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A Transgenic Cereal with Enhanced Folate: Rice Expressing Wheat HPPK/DHPS

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Summary
- Folate deficiency is a global health problem, however fortification and/or supplementation is problematic especially in the developing world.
- Cereals are widely consumed, but particularly poor in folate.
- The introduction of a single gene encoding an enzyme acting at a central point in the folate pathway (HPPK, DHPS) is able to elevate total folate levels in transgenic cereal plants.

Introduction
Folate is a B-group vitamin critical for normal cellular function and division. Insufficient intake causes megaloblastic anaemia and there are strong linkages to cardiovascular disease, various cancers and cognitive decline. Low levels prenatally can lead to spina bifida and anaencephaly. Vertebrates are unable to synthesize folate de novo, accordingly plant foods are our primary source. Unfortunately cereals which provide over half the worlds population with over 80% of their diets are particularly poor in folate. In the developing world fortification programmes are logistically difficult, a practicable alternative is the use of metabolic engineering to create a cereal crop plant expressing high levels of folate. Here we show plants transgenic for wheat 6-hydroxymethyl-7,8 dihydropterin pyrophosphokinase/7,8-dihydropterate synthase (HPPK,DHPS) can give elevated folate levels.

Results and Discussion
Our previous work showed the key enzymes of the folate pathway are expressed in all major tissues of the wheat plant including the seed; evidence of de novo folate synthesis throughout the plant. We confirmed these genes are highly conserved, validating our strategy of introducing a selected wheat gene into rice to enhance folate. HPPK,DHPS is a bifunctional enzyme which operates at a central point on the folate production pathway (Fig 1). Selecting this enzyme for introduction avoids disruption in feeder pathways and avoids the need to adjust transport protein levels. Additionally, the use of only a single enzyme provides a technology easily transferable to other species.

HPPK,DHPS was stably transformed into Jarrah, an Australian variety of Oryza Sativa under the control of the maize ubiquitin promoter via particle co-bombardment with a construct selecting for hygromycin resistance (Fig 2). All resistant primary lines which tested positive for the presence of the transgene by PCR expressed higher total folates than control plants. T0 leaf tissue showed nearly double that of control (1.2 ± 0.06 μg g and 2.1 μg g respectively, (Fig 3). Additionally, QRT-PCR identified rice HPPK,DHPS expression in seed from all positive transgenic plant lines, in a range from 0.05 to 1.99 fold increase relative to the housekeeping genes GAPDH and actin (Fig 4).

Figure 1. Schematic of folate pathway in plants. HPPK,DHPS shown in bold red. ADC, amino-deoxychoric acid; gABA, panto-aminobenzoic acid; HMDIP, hydroxymethyl-dihydropterin; THF, folate; DHM, dihydropterin; HPPK, dihydropterin phosphokinase; DHPS, dihydropterate synthase.

Figure 2. Schematic of expression constructs. A. Ubiquitin promoter. B. Hygromycin resistance (hph) is driven by the core cauliflower mosaic virus 35S promoter. Nos and 35S T are the transcriptional terminators of the nopaline synthase gene of Agrobacterium tumefaciens and the cauliflower mosaic virus 35S gene respectively.

Figure 3 Total folates in T0 transgenic plants. *** Statistically significant at P = 0.001

Figure 4. HPPK,DHPS transcript is produced by seed of T0 transgenic plants. Seed from primary lines were examined by QRT-PCR. Transcript expression is shown relative to Actin and GAPDH abundance.