A molecular toolbox for xanthophyll genes in wheat

Crawford AC[1], Shaw K[1], Stefanova K[1], Lambe W[1], Ryan K[2], Wilson R[1], Barclay I[1], McLean R[1], Moore C[1], Francki M[1]
[1] Department of Agriculture and Food, Western Australia. [2] Centre for Comparative Genomics, Murdoch University, Western Australia

BACKGROUND

The biological accumulation of xanthophyll compounds responsible for differences in yellow colouration of the wheat endosperm is of considerable commercial interest for specific end use products. The xanthophyll biochemical pathway is comprised of around nine major gene groups and associated enzymes involved in the synthesis of various xanthophyll or carotenoid components. The current project seeks to establish a molecular toolbox for exploring the chromosomal location and expression of xanthophyll related genes. Identification of target genes has involved online database searches for gene ontologies and similarity searching with rice or arabidopsis to retrieve wheat sequences. To include the possibility of multiple genes within each group, primer design was based upon the alignment of sequences from related grain species and validated by phylogenetic assessment. This work will assist in the identification of gene candidates for QTL and allelic variation for xanthophyll content and flour colour in Australian wheat germplasm. To date, preliminary results indicate possible involvement of Rab geranylgeranyl transferase I α-subunit genes.

BIOINFORMATICS

Sequences were obtained by searching the NCBI database for key terms associated with gene groups in the xanthophyll/carotenoid biosynthesis pathway. Unigene matches for wheat, rice and Arabidopsis in particular were investigated as sources of complete mRNA coding sequences. BLASTn analyses (more dissimilar sequences) of these sequences against *nr* and *est_others* databases were done to detect additional sequences, and to investigate the presence of closely related gene groups or families with up to 40% similarity to the reference sequence. The BLAST tree option was used to select representative sequences from gene clusters, with the longest sequence chosen in most cases. Initial BLAST results were scanned for wheat sequences in other regions of the reference sequence not aligned in the tree, and the process repeated. Related wheat unigene clusters were downloaded, and in some cases where few sequences were available, representative contigs assembled. Wheat unigene clusters were screened for probe sequences in the USDA database of binned wheat probes. This yielded information on wheat xanthophyll genes already designated a chromosomal location.

Sequences were downloaded to Vector NTI 10.3.0 2006 (Invitrogen Corporation). Alignments for each gene group were created, for the purposes of phylogenetic analyses and primer design. A phylogenetic approach was used to validate relationships among gene sequences, using MEGA version 3.1 (Kumar, Tamura, Nei 2004). Corresponding chromosome regions for rice EST sequences were also downloaded to determine the location of possible intron sequences within wheat transcripts. PCR primers were designed from conserved regions, with the intention of amplifying approximately 150bp of coding sequence for gene expression analyses, and spanning intron sequences to maximise identification of variation for genetic mapping. A summary of gene groups targeted for analysis and their known chromosomal location is provided in Table 1.

Table 1. Xanthophyll/carotenoid gene groups targeted for gene mapping and expression in wheat.

Gene	Wheat Unigene	Location
Geranylgeranyl transferase II β-subunit	Ta.8060	7AS, 7BS, 7DS[1]
Geranylgeranyl transferase I β-subunit	Ta.52355	3BS, 3DS[2]
Geranylgeranyl transferase I α-subunit	**Ta.36809**	**7BL, 7DL[5]**
Rab escort protein	Ta.48571	3B[2]
Geranylgeranyl pyrophosphate synthase	Ta.49323	*in progress*
	Ta.61207	*in progress*
Phytoene synthase	Ta.41960	*in progress*
	Ta.20776	7AL[3]
Phytoene desaturase	Ta.28286	4AS, 4BL, 4DL[1,4]
ζ-Carotene desaturase	Ta.49794	2A, 2B[4]
Lycopene ε-cyclase	ND	*in progress*
Lycopene β-cyclase	Ta.12691	*in progress*
β-Carotene hydroxylase	Ta.39180	*in progress*
	Ta.53923	*in progress*
ε-Carotene hydroxylase	Ta.35385	6AL, 6BL, 6DL[1]
Zeaxanthin epoxidase	Ta.53974	*in progress*
Violaxanthin deepoxidase	Ta.275	*in progress*
Neoxanthin synthase	ND	*in progress*

[1]USDA [2]Ryan 2005 [3]He et al. 2007 [4]Cenci et al. 2004 [5]This study

DELETION MAPPING AND QTL ALIGNMENT

Wheat deletion lines are currently being screened for candidate genes involved in xanthophyll biosynthesis. Preliminary results mapped the (Rab) geranylgeranyl transferase I α-subunit gene to the distal bin of the long arms of chromosomes 7B and 7D (Figure 1). The DNA sequence of the 563bp fragment from 7BL has been cloned, with the putative coding region of 165bp identical to wheat EST CJ723430. The coding region flanks an intron sequence of 398bp, the position of which is conserved across a rice RGGT gene ortholog (Figure 2).

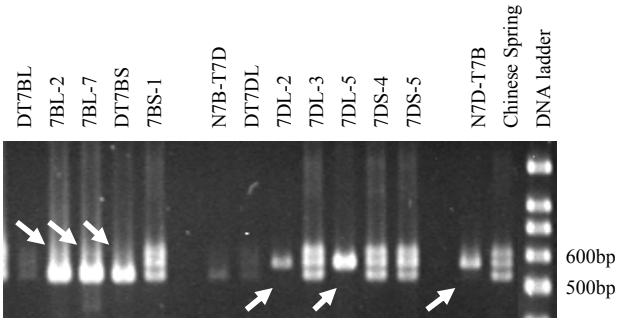

Figure 1. PCR of putative (Rab) geranylgeranyl transferase I α-subunit on wheat ditelosomic and deletion lines (chromosome 7 subset) located to distal ends of the long arms of chromosomes 7B and 7D (arrows indicate missing bands).

A genetic map of 480 SSR, DArT and Stm markers was generated from the doubled haploid population, Carnamah/WAWHT2046. The population consisted of 121 individuals and was grown in the field at one location in 2002 (Wongan Hills, WA) and two locations in 2003 (Wongan Hills and Merredin, WA). Grain from individuals were milled using a Quadramat Junior Mill and b* measurements of flour samples taken using a Minolta CR-400 Chroma meter. Xanthophyll was ethanol extracted from flour samples as described by Mares and Campbell (2001) and measured by absorbance at 436 nm on a Perkin Elmer 25 UV/VIS Spectrometer.

Pearson's correlation co-efficient for phenotypic values of b* and xanthophyll content ranged from r=0.733 to r=0.787 in different environments and were highly significant (P<0.0005). QTL analysis identified a region of the long arm of chromosome 7B accounting for 19% and 20% of the total variation for xanthophyll content and b*, respectively. The QTL for xanthophyll and b* were co-located in the marker interval 506acag-gwm537 (Figure 3) and highly significant (P<0.01) for each trait. The QTL were consistently detected in all environments. The QTL were anchored to the distal region of the long arm using 6 markers flanking the QTL (Figure 3). The region containing the QTL was mapped to the same deletion bin as the (Rab) geranylgeranyl transferase I α-subunit gene (Figure 3). Work is currently in progress to identify nucleotide sequence variation in the RGGT gene between parents of the doubled haploid mapping population, Carnamah and WAWHT2046, and whether it maps within the region delineating the QTL for b* and xanthophyll content. If so, then RGGT is a potential candidate gene encoding enzymes involved in the biosynthetic pathway that may contribute to phenotypic variation for xanthophyll content and flour b*. Any sequence differences for this gene may be associated with varying roles in vesicle trafficking and enzyme secretion (Wojtas 2007).

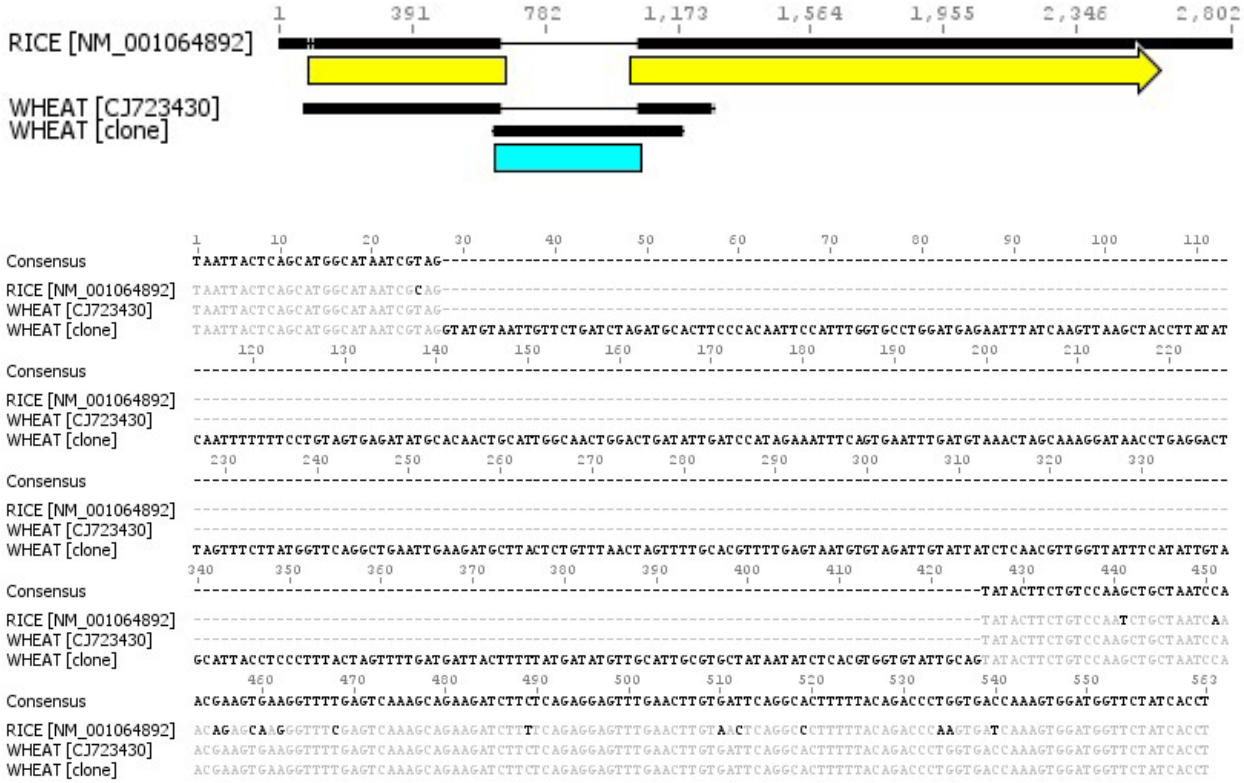

Figure 2. Schematic diagram (top) of cloned putative Rab-geranylgeranyl transferase I α-subunit gene fragment compared with existing wheat and rice mRNA sequences (yellow block - rice coding region, blue block - wheat intron sequence). Corresponding alignment (bottom) of cloned fragment with sequence differences highlighted.

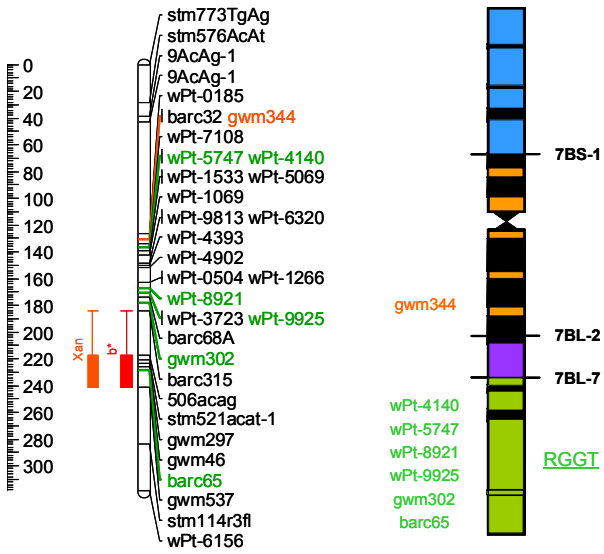

Figure 3. Genetic map of chromosome 7B from the Carnamah/WAWHT2046 population with QTL position for xanthophyll content and b* represented by orange and red bars, respectively. The centimorgan scale is shown to the left of the genetic map. The karyotype is shown on the right with markers in deletion bins (colour coded) that have also been placed on the genetic map. The position of RGGT is shown in the distal bin on the right of the deletion map.

ACKNOWLEDGEMENTS

This project was partly supported by the GRDC project CWQ00009 and CWQ00013 in the Australian Winter Cereal Molecular Marker Program. The authors wish to thank Natalie Parry and Tash Teakle for technical assistance in genetic map construction.

REFERENCES

USDA (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

Cenci A., Somma S., Chantret N., Dubcovsky J., Blanco, A. PCR identification of durum wheta BAC clones containing genes coding for carotenoid biosynthesis enzymes and their chromosome localization. Genome 47: 911-917

He X.Y., Zhang Y.L., He Z.H., Wu Y.P., Xiao Y.G., Ma C.X., Xia X.C. (2008). Characterization of phytoene synthase 1 gene (Psy1) located on common wheat chromosome 7A and development of a functional marker. Theor. Appl. Genet. 116: 213-221.

Kumar S., Tamura K., Nei M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5:150-163.

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

Ryan K (2005). Validation of flour colour in Western Australian adapted wheat: comparative genomics, moelcular markers and QTL analysis. PhD dissertation, Murdoch University, Western Australia.

Wojtas M., Swiezewski S., Sarnowski T.J., Plochocka D., Chelstowska A., Tolmachova T., Swiezewska E. (2007). Cloning and characterization of Rab Escort Protein (REP) from Arabidopsis thaliana. Cell. Biol. Int. 31(3): 246-251.