SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Plant Genetics

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

Raphaël Kiekens

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Molecular analysis of the dihydrodipicolinate synthase gene family of leguminous plants

Promotor:
Prof. dr. ir. Geert Angenon

The defense will take place on

Thursday, June 15, 2023 at 17h in D2.01

Members of the jury

Prof. dr. Wim Vranken (VUB, chair)
Prof. dr. ir. Eveline Peeters (VUB, secretary)
Prof. dr. Joske Ruytinx (VUB)
Prof. dr. Jean-Pierre Hernalsteens (VUB)
Prof. dr. Sofie Goormachtig (UGent)
Prof. dr. Elizabeth Kairuz Hernández-Díaz (Universidad Central "Marta Abreu" de las Villas, Cuba)

Curriculum vitae

Raphaël obtained a master's degree in chemical engineering (HOGENT, 2002) and molecular biotechnology (UGent, 2003). After that he was a researcher at the department of Plant Biotechnology and Bioinformatics between 2004 and 2009 at the VIB (UGent). Next he was lecturer in bio-informatics and statistics at Howest between 2009 and 2014. In 2015 he started his PhD as an assistant for Prof. Dr. ir. Geert Angenon at the Plant Genetics Lab of the VUB. His main results have been published in one research article and has in addition significantly contributed to three other published research articles for the Plant Genetics Lab. He was also a member of the Academic Council and Research Council of the VUB between 2019 and 2021. Currently he is information manager at the CaLi Business center (BUCE) of the VUB.

Abstract of the PhD research

DHDPS is a key enzyme in the aspartate-derived lysine biosynthesis pathway in plants and negatively feedback-regulated by lysine itself. Recently, DHDPS isoforms with novel regulatory properties were demonstrated in the model legume *Medicago truncatula* and hypothesized to be involved in abiotic and biotic stress responses. Based on the findings in *M. truncatula*, the main objective was to investigate further to what extent DHDPS isozymes with potentially novel molecular properties are present in legumes and how we can exploit this knowledge, including novel biofortification strategies.

To achieve this, the research was layered in three-fold. One, was an *in silico* analysis including a phylogenetic - and promoter analysis in land plants and legumes respectively, preceding a large scale RNA-seq re-analysis in *Lotus japonicus, M. truncatula* and *G. max*. Two, we explored the naturally occurring genetic variation of the *DHDPS* genes in the model legume *M. truncatula,* in addition to a *DHDPS* candidate gene-association analysis with root growth under normal or salt stress conditions and DHDPS activity or sensitivity to Lys feedback inhibition as our phenotypes of interest. Three, a mutation analysis of various plant DHDPS isozymes, measuring the effect of specific single amino acid changes on lysine feedback inhibition-sensitivity of the purified proteins *in vitro.* The *in silico* analysis established the existence of a legume-specific class of *DHDPS*, hereby termed as the *DHDPS* B-type, distinguishable from the *DHDPS* A-type commonly present in all land plants. In addition, we provide strong evidence that the *DHDPS* gene family is linked to specific abiotic and biotic stress responses in the plant. An interesting finding is the upregulation of a subgroup of *DHDPS* B-type genes specific upon root interaction with *Glomus intraradices,* a symbiotic arbuscular mycorrhizal fungus. Mutation of the highly conserved histidine to a lysine at position 80 of the protein (*Nicotiana sylvestris* numbering) renders the sensitive A-type DHPDS, insensitive to lysine feedback inhibition, however reducing hereby the total activity of the protein. This mutation has high potential of use in creating crops with a higher free lysine content.

Together these results contribute significantly to the knowledge of the genetics and molecular properties of the *DHDPS* gene family in leguminous plants.