptake by breeders. The most important question may be 'is the cost of MAS met with corresponding increases in genetic gain and/or economic efficiency?'

6) Current wheat breeders are not trained in molecular breeding or convinced of its benefits

Crop improvement through plant breeding is a traditional science, relying on genetic principles and selection methodologies largely developed over the past one and a half centuries. During that time, breeders have successfully adopted the benefits offered by various disciplines of science and technology (Hollamby 2001), including; mechanisation, computerisation, statistical methodology, out-of-season breeding nurseries, bioassays, and robotics. However, it would be fair to conclude that breeders are generally cautious implementers of new technologies, being careful not to upset what is in reality is a finely tuned logistical operation. Only once the benefits of a system are proven, have they been fully integrated into breeding programmes. This generates some new important questions. Have the benefits of MAS been sufficiently demonstrated to breeders to warrant changes to systems that currently operate effectively? Secondly, how are markers best integrated into a breeding programme? Finally, do the benefits of MAS vary with the method or stage at which they are implemented? These questions should be adequately answered before breeders divert expenditure from traditional breeding techniques to genotypic based selection.

1.4 Aims of the Thesis and the Research Papers that Address Them

MAS may accelerate the rate of genetic gain achieved in a wheat breeding programme. However a number of questions remain concerning the molecular strategies that are best employed by breeders. In addition, there is a lack of genetic knowledge concerning the specific genotype-environment system that controls the profitability of the wheat industry in southern Australia. Consequently, the aims of this thesis are to:

1) Gain an improved understanding of the genetic basis to economically important complex traits in the southern Australian environment

2) Investigate MAS methodologies that can apply genetic knowledge to improve the rate of genetic gain within southern Australian wheat breeding programmes.

The elite southern Australian breeder's line 'Stylet' will be used as the genetic basis for this study. 'Stylet' was bred at the Roseworthy breeding programme of the University of Adelaide under the direction of Gil Hollamby. The main objectives, and achievements, of the 'Stylet' breeding strategy included wide adaptation, rust resistance, cereal cyst nematode resistance and good end-use quality. 'Stylet' is the highest yielding varietal representative of the highly successful 'Spear' lineage of wheat varieties that also includes the widely grown wheat varieties 'Dagger', 'Frame', 'Yitpi', 'Pugsley' and 'Correll'. However before 'Stylet' could be released to growers, leaf, stem and stripe rust strains developed that were virulent to the

resistance genes carried by ‘Stylet’. Consequently, a large number of populations were developed by the Australian Grain Technologies wheat breeding programmes in an effort to produce a rust resistant ‘Stylet’ derivative. In order to best utilise these (and related) populations it would be beneficial to dissect the genotype-environment system underpinning ‘Stylet’s’ grain yield in southern Australia. In addition, this literature review has shown that further research is required to examine the use of MAS within pragmatic wheat breeding. Due to time constraints, it will not be possible to include the outcomes from this genetic analysis when investigating MAS methodologies. Instead, marker-trait associations developed by others will be used to develop a superior end-use quality and rust resistant ‘Stylet’ derivative using various genotypic based selection strategies.

The specific aims of this thesis are outlined in Table 3, along with the publications included in this thesis that address them. Briefly, a doubled haploid linkage mapping population (182 individuals) developed from a cross between ‘Stylet’s’ parents ‘Trident’ and ‘Molineux’ (Ranjbar 1997) will be used to dissect several traits that are economically important to southern Australia. These include; end-use quality, ear-emergence and grain yield. As shown in this review, each of these traits are genetically complex and have a significant impact on the profitability of wheat production systems. It would be desirable to better understand the genetic basis to these traits in order to facilitate improved genetic gain through MAS. Genotypic based selection strategies aimed at improving the end-use quality and rust resistance characters of ‘Stylet’ will also be investigated. Computer aided simulation and practical breeding will be used to compare various methods of MAS and their

relative effectiveness within a back-cross population between ‘Stylet’ and the elite end-use quality and rust resistant variety ‘Annuello’.

Table 3. The aims of this thesis, and a description of the publications that address them.

Aim	Description	Publication Title	Reference
Gain an improved understanding of the genetic basis to economically important complex traits in the southern Australian environment	Genetic dissection of complex traits underlying the productivity of a southern Australian genotype-environment system. Including:		
	End-use quality	The genetic control of milling yield, dough rheology and baking quality of wheat	(Kuchel et al. 2006b)
	Time to ear-emergence	Identification of genetic loci associated with ear-emergence in bread wheat	(Kuchel et al. 2006a)
	Grain yield	Genetic dissection of grain yield in bread wheat. I. QTL analysis	(Kuchel et al. 2007b)
	Genotype-by-environment interaction for grain yield	Genetic dissection of grain yield in bread wheat. II. QTL-by-environmental covariable interactions	(Kuchel et al. 2007c)
Investigate MAS methodologies that can apply genetic knowledge to improve the rate of genetic gain within southern Australian wheat breeding programmes.	Simulation based comparison of phenotypic selection and various strategies of MAS: aimed at a specific breeding outcome for southern Australia (a rust resistant 'Stylet' derivative).	Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy	(Kuchel et al. 2005)
	Analysis and validation of MAS in the same breeding population used for computer simulation.	The successful application of a marker-assisted wheat breeding strategy	(Kuchel et al. 2007a)

1.5 References

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Chapter 2

Research Articles

The genetic control of milling yield, dough rheology and baking quality of wheat

End-use quality is a key driver of farm profitability and is consequently a major selection target for many wheat breeding programmes. Currently, genetic selection for end-use quality is largely limited to the selection of favourable glutenin, puroindoline and granule bound starch synthase alleles. However these loci do not explain all of the genetic variation in wheat quality. Consequently, it would be beneficial to further examine the genetic basis to end-use quality in Australian wheat cultivars. This may enable marker-assisted selection to be extended to a greater proportion of the genetic variance underlying wheat quality. This paper addresses the first of the two aims of this thesis; *to gain an improved understanding of the genetic basis of economically important traits in southern Australia.*

Kuchel, H., Langridge, P., Mosionek, L., Williams, K. and Jefferies, S.P. (2006) The genetic control of milling yield, dough rheology and baking quality of wheat. *Theoretical and applied genetics v.112 (8) pp. 1487 – 1495, May 2006*

Identification of genetic loci associated with ear-emergence in bread wheat

In southern Australia, wheat is generally planted in late autumn or early winter, it flowers in early to mid spring and is then harvested in early summer. This agronomic practice has been adopted to ensure the crop is able to generate sufficient biomass over winter when water is usually plentiful, flower late enough to avoid substantial reproductive damage by frosts and still mature before the hot and dry desiccating conditions of summer limit crop yields. The introduction of photoperiod insensitive spring wheat cultivars into Australia is thought to have contributed to this environmental adaptation profile. However the complete genetic basis to flowering time, and therefore adaptation, in Australian wheat cultivars has not been fully explained. It would be desirable to have a clear understanding of the genetic basis to phenological development within a southern Australian genotype-environment system. This paper presents a QTL based dissection of time to ear-emergence in the 'Trident/Molineux' doubled haploid population and addresses the first of the two aims of this thesis; *to gain an improved understanding of the genetic basis of economically important traits in southern Australia.*

Kuchel, H., Hollamby, G., Langridge, P., Williams, K. and Jefferies, S.P. (2006)
Identification of genetic loci associated with ear-emergence in bread wheat.
Theoretical and applied genetics v.113 (6) pp. 1103 - 1112, October 2006

Genetic dissection of grain yield in bread wheat. I. QTL analysis

Grain yield, along with end-use quality, is a major determinant of farm income. Wheat breeders have achieved considerable genetic improvement of grain yield within southern Australia, although it has been hindered by moderately low heritability, large genotype-by-environment interaction and expensive assay systems. A dissection of the genetic basis to grain yield in southern Australia may help to determine the adaptive function of agronomic loci controlling plant height, phenology and disease resistance. In addition, it may be possible to identify and tag with molecular markers the QTL that are responsible for improvements in grain yield independent of these traits. This paper presents a QTL based dissection of grain yield in the 'Trident/Molineux' doubled haploid population and addresses the first of the two aims of this thesis; *to gain an improved understanding of the genetic basis of economically important traits in southern Australia.*

Kuchel, H., Williams, K., Langridge, P., Eagles, H. and Jefferies, S.P. (2007) Genetic dissection of grain yield in bread wheat. I. QTL analysis.
Theoretical and applied genetics v.115 (8) pp. 1029 - 1041, November 2007

Genetic dissection of grain yield in bread wheat. II. QTL-by-environmental covariable interactions

Characterisation of the grain yield effects of agronomic genes, and the identification of grain yield QTL independent of these traits may help to improve the rate of genetic gain for grain yield. This paper uses the grain yield genes/QTL identified in the previous paper and characterises their interaction with various environmental variables. This information should help breeders to identify which loci, when selected with molecular markers, are likely to lead to improvements in both yield and yield stability. This paper addresses the first of the two aims of this thesis; *to gain an improved understanding of the genetic basis of economically important traits in southern Australia.*

Kuchel, H., Williams, K., Langridge, P., Eagles, H. and Jefferies, S.P. (2007) Genetic dissection of grain yield in bread wheat. II. QTL-by-environment interaction. *Theoretical and applied genetics v.115 (7) pp. 1015 - 1027, November 2007*

Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy

An improved understanding of the genetic basis to economically important traits could lead breeders to shift from phenotypic to genotypic selection through the application of marker-assisted selection. Genotypic selection can be performed at any growth stage, is not limited by the quantity of seed available, nor is it influenced by environmental variation. However it is likely that the benefits of maker-assisted selection will vary depending on the traits being manipulated and the complexity of the genetics underlying them. The aim of this paper was to investigate the potential benefits of marker-assisted selection when applied to a specific breeding strategy. This was undertaken using a computer-based simulation, coupled with a spreadsheet-based economic analysis. This paper addresses the second of the two aims of this thesis; *to investigate MAS methodologies that can apply genetic knowledge to improve the rate of genetic gain within southern Australian wheat breeding programmes.*

Kuchel, H., Ye, G., Fox, R. and Jefferies, S.P. (2005) Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy.
Molecular Breeding v. 16 (1) pp. 67 - 78, August 2005

The successful application of a marker-assisted wheat breeding strategy

Computer-based simulation can be used to predict outcomes, or to aid in strategy design, but in order to demonstrate the true potential of marker-assisted selection technology, validation in a practical breeding program is required. An appropriately designed practical marker-assisted selection strategy may be able to confirm previously published marker-trait associations, and validate computer-based simulations. This paper presents a marker-assisted breeding strategy based on the same population used for computer-based simulation and addresses the second of the two broad aims of this thesis; *to investigate MAS methodologies that can apply genetic knowledge to improve the rate of genetic gain within southern Australian wheat breeding programmes.*

Kuchel, H., Fox, R., Reinheimer, J., Mosionek, L., Willey, N., Bariana, H. and Jefferies, S.P. (2007) The successful application of a marker-assisted wheat breeding strategy.
Molecular Breeding v. 20 (4) pp. 295 - 308, November 2007

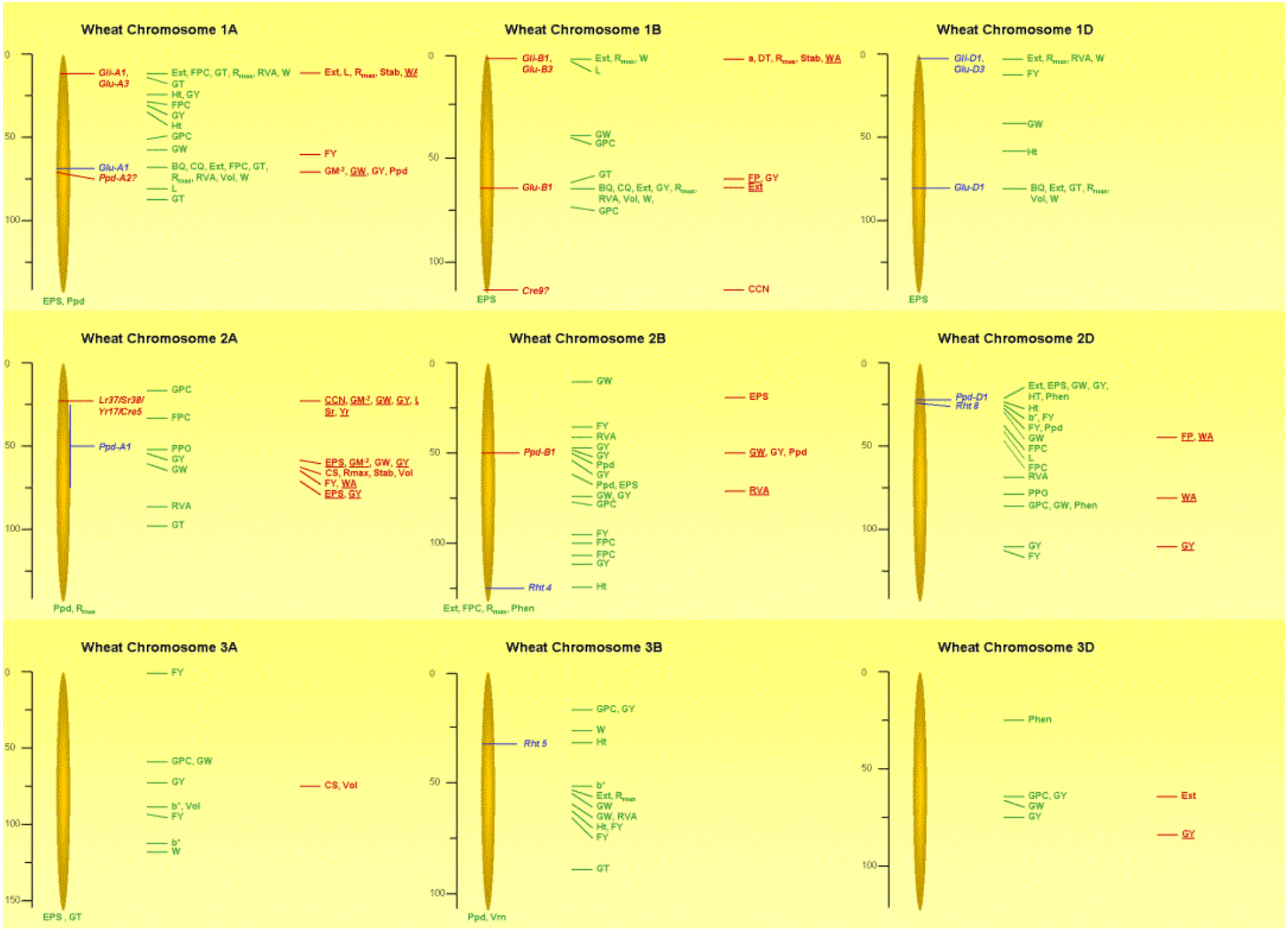
Chapter 3

General Discussion

Quantitative Trait Loci and Marker Assisted Breeding Strategies

3.0 Quantitative Trait Loci and Marker Assisted Breeding Strategies

A total of 89 genetic associations with the expression of economically important traits including end-use quality, phenology, grain yield and grain yield components were identified in this study (Kuchel et al. 2006a; Kuchel et al. 2006b; Kuchel et al. 2007b; Kuchel et al. 2007c). Some of these quantitative trait loci (QTL) are coincident with previously characterised genes and QTL, while many have not been described previously (Figure 1). Computer simulation and practical implementation of a specific marker-assisted selection (MAS) breeding strategy has highlighted the benefits that genotypic selection may yield for wheat breeding programmes (Kuchel et al. 2005; Kuchel et al. 2007a).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50
100
Gll-A1, Glu-A3
Glu-A1
Ppd-A2?
EPS, Ppd
Ext, FPC, GT, Rmax, RVA, W
GT
Ht, GY
FPC
GY
Ht
GPC
GW
BQ, CQ, Ext, FPC, GT, Rmax, RVA, Vol, W
L
GT
Ext, L, Rmax, Stab, WA
FY
GM-2, GW, GY, Ppd
Wheat Chromosome 1B
0
50
100
Gll-B1, Glu-B3
Glu-B1
Cre9?
EPS
Ext, Rmax, W
L
GW
GPC
GT
BQ, CQ, Ext, GY, Rmax, RVA, Vol, W,
GPC
a, DT, Rmax, Stab, WA
FP, GY
Ext
CCN
Wheat Chromosome 1D
0
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Gll-D1, Glu-D3
Glu-D1
EPS
Ext, Rmax, RVA, W
FY
GW
Ht
BQ, Ext, GT, Rmax, Vol, W
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50
100
Lr37/Sr38/ Yr17/Cre5
Ppd-A1
Ppd, Rmax
GPC
FPC
PPO
GY
GW
RVA
GT
CCN, GM-2, GW, GY, L
Sr, Yr
EPS, GM-2, GW, GY
CS, Rmax, Stab, Vol
FY, WA
EPS, GY
Wheat Chromosome 2B
0
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Ppd-B1
Rht 4
Ext, FPC, Rmax, Phen
GW
FY
RVA
GY
GY
Ppd
GY
Ppd, EPS
GW, GY
GPC
FY
FPC
FPC
GY
Ht
EPS
GW, GY, Ppd
RVA
Wheat Chromosome 2D
0
50
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Ppd-D1
Rht 8
Ext, EPS, GW, GY, HT, Phen
Ht
b*, FY
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FPC
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GPC, GW, Phen
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WA
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Wheat Chromosome 3A
0
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EPS , GT
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CS, Vol
Wheat Chromosome 3B
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Rht 5
Ppd, Vrn
GPC, GY
W
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b*
Ext, Rmax
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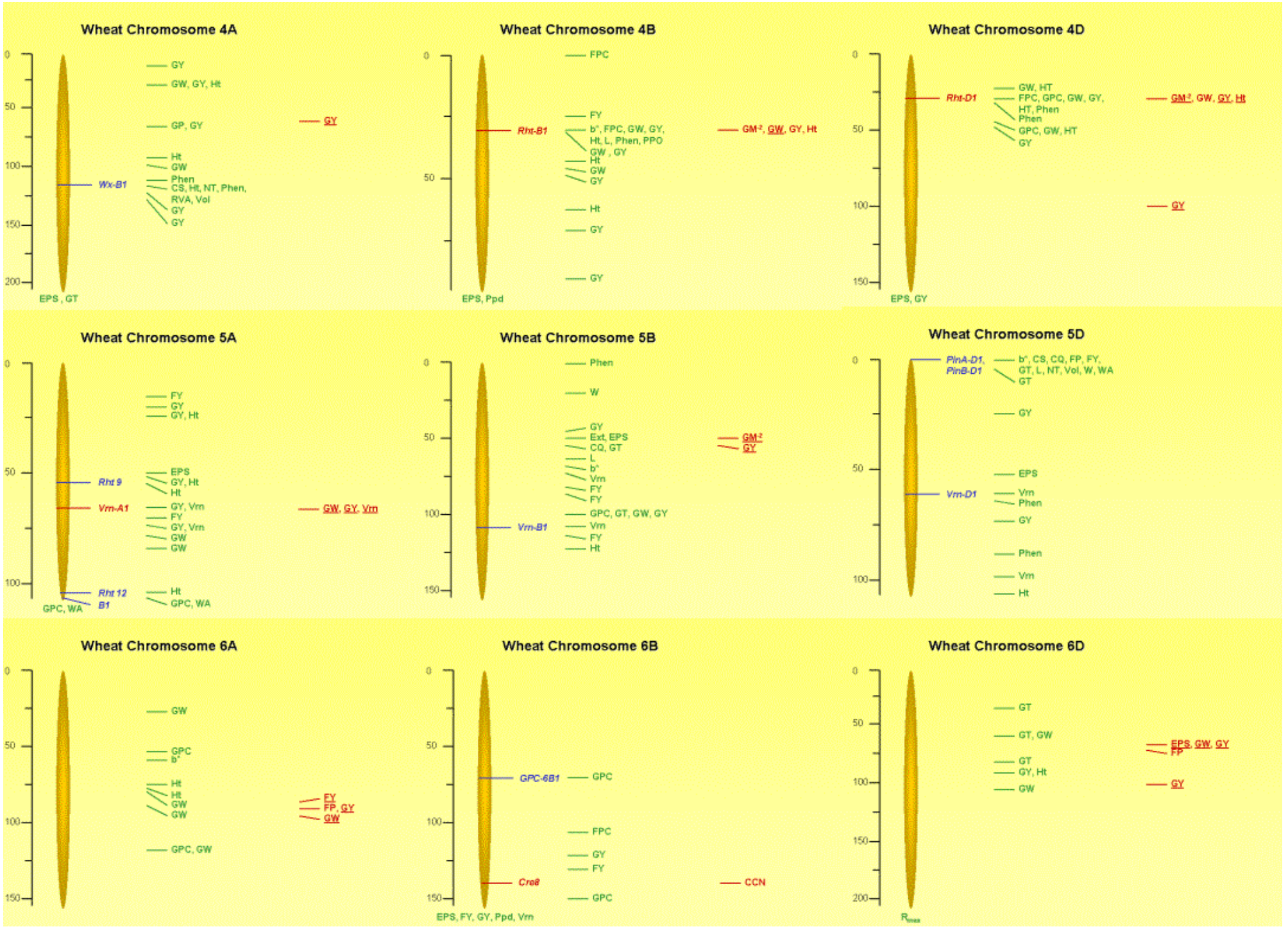
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EPS , GT
Wx-B1
GY
GW, GY, Ht
GP, GY
Ht
GW
Phen
CS, Ht, NT, Phen,
RVA, Vol
GY
GY
GY
Wheat Chromosome 4B
0
50
EPS, Ppd
Rht-B1
FPC
FY
b*, FPC, GW, GY,
Ht, L, Phen, PPO
GW , GY
Ht
GW
GY
Ht
GY
GY
GM-2, GW, GY, Ht
Wheat Chromosome 4D
0
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100
150
EPS, GY
Rht-D1
GW, HT
FPC, GPC, GW, GY,
HT, Phen
Phen
GPC, GW, HT
GY
GM-2, GW, GY, Ht
GY
Wheat Chromosome 5A
0
50
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GPC, WA
Rht 9
Vrn-A1
Rht 12
B1
FY
GY
GY, Ht
EPS
GY, Ht
Ht
GY, Vrn
FY
GY, Vrn
GW
GW
Ht
GPC, WA
GW, GY, Vrn
Wheat Chromosome 5B
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150
Vrn-B1
Phen
W
GY
Ext, EPS
CQ, GT
L
b*
Vrn
FY
FY
GPC, GT, GW, GY
Vrn
FY
Ht
GM-2
GY
Wheat Chromosome 5D
0
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Vrn-D1
PinA-D1,
PinB-D1
b*, CS, CQ, FP, FY,
GT, L, NT, Vol, W, WA
GT
GY
EPS
Vrn
Phen
GY
Phen
Vrn
Ht
Wheat Chromosome 6A
0
50
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GW
GPC
b*
Ht
Ht
GW
GW
GPC, GW
FY
FP, GY
GW
Wheat Chromosome 6B
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50
100
150
EPS, FY, GY, Ppd, Vrn
GPC-6B1
Cre8
GPC
FPC
GY
FY
GPC
CCN
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GY, Ht
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EPS, GW, GY
FP
GY

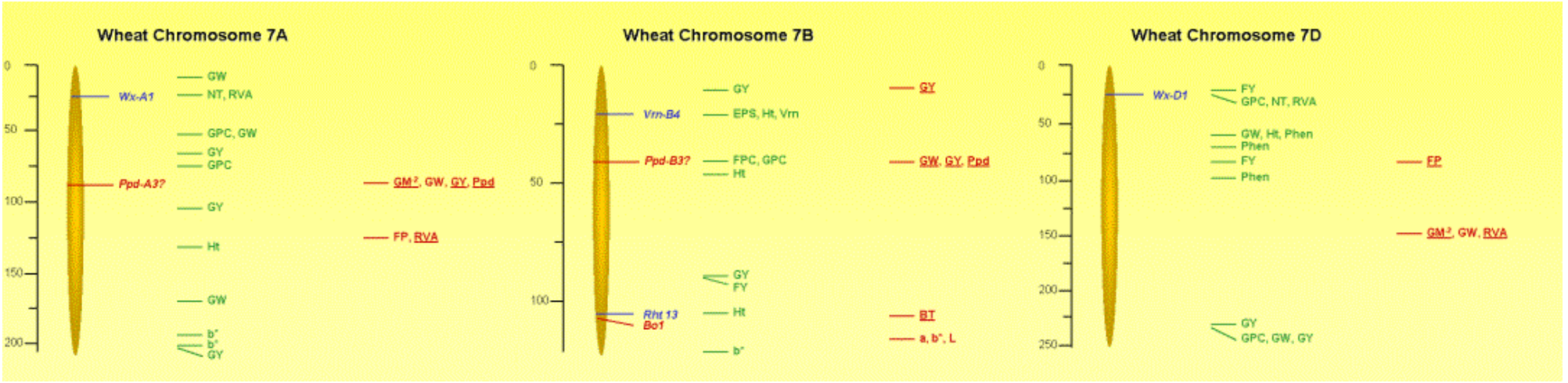

Figure 1. A summary of the chromosomal location of QTL (and if identified, genes believed to be underlying these QTL) mapped in this thesis (presented in red), and the location of QTL (in green) and genes (in blue) collated from relevant literature (see Chapter 1). The location of new loci identified in this study have also been included on the map in red followed by a question mark, indicating their putative nature and nomenclature. Some QTL (for example CCN resistance on chromosomes 1B, 2A, and 6B, and boron tolerance on 7B) were not detected as part of this thesis, but have been included from the work of Williams et al. (2006) and H. Kuchel (unpublished data) for completeness. Abbreviations for QTL are described in Table 1 of Chapter 1.

3.1 Marker-Assisted Selection of QTL Involved in the Control of Complex Traits

This study has shown that MAS for genes controlling rust resistance, agronomic, and end-use quality traits can increase the rate of genetic gain achieved in a breeding programme, whilst also improving its efficiency (Kuchel et al. 2005; Kuchel et al. 2007a). However, questions are often raised about whether or not the success observed with MAS when manipulating simply inherited traits, can be duplicated for QTL found to influence complex traits (Anonymous 2004) such as those identified in this study. Some may also point to the potential redundancy of MAS for many simply inherited traits by highlighting the success with which these traits have already been routinely manipulated using traditional methods. However, the results presented here have demonstrated that an optimum selection strategy may not utilise MAS *or* phenotypic selection but a combination of both. In fact, it appears that targeting the application of MAS within a selection strategy has the capacity to dramatically improve the rate of genetic gain and efficiency of phenotypic selection events within a breeding programme (Kuchel et al. 2005).

One of the prime objectives of MAS is to efficiently select the genotype for complex traits. However the largest limitation to the application of MAS for these traits has been gaining a thorough understanding of their genetic basis. Here the genetic dissection and subsequent characterisation of grain yield in a southern Australian genotype-environment system has been presented (Kuchel et al. 2007b; Kuchel et al. 2007c). One QTL in particular, *QGyld.agt-1B,* was shown to be associated with variation in grain yield over a large number of locations and showed only relatively minor interactions with the environment. Consequently, this locus may be a prime candidate for MAS of the complex trait, grain yield. However, given the modest improvements that are contributed by this locus (4.8% increase in grain yield),

some may claim that the use of MAS for this locus is not cost effective. In contrast to MAS for this 'minor' grain yield QTL, the marker-assisted introgression of a 'major' stripe rust resistance gene may alter a variety from susceptible to resistant. Superficially, this could be considered a more effective use of molecular markers. However, a more appropriate means of comparing response to selection is on an economic basis. The description of 'minor' and 'major' genes may be accurate genetically, but perhaps misleading economically.

In southern Australia, a stripe rust susceptible variety may need to be treated with fungicide twice to protect its grain yield potential. Consequently, the economic value of resistance can be calculated at about $15 per hectare (depending on what fungicide is used and if it is applied concurrently with other chemicals) (P. Hooper, personal communication 2006). In comparison, if an average production of two tonne per hectare and a value of $200 per tonne is assumed (a conservative assumption), the value of the 'Molineux' allele at the *QGyld.agt-1B* locus can be estimated to be in the region of around $20 per hectare. Consequently, the economic impact of selecting a 'major' gene for rust resistance, or a 'minor' gene for grain yield is similar. As breeders move toward MAS implementation for complex traits, it may be appropriate to consider adopting some concepts developed in animal breeding. Economic breeding values have been used to summarise the parental value of breeding stock for many years (Cameron 1997). Recently, this concept has been extended to include both phenotypic and genotypic measures of breeding value (Lahav et al. 2006). An economic based selection index such as this may help breeders to rationalise their breeding objectives from complex traits to individual genes.

Possibly the largest constraint to the effective manipulation of a genetically minor, but economically major gene such as *QGyld.agt-1B*, is the difficulty faced

when characterising parents and determining linkage phase between the QTL and selectable markers. The magnitude of this problem may be reduced through the use of flanking markers, but the most desirable solution is the development of diagnostic or 'perfect' markers.

3.2 Review of Experimental Procedures and Recommendations for Future Research

A thorough characterisation of the phenotypic effects of major genes previously reported in the literature has been an important output of this study. However, the study has highlighted the fact that segregation of these major genes in a mapping population can complicate the detection of minor genes/QTL. For example, end-use quality was influenced by the genes *Glu-A3*, *Glu-B1* and *Glu-B3*, and these appeared to hinder the detection of minor genetic associations for dough rheology traits. For grain yield, the height reducing genes (*Rht-B1* and *Rht-D1*) and the 'VPM' derived disease resistance locus (*Lr37/Sr38/Yr17*) explained 31% of the variance in the multi-environment trial mean for grain yield (data not shown). In many crosses made by wheat breeders, the same alleles at these major loci are likely to be carried by both parents. Consequently, the effects of unknown, and therefore perhaps unfixed QTL would be of more interest when attempting to achieve additional genetic progress through MAS. Whenever possible, statistical methods were used in this study to reduce the confounding effects of major genes. However in future, research of this nature would benefit from the use of populations not segregating for previously identified major genes. Ideally, such a population would carry "non-yield limiting" alleles at such loci. For example, based on the results from this study, a population used to study the inheritance of grain yield should be fixed for semi-dwarf stature,

early-medium maturity, rust resistance and the 'Molineux' allele at *QGyld.agt-1B*. This phenotype would be most likely to facilitate maximum expression of as yet unknown minor QTL for grain yield. Additionally, this could provide a genetic background more relevant to breeding populations and would therefore provide an accurate assessment of the breeding value attributable to a QTL.

The QTL-by-environmental covariable study presented in this thesis supported the results observed by Crossa et al. (1999), Campbell et al. (2004) and Malosetti et al. (2004) who found that of the climatic variables tested, maximum temperature had the greatest effect on the expression of grain yield QTL. However all environmental covariables assessed showed interactions with grain yield QTL.

Studies of the interactions between QTL and stripe rust severity have helped to characterise the routes by which some QTL may have influenced grain yield. This highlights the potential of extending such research beyond simple climatic characteristics into a more complex environmental analysis. For example, a simple extension of this analysis could be to retrospectively sample locations for soil related characters such as pH, sodicity and soil structure (ie clay content). These are unlikely to have changed substantially over the 2-5 years following the grain yield analyses at these locations. Characteristics such as *Heterodera avenae* and *Pratylenchus neglectus* densities, and macro- and micro-nutrient levels would also be worthwhile targets for investigation. However the passage of time would most likely reduce the validity of retrospective sampling for these traits.

Despite the experimental limitations outlined above, a number of new QTL associated with important economic traits were identified in this study but require further investigation. In particular, it would be useful to characterise the molecular and biochemical/physiological basis of the phenotypes associated with; *QRmx.agt-2A*,

QBvol.agt-3A, *QEps.agt-2AS*, *QEps.agt-6D*, *QPpd.agt-1A*, *QPpd.agt-7A*, *QPpd.agt-7B*, *QGyld.agt-1B* and *QGyld.agt-4D*.

Computer simulation of 'Annuello/2*Stylet' MAS breeding strategies showed that the use of loosely linked markers may be beneficial under some circumstances (Kuchel et al. 2005). In contrast, when this cross and selection strategy was actually undertaken, the results suggested that the development of closely linked markers for QTL will result in far greater rates of genetic gain (Kuchel et al. 2007a). In order to achieve effective MAS of the QTL from this study, or accurate parental genotypic classification for use in cross prediction, it would be beneficial to identify closely linked markers, or more preferably, clone the genes underlying these QTL. Figure 2 illustrates a process by which the cloning of genes underlying each of the QTL could be achieved. Briefly, new populations could be produced for each QTL being targeted, by inter-crossing 'Trident/Molineux' (T/M) DH lines with genotypes that differ for only one QTL/gene affecting the trait of interest (assuming that these DHs exist). The objective would be to fix all other QTL/genes associated with the trait, allowing the trait to be assessed in a semi-qualitative (rather than quantitative) manner. Similar approaches, utilising crosses with backcross derived near-isogenic lines or chromosome substitution lines, have been used previously to simplify the genetics underlying complex traits (Kota et al. 2006; Lagudah et al. 2006).

Positional cloning in wheat has been largely limited to discrete genes (Keller et al. 2005). Yan et al. (2003) and Yan et al. (2004) reported the cloning of two genes involved in the control of vernalisation sensitivity, while the domestication gene *Q* was cloned by Faris et al. (2003). Three disease resistance genes in wheat have also been successfully cloned, *Pm3b* (Yahiaoui et al. 2004), *Lr10* (Feuillet et al. 2003) and

Lr21 (Huang et al. 2003). Lastly, in 2006 Uauy et al. reported the positional cloning of the high grain protein content gene.

Of the new QTL identified in this study, success may be most likely for *QPpd.agt-7A* and *QPpd.agt-7B*, where a photoperiod responsive QTL in rice (*Hd1*) on chromosome 6, homoeologous to the position of these QTL (Laurie 1997; Kuchel et al. 2006a), has been finely mapped, and the gene responsible (*Se1*) for the variation cloned (Yano et al. 2000). More recently, Yan et al. (2006) have identified a gene associated with vernalisation response on a syntenous region of chromosome 7B in wheat. For traits such as grain yield and some end-use quality traits which tend to suffer from particularly high experimental error and environmental interaction, it is likely that data will need to be collected from multiple environments and perhaps years. However the benefits to the rate of achievable genetic gain would warrant such scientific investment. This could prove cost prohibitive for the end-use quality traits which are particularly expensive to measure. However, in these cases, a step-wise process could be used for the phenotypic measurement. A sub-population (50 individuals for example) could be assessed using a grain composite from two or three locations, which may enable the chromosomal region flanking the QTL to be narrowed. The remaining 450 individuals could then be typed with markers located within the narrowed region surrounding the QTL to determine those that have undergone recombination within this region. Further (rigorous) phenotypic assessment would then be limited to the lines carrying the key recombination events. Consequently, it may be possible to fine map or even clone the genes responsible for an end-use quality QTL by processing a relatively low number of samples.

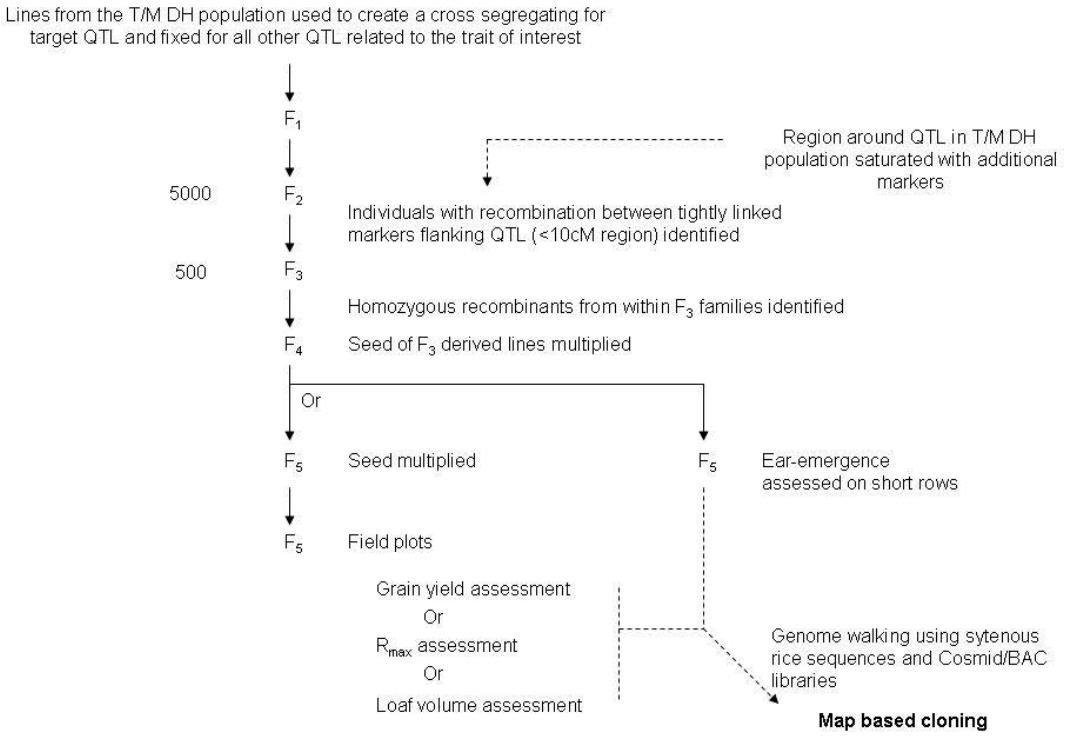

Figure 2. A schematic of the methodologies that may be used to fine map/clone the genes responsible for the QTL identified in this study

3.3 An Assessment of the Genetic Basis to the Elite Performance of the Variety 'Stylet' and its Implications on Breeding

'Stylet' was an elite variety, but was not released commercially because of changes in rust pathotypes in Australia that rendered the variety susceptible to all three rust diseases. However, due to its wide adaptation 'Stylet' became a very important parent within the South Australian based Australian Grain Technologies breeding programme. The aim of the original breeding strategy that resulted in 'Stylet' was to develop a CCN resistant and improved end-use quality version of the widely adapted variety 'Trident' (G.J. Hollamby, personal communication 2001). In this study, detailed QTL analysis of a mapping population created between 'Stylet's'

parents ‘Trident’ and ‘Molineux’ identified a wide range of favourable QTL alleles from both parents. An ideal molecular ideotype can be produced by the combination of these favourable alleles, and this ideotype can in turn be compared with the genotype of ‘Stylet’ (Figure 3).

Given that ‘Stylet’ is a product of the backcross ‘Molineux/2*Trident’ it is not surprising that a large number of the favourable QTL from ‘Trident’ were incorporated into this variety. The CCN resistance gene *Cre8* was successfully introgressed from ‘Molineux’ into ‘Stylet’ (the major target of the breeding strategy). However, the improved quality glutenin alleles on chromosomes 1A and 1B, and a secondary CCN resistance locus on 1B (Williams et al. 2006) were not inherited by ‘Stylet’. Also, although the breeding programme used to select ‘Stylet’ had a large focus on improved grain yield (GJ Hollamby, personal communication 2001), the *QGyld.agt-1B* allele from ‘Molineux’ was not incorporated into this variety. This may call into question the importance of the *QGyld.agt-1B* QTL in adaptation to southern Australia. Equally, this result may highlight the extent of environmental variation that limits response to selection for grain yield when selected phenotypically. Although ‘Stylet’ represents the best line derived from a series of crosses based on ‘Trident’ and ‘Molineux’ from the South Australian breeding programme, this work shows that it does not represent the best combination achievable from this parental combination.

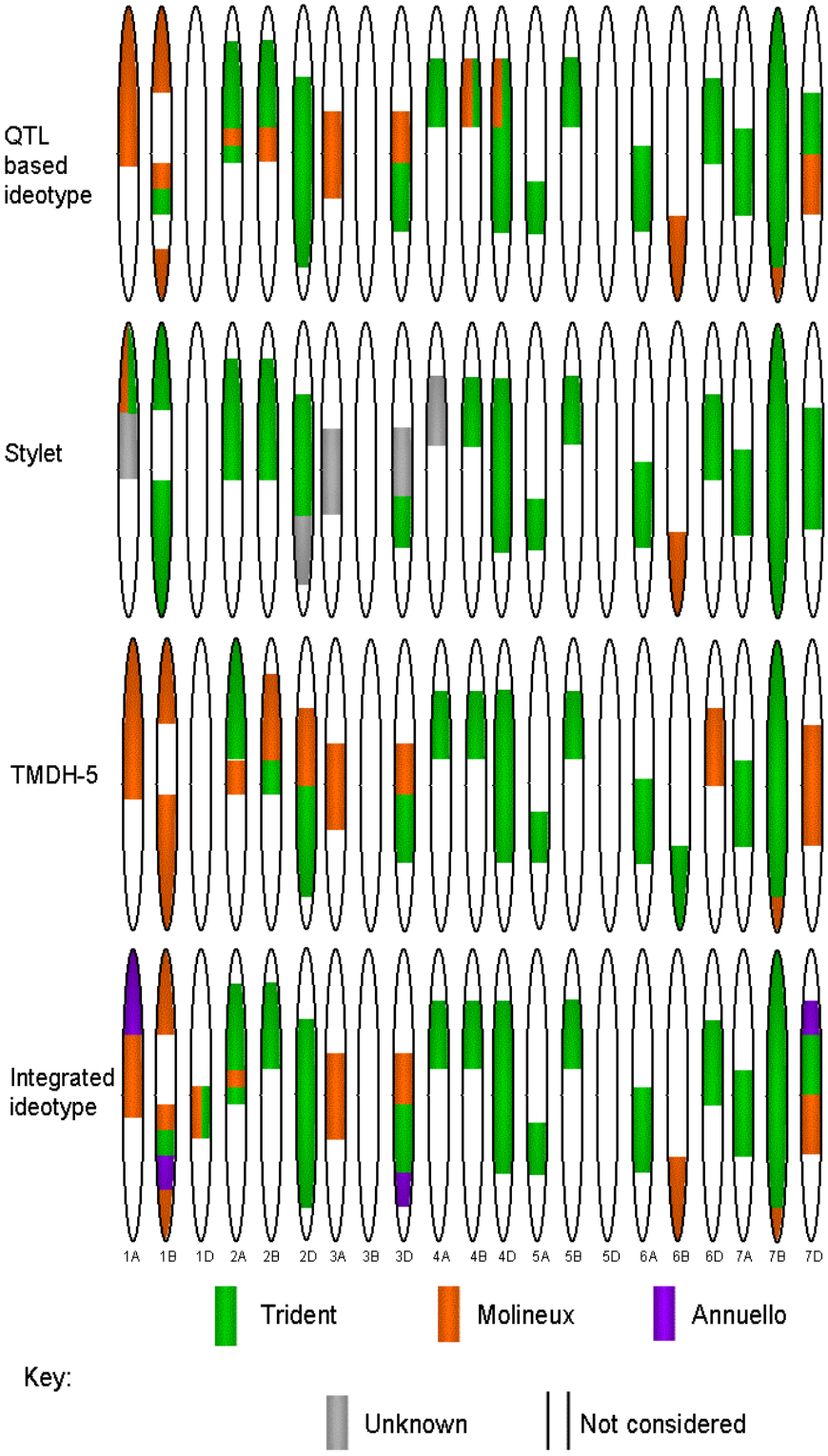

Figure 3. Graphical representation of the ideal QTL based genomic ideotype (as determined from QTL studies Kuchel et al. (2006a), Kuchel et al. (2006b), Kuchel et al. (2007b) and Kuchel et al. (2007c), the elite variety 'Stylet', and an elite DH from the T/M DH population (TMDH-5). Finally, an integrated genomic ideotype is proposed that combines each of the QTL from this study and the rust resistance and quality genes from 'Annuello' targeted in Kuchel et al. (2007a) and Kuchel et al. (2005).

The expected frequency of individuals with the QTL based ideotype (as characterised in Figure 3), in a non-selected population of fixed lines from a 'Molineux/2*Trident' cross, can be calculated as less than 1 in 10^{11} (calculations not shown). A figure such as this, puts into perspective, the success (or good fortune) of the breeding strategy that produced 'Stylet'. However, it also highlights the importance of developing breeding strategies, such as the MAS based strategy outlined in Kuchel et al. (2005) and Kuchel et al. (2007a), that are capable of improved efficiency and genetic improvement through accurate selection within early generation segregating populations thereby improving the probability of success.

3.4 A Breeding Strategy Incorporating the Knowledge Gained from this Study

A search of the T/M DH population used in this study identified an individual, 'TMDH-5' that partially resembled the "ideal" molecular ideotype proposed in Figure 3. As with 'Stylet', a number of favourable alleles were not inherited by this line. However, a comparison of the genotypes of 'Stylet' and 'TMDH-5' indicate that it may be possible to obtain the desired ideotype by making a cross between them. Only one locus, the chromosome 2A RVA QTL would be unable to be incorporated within lines derived from this cross. Unfortunately, such a cross would be purely academic, as rust pathotypes have developed in Australia that are virulent on each of the genes at the *Lr37/Sr38/Yr17* locus. Consequently, a more useful breeding strategy may attempt to combine this cross with an elite line generated from the work of (Kuchel et al. 2007a). An 'Annuello/2*Stylet' DH ('CO6476') that possesses the *Lr34/Yr18*, *Lr46/Yr29* and *Lr24/Sr24* resistance alleles, and the *Glu-A3b* allele from 'Annuello' whilst retaining many of the favourable QTL captured in 'Stylet', could be used as a

donor of improved rust resistance and end-use quality. The following breeding strategy (Figure 4) is suggested as a means of producing a variety, based on 'Stylet', that should possess improved grain yield, end-use quality and rust resistance (integrated ideotype in Figure 3). This strategy relies heavily on the conclusions drawn from Kuchel et al. (2005) and Kuchel et al. (2007a). A substantial investment of resources in MAS during the early stages of this strategy is suggested. Kuchel et al. (2005 & 2007a) concluded that allele enrichment through MAS in segregating populations is likely to result in large genetic gains. Given the number of QTL/genes being selected in this cross, it is very unlikely that a TC_1F_1 individual would be identified carrying each of the targeted loci from 'TMDH-5' and 'CO6476'. Consequently, the recombinant F_2 system suggested by Howes et al. (1998) is recommended in order to reduce cost.

Although the number of individuals required to achieve the desired genetic outcome are indicated alongside the strategy (Figure 4), the effects of linkage have not been taken into account when calculating these figures, and the numbers are presented as a guide only. Computer simulation of this cross would allow the optimisation of MAS events, taking into account linkage between targeted genes, and the impact of MAS on the effectiveness of phenotypic selection at later generations. Although it should be possible, a breeding strategy aimed at achieving the desired ideotype within one breeding cycle, is ambitious. It would require DNA to be extracted from at least 20,000 individuals, and even if step-wise MAS was used, around 150,000 marker assays would be required. Consequently, the molecular costs associated with this strategy would probably reach $150,000 - $200,000 (Kuchel et al. 2005). This strategy is an example of what could be achieved within a single

population if sufficient genetic knowledge is available for the parents being manipulated and if substantial resources are available for MAS.

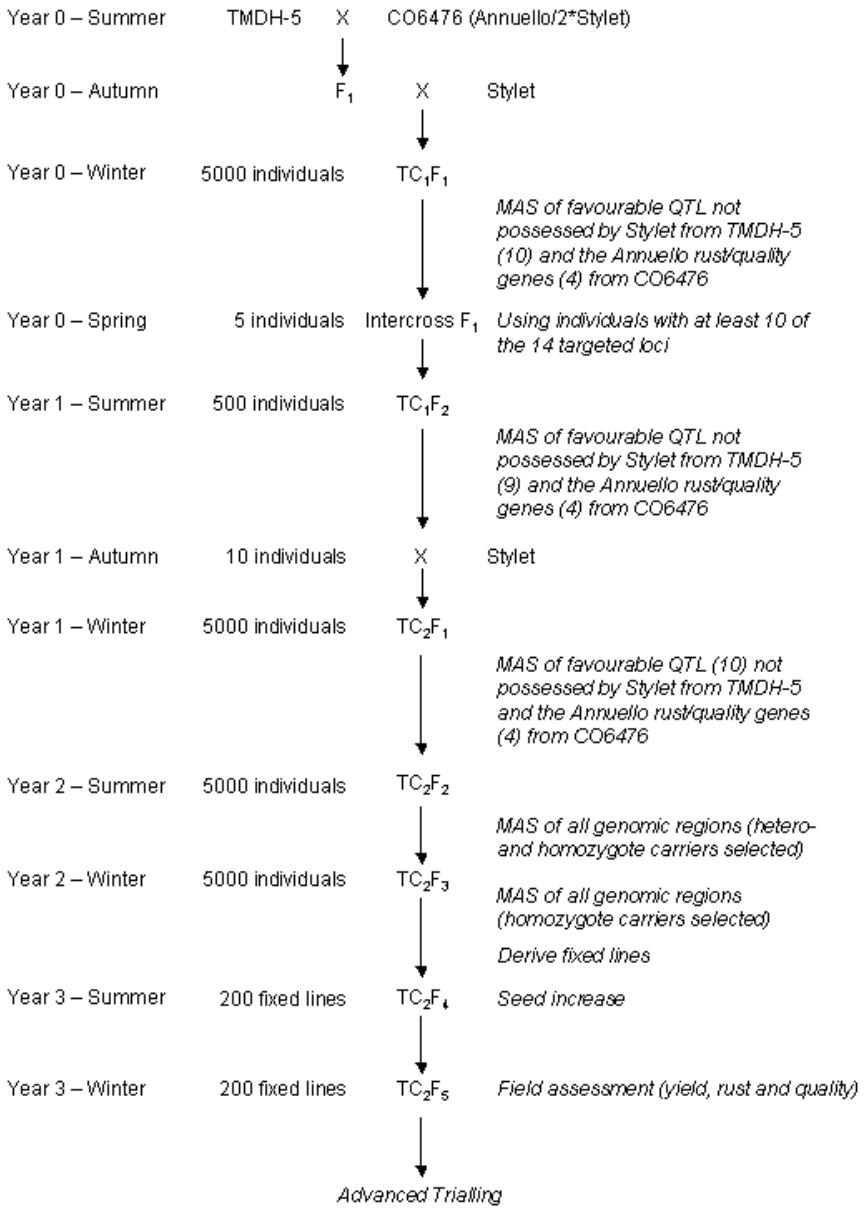

Figure 4. A breeding strategy is proposed to incorporate the favourable QTL identified within the T/M DH mapping population (Kuchel et al. 2006a; Kuchel et al. 2006b; Kuchel et al. 2007b; Kuchel et al. 2007c) and additional rust resistance and end-use quality genes from 'Annuello'. This strategy has been designed using conclusions from the analysis of MAS strategies undertaken as part of this study (Kuchel et al. 2005; Kuchel et al. 2007a).

The crossing and selection strategy outlined above implements the results from the genetic analyses undertaken during this study (Kuchel et al. 2006a; Kuchel et al. 2006b; Kuchel et al. 2007b; Kuchel et al. 2007c) and the MAS strategies developed as part of this research programme (Kuchel et al. 2005; Kuchel et al. 2007a). If this strategy is followed (after computer simulation assisted optimisation), a series of elite lines could be generated that carry all but one of the favourable QTL/genes identified in this study. The resultant lines would be semi-dwarf, early maturing, carry multiple disease resistance loci, possess elite baking properties through the combination of favourable glutenin alleles and end-use quality QTL, and carry desirable grain yield and grain weight QTL identified in this study (Table 1). Without the results from this study, this aggressive MAS breeding strategy would be inconceivable. It is possible that alternative strategies could be proposed to achieve the same outcome. However these would have to encompass multiple crossing and phenotypic selection cycles, and even then the likelihood of accumulating each of the QTL targeted by phenotypic selection would be so low that most breeders would not consider it a viable option. Ultimately, MAS allows breeders to design and implement breeding strategies that they would not otherwise consider feasible.

Table 1. A list of the QTL/gene alleles expected to be carried by the 200 wheat lines generated by the MAS breeding strategy outlined in Figure 4.

Agronomic			Grain yield and yield components			Disease resistance			End-use quality		
QTL/gene	Loc[1]	Allele	QTL/gene	Loc	Allele	QTL/gene	Loc	Allele	QTL/gene	Loc	Allele
Rht-B1b	4BS	'Trident'	*QGyld.agt-1B*	1BS	'Molineux'	*Lr37/Sr38/Yr17/Cre5*	2AS	'Trident'	Glu-A3	1AS	'Annuello'
Rht-D1b	4DS	'Trident'	*QGyld.agt-2D*	2DL	'Trident'	*Lr34/Yr18*	7DS	'Annuello'	*Glu-D1*	1DL	'Trident' or 'Molineux'
QPpd.agt-1A	1AL	'Molineux'	*QGyld.agt-3D*	3DL	'Trident'	*Lr46/Yr29*	1BL	'Annuello'	*QRmx.agt-2A*	2AS	'Molineux'
QEps.agt-2AL	2AL	'Trident'	*QGyld.agt-4A*	4AS	'Trident'	*Lr24/Sr24*	3DL	'Annuello'	*QBVol.agt-3A*	3AS	'Molineux'
QEps.agt-2AS	2AS	'Trident'	*QGyld.agt-4D*	4DL	'Trident'	*Cre8*	6BL	'Molineux'	*QExt.agt-3D*	3DS	'Molineux'
Ppd-B1	2BS	'Molineux'	*QGyld.agt-5B*	5BS	'Trident'	*QCre.srd-1B*	1BL	'Molineux'	*Qb*.agt-7B*	7BL	'Molineux'
Vrn-A1	5AL	'Trident'	*QGyld.agt-6A*	6AL	'Trident'				*QFpc.agt-7D*	7DS	'Trident'
QEps.agt-6D	6DS	'Trident'	*QGyld.agt-6D*	6DS	'Trident'						
QPpd.agt-7A	7AS	'Trident'	*QGyld.agt-7B*	7BS	'Trident'						
QPpd.agt-7B	7BS	'Trident'	*QGno.agt-5B*	5BS	'Molineux'						
Bo1	7BL	'Trident'	*QTgw.agt-7D*	7DL	'Molineux'						

[1]The chromosomal location of the QTL/gene

3.5 Conclusion

This study has investigated the genetic basis of a series of complex and economically important traits in a southern Australian environment. The elite breeder's line 'Stylet' was used as the basis of this research. The first aim was to investigate the genes/QTL underlying end-use quality, phenology and grain yield within a population created from the parents of 'Stylet', namely 'Trident' and 'Molineux'. The second aim was to examine a series of MAS strategies aimed at producing a rust resistant and end-use quality elite backcross derivative of 'Stylet'. This series of research papers has:

- Identified genomic regions associated with various end-use quality traits, including milling quality, dough rheology and baking potential.

- Located and characterised the phenological action of ear-emergence QTL in the 'Trident' × 'Molineux' DH population.

- Determined the effects of plant height, ear-emergence, and rust resistance genes/QTL on grain yield in the southern Australian wheat belt.

- Located novel QTL associated with grain yield that are apparently unrelated to plant height, ear-emergence and rust resistance.

- Characterised the interaction of grain yield QTL and specific environmental features including stripe rust infection, temperature, rainfall and latitude.

- Utilised computer based simulation to assess the effectiveness and efficiency of various complex MAS regimes for a specific cross.

- Generated elite rust resistant and superior end-use quality derivatives of the elite breeder's line 'Stylet' using MAS.

These findings demonstrate the benefits of comprehensive genetic analyses of complex traits in wheat. The results from this thesis should provide southern Australian breeders with the tools required to begin detailed MAS of economically important traits. The conclusions from the MAS simulation and application studies within this thesis have highlighted the economic and genetic benefits of utilising genotypic selection within early generation segregating populations. When combined with the genetic knowledge generated through work on end-use quality, phenology and grain yield traits, it is expected that MAS should improve the rate of genetic gain that may be achieved for wheat growers. Armed with a thorough understanding of the genetic basis of one's target traits, and equipped with the tools required to manipulate those genetic loci, breeders may be prompted to ask; "Should I be spreading my breeding programme's resources across a large number of crosses and selecting within them using phenotypic selection, or would my budget be better allocated to just a few crosses that are then highly leveraged with detailed MAS?"

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Appendix 1

Additional Research Articles Arising

Williams, K., Willsmore, K., Olson, S., Matic, M. and Kuchel, H. (2006) Mapping of a novel QTL for resistance to cereal cyst nematode in wheat.
Theoretical and applied genetics v.112 (5) pp. 1480-1486, May 2006

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