

# **Variation of flour colour in Western Australia adapted wheat: comparative genomics, molecular markers and QTL analysis**

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## **Declaration & List of Papers Published**

I declare that this thesis is my own account of my research and contains as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

Papers that have been published from the research described in this thesis are:

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## Abstract

The yellowness of flour colour ranges is an important quality trait in wheat for end-use products and is determined by the accumulation of carotenoids in the endosperm. The aims of this study were to develop EST-based molecular markers for genes encoding enzymes of the carotenoid biosynthetic pathway leading to xanthophyll accumulation and identify quantitative trait loci for flour colour ( $b^*$ ) and xanthophyll content in Western Australian adapted germplasm.

A novel bioinformatic strategy was developed to identify rice genes encoding key enzymes of the carotenoid biosynthetic pathway and to predict wheat orthologues on the short arm of chromosome 3 or long arm of chromosome 7. The bioinformatic strategy involved the identification of rice carotenoid genes on BAC/PAC contigs aligned to wheat mapped ESTs. Rice genes predicted to have wheat orthologues were selected based on ESTs mapping to regions on wheat homoeologous chromosomes 3 and 7 known to be involved in flour colour. The rice genes predicted to have wheat orthologues were *Geranylgeranyltransferase I  $\beta$ -subunit (GGT-Ibeta)* and *Rab geranylgeranyltransferase component A (RGGT-A)* on the short arm of chromosome 3, *Lycopene  $\beta$ -cylcase (LBC)* on the long arm of chromosome 3 and *Lycopene  $\epsilon$ -cylcase (LEC)* on the long arm of chromosome 7.

The prediction of these wheat orthologues provided the basis for development of EST-based molecular markers for detecting variation in xanthophyll content. Wheat ESTs with unknown chromosomal locations and having the highest similarity to *GGT-Ibeta*, *RGGT-A* and *LBC* were selected for the development of molecular markers. No EST homologues were identified for *LEC* and therefore this gene was not further considered. Orthology was confirmed by sequencing and deletion lines were used to confirm chromosomal locations. Two partial orthologues of *GGT-Ibeta* were identified on the short arms of chromosomes 3B and 3D. A partial orthologue of *RGGT-A* was mapped to the proximal regions of the short and long arms of chromosome 3B. At least two or more orthologues of *LBC* were identified from nullisomic-tetrasomic lines. An EST-based molecular marker for *GGT-Ibeta* was found to be involved in minor variation of xanthophyll content in a Westonia\*2/Janz doubled haploid population.

QTL analysis from three doubled haploid populations indicated variation in WA-adapted germplasm may be due to different alleles controlling flour colour. QTLs for

b\* and xanthophyll content were found to coincide on the short arms of chromosomes 3A, 4D, and 7B and the long arm of chromosomes 7A and 7B in WA-adapted germplasm. Homoeologous expression of regions controlling variation in b\* and xanthophyll content on the long arm of chromosomes 7A and 7B suggests the shut-down of genes in the same region on chromosome 7D. The main outcome of this study is flour colour and identification of gene orthologues in wheat controlling variation in xanthophyll content is complex most likely because of the interaction of the carotenoid biosynthetic pathway with other pathways.

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## Glossary

**ABA** abscisic acid.

**ABA** abscisic acid locus.

**ADR** Australian Durum wheat.

**AFLP** Amplified fragment length polymorphisms.

**allele** One of the different forms of a gene that can exist at a single locus.

**ASW** Australian Standard White.

**AWB** Australian Wheat Board

**BAC** Bacterial artificial chromosome; an F plasmid engineered to act as a cloning vector that can carry large inserts.

**BCH** *β-Carotene hydroxylase*.

**BLAST** Basic Local Alignment Search Tool.

**BLASTN** comparison of nucleotide sequence databases.

**BLASTP** comparison of protein sequence databases.

**BLASTX** comparison of nucleotide query sequence translated in all reading frames against a protein sequence database.

**BSA** Bulk segregant analysis.

**candidate gene** A sequenced gene of previously unknown function that, because of its chromosomal position or some other property, becomes a candidate for a particular function such as disease determination.

**Ccs** *Capsanthin-capsorubin synthase* gene.

**cDNA (complementary DNA)** Synthetic DNA transcribed from a specific RNA through the action of the enzyme reverse transcriptase.

**CIE b\*** Commission Internationale l'Eclairage whiteness/yellowness value.

**CIE L\*** Commission Internationale l'Eclairage L\* (brightness) value.

**CIELAB** Commission Internationale l'Eclairage L\* a\* b\* colour space.

**CO<sub>2</sub>** Carbon dioxide

**crtE** *Lycopene ε-cyclase* gene from *Erwinia uredovora*.

**CrtL-b** *Lycopene β-cyclase* gene.

**CrtL-e** *Lycopene ε-cyclase* gene.

**CrtR** *β-Carotene hydroxylase* gene.

**DAWA** Department of Agriculture Western Australia.

**dbEST** Expressed sequence tag database.

**deletion** Removal of a chromosomal segment from a chromosome set.

**DH** Doubled haploid.

**DMADP** dimethylallyl diphosphate.

**DNA (deoxyribonucleic acid)** A double chain of linked nucleotides (having deoxyribose as their sugars); the fundamental substance of which genes are composed.

**DPA** Days post anthesis.

**environment** The combination of all the conditions external to the genome that potentially affect its expression and its structure.

**EST** Expressed sequence tag(s).

**FAD** Flavin adenine dinucleotide.

**FAO** Food and Agricultural Organization of the United Nations

**FDP** Farnesyl diphosphate.

**ha** Major locus controlling the recessive grain hardness trait.

**GDP** Geranyl diphosphate.

**GDPS** Rab Geranylgeranyl transferase-like protein.

**gene** The fundamental physical and functional unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that makes possible transcription.

**gene family** A set of genes in one genome all descended from the same ancestral gene.

**genome** The entire complement of genetic material in a chromosome set.

**genotype** The specific allelic composition for a certain gene or set of genes.

**GGH** Geranylgeranyl hydrogenase (reductase).

**GGPP** Geranylgeranyl pyrophosphate.

**GGPP** Geranylgeranyl pyrophosphate synthase.

**GGT-Ibeta** Geranylgeranyltransferase I  $\beta$ -subunit.

**GRDC** Grains Research and Development Corporation of Australia

**HIF** heterogenous inbred families.

**HMW-GS** high-molecular-weight glutenin subunit.

**IDP** Isopentyl diphosphate.

**ITMI** International Triticeae Mapping Initiative.

**L** Long arm of chromosome.

**LBC** *Lycopene  $\beta$ -cyclase*.

**LEC** *Lycopene  $\epsilon$ -cyclase*.

**LHC** light harvesting complex.

**library** A collection of DNA clones obtained from one DNA donor.

**linkage** The association of genes on the same chromosome.

**linkage group** A group of genes known to be linked; a chromosome.

**linkage map** A chromosome map; an abstract map of chromosome loci, based on recombinant frequencies.

**LMW-GS** low-molecular-weight glutenin subunit.

**locus** (plural **loci**) The specific place on a chromosome where a gene is located.

**MAS** Marker assisted selection.

**molecular markers** genetic markers; alleles used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome, a gene or a trait.

**NIL** Near isogenic line.

**NS** *Neoxanthin synthase*.

**Nulli-tetra** Nullisomic-tetrasomic line.

**NWMMP** National Wheat Molecular Marker Project.

**PAC** Phage P1 artificial chromosome.

**PCR** *See Polymerase chain reaction.*

**PDS** *Phytoene desaturase*.

**phenotype** (1) The form taken by some character (or group of characters) in a specific individual. (2) The detectable outward manifestations of a specific genotype.

**Polymerase chain reaction** A method for amplifying specific DNA segments which exploits certain features of DNA replication.

**polymorphism** The occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome.

**PPDP** Prephytoene diphosphate.

**PPO** Polyphenol oxidase.

**PHST** Pre-harvest sprouting tolerance.

**PSII** Photosystem II.

**PYS** *Phytoene synthase*.

**Quantitative trait loci** loci controlling genetic variation of a quantitative trait.

**QTL** *See Quantitative trait loci*.

**RAPD** Randomly amplified polymorphic DNA.

**RFLP** Restriction fragment length polymorphisms.

**RGGT-A** *Rab geranylgeranyltransferase* component A.

**Rht** Semi-dwarfing (restricted height) gene.

**RIL** Recombinant inbred line.

**S** Short arm of chromosome.

**SNP** Single nucleotide polymorphisms.

**SSR** Simple sequence repeats ( or microsatellites).

**STS** Sequence tagged site.

**TBLASTN** comparison of a protein query sequence against a nucleotide sequence database translated in all reading frames.

**TBLASTX** comparison of six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

**TIGR** The Institute for Genomic Research

**VDE** *Violaxanthin deepoxidase*.

**Vp14** *Violaxanthin-cleavage* gene (*Zea mays*).

**WA** Western Australia.

**WSN** White salted noodles.

**YAC** yeast artificial chromosome.

**YAN** Yellow alkaline noodles.

**ZDS** *ζ-Carotene desaturase*.

**ZE** *Zeaxanthin epoxidase*.



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