Genetic control of Apigenin di-C-glycoside biosynthesis in bread wheat grain and their role as yellow pigments of Asian alkaline noodles

Submitted by

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This book is An Answer to Prayers of a Long list of Believers

I have been very blessed and loved with all of your spiritual supports, for His guidance and protections, and sincerely will not have enough to thank you all.....

May you all be blessed and loved, too

As I have been always,

yasmein

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Summary

Colour is an important determinant of quality and customer appeal for Asian noodles that are made from bread wheat (*Triticum aestivum* L.). The Asian noodle market represents approximately one third of wheat exports from Australia and as a consequence maintaining and improving colour for noodles is an important research and breeding objective. The focus of this project is yellow alkaline noodles (YAN) prepared using wheat flour and alkaline salts, sodium and potassium carbonate, and for which a bright yellow colour is desired. Xanthophylls, primarily lutein, and apigenin di-C-glycosides (ACGs) have been shown to be important components of this yellow colour. ACGs were of particular interest since, in contrast to lutein, the content in flour could be increased without adverse effects on colour of other end-products. There was little information either on the genetic variation for ACG content or the mechanism and genetic control of biosynthesis which was surprising in view of their putative role in a wide range of plant processes, food colour and flavour, and possibly human health.

The aims of this project were to provide new information on the role of ACGs in YAN colour and genetic regulation of their biosynthesis. To achieve this aims: genetic variation in grain ACG traits in bread wheat and related species was surveyed, the quantitative contribution of ACG to the yellow colour of YAN was determined and compared to lutein, QTL for ACG content and composition were located, and candidate genes associated with variation in ACG composition identified.

Substantial variation in both grain ACG content and the ratio, ACG1/ACG2, were identified within bread wheat cultivars and related species. Genotype controlled the

major portion of the variation. ACG content appeared to be a multigenic trait whereas variation in ACG1/ACG2 was associated with a limited number of chromosomes, in particular chromosomes 1B, 7B and 7D. In the absence of chromosome 7B (Chinese Spring 7B nullisomics) there was a substantial increase in ACG1/ACG2, i.e. a relative increase in the glucose-containing isomer, possibly indicating the presence of a *C*-glycosyltransferase on 7B with specificity for UDP-galactose. A similar phenotype observed in some wheat cultivars could be explained by a deletion or mutation of a gene controlling this enzyme. The results suggest that it should be possible to manipulate both ACG content and composition through breeding.

Only 30% of ACG (means 19.3μg/g) is recovered in flour, which contributed to 1 to 3 CIE b* units to the part of the yellow colour of yellow alkaline noodles (YAN) that develops specifically in the presence of alkali. The relatively low recovery of ACG in flour contrasts with the high recovery of lutein (90%, with means 1.011μg/g). Since the difference between white salted noodles (WSN) and YAN is approximately 6 b* units, this would indicate that another unidentified compound(s) is responsible for the difference. Potential for ACG0-based improvement of bread wheat cultivars for YAN yellowness is likely to be limited by the range of genetic variation, the location of ACG in grain tissues that are largely discarded during milling and the lack of correlation between grain and flour ACG content. Moreover, the observed variation in ACG recovery in small scale milling was not reflected in larger scale milling anticipated to better represent commercial practice. The improvement in flour recovery and the amount of ACG recovered in the flour were not significant and not enough to achieve the yellowness of commercial noodles. Selection that requires larger scale milling is costly, time consuming and not applicable to early generation screening. In this context,

further work on QTL associated with variation in ACG content and development of marker-assisted-selection would be very useful.

Addition of thirteen new markers to the QTL region for ACG trait on chromosome 7BS in a Sunco/Tasman doubled haploid population reduced the size of the QTL interval from 28.8cM to approximately 5.5cM. In this revised 7BS map, the major QTL for ACG1 and ACG2 content as well as ACG1/ACG2 ratio were detected within 4.7cM of SSR marker *Xwmc76*. The QTL region linked to *Xwmc76* was shown to be syntenic with a region in rice chromosome 6S between AP005387 and AP005761 and a region on *Brachypodium* chromosome 1. Based on these comparisons, the most likely candidate gene associated with variation in ACG composition appeared to be a glycosyltransferase. Alternate alleles at the 7BS QTL may be associated with amino acid changes within the *C*-glycosyltransferase that shift the substrate specificity from galactose (ACG2, Tasman) to glucose (ACG1, Sunco). Alternatively, based on a comparison of Chinese Spring nullisomic-tetrasomic lines where nullisomic 7B was associated with a phenotype similar to Sunco, it is possible that Sunco contains a null allele. Other candidate genes located on the same chromosome that could potentially be involved in ACG biosynthesis were identified and included a sugar transporter, which could determine the relative sizes of the available pools of UDP-glucose and UDP-galactose, an epimerase required for inter-conversion of these sugars, other glycosyltransferases and a flavone-2-hydroxylase (F2H) involved in the first committed step in the pathway to ACG.

Research approaches that could be used to validate the role of the candidate gene are discussed along with other options for improving the colour of wheat cultivars for the

YAN market. Options for utilizing ACG as yellow pigment of noodles might include incorporating the embryo or seed coat materials (pollard and bran) into the flour after milling and genetic modification of bread wheat to achieve ACG expression in the starchy endosperm.

Statement of Authorship

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Grace Yasmein Wijaya

November 2012

List of Publications

Wijaya GY, Asenstorfer R & Mares D 2010, 'Flavone-*C*-diglycosides and lutein in wheat grain: their contribution to the yellow colour of alkaline noodles', in: C Blanchard, D Pleming & H Taylor (eds), *Cereal 2009: Proceedings of the 59th Australian Cereals Chemistry Conference*, Wagga Wagga, New South Wales, Australia, 27-30 September 2009, Royal Australian Chemistry Institute, pp. 162-164.

Wijaya GY, Asenstorfer RA & Mares DJ (2010), 'Genetic control of *C*-glycosylation in the biosynthesis pathway of flavone-*C*-diglycosides, yellow colour substance of alkaline noodles, in bread wheat'. *Molecular Life- from discovery to Biotechnology*, *Ozbio 2010 (ASBMB conference in conjuction with the 12th IUBMB conference and FAOMB)*, Melbourne, 26 September-1 October 2010.

Wijaya GY, Mather DE, Brave M, Wu H, Walker AR, Chalmers KJ & Mares DJ 2012, 'QTL associated with variation in apigenin-*C*-diglycoside content and composition of bread wheat (*Triticum aestivum* L.) grain and the identification of candidate genes', *Molecular Mapping and Marker assisted Selection International Conference*, Vienna, 8-11 February 2012.

Wijaya GY & Mares DJ 2012, 'Apigenin di-*C*-glycosides (ACG) content and composition in grains of bread wheat (*Triticum aestivum*) and related species', *Journal of Cereal Science* 56 (2): 260-267.

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List of Abbreviations

2-ODDs	2-oxoglutarate-dependent dioxygenases
ACG	apigenin di-*C*-glycosides
ACG1	apigenin-6*C*-arabinoside-8*C*-glucoside (isoschaftoside)
	apigenin-6C-glucoside-8*C*-arabinoside (schaftoside)
ACG2	apigenin-6*C*-arabinoside-8*C*-galactoside
	apigenin-6*C*-galactoside-8*C*-arabinoside
AFLP	amplified fragment length polymorphism
AMD	age-related macular degeneration
ANR	anthocyanidin reductase
ANS	anthocyanidin synthase
AS	aureusidin synthase
BAC library	bacterial artificial chromosome library
bHLH	basic/helix-loop-helix
Bz-W22	*O*-glycosyltransferases: UDP-glucosyl and glucuronosyl transferase
	of maize bronze alleles
cDNA	complimentary DNA, synthesise from mRNA
CHI	chalcone isomerase
CHS	chalcone synthase
CIE	Commission Internationale d'Eclairage
DArT	diversity arrays technology
DFR	dihydroflavonol-4-reductase
EBG	early biosynthetic genes
EST	expressed sequence tags

F2H	2*S*-flavanone 2-hydroxylase
F3H	flavanone-3β-hydroxylase
FCGT	flavonoid *C*-glycosyltranferase
FLS	flavonol synthase
FS1	flavone synthase 1
FS2	flavone synthase 2
GAT	UDP-glucuronic acid:flavonol-3-O-glucuronosyltransferase
gDNA	genomic DNA
GT	glycosyltransferases
HPLC	high performance liquid chromatography
IFS	isoflavones synthase
LAR	leucoanthocyanidin reductase
LBG	late biosynthetic genes
LOX	lipoxygenase
OMT	*O*-methyltransferases
OsCGT	*C*-glycosyltransferase of rice
PAC clone	P1 artificial chromosome clone
PPO	polyphenol oxidase
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
RT	rhamnosyl transferase
RT-PCR	reverse transcription polymerase chain reactions
SDR	short-chain dehydrogenase/reductases
SNP	single nucleotide polymorphism
SSR	simple sequence repeat

UDP-galactose	uridine 5′-diphosphogalactose
UDP-glucose	uridine 5′-diphosphoglucose
UFGT	UDP glycose:flavonoid-3-O-glycosyltransferase UDPG flavonol 3-*O*-glucosyl transferase
VvGT1	UDP-glucose:flavonoid 3-O-glycosyltransferase with ability to transfer UDP galactose in *Vitis vitifera*
VvGT5	UDP-glucuronic acid:flavonol-3-O-glucuronosyltransferase (GAT) of *Vitis Vitifera*
VvGT6	UDP-glucose/UDP-galactose:flavonol-3-O-glucosyltransferase/galactosyltransferase of *Vitis Vitifera*
WSN	white salted noodles
YAN	yellow alkaline noodles
Є-LCY	Є-cyclase