

The Research Group
Plant Genetics

has the honor to invite you to the public defence of the PhD thesis of

Ramon de Koning

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Unravelling the raffinose family oligosaccharides metabolic pathway in common bean: From gene discovery to gene editing for improved nutrition

Promotor:

Prof. Dr. ir. Geert Angenon (VUB)

The defence will take place on
Thursday, October 5, 2023 at 17h in auditorium I.2.01

The defense can also be followed via
livestream. Please contact
Ramon.de.Koning@vub.be for more details.

Members of the jury

Prof. Dr. Joske Ruytinx (VUB, chair)
Prof. Dr. ir. Frédéric Leroy (VUB, secretary)
Prof. Dr. Stefan Weckx (VUB)
Prof. Dr. Harry Olde Venterink (VUB)
Dr. Francesca Sparvoli (Institute of Agricultural
Biology and Biotechnology)
Dr. Laurens Pauwels (UGent)

Curriculum vitae

Ramon de Koning obtained the degree of Master of Science in Bioengineering Sciences: Cell and Gene Biotechnology at the Vrije Universiteit Brussel in 2016. After graduating, he started as PhD researcher and teaching assistant in the Research Group of Plant Genetics.

Ramon's research resulted in five peer-reviewed journal articles. Furthermore, he has been active in the field of science communication and was board member of the GeneSprout initiative for 4 years. During his PhD trajectory, he supervised six master thesis students and gave eight different practical courses for bachelor and master students.

Abstract of the PhD research

Common bean seeds are vital for human nutrition due to their rich content of proteins, carbohydrates, vitamins, minerals, and dietary fibers. However, they also contain anti-nutritional factors, including raffinose family oligosaccharides (RFOs), which are indigestible by humans and monogastric animals, leading to flatulence and digestive disturbances after consumption. The primary objectives of this study were to elucidate the RFO metabolic pathway in common bean and subsequently develop a strategy to reduce the RFO content in seeds without negatively impacting plant development and functioning, thereby improving nutritional quality and alleviating associated digestive issues.

An extensive analysis of annotation, genetic structure, and expression patterns of all galactinol and RFO biosynthesis genes in common bean was conducted. By re-analysing publicly available RNA-seq data, two expression atlases were created, revealing tissue-specific expression patterns of these genes. This information allowed for the identification of key galactinol and RFO synthase genes involved in RFO accumulation in seeds and contributed to a comprehensive understanding of the RFO metabolic pathway. The role of galactinol and RFOs during drought and salt stress was also examined by assessing differential gene expression and measuring the galactinol and RFO content in common bean during abiotic stress. The findings indicate that both galactinol and raffinose contribute to plant protection against abiotic stress, with the *galactinol synthase 3* isoform playing a specific role in the drought stress response.

To lower the RFO content in common bean, the CRISPR/Cas9 gene-editing technology was utilized. Given the time-consuming nature of stable common bean transformation, a hairy root transformation system was developed to rapidly evaluate the efficiency of several sgRNAs targeting RFO biosynthetic genes and assessing the influence of different promoters driving Cas9 expression. The study compared *in silico* predicted editing efficiencies and mutations of designed sgRNAs with *in planta* data, finding two computational models (Lindel and inDelphi) relatively reliable for predicting sgRNA efficiency and induced mutation types. Upon identifying the optimal sgRNA and promoter combinations, stable transformation and regeneration protocols for common bean were optimized, significantly enhancing callus production and achieving a 67% transformation efficiency. This method can be employed in future research to generate common bean plants with reduced RFOs in the seed.