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Antinutrient biosynthesis

Dreaming of clean bean protein

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The antinutrient pyrimidines vicine and convicine, causative agents of favism in faba bean, have surprising biosynthetic origins in purine biosynthesis. This discovery is a step forward towards the generation of antinutrient free faba bean varieties.

Leguminous crops hold considerable potential in the quest for sustainable and nutritious food production. They are protein-rich and a key facet of a shift towards reduced-meat, plant-based diets, which the Intergovernmental Panel on Climate Change (IPCC) see as a major opportunity for mitigating climate change[1].

The faba bean (*Vicia faba* L.), also known as fava or broad bean, is a high yielding legume that can be grown in temperate climates. Greater adoption of faba bean in European agriculture could ease reliance on imported soybean for protein-rich food and feed, the cultivation of which contributes to tropical deforestation. However, faba bean contains the anti-nutritional pyrimidine glycosides vicine and convicine, which can cause severe haemolytic anaemia in individuals with favism, a glucose-6-phosphate dehydrogenase-deficiency present in approximately 5% of the world population[2].

A fear of favism may have given the great philosopher Pythagoras a strong aversion to the bean, which reportedly led to his death when he refused to cross a faba bean field when fleeing from an angry mob (ref). Today, concerns remain around the bean's toxicity, and consequently there is an international effort to develop anti-nutrient free faba bean which will enable more widespread cultivation of this promising crop[3].

In this issue of *Nature Plants*, Björnsdotter *et al* shed light on the metabolic process leading to vicine and convicine biosynthesis in faba bean (Figure) (ref). This study paves the way for the development of antinutrient free faba bean varieties.

First, a paired metabolomic and transcriptomic dataset was assembled across multiple tissue types, and gene-to-metabolite correlations pinpointed 20 candidate genes highly correlated with vicine. One candidate stood out as it was also highly expressed in whole seeds. This gene, VC1, was found to encode a protein containing two domains, RibA and RibB, which catalyse non-adjacent steps in riboflavin biosynthesis.

The VC1 sequence matched previously identified SNPs that correlated with normal and low vicine cultivars. Furthermore, a fine mapping population generated by crossing these cultivars highlighted a region that co-segregated with the aforementioned SNPs. Syntenic regions in related species with sequenced genomes were shown to contain VC1 homologs. Analysis of low-vicine/convicine cultivars showed they carried a *vc1* allele containing an AT-insertion causing a frameshift rendering the enzyme truncated and inactive.

To functionally validate the role VC, in the absence of gene silencing or transformation methods, the researchers turned to hairy-root expression system, a useful tool in non-model plants. The VC1 sequence from a normal vicine producing line boosted vicine biosynthesis in hairy roots derived from a low producing line. Altogether this data supports VC1 involvement in vicine/convicine phenotype and biosynthesis.

The VC1 protein contains RibA and RibB domains, which are involved in riboflavin biosynthesis. Of these, RibA appears to be involved vicine/convicine biosynthesis as judged by the aforementioned frame-shift mutation insertion which impacted the C-terminal RibA domain. RIbA encodes a GTP cyclohydrolase II, which catalyses the hydrolysis of GTP, a purine nucleotide, to DARPP (), an activity of VC1 that was verified *in vitro*. The involvement of this enzyme in pyrimidine glycoside biosynthesis

was somewhat surprising, as it was naturally expected that these compounds would be derived from pyrimidine precursors and not purines.

The origin of convicine/vicine pyrimidines from a purine precursor is a remarkable biosynthetic twist and was validated by elegant isotope labelling experiment, a highlight of the manuscript. Isotopic labelling involves feeding the plant putative biosynthetic precursors labelled with stable isotopes, and then searching for the labelling pattern in the target specialized metabolite using mass spectrometry. Feeding labelled GTP to faba bean led to the accumulation of labelled vicine and convicine, thereby linking the proposed precursor to the products.

In a tantalising extension to this result, the same experiment was performed with bitter melon (*Momordica charantia*), a phylogenetically distant species that also accumulates vicine, presumably through independent evolution. Again, labelled vicine was observed after feeding labelled GTP, indicating that vicine biosynthesis in bitter melon has similar purine origin.

The work represents a major breakthrough elucidating vicine/convicine biosynthesis and may lead to the development of new faba bean cultivars. Particularly impressive is how, in the absence of a sequenced genome or mature transformation methods, the smart combination of methods has still led to improved genomic understanding of the trait and the gene's functional validation in the plant.

Of course, there are a number of question that have arisen due to this work. Most notably, the complete biosynthetic pathway from GTP to vicine/convicine has not yet been elucidated, though the gene-to-metabolite correlation data provides an excellent starting point for this.

The relationship between the riboflavin pathway and convicine/vicine biosynthesis is particularly interesting. For instance, does VC1 act across both pathways or is there is there a paralog present dedicated to riboflavin biosynthesis? Why are trace levels of vicine/convicine present in *vc1* lines? What impact would knocking-out vicine/convicine biosynthesis have on flux through riboflavin biosynthesis?

Intriguingly, riboflavin biosynthesis lacks feedback control and features reactive intermediates that are toxic if they accumulate. It has been shown that enzymes from "directed overflow metabolism" can shuttle reactive intermediates away from the pathway by converting them into non-reactive by-products [4]. There is a possible connection between riboflavin directed overflow metabolism and the pyrimidine glycoside biosynthesis, with potential implications for flux balance and our understanding of how plant specialised metabolite pathways can evolve.

This study has revealed the key role of VC1 in vicine/convicine biosynthesis. The work is a remarkable example of a multi-disciplinary approach to elucidating plant biosynthetic pathways, combining robust multi-omics data analysis with gene mapping populations, recombinant expression and isotope labelling. The authors have revealed a surprising purine origin for the pyrimidine glycosides. Whilst perhaps coming too late for Pythagoras, this work represents a great stride towards the engineering or breeding of faba bean varieties free of anti-nutrients.

Figure legend. Biosynthetic origin of vicine and convicine from GTP as reported by Björnsdotter *et al*. Pyrimidine glycosides vicine and convicine from faba beans are the cause of haemolytic anaemia in individuals with favism. Rather than the expected origin from pyrimidines, vicine and convicine are found to originate from a purine, GTP. The enzyme VC1, an isoform of an enzyme involved in riboflavin biosynthesis, contains a RibA domain, which catalyses the first step from GTP to vicine/convicine.

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