QTL Mapping of Flour Color in a Hexaploid Wheat Doubled Haploid Population Using Diversity Array Technology

A. Sadeque[1,]* and M.A. Turner[2]

[1]Department of Industry and Investment, Narrabri, NSW, Australia
[2]The University of Sydney, Plant Breeding Institute, Cobbitty, Australia

**Corresponding author. Email: abdus.sadeque@industry.nsw.gov.au*

Abstract

QTL (Quantative Trait Loci) mapping of flour color was investigated in a doubled haploid (DH) population that was derived from a hybrid between a wheat line DM5637*B8 and an Australian wheat variety H45. The population was grown at The University of Sydney, Plant Breeding Institute site at Narrabri, and analysed, for two seasons. The Diversity Array Technology (DArT) approach was employed to genotype this DH population. QTL analyses identified five genomic regions (on chromosomes 1A, 2B, 3B, 4B and 5D) that controlled flour yellowness (b*). A highly significant (P<0.001) association between the QTL on chromosome 2B (Qb*.dmh45-2B) and flour yellowness was identified in both seasons. QTL on chromosomes 4B (Qb*.dmh45-4B) and 5B (Qb*.dmh45-5B) that were significantly (P<0.01) associated with flour yellowness were also identified in both seasons. This information could be applied to generate wheat cultivars that have enhanced flour yellowness that is better for yellow alkaline noodle production but not too yellow for bread production.

Keywords: QTL mapping, wheat, doubled haploid (DH), flour colour

Introduction

Wheat products such as noodles and bread are made from Australian prime hard wheat. In the domestic market a large percentage of wheat is used as an input in bread production. However, a substantial amount is also exported for production of Asian yellow alkaline noodles (YAN), which are an important part of the diet in many Asian countries. Flour yellowness is related to the yellow color of bread and Asian noodles (Miskelly, 1984) and YAN and bread producers have different desires in regard to flour yellowness. Yellow alkaline noodles that are bright and have stable yellow color are preferred by consumers of Asian noodles, whilst bread that is white is preferred by consumers domestically. Xanthophyll compounds are predominantly responsible for the yellow color of wheat flour and these products. However YAN color is also influenced by other compounds such as flavones, which are colorless phenolic compounds that become yellow when exposed to alkaline conditions such as those of doughs used for YAN production (Kruger et al., 1992).The efficient development of wheat with optimised yellow flour color requires an understanding of the genetic control of important quality characteristics of wheat end products. Quantitative Trait Loci (QTL) are genetic factors that are responsible for a part of the observed phenotypic variation for a quantitative trait (polygenic trait) (Acquaah, 2007). There is a limited number of reports on the mapping of flour color in Australian wheats. QTLs that control flour color were identified on chromosomes 3A and 7A in a recombinant population of hexaploid wheat. These loci accounted for 13% and 60% of the observed phenotypic variation, respectively (Parker et al., 1998). Mares and Campbell (2001) investigated three doubled haploid (DH) mapping populations (Cranbrook x Halberd, CD87 x

Katepwa and Sunco x Tasman) and identified a number of QTLs for flour color (b*). Four QTLs were identified in the CD x Katepwa population on chromosomes 2D, 3A, 6A, and 7B. QTLs on 3B, 5D, and 7A were identified in the Cranbrook x Halberd population, and in Sunco x Tasman QTLs on 3B, 4B, 5B and 7A were consistent across sites.

Diversity Arrays Technology (DArT) is a microarray-based DNA marker technique for genome wide discovery and genotyping of genetic variation (Wittenberg et al., 2005). This approach has high-throughput capability and is able to discover hundreds of polymorphic markers in a single experiment. Diversity arrays technology was developed to provide a practical and cost-effective whole-genome fingerprinting tool (Jaccoud et al. 2001) for species which are complex in nature and lack prior DNA sequence information (Wenzl et al., 2004). Hence, the present investigation aimed to identify the QTLs and DArT markers associated with flour color that would accelerate the development of wheat lines having desired flour color in Australian wheat populations.

Materials and Methods

Plant Materials

A doubled haploid population (n=187) that was generated from a hybrid between DM5637*B8 (AUS1408/Sunco), a line which has low polyphenol oxidase activity (PPO), is sprouting tolerant and exhbits low blackpoint incidence, and Australian cultivar H45 (Ciano67/2* Olympic/3/ WW80/3*Anza//Kalyonsona/Bluebird) was used in this study. A subset of 48 lines and parents were grown during the 2005 and 2006 growing seasons, at The University of Sydney, Plant Breeding Institute site at Narrabri. The field trials were grown in a RCB design with two replicate 2 m x 6 m plots.

Milling and Flour Color Measurement

Grain of the lines was milled using a Quadrumat Junior Mill (Brabender® OHG DUISBURG, Germany). The flour extraction rate was adjusted to 60 percent. The moisture and protein content of grain samples was measured using Near Infrared Reflectance (NIR; Perten Instruments, Type 9100/01, Germany) They were conditioned to 15% overnight by adding the appropriate amount of water before milling. Flour and bran yield was recorded for each sample. The flour color values (CIE L*, a*, b*) were recorded with a chromameter (model 300, Minolta Camera Co., Ltd., Osaka, Japan) with a 50mm diameter measuring tube using a white tile background. The L* axis runs from top to bottom. The maximum for L * is 100, which represent a perfect reflecting diffuser. The minimum for L* is zero, which represents black. The* and b* axes have no specific numerical limits. Positive and negative values of a* are red and green, respectively. Positive and negative values of b* are yellow and blue, respectively.

DNA Isolation

Genomic DNA extractions were performed using a modified CTAB (Cetyltrimethylammonium bromide) protocol based on that of Doyle et al. (1990). 2.5 µg of restriction quality DNA of each DH genotype and the parents of the DM5637*B8 x H45 population were prepared. Genomic DNA was extracted from fresh leaf tissue (0.5 to 1 g) of a bulk of four plants. Leaves from three weeks old plants were taken. Leaves that were dry, disease free and free from sticking soil particles were selected for DNA extraction. CTAB extraction buffer was prepared with 2% (w/v) CTAB (sigma), 1.4 M NaCl, 0.2% mM EDTA, 100 mM Tris-HCl, (pH 8.0).

DArT Marker Analyses

2.5 µg of restriction quality DNA of each DH genotype (n=92) and the parents of the population DM5637*B8 x H45 was sent to Triticarte Pty. Ltd., Canberra, Australia (http://www.triticarte.com.au) for whole genome profiling. The 92 lines were randomly selected from the population and included the 48 lines that were analysed for flour color. The loci were scored as present (1) or absent (0) and the locus designations were as used by Triticarte Pty. Ltd. The P value reflects how well the two phases (present=1 vs absent=0) of the marker are separated in the sample and P is based on ANOVA which is an estimate of marker quality. Markers with P values 80, 77-80, and 75-77 are considered to be extremely reliable and provide useful information. DArT markers are labelled with a prefix ‘wPt’, followed by number corresponding to a particular clone in the genomic representation, where w stands for wheat, P for *Pst*I (primary restriction enzyme used) and T for *Taq*I (secondary restriction enzyme) (Wenzl et al., 2004; Huttner et al., 2006).

Linkage Map Construction and QTL Mapping

The initial linkage mapping was performed with Cartablanche software, version 1.5.0(111), Keygene Products B.V. Linkage groups were further reassessed and reconstructed with Map Manager (QTXb20). The assignment of markers in linkage groups for individual chromosome relied on the consensus map of the Triticarte Ltd. and ongoing construction of consensus map for the DArT assay (Akbari et al. 2006; Howes pers. comm.; http://www.triticarte.com.au)

Interval mapping was performed at P=0.01 for marker-trait association using Map Manager QTXb20 (Manly et al. 2001). Single marker regression and simple interval mapping tools were used for this purpose. The marker regression function (P=0.01) was performed to find single marker loci linked with the quantitative data. The analysis was carried out on data from the two individual environments/years and the pooled data from both environments. The Likelihood Ratio Statistic (LRS) thresholds in the regression analyses P<0.01 and P<0.001 were used for declaring significant and highly significant levels, respectively. QTL effects were calculated by using the permutation function. This function provided an approximation to a permutation test according to the method described by Piepho (2001).

Results

Evaluation of Flour Color (CIE b*)

Flour yellowness (b*) was significantly (r^2=0.55, P<0.001) correlated across seasons (Figure 1). The range of b* values among lines was similar across seasons (data not shown, Sadeque 2008). In 2005 b* ranged from 7.37 to 10.21, and in 2006 it ranged from 7.91 to 11.06. The population means (b* values) were 8.68 and 9.60 for 2005 and 2006, respectively.

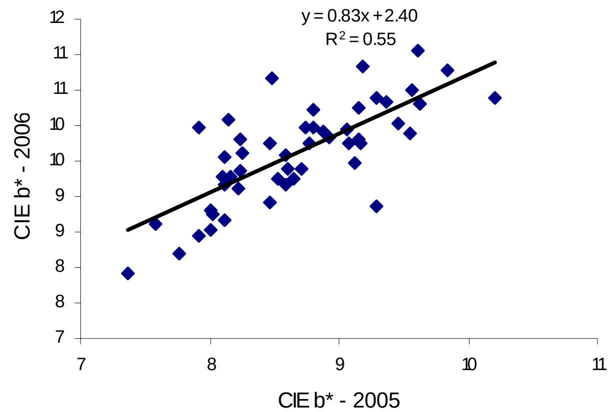

Figure 1 Relationship of flour color traits CIE b* between years (2005 and 2006) for a set of lines from the DM5637*B8 x H45 DH population (n=50).

DArT Marker Screening

DNA of 92 lines and the two parents of the DM5637*B8 x H45 population were analysed by DArT. Three hundred and eighty four polymorphic DArT markers were obtained with an overall call rate of 94.28%. The overall marker P values were extremely reliable and ranged between 73.87 and 98.84 with a mean of 89.63. Only 17 markers had P values below 77. The distribution of markers among the genomes were not uniform, viz., 75, 202, and 79 markers were assigned to chromosome of the A, B and D genomes, respectively. Twenty eight markers were unassigned. DArT analysis mapped between 2 and 51 polymorphic markers to each of the chromosomes. Less than 10 polymorphic markers were assigned to linkage groups on chromosomes 2A, 3A, 4A, 4D, 5A, 4D, 5D and 6D (data not shown; See Table 3.3 in Sadeque, 2008).

Linkage Group Construction

Of the 384 polymorphic markers that were identified 317 markers were assembled into 30 linkage groups. Sixty seven markers could not be assigned to any linkage groups. Linkage groups could not be formed for chromosome 3A, 4D, 5A, 5D and 6D due to an insufficient number (<3) of linked markers. Eight linkage groups were assigned to chromosomes in the A genome, 13 linkage groups were assigned to chromosomes in the B genome, and only 7 linkage groups were assigned to the D genome. Two linkage groups could not be assigned to chromosomes and were designated mixed groups (data not shown; See Table 3.3 in Sadeque, 2008). A total map distance of 882.9cM was developed.

QTL Analyses

QTL analyses identified five genomic regions (chromosomes 1A, 2B, 3B, 4B and 5B) that controlled flour yellowness (b*) (Table 1, For Likelihood Ratio Statistic (LRS) and additive effect plots see Sadeque, 2008.). A highly significant QTL (*Qb*.dmh45-2B*) was located on chromosome 2B across the growing seasons. In 2005, the QTL on chromosome 2B (LRS=20.5) explained 35% of variation in flour b*. Marker wPt-6576 associated with flour b*. In 2006, chromosome 2B explained 35% of phenotypic variation with an LRS value of 20.6. QTL *Qb*.dmh45-4B* which appeared across the growing seasons explained 25% (LRS=10.9) and 23% (LRS=9.8) of phenotypic variation respectively (Table 1). Marker wPt-7569 within this QTL was associated with flour b*. Another significant QTL (*Qb*.dmh45-5B*) was also detected that explained 19% of observed variation across seasons. It was significant in season 2005, suggestive in 2006 and significant across seasons (LRS=10.9). Marker wPt-3457 within this QTL had a significant association with flour b*.

Two other QTLs (*Qb*.dmh45-1A and Qb*.dmh45-3B)* were identified in 2006 on chromosomes 1A and 3B that were associated with 23 and 17% of phenotypic variations in flour b*, respectively (Table 1). Marker loci wPt-9266, and wPt-0086 were identified to have associations of the highest significance with b* within the QTLs on chromosomes 1AS and 3B, respectively. Although these QTLs were only detected in the growing season of 2006, they were observed in both replications; and the pooled environment. The allele of the QTL on chromosome 3B that contributed to increasing b* was present in the H45 parent while other alleles that contributed to increased b* were donated by the parent DM5637*B8. In this experiment it was identified that both parents DM5637*B8 and H45 contributed towards increasing yellowness of flour (b*).

Table 1 Results of interval mapping of flour color (CIE b*) in a set of lines of the DM5637*B8 x H45 DH population.

Site-year	Chromosome location	QTLs	Closest marker	LRS value[1/]	PVE (%)[2/]	Parent (+ve PPO)
Narrabri-2005						
Flour b*	2B	*Qb*.dmh45-2B*	wPt-6576	20.5**	35	DM5637*B8
	4B	*Qb*.dmh45-4B*	wPt-7569	10.9*	25	DM5637*B8
	5B	*Qb*.dmh45-5B*	wPt-3457	10.5*	20	DM5637*B8
Narrabri-2006						
Flour b*	1A	*Qb*.dmh45-1A*	wPt-9266	12.7*	23	DM5637*B8
	2B	*Qb*.dmh45-2B*	wPt-6576	20.6**	35	DM5637*B8
	3B	*Qb*.dmh45-3B*	wPt-0086	8.7*	17	H45
	4B	*Qb*.dmh45-4B*	wPt-7569	9.8*	23	DM5637*B8
	5B	*Qb*.dmh45-5B*	wPt-3457	6.9*	13	DM5637*B8
Pooled						
Flour b*	1A	*Qb*.dmh45-1A*	wPt-9266	10.4*	19	DM5637*B8
	2B	*Qb*.dmh45-2B*	wPt-6576	25.8**	42	DM5637*B8
	3B	*Qb*.dmh45-3B*	wPt-0086	7.0*	14	H45
	4B	*Qb*.dmh45-4B*	wPt-7569	12.9*	29	DM5637*B8
	5B	*Qb*.dmh45-5B*	wPt-3457	10.9*	19	DM5637*B8

[1/] Likelihood statistic ratio; * and ** indicate significant and highly significant level at P<0.01 and P<0.001, respectively. Non asterisk LRS values indicate the suggestive level (P>0.05).
[2/] Phenotypic variation explained.

Discussion

In this study, the molecular mapping of wheat flour color (yellowness) was studied using the DArT approach. QTL analysis for flour yellowness (ie, color b*) identified associations of DArT markers in different chromosomal locations with the character. A new putative QTL for controlling flour color (b*) on chromosome 2B (*Qb*.dmh45-2B*) was identified in this study. QTL locations on chromosomes 3B, 4B and 5B (*Qb*.dmh45-3B*, *Qb*.dmh45-4B* and *Qb*.dmh45-5B*) are in agreement with the findings of Mares and Campbell (2001). They identified QTLs for flour b* on chromosomes 3B, 4B, 5B and 7A using a Sunco x Tasman DH population. The similarity between findings is not unexpected because Sunco is in the parentage of the DM5637*B8 line. However, these results are quite different to those of Parker et al. (1998) who detected molecular markers within two QTLs on chromosomes 7A and 3A that were associated with flour color in a recombinant inbred population of hexaploid wheat derived from two Australian cultivars Schomburgk x Yarralinka**.** In the current study, the QTLs on 2B, 4B and 5B were identified across the growing seasons and could be considered to be loci that consistently control flour color. The QTL that was identified on chromosome 2B explained 42% of the observed phenotypic variation indicating that the inheritance of flour yellowness of this population is predominantly contributed by this genomic region. The other two QTLs (*Qb*.dmh45-1A* and *Qb*.dmh45-3B*) that were identified were detected in both replications in2006 and upon analysis of pooled data. Some QTLs are common to all environments. Others are environment specific and are only expressed in particular environmental conditions. These are usually less favoured in breeding. Beside the putative QTL, the genomic regions detected by the other QTLs also together showed a large amount of phenotypic variations towards flour yellowness.

The results of the current study which used a set of lines of the DM5637* x H45 DH population confirmed earlier findings about the involvement of the genomic regions in controlling flour color. In addition, new genomic regions and associated DArT markers were identified that were linked to loci that control wheat flour color. The information obtained and the markers identified will expedite efforts to develop improved Australian cultivars that are suitable inputs for Asian noodle products.

Acknowledgments

The research was conducted in the University of Sydney Plant Breeding Institute, Cobbitty and financed by The Value Added Wheat CRC Ltd.

References

Acquaah, G. 2007. Principles of Plant Genetics and Breeding. Blackwell Publishing, U.K.

Huttner, E., P. Wenzyl, J. Carling, L. Xia, S. Yang, D. Jaccoud, V. Caig, M. Evers, G. Uszynsi, N. Howes, P. Sharp, P. Vaughan, B. Rathmell and A. Kilian. 2006. Triticarte: whole-genome profiling service for wheat and barley using Diversity Arrays Technology (DArT). Proceedings 13th Australasian Plant Breeding Conference. 18-21 April, 2006. Christchurch, New Zealand.

Jaccoud, D., K. Peng, D. Feinstein and A.Kilian. 2001. Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucl. Acids Res. 29:e25.

Kruger, J.E., R.R. Matsuo and K. Preston. 1992. A comparison of methods for the prediction of Cantonese noodle colour. Can. J. Plant Sci. 72: 1021-1029.

Manly, K.F., R.H. Cudmore and J.M. Meer. 2001. Map manager QTX, cross-platform software for genetic mapping. Mamm. Genome 12: 930-932.

Mares, D.J. and A.W. Campbell. 2001. Mapping components of flour and noodle colour in Australian wheat. Aust. J. Agric. Res. 52: 1297-1309.

Miskelly, D.M. 1984. Flour components affecting pasta and noodle colour. J Sci. Food Agric. 35: 463-471.

Parker, G.D., K.J. Chalmers, A.J. Rathjen and P. Langridge. 1998. Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 97**:**238-245.

Piepho, H.P. 2001. A quick method for computing approximate thresholds for quantitative trait loci detection. Genetics 157: 425-432.

Sadeque, A. 2008. Genetic mapping of noodle quality characters and rust resistance in hexaploid wheat. Ph.D. thesis. The University of Sydney, NSW, Australia.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93: 77-78.

Wenzl, P., V. Caig, J. Carlin, C. Cayla, M. Evers, D. Jaccoud, S. Patarapuwadol, G. Uszynski, L. Xia, S. Yang, E. Huttner and A. Kilian. 2004. Diversity Arrays Technology, a novel tool for harnessing crop genetic diversity. Proceedings 4th International Crop Science Congress.

Wittenberg, A.J., T. Lee, C. Cayla, A. Kilian, R.G.F Visser and H.J. Schouten. 2005. Validation of the high-throughput marker technology DArT using the model plant Arbidopsis thaliana. Mol. Gen. Genomics 274: 30-39

Manuscript received 11 February 2010, accepted 30 September 2010