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Susan A. Gillies
Southern Cross University

Shane R. McIntosh
Southern Cross University

Robert J. Henry
Southern Cross University

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Fortification of Plant Folate Genes in Rice

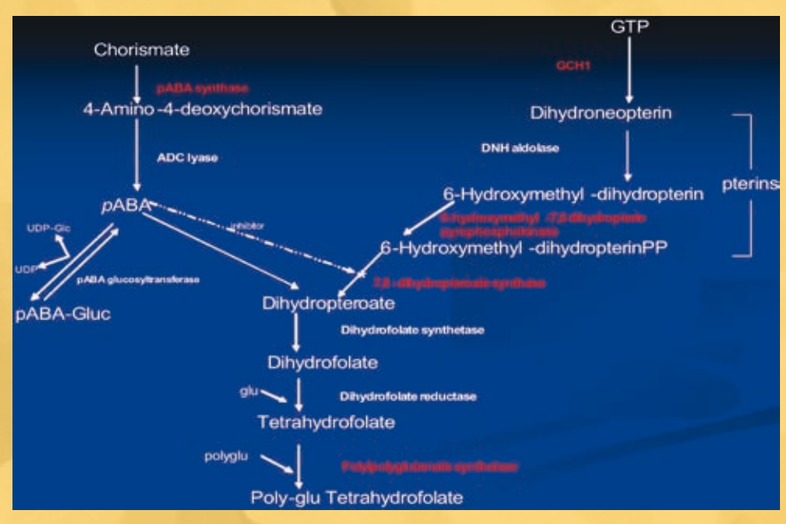
S. Gillies, S. McIntosh, R. Henry
Grain Foods CRC, Centre for Plant Conservation Genetics, Southern Cross University NSW 2480, Australia

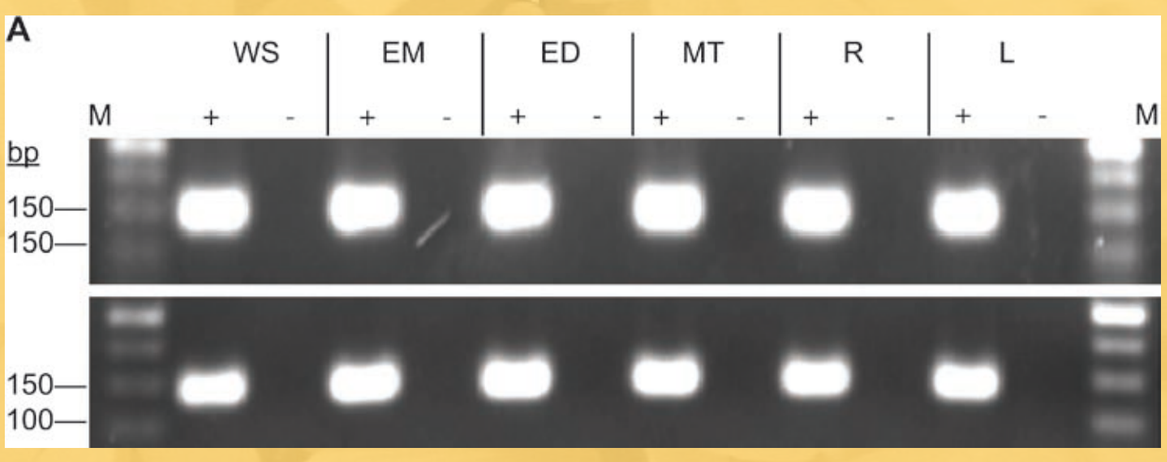
Tetrahydrofolate is a B vitamin that serves as a coenzyme critical in the methylation cycle and DNA synthesis. It has an important role in the pathogenesis of a broad spectrum of human disease including anemia, cardiovascular disease, thromboembolism, dementia and Alzheimers along with various cancers. Importantly low folate has been demonstrated to be a major cause of congenital neural tube defects (NTDs).

Folate cannot be synthesized by vertebrates, consequently plant food is the primary source. Rice however, which provides half the world's population with 80 percent of their diets, is particularly low in folate. The production of a rice crop yielding high level folate would benefit not only the developing world, but also the developed world as a supplement to fortified wheat products, particularly as the number of persons avoiding wheat products is rising markedly.

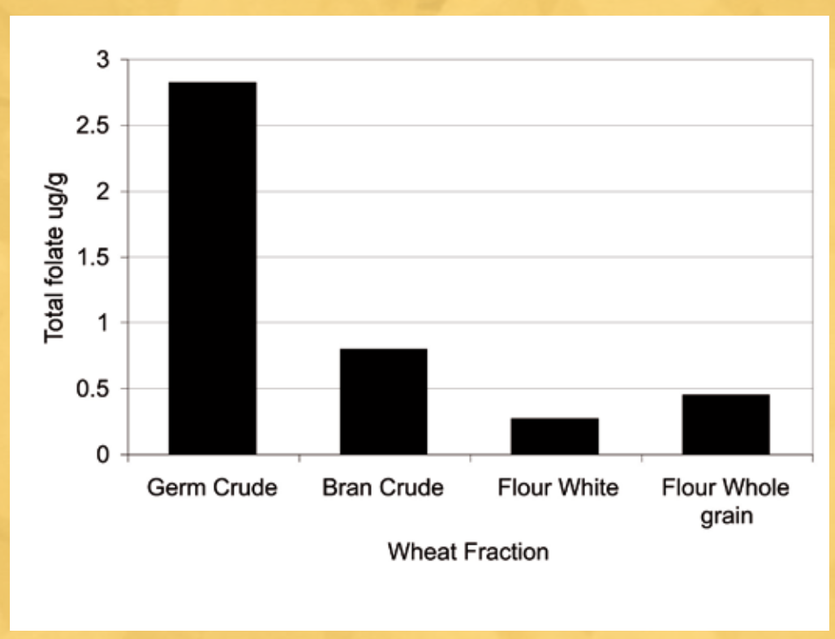
We have identified and isolated four genes essential to the production of folate from wheat, a cereal with higher endogenous folate levels than rice. These include GTP cyclohydrolase1 (GCH1); HPPK/DHPS (6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK), 7,8-dihydropteroate synthase (DHPS); 4-amino-4-deoxychorismate synthase (pABAS) and tetrahydrofolypolyglutamate synthase (FPGS). Each of these will be transformed into rice plants either singly or in combination via particle bombardment. The use of wheat genes, a closely related cereal, should alleviate problems seen with introduction of genes from distantly related species where poor gene homology results in a less functional pathway in the host plant.

Folate Biosynthesis in plants
A simplified schematic of the folate biosynthesis pathway where GTP and chorismate are committed to the pathway through the action of GCH1 and pABAS. Isolated genes are highlighted in red.





GTP cyclohydrolase 1 and pABA synthase tissue distribution in wheat.
Total RNA extracted from 16-20 dpa whole seed (WS), embryos (EM), endosperm (ED), maternal tissues (MT), roots (R) and leaves (L) were assayed for the presence of GTP cyclohydrolase 1 and pABA synthase mRNA transcripts by reverse transcription PCR (upper and lower panels respectively). Root and leaf material isolated from four week old plants was included to examine the broader patterns of expression in plants. Total RNA was subject to cDNA synthesis in the presence (+) or absence (-) of reverse transcriptase to indicate genomic DNA contamination.



Folate partitioning in mature wheat.
Comparison of total folate levels in standard commercial milling fractions expressed as µg per g dry weight. Fractionation of wheat highlights the partitioning of folate in the mature wheat grain. It is this partitioning that underpins nutritional losses arising through modern milling practices.

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