Theor Appl Genet (1998) 97: 238–245 © Springer-Verlag 1998

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Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.)

Received: 30 September 1997 / Accepted: 4 February 1998

Abstract An RFLP map constructed using 150 single seed descent (SSD) lines from a cross between two hexaploid wheat varieties ('Schomburgk' × 'Yarralinka') was used to identify loci controlling flour colour. Flour colour data were obtained from field trials conducted over two seasons at different sites. The estimated heritability of this trait was calculated as 0.67. Two regions identified in the preliminary analysis on chromosomes 3A and 7A, accounted for 13% and 60% of the genetic variation respectively. A detailed analysis of the major locus on 7A was conducted through fine mapping of AFLP markers identified using bulked segregant analysis (BSA). Seven additional markers were identified by the BSA and mapped to the region of the 7A locus. The applicability of these markers to identify wheat lines with enhanced flour colour is discussed.

Key words Wheat · Flour colour · QTL mapping · RFLP · AFLP

Introduction

Flour colour is one of the most important considerations in the assessment of flour quality and is of particular importance in determining the quality of the end product. White flour, lacking significant pigmentation, is considered a pre-requisite for bread production and flour is often bleached with bezoyl peroxide before use to eliminate colour defects (Kruger and Reed 1988). Alternatively, flour with high levels of yellow pigmentation is preferred for Chinese alkaline noodle production, and in many Asian countries noodles are prepared from flour specifically selected to enhance the colour of the final product (Kruger et al. 1994). Yellow alkaline noodles and white salted noodles are generally made from wheats with mixed grain hardness varying between soft and hard classes, with protein levels ranging from 10 to 12% (Simmonds 1989).

Flour carotenoids, principally xanthophyll and flavone compounds, are responsible for the yellow colour of the flour (Miskelly 1984). The natural colour of the flavones is advantageous for the production of noodles in Japan and South East Asia where traditionally an alkaline-salt mixture of sodium and potassium carbonates and phosphates, called 'Kansui', is added during production (Kruger et al. 1992). The highly alkaline pH (between 9 and 11) of the dough mixture causes the disassociation of flavones from the polysaccharides, which enhances the yellow pigmentation. As well as enhancing the colour, the alkaline salts contribute to the flavour of the noodles and toughen the dough, improving the bite texture of the final product.

In commercial noodle production, alkaline salts are frequently substituted with a sodium hydroxide solution. The addition of increasing concentrations of alkali results in noodles that have a brighter, more intense, yellow colour than noodles made with salted dough. However, alkali treatment produces a deterioration of dough quality, particularly the loss of gluten elasticity and pasting properties which may result in an undesirable soft textured product (Terada et al. 1981). An increase in flour pigment levels would lower the concentrations of alkali required to produce the desired yellowness, resulting in an improvement in noodle texture.

Communicated by G.E. Hart

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Miskelly et al. (1984) detected a significant positive correlation between flour colour and noodle sheet yellowness in both Japanese and Chinese noodles. This finding offers the opportunity to enhance the yellow pigmentation of the dough by breeding for improved flour colour. While significant genetic diversity exists within cultivated wheat for flour colour (Moss 1967), this trait is expressed as a quantitative character and has proved difficult to manipulate in breeding programs. Selection for quantitative-trait characters is often difficult due to variation associated with environmental factors that adversely affect accurate measurement of the trait. Indirect selection for linked markers offers an alternative approach that avoids multiple evaluations in replicated trials over sites and seasons.

The development of molecular-marker techniques has provided an additional tool for the construction of genetic maps in many crop species including wheat (Devos and Gale 1993). Using these maps and their corresponding mapping populations, the number, significance and location of quantitative trait loci (QTLs) associated with a variety of phenotypic characteristics have been identified (Paterson et al. 1988). In rice, QTLs for yield related traits, blast resistance and root morphology have been located on genetic linkage maps (Wang et al. 1994; Champoux et al. 1995; Xiao et al. 1995). QTLs associated with important traits have also been identified in other cereals including agronomic traits, grain yield and disease characters in barley (Backes et al. 1995; Thomas et al. 1995; Oziel et al. 1996), as well as resistance to pre-harvest sprouting, kernel hardness and grain protein content in wheat (Anderson et al. 1993; Blanco et al., 1995; Sourdille et al. 1996). Marker-assisted strategies may be useful for dissecting the genetic control of quantitative phenotypes such as flour colour. The goal of the present research is to identify associations between QTLs for flour colour and molecular markers so that such markers may be used as indirect selection criteria in wheat breeding programs.

Material and methods

Plant material

A recombinant inbred population derived from hexaploid wheat that segregated for flour colour was used in this analysis. This population consisted of 150 F_4-derived lines developed through single seed descent (SSD) from a cross between the Australian cultivars 'Schomburgk' and 'Yarralinka'. The mapping population was sown in field experiments with the University of Adelaide's wheat breeding trials at sites in South Australia during the 1995 and 1996 seasons. The 1995 trial was sown at Winulta while the 1996 trial was sown at Walkers Flat. Both sites have a similar soil type of sandy loam over clay. A randomised complete blocks design was used in both years. Limited seed restricted the 1995 trial to one replicate while two replicates were sown in 1996. Wheat nullisomic-tetrasomic lines obtained from Mr. Terry Miller (John Innes Centre, Norwich) were used to assign linked markers to specific chromosomes.

Flour colour evaluation

One hundred grams of the harvested seed from each line was milled on a Quadramant Junior Mill (Bass 1988) and flour samples were scored for colour using a Minolta Chroma CR-200 meter (Symons and Dexter 1991; Wutscher and McCollum 1993). This probe uses diffuse illumination produced by a pulsed xenon arc lamp and is commonly employed for measuring colour in a variety of products ranging from wheat milling products to soil (Wutscher and McCollum 1993; Symons and Dexter 1996). Colour readings are expressed as L* (lightness), a* (red-green chromaticity) and b* (yellow-blue chromaticity), with the b* values providing a measure of the yellowness of the flour sample.

RFLP analysis

A total of 217 RFLP markers were screened for their ability to identify polymorphisms between the parents. These DNA clones were obtained through the Australian Triticeae Mapping Initiative and were selected to give uniform genome coverage. The majority of these markers have been mapped in wheat and their chromosomal locations identified (Gale et al. 1995; Nelson et al. 1995 a–c; Van Deynze et al. 1995; Marino et al. 1996). DNA extraction, restriction-enzyme digestion, Southern blotting and hybridisation were carried out as described by Guidet et al. (1991). Total genomic DNA was digested with *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV or *Hin*dIII. Southern blots of the 150 wheat lines were hybridised to RFLP probes and the results scored as 'Schomburgk' allele, 'Yarralinka' allele, or heterozygous according to the genotype at each locus.

AFLP analysis

The AFLP method developed by Vos et al. (1995) was followed with some modifications. Genomic DNA (1 μg) was digested with the restriction endonucleases *Pst*1 and *Mse*1. Double-stranded adaptors were then ligated to the ends of the restriction fragments followed by ethanol precipitation and resuspension in 60 μl of 0.1 M TE. Pre-amplification was performed using primers specific for the *Pst*1 and *Mse*1 adaptors including one selective nucleotide, followed by selective amplification using similar primers with three selective bases. A total of eight *Pst*1 and eight *Mse*1 primers were used giving a total of 64 possible primer combinations (Table 1). The pre-amplification mix was diluted 1 : 5 in water before being used in the selective amplification step. Pre-amplification PCR conditions consisted of 20 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min. PCR reaction conditions for selective amplification consisted of one cycle at 94°C for 30 s, 65°C for 30 s and 72°C for 1 min followed by nine cycles over which the annealing temperature was decreased by 1°C per cycle with a final step of 25 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min. The *Pst*1 primer used in selective amplification was end-labelled with [γ^{32}P] ATP (Feinberg and Vogelstein 1983). Amplified fragments were separated on 6% denaturing polyacrylamide gels. The gels were transferred to 3MM paper for drying and auto-radiography was carried out with Fuji RX medical X-ray film at room temperature for 24–48 h.

Microsatellite analysis

Thirty one wheat microsatellite (WMS) primer pairs, developed by Plaschke et al. (1995), and Röder et al. (1995), were analysed. The

Table 1 Selective *Pst*1 and *Mse*1 primers used to screen bulks of F_4 SSD lines for polymorphisms associated with flour colour (selective bases in bold). A total of 64 possible combinations were used with P1M1 representing the first combination, P1M2 the second combination and P8M8 the 64th combination

*Pst*1 selective primers	Primer designation	*Mse*1 selective primers	Primer designation
GACTGCGTACATGCAG**AAC**	P1	GATGAGTCCTGAGTAA**CAA**	M1
GACTGCGTACATGCAG**AAG**	P2	GATGAGTCCTGAGTAA**CAG**	M2
GACTGCGTACATGCAG**ACA**	P3	GATGAGTCCTGAGTAA**CAT**	M3
GACTGCGTACATGCAG**ACC**	P4	GATGAGTCCTGAGTAA**CTG**	M4
GACTGCGTACATGCAG**ACG**	P5	GATGAGTCCTGAGTAA**CCA**	M5
GACTGCGTACATGCA**GACT**	P6	GATGAGTCCTGAGTAA**CCT**	M6
GACTGCGTACATGCAG**AGC**	P7	GATGAGTCCTGAGTAA**CGA**	M7
GACTGCGTACATGCAG**AGG**	P8	GATGAGTCCTGAGTAA**CTA**	M8

polymerase chain reaction (PCR) contained 20 mM of Tris-HCl (pH 8.4), 50 mM of KCl, 100 µM of each dNTP, 1.5 mM of $MgCl_2$, 900 nM of each primer, 0.5 units of *Taq* polymerase and 80 ng of genomic DNA in a total volume of 25 µl. Temperature conditions for amplification were 95°C for 3 min, followed by 35 cycles of 96°C for 1 min, 55°C or 58°C for 1 min 30 s, 72°C for 1 min 50 s with a final step of 72°C for 10 min and 25°C for 5 min. Amplified products were separated on 7% non-denaturing polyacrylamide gels and stained with ethidium bromide as described by Röder et al. (1995).

Bulked segregant analysis

Bulked segregant analysis (Michelmore et al. 1991) was performed to develop additional markers for fine mapping the major locus on chromosome 7A in order to identify tightly linked flanking markers which could be used in marker-assisted selection. Two bulks were constructed by combining aliquots of DNA (2 µg) from two sets of ten SSD lines representing the two extremes of the distribution for flour colour with alternate alleles at the *Xcdo347 7A* locus. The bulks were screened with the 64 AFLP primer combinations to identify polymorphisms.

Genetic analysis

Data obtained from scoring segregation patterns of DNA markers among recombinant inbreds were analysed with MapManager QT (Version 8.0) software (Manly and Elliott 1993) using the Kosambi mapping function (Kosambi 1944; Lander et al. 1987). Analysis of variance (ANOVA) was performed with the computer program Genstat 5 (Lane et al. 1988) with the three field data sets treated as replicates. The results of the ANOVA were used to obtain an estimate of the broad-sense heritability (H^2) of flour colour (Hartl et al. 1988). Broad-sense heritabiltiy was calculated as: $H^2 = \sigma_g^2/\sigma_t^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$ where σ_g^2 is the genotypic variance, $\sigma_e^2$is the environmental variance and σ_t^2 is the total variance for the trait. The genotypic variance (σ_g^2) can be calculated from the value of the mean squares between the SSD lines: m.s. $= \sigma_e^2 + b\sigma_g^2$ where m.s. is the mean squares, σ_e^2 is the residual mean squares and b is the number of replicates. The amount of genetic variation accounted for by a particular QTL is calculated by dividing the phenotypic variation by H^2.

The associations between markers and QTLs for flour colour was tested with a single-point regression analysis using MapManager QT based on the likelihood ratio statistic. Single-point regressions served as the primary method of detecting associations between markers and QTLs. Groups of two or more closely linked markers with significant associations were assumed to identify the same QTL. A second method, interval mapping, which searches for the effects of a QTL using sets of linked markers (Lander and Botstein 1989) was employed to obtain further information on QTLs identified by single-point analysis. A LOD score of 3.0 was chosen as the threshold for detecting QTLs (Lander and Botstein 1989). A graphical display of the QTL associations was generated using Qgene (Nelson 1997).

Results

Partial map construction

A partial genetic linkage map was constructed based on the segregating RFLP, microsatellite and AFLP data from the 150 SSD lines at a LOD threshold of 3. Of the 217 RFLP probes screened, 70 (32%) identified polymorphisms between the two parents, while 11 (33%) of the 31 microsatellite markers tested identified a polymorphism between the parents. AFLP primer combinations that identified polymorphisms between the bulks were also screened on the 150 SSD lines, identifying 5.2 polymorphisms on average per primer pair. A total of 147 RFLP, microsatellite and AFLP marker loci were used to create the partial map (data not shown).

Analysis of agronomic traits

The two parents of the population differed significantly in their flour colour scores (see Table 3). The distribution of flour colour scores from the three field data sets is shown in Fig. 1, including the means for the parents. The distribution of the SSD lines in the mapping population appeared to be bimodal in the 1995 trial (Fig. 1 A) and more normal in the 1996 trial (Fig. 1B and C). The potential association of flour colour with markers was tested with single-point regression analysis. Three marker loci *Xcdo347–7A, Xwg232–7A.1* and *Xbcd828–3A* were found to be significantly associated with flour colour. The chromosomal locations of the segregating bands detected by these markers were identified using nullisomic-tetrasomic wheat lines.

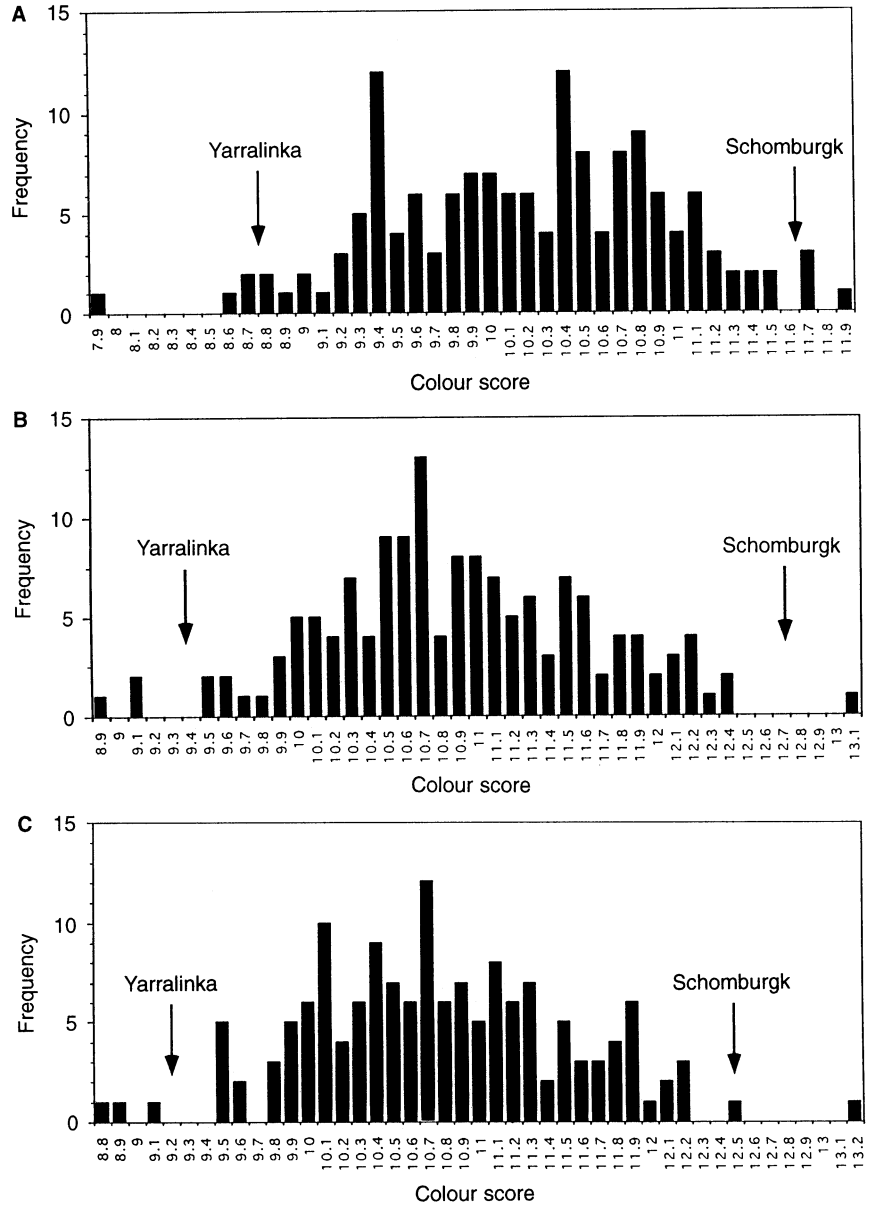

Fig. 1A–C Histograms of frequency of F_4 and F_5 SSD lines versus average flour- colour score for samples collected from field trials grown over two seasons at different sites. Three data sets were obtained from the trials: (**A**) 1995, (**B**) 1996 Replicate 1 and (**C**) 1996 Replicate 2. Parental means are indicated by *arrows*.

Associations of flour colour with the linked markers on chromosome 7A (*Xcdo347–7A* and *Xwg232–7A.1*) and 3A (*Xbcd828–3*A) were highly significant ($P \leqslant 0.001$) over both seasons at the two sites (Table 2). Individually these markers accounted for an average of 37% and 9% of the phenotypic variation observed for flour colour. In order to obtain an estimate of the broad-sense heritability of flour colour, ANOVA was performed with the three data sets treated as replicates (Table 3). From the ANOVA, the heritability of the trait was calculated as 0.68. Based on these results the amount of genetic variation accounted for by the RFLP markers on chromosomes 7A and 3A is estimated at 54% and 13%, respectively.

Bulked segregant analysis

To identify additional markers linked to the QTL for flour colour and to enable interval mapping of the region around *Xcdo347–7A*, a bulk segregant analysis was conducted. DNA samples from 20 individual SSD-lines representing the two extremes (ten individuals per extreme) of the distribution for flour colour and having alternate alleles at the *Xcdo347–7A* locus were analysed. Of the 64 primer combinations tested to identify AFLP markers linked to *Xcdo347–7A*, six primer combinations produced amplification products that were

Table 2 Summary of three RFLP and seven AFLP markers linked to a major locus on chromosome 7A associated with flour colour ($P \leqslant 0.001$). The likelihood ratio, the percentage of phenotypic variation accounted for, and the regression coefficients are reported for each marker in three field-data sets

Marker	Likelihood ratio statistic			% Variation			Regression coefficient		
	1995	1996 rep1	1996 rep2	1995	1996 rep1	1996 rep2	1995	1996 rep1	1996 rep2
Xbcd828–3A	9	8	12	7	6	8	− 0.37	− 0.35	− 0.43
Xwg232–7A.1	26	29	22	17	19	15	− 0.62	− 0.65	− 0.59
Xcdo347–7A	36	43	45	27	32	33	− 0.78	− 0.84	− 0.90
Xwua18–7A.9	40	57	55	24	34	32	− 0.73	− 0.85	− 0.84
Xwua35–7A.1	45	58	57	27	34	33	− 0.77	− 0.88	− 0.88
Xwua16–7A.5	45	58	56	28	35	34	− 0.78	− 0.89	− 0.89
Xwua26–7A.4	43	57	55	26	34	32	− 0.74	− 0.87	− 0.85
Xwua39–7A.3	37	45	44	24	29	27	− 0.71	− 0.79	− 0.80
Xwua2–7A.4	26	34	33	17	22	21	− 0.62	− 0.72	− 0.71
Xwua56–7A.5	24	32	34	16	21	22	− 0.58	− 0.70	− 0.72

Table 3 Analysis of variance results for flour colour scores from 150 segregating F_4 and F_5 SSD lines sown over two seasons at different field sites

Source of variation	*df*	Sums of squares	Mean squares	Variance ratio	*F* pr.
Between parents	1	219.6	219.6	3219	< 0.001
Between lines	149	198.0	1.33	7.26	< 0.001
Residual	290(8)	53.07	0.183		

present in only one of the bulks. Three of these bands were associated with the Schomburgk allele and three with the Yarralinka allele. A seventh polymorphism was also identified which was co-dominant. Linkage between the *Xcdo347–7A* locus and the amplified products was confirmed by monitoring the segregation products of each informative primer combination in the 150 SSD lines. All seven polymorphic amplification products were linked to flour colour and the *Xcdo347–7A* locus on chromosome 7 when mapped on the whole population. The seven AFLP primer combinations also identified additional polymorphisms not associated with the *Xcdo347–7A* locus which were scored and included in the mapping data set. The flour colour data was re-analysed after the additional AFLP markers had been added to the partial map, but no other QTLs were identified.

Interval analysis

When flour colour scores from the field-trial data were subjected to interval analysis using QGENE software (Nelson 1997), only one QTL on 7A was detected (LOD > 3.0). The LOD score plots of the three data sets were drawn to localise the genes for flour colour more precisely (Fig. 2). The AFLP locus *Xwua16–7A.5* was the most closely associated with the flour colour locus (0.7 cM). The average percentage of phenotypic variance explained by this QTL was 41%, which is equivalent to 60% of the genetic variance based on the estimated heritability.

Discussion

The primary goal of this research was to identify associations between QTLs for flour colour and molecular markers so that such markers could be used as an indirect selection criterion in wheat breeding programs. Using a SSD population of 150 lines, a total of ten marker loci were identified that showed significant associations with QTLs for flour colour located on chromosomes 3A and 7A.

The benefit of using a single seed descent population is that the genetic structure of the population is predominantly fixed. Any heterozygotes that are scored are detected by the MapManager software. Since genotypes are predominantly fixed, SSD lines are valuable for assessing the environmental impact on trait expression. In our study, the estimated heritability of flour colour was calculated as 0.68, indicating that a large part of the expression of this trait is genetically controlled, making it easier to manipulate at the genetic level in a breeding program. We identified two QTLs for flour colour in this population but only the QTL on chromosome 7A was highly significant in all three replicates. This may indicate that the QTL on chromosome 3A is more sensitive to environmental effects.

All of the regression coefficients of the marker loci linked to yellow flour colour were negative (Table 2). This suggests that the 'Schomburgk' parent was the source of all the increasing effects on flour colour, and largely explains the lack of transgressive segregation observed in the progeny.

Seventy four percent of the genetic variation could be explained by the detected QTLs, with the QTL at

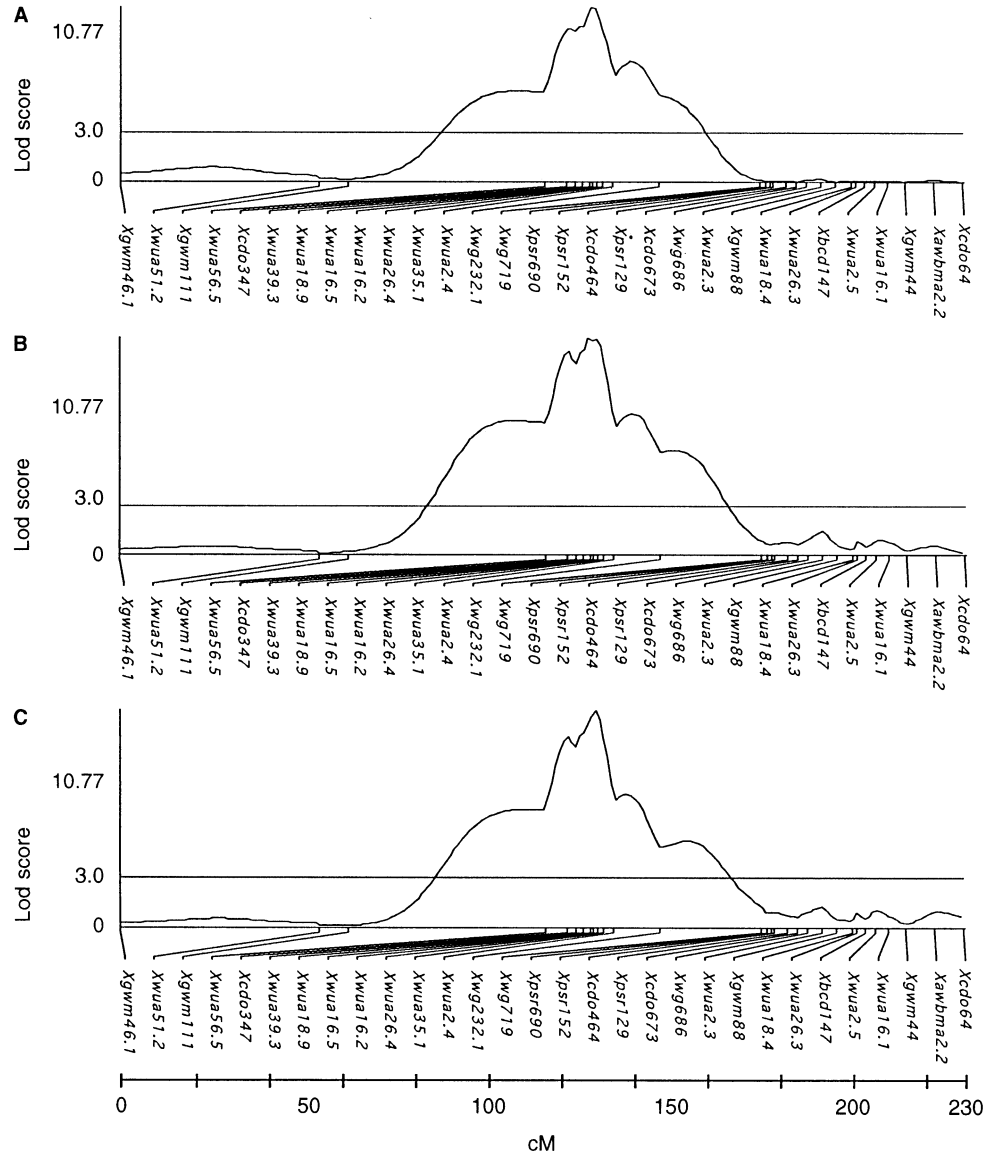

Fig. 2A–C LOD plots of chromosome 7A showing the location of a major locus associated with flour colour. Data were obtained from 150 F_4 and F_5 SSD lines sown in field trials over two seasons at different sites. Results include data from (**A**) 1995, (**B**) 1996 Replicate 1 and (**C**) 1996 Replicate 2. The AFLP marker prefix *Xwua* indicates a locus detected by a primer combination at the University of Adelaide's Waite Institute. The *number* following the prefix corresponds to the primer combination used to identify the locus

Xwua16–7A.5 alone accounting for 60%. The large amount of variation explained by this QTL would suggest that the inheritance of this trait is predominantly controlled by this locus. The inability to explain the remaining genetic variation may result from the moderate size of our mapping population, which did not allow for low effect QTLs to be identified, or the incomplete coverage of the wheat linkage map.

Ideally a comprehensive map of the wheat genome would be required for complete analysis but this is time consuming to develop. The AFLP technique (Vos et al. 1995; Zabeau and Vos 1993) has the capacity to assay a much greater number of loci for polymorphism than other currently available PCR-based techniques, and is suitable for detailed mapping exercises. Initially, we used a combination of locus specific RFLP and microsatellite markers to construct a partial genetic linkage map. Bulk segregant analysis and AFLP markers were used to identify markers closely linked to a major QTL identified on chromosome 7. This approach resulted in more markers being identified in the region around *Xcdo347–7A* and rapidly increased the number of markers scored in the population. Bulked segregant analysis using AFLP markers has proved to be an effective method of identifying markers closely linked to traits of interest. The ability to rapidly identify and map additional markers tightly linked to the *Xcdo347–7A* locus enabled a detailed map of this chromosomal region to be constructed. The combination of RFLP and AFLP markers proved to be an efficient

way of identifying markers closely linked to the trait of interest.

Thomas et al. (1995) reported on the suitability of converting AFLP markers to allele-specific markers and there is clearly the potential to apply this to the markers identified in our study. The identification of molecular markers linked to flour colour has the potential to accelerate wheat breeding for this particular trait. Once suitable genomic regions associated with flour colour have been identified, breeders will be able to select for quantitative traits at the genetic level rather than relying on phenotypic expression. Previously, the early selection of traits at the phenotypic level has been limited due to the small amount of grain available on an individual plant basis. The selection of plants based on their molecular profile will allow the breeder to readily combine preferred alleles at selected loci to maximise expression of the trait.

Although the markers described here are likely to be effective in monitoring the yellow flour colour locus from the variety 'Schomburgk', it is not known if desirable yellow colour from other sources is controlled by the same locus. In this case, the absence of transgressive segregation and the apparent simplicity of inheritance suggests that the 'Schomburgk' locus will have broad applicability. However, future testing is required to confirm this. The behaviour of the allele from Yarralinka that is associated with whiteness should also be further assessed. In some breeding programs this trait is also important but it will again be necessary to analyse the behaviour of the Yarralinka allele in different genetic backgrounds.

References

Anderson JA, Sorrells ME, Tanksley SD (1993) RFLP analysis of genomic regions associated with resistance to preharvest sprouting in wheat. Crop Sci 33 : 453–459

Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localisation of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (Hordeum vulgare L.) Theor Appl Genet 90 : 294–302

Bass EJ (1988) Wheat flour milling. In: Pomeranz Y (ed) Wheat: chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, Minnesota, USA, vol II, pp 1–68

Blanco A, De Giovanni C, Laddomada B, Sciancalepore A, Simeone R, Devos KM, Gale MD (1995) Quantitative trait loci influencing grain protein content in tetraploid wheats Plant Breed 115 : 310–316

Champoux M, Wang G, Sarkarung S, Huang N, Mackill DJ, O'Toole JC, McCouch SR (1995) Locating genes associated with root morphology on the molecular linkage map of rice. Theor Appl Genet 90 : 969–981

Devos K, Gale MD (1993) The genetic maps of wheat and their potential in plant breeding. Outlook Agric 22 : 93–99

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragments to high specific activity. Anal Biochem 132 : 6–13

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. Proceedings of the 8th Int Wheat Genet Symp, China Agricultural Scientech Press, Beijing, China. pp 29–40

Guidet F, Rogowsky R, Taylor C, Song W, Langridge P (1991) Cloning and characterisation of a new rye-specific repeat sequence. Genome 34 : 81–87

Hartl DL, Freifelder D, Snyder LA (1988) Quantitative genetics. In: Basic genetics. Jones and Bartlett Publishers, Inc., California, USA, pp 222–259

Kosambi DD (1944) The estimation of map distances from recombinational values. Ann Eugen 12 : 172–175

Kruger JE, Reed G (1988) Enzymes and colour. In: Pomeranz Y (ed) Wheat: chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, Minnesota, USA, vol II, pp 441–487

Kruger JE, Matsuo RR, Preston K (1992) A comparison of methods for the prediction of Cantonese noodle colour. Can J Plant Sci 72 : 1021–1029

Kruger JE, Anderson MH and Dexter JE (1994) Effect of flour refinement on raw cantonese noodle colour and texture. Cereal Chem 71 : 177–182

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps Genetics 121 : 185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1 : 174–181

Lane P, Galway N, Alvey N (1988) Genstat 5: an introduction. Oxford University Press, UK

Manly KF, Elliott RW (1993) RI manager, a microcomputer program for analysis of data from recombinant inbred strains. Mammal Genome 1 : 123–126

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39 : 359–366

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specifiec genomic regions by using segregating populations. Proc Natl Acad Sci USA 88 : 9828–9832

Miskelly DM (1984) Flour components affecting paste and noodle colour. J Sci Food Agric 35 : 463–471

Moss HJ (1967) Yellow pigment content of some Australian flours. Aust J Exp Agric Anim Husb 7 : 463–464

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3 : 229–235

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995 a) Molecular mapping of wheat. Homoeologous group 2. Genome 38 : 516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995 b) Molecular mapping of wheat. Homoeologous group 3. Genome 38 : 525–533

Nelson JC, Sorrells ME, Van Deynze AE , Lu Y-H, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995 c) Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141 : 721–731

Oziel A, Hayes PM, Chen FQ, Jones B (1996) Application of quantitative trait locus mapping to the development of winter-habit malting barley. Plant Breed 115 : 43–51

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms Nature 335 : 721–726

Planchke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91 : 1001–1007

Röder MS, Plaschke J, König SU, Börner A, Sorrels ME, Tanksley, S.D. Ganal, MW (1995) Abundance, variability and

chromosomal location of microsatellites in wheat. Mol Gen Genet 246 : 327–333

Simmonds D (1989) Wheat production in Australia. In: Wheat and wheat quality in Australia. CSIRO Australia, pp 1–28

Sourdille P, Perretant MR, Charmet G, Leroy P, Goutier MF, Joudrier P, Nelson JC, Sorrells ME, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93 : 580–586

Symons SJ, Dexter JE (1991) Computer analysis of fluorescence for the measurement of flour refinement as determined by flour ash content, flour grade colour, and tristimulus colour measurements. Cereal Chem 68 : 454–460

Symons SJ, Dexter JE (1996) Aleurone and pericarp fluorescence as estimators of mill stream refinement for various Canadian wheat classes. J Cereal Sci 23 : 73–83

Terada M, Minami J, Yamamoto T (1981) Rheological properties of dough made from flour exposed to gaseous ammonia. Cereal Chem 58 : 101–105

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant Jour 8 : 785–794

Van Deynze AE, Dubdovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group-1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38 : 45–59

Vos P, Hogers R, Blecker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23 : 4407–4414

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136 : 1421–1434

Wutscher HK, McCollum TG (1993) Rapid objective measurement of soil colour with a tristimulus colorimeter. Commun Soil Sci Plant Anal 24 : 2165–2169

Xiao JH, Li JM, Yuan LP, Tanksley SD (1995) Dominance is the major genetic basis of hetrosis in rice as revealed by QTL analysis using molecular markers. Genetics 140 : 745–754

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application 92402629.7; Publication number EP 0534858 A1