***VC2* regulates baseline vicine content in faba bean**

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Faba bean (*Vicia faba*) is one of the highest-yielding cool-season legume crops and provides a valuable source of vegetable protein for human consumption in temperate climatic regions. However, its use for human nutrition is limited by the seed vicine and convicine (v-c) contents, which can induce favism in individuals with glucose-6-phosphate dehydrogenase deficiency. A bifunctional riboflavin gene, *VC1*, was identified as a major biosynthetic pathway gene. Although a 2 bp insertion in this gene results in a loss of function, this inactivation only partially eliminates v-c biosynthesis, thus indicating the involvement of other genes.

Here, we demonstrate that a novel *V. faba* riboflavin gene, *VC2*, is responsible for the residual v-c contents in faba bean. *VC2* shares nearly identical functional domains with *VC1* and has GTP cyclohydrolase II activity, catalyzing the conversion of GTP into an intermediate molecule in the biosynthetic pathway. Gene expression analysis revealed that *VC2* has a minor effect, accounting for approximately 5-10% of total riboflavin gene transcripts which significantly correlates with the baseline contents in low v-c cultivars. Our results illustrate that genotypes carrying the 2 bp inactivating insertion in *VC1* still have residual v-c levels due to *VC2* activity. Furthermore, *VC1* exhibits multiple alleles and copy number variations, complicating molecular marker development. Conversely, single nucleotide polymorphisms within *VC2* provide a reliable alternative for marker-assisted selection in faba bean breeding.

In conclusion, our study elucidates the complex genetic regulation of v-c biosynthesis and provides valuable insights to facilitate its elimination in faba bean.

**Theme**: Metabolism and quality traits