

Flour yield QTLs in three Australian doubled haploid wheat populations

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Abstract. Flour yield quantitative trait loci (QTLs) were identified in 3 Australian doubled haploid populations, Sunco × Tasman, CD87 × Katepwa, and Cranbrook × Halberd. Trial data from 3 to 4 sites or years were available for each population. QTLs were identified on chromosomes 2BS, 4B, 5AL, and 6BL in the Sunco × Tasman population, on chromosomes 4B, 5AS, and 6DL in the CD87 × Katepwa population, and on chromosomes 4DS, 5DS, and 7AS in the Cranbrook × Halberd population. In the Sunco × Tasman cross the highest genetic variance was detected with the QTL on chromosome 2B (31.3%), in the CD87 × Katepwa cross with the QTL on chromosome 4B (23.8%), and in the Cranbrook × Halberd cross with the QTL on chromosome 5D (18%). Only one QTL occurred in a similar location in more than one population, indicating the complexity of the flour yield character across different backgrounds.

Additional keywords: *Triticum aestivum*, doubled haploid lines, QTL mapping.

Introduction

Empirical breeding and cultural advances of farmers and plant breeders over many millennia have resulted in increases in the productivity of domestic crop plants. The driver has been the need to provide food, feed, and fibre sufficient for an ever-increasing global human population. Gains resulting from cultural practices are, however, limited and future gains in productivity may rely almost entirely on genetic improvements (Stuber *et al.* 1999). Plant breeders may, therefore, need to deploy new technologies such as marker-assisted selection to more efficiently improve the yield potentials of crop plants.

Increased flour yield is one of the most important traits in wheat breeding programs in Australia for both domestic and international markets. Marker-assisted breeding for flour yield and flour quality traits is a major challenge because these traits are controlled by multiple-interactive and environmentally dependent quantitative trait loci (QTLs) that may have low heritability (Yin *et al.* 2003). Location, genotype, and genotype × environment interaction effects are highly significant for flour yield (Kato *et al.* 2000; Campbell *et al.* 2001; Groos *et al.* 2003).

To date only a few studies have reported flour yield QTLs in bread wheat populations (Parker *et al.* 1999;

Campbell *et al.* 2001; Smith *et al.* 2001). In a cross between Schomburgk and Yarralinka, using 150 F₄-derived single-seed descent lines, chromosomes 3A, 5A, and 7D were significantly associated with flour yield (Parker *et al.* 1999). The QTL identified on chromosome 3A was associated in all 3 datasets available for the study, whereas chromosomes 5A and 7D were associated with flour yield in 2 out of the 3 datasets. In this study a large proportion of the genetic variation was accounted for by the QTLs, suggesting that a significant proportion of flour yield variation was being controlled by a few loci with fairly large effects.

The hardness locus *Pinb* on chromosome 5D had a strong influence on flour yield in a cross between soft and hard wheat (NY18/CC) (Campbell *et al.* 2001). Significant associations between flour yield and loci on 1B, 3B, and 6B were also identified in this study. When the analysis was controlled for the effects of *Pinb*, significant additional loci were observed on 3A and 4AL.

Smith *et al.* (2001) have discussed statistical issues involved in the analysis of quality traits and presented a method of analysis that allowed for variation arising from the field and laboratory phases. One field trial each of the Cranbrook × Halberd population and the CD87 × Katepwa population was used to illustrate the technique. Using a

mixed modelling multiple regression approach and early versions of the population maps, QTLs were identified on chromosomes 3B, 5B, and 7D in the Cranbrook \times Halberd cross and on chromosomes 1D, 5B, and 6B in the CD87 \times Katepwa cross.

Several Australian doubled haploid populations were developed for the GRDC-funded National Wheat Molecular Marker Program (1996–2001), now known as the Australian Winter Cereals Molecular Marker Program (AWCMMP). These populations were established in order to identify genes associated with quality, agronomic, and disease characteristics (Kammholz *et al.* 2001). Linkage maps were constructed for these populations (Chalmers *et al.* 2001) with a number of laboratories contributing data towards the maps. Lehmsiek *et al.* (2005) have reported on the subsequent amendment and refinement of these maps. The aim of the current study was to integrate the revised maps with the more extensive flour yield datasets now available for the AWCMMP populations so that potential markers for flour yield, applicable in marker-assisted selection, could be evaluated.

Materials and methods

Plant material

Three wheat, *Triticum aestivum* L., doubled haploid (DH) populations, Sunco \times Tasman (S \times T), CD87 \times Katepwa (CD \times K), and Cranbrook \times Halberd (C \times H) were used in this study. Both the S \times T and CD \times K populations were produced at the Leslie Research Centre, Toowoomba, Queensland, and consist of 180 DH lines (Kammholz *et al.* 2001). The C \times H population was produced by Dr R. Islam, Adelaide University, and consists of 161 DH lines (Chalmers *et al.* 2001). The S \times T population was grown at Roma (Qld) in 1998 (Roma98) and 2000 (Roma00), Roseworthy (South Australia) in 1999 (Rosw99) and Stow (SA) in 2000 (Stow00). Field trials for the CD \times K population were conducted at Roma in 1998 (Roma98) and 1999 (Roma99), Wongan Hills (Western Australia) in 1999 (Wong99), and Horsham (Victoria) in 2000 (Hors00). The Roma98 and Roma99 trials were split into quick- and mid-maturing lines and grown in separate trials. The C \times H DH population was grown at Roma in 1997 (Roma97) and Stow in 1997 (Stow97) and 1998 (Stow98). The plots at Roma were grown in 7 rows with 25-cm gaps between rows and 40-cm gaps between plots. The total plot harvest area was 8.75 m². The trial at Horsham was grown in 6 rows with 18-cm gaps between rows and 40-cm gaps between plots. The total plot harvest area was 6.3 m². The other trials were sown as 6-row plots with 18 cm between rows and 35 cm between plots, having total plot areas of 4.0 m².

Two replicates were grown for most field trials (exceptions are Wong99, Stow97, and Stow98, which were not replicated) and the parental lines and a selection of other varieties were included in the trials to be used as check plots in spatial analysis.

Flour yield data and QTL analysis

A standard method was used to mill the samples of the 3 different populations. Flour yield (% flour) was obtained by milling grain samples of approximately 600 g through a Bühler MLU-202 pneumatic mill by approved AACC method 26-21A (AACC 2000). Prior to milling, the samples were conditioned to a moisture content of 15%. A differential conditioning regime, depending on the grain hardness, was used for the C \times H population to determine the amount of water needed to

condition the samples. The harder the grain the higher the conditioning level required.

A method of statistical analysis presented by Smith *et al.* (2001) was used to allow for variation arising from the laboratory phases and thus to ensure the reproducibility of the flour samples.

Heritability, defined as being the proportion of total variation explained by genetic effects, was calculated using the approximation given in Cullis *et al.* (2006).

The construction and revision of the linkage maps are described in Lehmsiek *et al.* (2005). QTL analysis was performed using the multi-site method of Verbyla *et al.* (2003) where QTL effects are fitted as random effects. Non-genetic effects due to field and laboratory variation were also accounted for in the analysis. This method produced both an overall LOD score for the amount of genetic variation across sites due to a QTL, as well as LOD scores for the strength of the QTL effect at each site. QTL figures were produced with MapChart version 2.1 (Voorrips 2002).

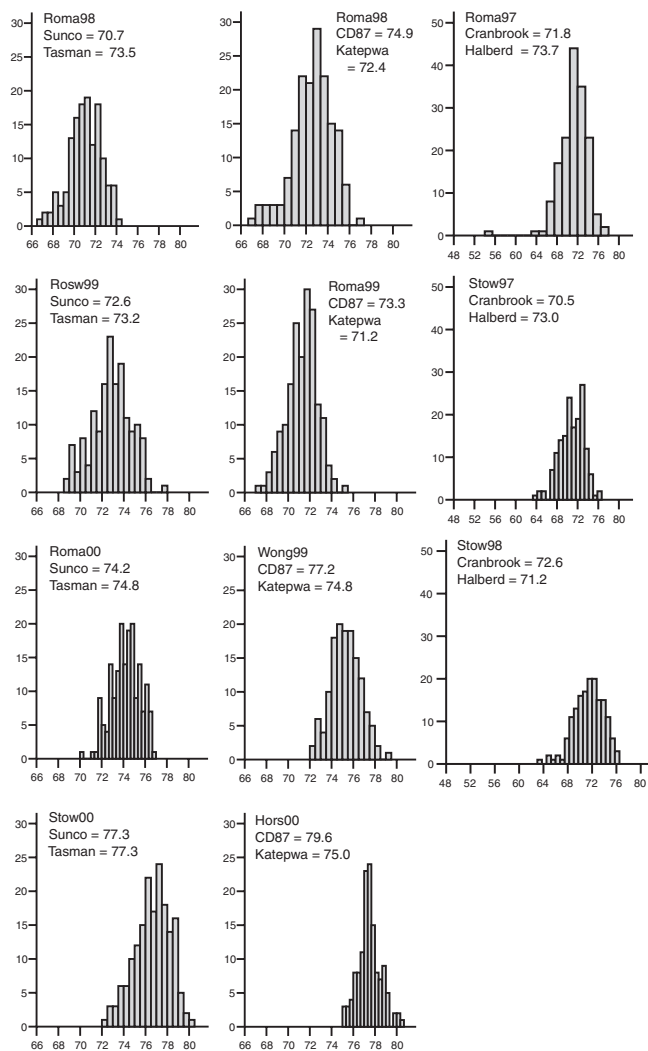


Fig. 1. Frequency and distribution of flour yield. The y-axis in all graphs is the number of DH lines; the flour yield trait values are indicated on the x-axis. Flour yield of the parental lines is presented.

Results

The assumption that flour yield is normally distributed appears reasonable for all populations and sites (Fig. 1). Large shifts in flour yield ranges could be observed within different years and environments, especially in the S × T and CD × K populations where means ranged between 71 and 77. A significant range among the progeny was observed, although in most instances the average flour yield of the parental lines differed by only a small amount. In most trials, the parental line Tasman produced a higher flour yield than Sunco, CD87 produced a higher yield than Katepwa, and Halberd produced a higher yield than Cranbrook. The minimum and maximum values among the DH lines for each population significantly exceeded the parental values thus indicating transgressive segregation and suggesting that flour yield was improved by alleles from both parents.

Similar heritability estimates were observed for each population and each site (Table 1). These heritability values are estimations of the proportion of total variation that is due to genetic effects.

QTL analysis

Chalmers *et al.* (2001) reported on the initial construction of the linkage maps of the 3 crosses used in this study. These maps have since been extended and improved by adding new markers, re-ordering markers, and editing the marker data for double crossovers (Lehmensiek *et al.* 2005).

Flour yield data for 4 sites were available for the S × T and CD × K populations and for 3 sites for the C × H population.

LOD scores for the strength of a QTL effect at each site were calculated, as well as multi-site (overall) LOD scores for the amount of genetic variation across all sites due to a QTL (Table 1). To give an idea of the most significant QTLs identified, QTLs that had a multi-site LOD score greater than 2.5 and at least 2 single-site LOD scores greater than 2 are indicated in Table 1.

In the S × T population, QTLs were identified on chromosomes 2BS, 4B, 5AL, and 6BL (Table 1, Fig. 2). The QTL on chromosome 2B had the highest overall LOD score (13.1). This QTL explained 14.4–31.3% of the genetic variance across different sites. The QTL on chromosome 4B had an overall LOD score of 6.9 and a genetic variance of 4.7–9.9%. Associations between flour yield and markers on chromosome 5AL produced an overall LOD score of 4.5 and 3.8–11% of the genetic variance was explained by this QTL across different sites. An overall LOD score of 7.1 was observed for the QTL on chromosome 6BL and this QTL explained 6.3–13.7% of the genetic variance. The QTL on chromosome 2B was contributed by Sunco, whereas the other 3 QTLs were contributed by Tasman.

Three QTLs, located on chromosomes 4B, 5AS, and 6DL, were identified in the CD × K population (Table 1, Fig. 3). The QTL on chromosome 4B was contributed by Katepwa, whereas the other 2 QTLs were contributed by CD87. The QTL on chromosome 4B had the highest overall LOD score (8.8) and explained 13.6–23.8% of the genetic variances across different sites. The QTLs on chromosomes 5AS and 6DL had overall LOD scores of 4.3 and 2.7, respectively. The highest genetic variance explained by the QTL on 5A

Table 1. Summary of chromosome arms bearing QTLs identified for each site in the S × T, CD × K, and C × H populations

LOD scores are given together with the percent genetic variance (in parentheses) explained by each QTL. The parental lines contributing the QTL are indicated in the second column (S, Sunco; T, Tasman; CD, CD87; K, Katepwa; C, Cranbrook; H, Halberd). Heritability estimates are given for each population and site

Chromosome	Parent	Sites				
		Roma98	Rosw99	Roma00	Stow00	Multi-site
S × T						
2BS	S	1.8 (3.8)	12.2 (23.3)	6.7 (14.4)	>15 (31.3)	13.1
4B	T	2.1 (4.7)	–	3.2 (6.8)	6.6 (6.9)	6.9
5AL	T	4.2 (10.8)	5.9 (11.0)	1.7 (1.1)	4.6 (3.8)	4.5
6BL	T	6.3 (13.7)	3.8 (6.3)	–	6.7 (9.8)	7.1
Heritability		0.835	0.854	0.861	0.833	
CD × K						
		Roma98	Roma99	Wong99	Hors00	Multi-site
4B	K	–	–	7.6 (13.6)	10.7 (23.8)	8.8
5AS	CD	–	2.2 (4.1)	2.2 (2.2)	6.4 (12.9)	4.3
6DL	CD	1.8 (4.3)	4.4 (9.6)	2.9 (5.5)	1.1 (2.3)	2.7
Heritability		0.784	0.851	0.869	0.792	
C × H						
		Roma97	Stow97	Stow98		Multi-site
4DS	H	3.9 (6.9)	6.6 (11.7)	1.3 (3.2)		4.5
5DS	H	10.0 (18.0)	6.9 (12.7)	2.1 (5.2)		7.2
7AS	C	3.2 (6.8)	3.1 (6.2)	–		2.6
Heritability		0.901	0.865	0.876		

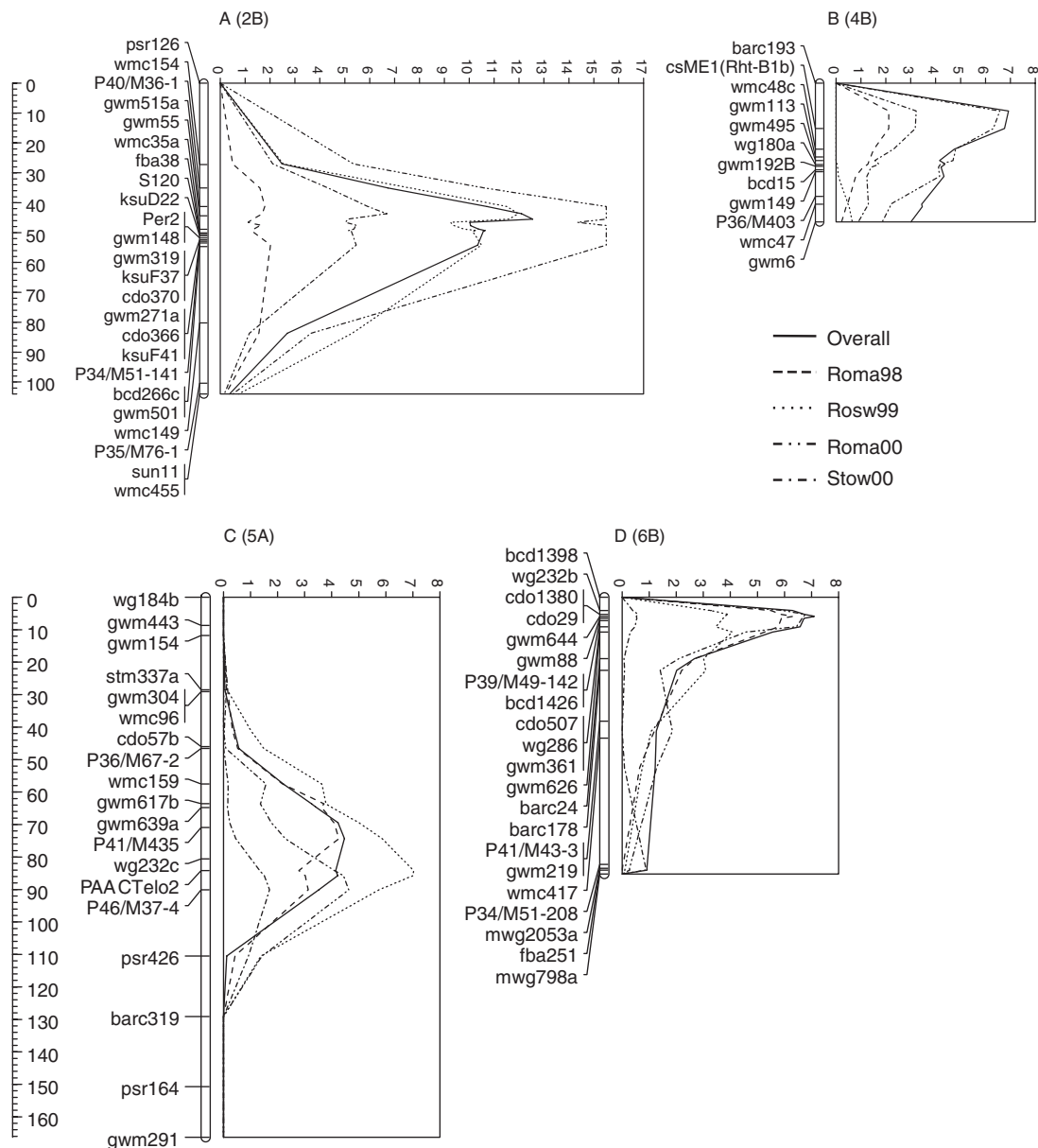


Fig. 2. Map location of flour yield QTLs of the S × T cross. Chromosome numbers are given in parentheses. LOD scores are indicated on the x-axis and a scale of genetic distance (in centimorgans) is provided.

was 12.9%, whereas the highest genetic variance explained by the QTL on 6DL was 9.6%. With 1 out of the 4 sites (Roma98) of this population, no significant QTL results were produced.

In the C × H population the overall LOD scores for the QTLs identified on chromosomes 4DS, 5DS, and 7AS were 4.5, 7.2, and 2.6, respectively (Table 1, Fig. 4). Between 3.2 and 12% of the genetic variance was explained by the QTL on chromosome 4DS and 6.2–6.8% of the genetic variance was explained by the QTL on 7AS. The QTL on chromosome 5D was associated with the hardness locus *Pina* and explained 5.2–18% of the genetic variance across different sites. This

QTL and the QTL on chromosome 4DS were contributed by Halberd, whereas the QTL on 7AS was contributed by Cranbrook.

In addition to the QTLs in Table 1, there were several QTLs that fulfilled the criterion of a multi-site LOD score of >2.5 but that showed a LOD score of >2 at only one site. These were identified on chromosome 2DS (overall LOD = 4.4) in the CD × K population and on chromosome 2BS (overall LOD = 3.6) and 2DS (overall LOD = 4.7) in the C × H population. The QTLs on chromosome 2D identified in these 2 populations were located in the same chromosomal region. In the CD × K cross the QTL on 2D explained 9.5% of

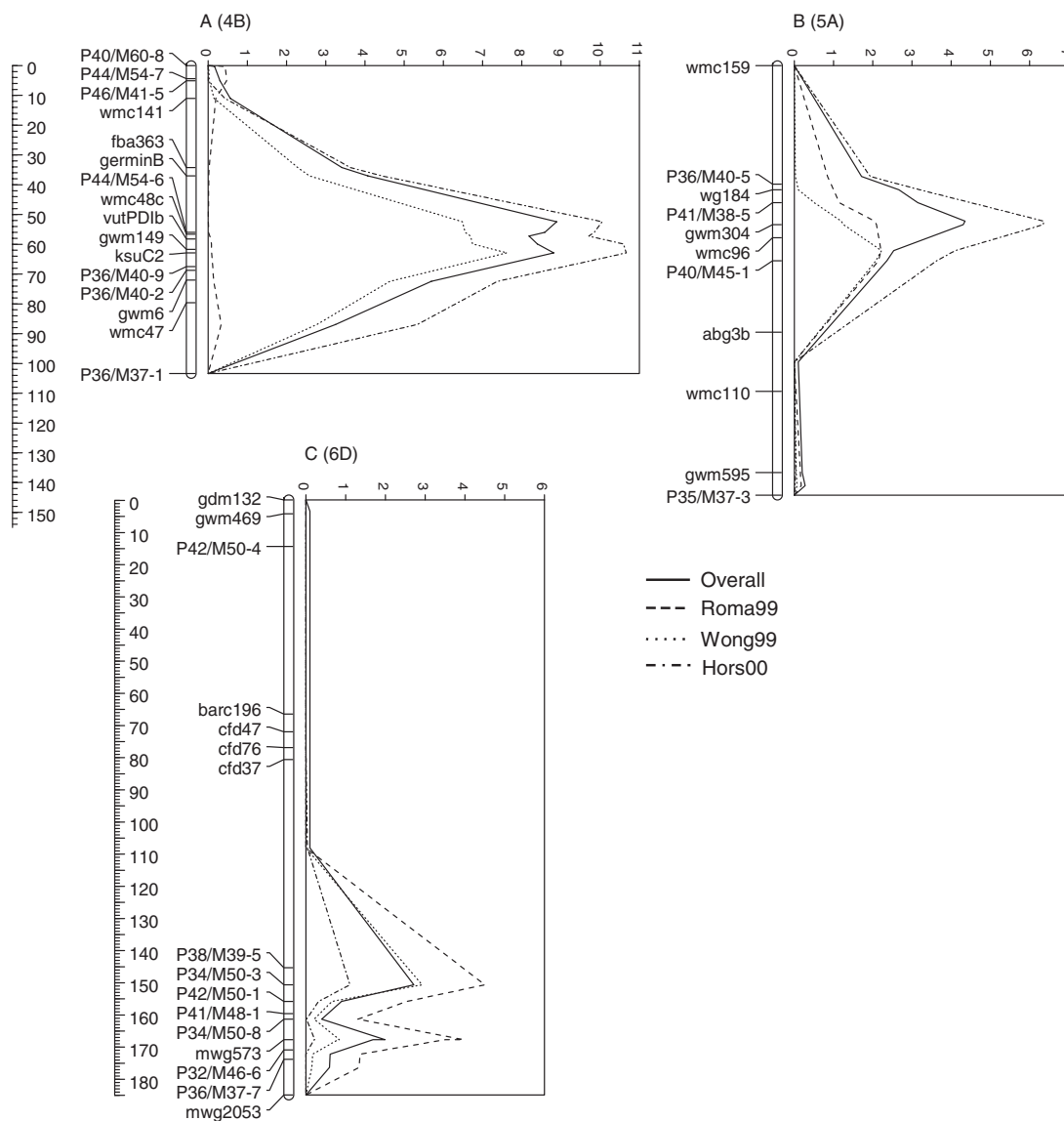


Fig. 3. Map location of flour yield QTLs of the CD × K cross. Chromosome numbers are given in parentheses. LOD scores are indicated on the x-axis and a scale of genetic distance (in centimorgans) is provided. Roma98 did not produce any significant QTL results and is, therefore, not included in the figure.

the genetic variance, whereas in the C × H cross it explained 6.8% of the genetic variance. The QTL on 2B in the C × H cross explained 4.8% of the genetic variance. A QTL on 2BS was also identified in the S × T population; however, this QTL was located on a translocation from *Triticum timopheevii*. Further investigation is required to determine if this is an example of synteny between related species.

Discussion

Flour yield ranges varied significantly among sites of each population, reflecting the importance of environmental factors in flour yield. In all 3 populations the variation in the population extended well beyond the variation in parental

scores thus suggesting that both parental lines contain alleles associated with high and low flour yield. This was confirmed by QTL analysis.

Four flour yield QTLs were identified in the S × T population, and 3 each in the CD × K population and the C × H population. Of these, only the QTLs on chromosome 4B of the S × T and the CD × K population seemed to be located in the same region. Additionally, QTLs were identified in the same region on chromosome 2D in the CD × K and C × H crosses. These QTLs were, however, only identified at a single site. The QTLs on chromosome 4B in the S × T and CD × K population were located in the centromere region. Dwarfing genes have been identified just above the

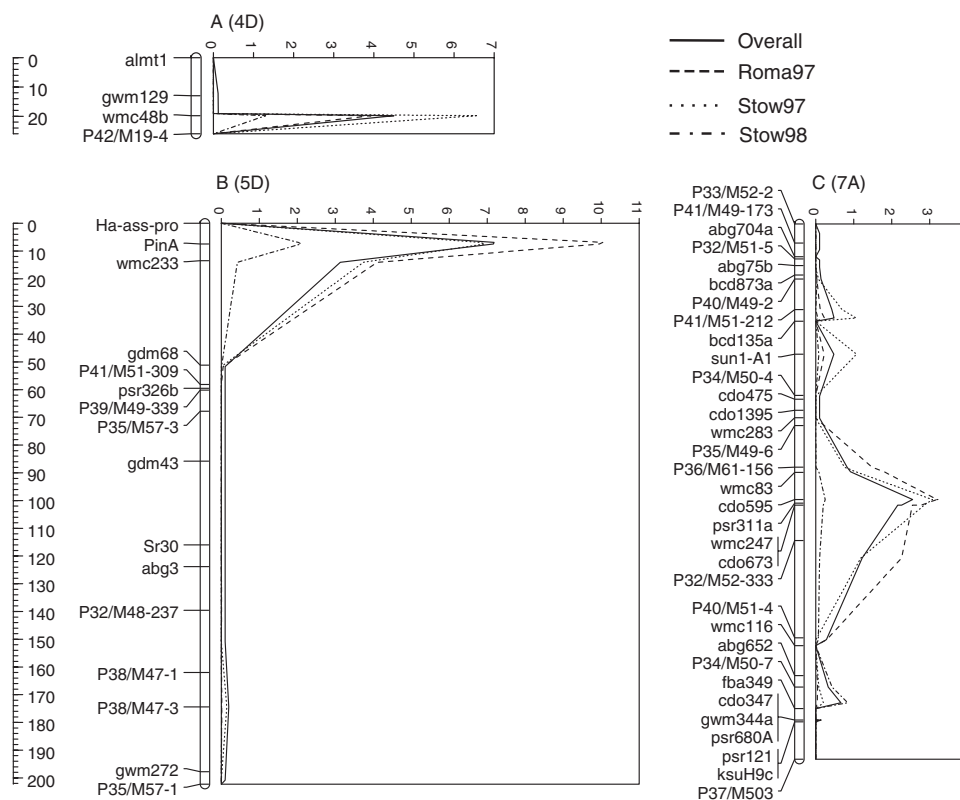


Fig. 4. Map location of flour yield QTLs of the C \times H cross. Chromosome numbers are given in parentheses. LOD scores are indicated on the x-axis and a scale of genetic distance (in centimorgans) is provided.

centromere region of chromosome 4B (locus *csME1* (*Rht1*), Fig. 2B) in Sunco and on chromosome 4DS in Tasman (*Rht2*) (Ellis *et al.* 2002). In the S \times T population the flour yield QTL in the region between marker *barc193* and marker *wmc48* on chromosome 4B was contributed by Tasman, which carries the *rht1* (tall) allele. This is not in agreement with suggestions that dwarfing alleles are associated with large increases in flour yield (Evans 1998). We conclude that the *Rht1* allele is not identical with the identified flour yield QTL in this population. Plant height QTLs have been identified on 4BS, 4BL, 2DS, 3AL, and 5AL in the C \times H population (Rebetzke *et al.* 2001), with Cranbrook having the semi-dwarf and Halberd the tall phenotype. These regions were also not associated with flour yield QTLs in our study.

In the S \times T and CD \times K populations a negative association between flour yield and the SKCS (Single-Kernel Characterisation System) hardness index can be inferred, since both traits are strongly linked to SSR marker *wmc48* in the centromere region of chromosome 4B (Osborne *et al.* 2001). This relationship between flour yield and grain hardness has been noted previously (Martin *et al.* 2001).

The flour yield QTL identified on 4D in the C \times H cross is linked to the hardness index locus previously identified by Osborne *et al.* (2001). In addition to the hardness gene,

Pina on the short arm of chromosome 5D, Partridge *et al.* (2002) identified a hardness-associated protein (Ha-ass-pro, Fig. 4) 8 cM distal to the *Pina* locus. The 5D flour yield QTL identified in the C \times H population, however, only seemed to be associated with the *Pina* locus and was contributed by the parental line Halberd. Two genes, *Pina-D1* and *Pinb-D1*, are thought to be components of the *Ha* (hardness) locus, where *Pina-D1a* and *Pinb-D1a* are the wild-type alleles resulting in soft grains and *Pina-D1b* and *Pinb-D1b* are the alleles with mutations resulting in hard grains (Giroux and Morris 1998; Hogg *et al.* 2004). Halberd has the *Pinb-D1b* allele and also carries the hardness-associated protein (Ha-ass-pro), whereas Cranbrook has the *Pina-D1b* allele (Osborne *et al.* 2001; Cane *et al.* 2004). Lower flour yield has been previously observed for *Pina-D1b* compared with *Pinb-D1b* (Martin *et al.* 2001). This is in agreement with our study where the parental line Halberd (having the *Pinb-D1b* allele) contributed positively towards the flour yield QTL at the 5D locus.

The flour yield QTL on chromosome 2B of the S \times T population is located on the translocation from *T. timopheevii* (Friebe *et al.* 1996; Kammholz *et al.* 2001), which has previously been associated with black point resistance (Lehmsiek *et al.* 2004). This region is also associated with

the presence of the rust resistance gene *Sr36* (Kammholz *et al.* 2001). As the translocation appears to segregate as one segment with almost no crossovers being observed (Lehmensiek *et al.* 2005), phenotypic (or marker-assisted) selection for *Sr36* could be an effective strategy for the co-selection of the flour yield and black point resistance QTLs in a commercial breeding program that includes the 2B introgression from *T. timopheevii*.

Using a multiple regression approach similar to the one used in our study, QTLs were previously identified on chromosomes 3B, 5B, and 7D in the C × H cross and on chromosomes 1D, 5B, and 6B in the CD × K cross (Smith *et al.* 2001). Only one C × H site and one CD × K site (Roma98) were available for this earlier analysis, which was based on early versions of the genetic maps. The C × H site used in the study by Smith *et al.* (2001) was not included in our analysis as this trial was a seed multiplication trial only. Our study indicated that none of the QTLs identified by Smith *et al.* (2001) was significant in subsequent years and other sites. This emphasises the importance of replicating sites in more than one environment in order to reliably identify QTLs associated with quality traits. In a recent study to identify QTLs for grain protein content, of 7 loci identified, only 1 QTL was detected in 7 out of the 8 environments trialled, 2 QTLs were detected in only 4 or 5 environments, whereas the remaining 4 QTLs were detected in only 2 of the 8 environments (Blanco *et al.* 2002). Nine different QTL regions associated with flour yield were identified in the 3 populations used in our study. Only 2 of these coincide with regions associated with flour yield in other studies (Campbell *et al.* 2001). The QTLs on chromosomes 5D and 6B identified by Campbell *et al.* (2001) were located in the same chromosomal region as the QTL on 5DS in the C × H and the QTL on 6BS in the S × T population. The lack of consistency of QTLs across different crosses and trial site/year indicates that for flour yield, QTL expression is highly dependent upon the genetic background and its interactions with the environment. Thus, breeding programs will need to base their marker selection strategies for this trait within pedigree groupings of regionally important core germplasm. For example, QTLs identified in this study, such as 2BS and 6BL in S × T, 4B in CD × K, and 5DS in C × H, were consistent in a number of environments and hence may be useful in future marker-assisted selection programs incorporating these sources. Future work may involve the validation of the identified markers on related populations developed within regional breeding programs.

Future studies could involve other components of milling quality such as ash content or Branscan. High bran is a variable that could potentially bias the QTL analysis and could thus be included in the analysis at the phenotypic level and/or at the genetic level using a bivariate analysis. It is important to understand which additional measurements need to be taken when focussing on one particular trait.

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