A molecular toolbox for xanthophyll genes in wheat

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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 particular were investigated as sources of complete Unigene matches for wheat, rice and Arabidopsis in the xanthophyll/carotenoid biosynthesis pathway. 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.

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 flanks an intron sequence of 398bp, the position of which is conserved across a rice RGGT gene ortholog (Figure 2).

1USDA 2Ryan 2005 3He et al. 2007 4Cenci et al. 2004 5This study
A genetic map of 480 SSR, DAfT 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 506aagc-gwm357 (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 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).

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.
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.

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Figure 3. Genetic map of chromosome 7B from the Carnamah/WAWHT2046 population with QTL position for xanthophyll content and \textit{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.

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