SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Plant Genetics

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

Mary Esther Muyoka Toili

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Insights into the molecular mechanism of the hard-to-cook defect towards genetic improvement of common bean (*Phaseolus vulgaris* L.) through CRISPR-Cas9 gene editing optimization

Promotors:
Prof. Dr. ir. Geert Angenon (VUB)
Prof. Dr. Stephen Githiri (Jomo Kenyatta University of Agriculture and Technology)

The defense will take place on
Tuesday, December 20, 2022 at 16h in U-residence Green Room

The defense can also be followed via livestream. Please contact Mary.Esther.Muyoka.Toili@vub.be for more details.

Members of the jury

Prof. Dr. Joske Ruytinx (VUB, chair)
Prof. Dr. Stefan Weckx (VUB, secretary)
Prof. Dr. Luc Leyns (VUB)
Prof. Dr. Godelieve Gheysen (UGent)
Prof. Dr. Steven Runo (Kenyatta University)

Curriculum vitae

Mary Esther Muyoka Toili obtained her Master's degree in Biotechnology at Jomo Kenyatta University of Agriculture and Technology (JKUAT-Kenya) in 2014, and in 2018, she commenced her PhD in Bioengineering Sciences at the Plant Genetics lab (VUB) as a VLIR-UOS-funded scholar.
Prior to her PhD, Esther taught Agricultural Biotechnology and Genetic Engineering undergraduate courses in JKUAT. During her PhD, Esther supervised three Master's students. Currently, her PhD research has led to the publication of one first author paper and three co-authored papers, published in international peer-reviewed journals. She has additionally presented her work in several forums.
Esther has also co-authored two books in the fields of biological (*Teaching secondary school biology*) and environmental science (*Environmental education for sustainability*).

Abstract of the PhD research

The hard-to-cook (HTC) defect is characterized by prolonged cooking of common bean (*Phaseolus vulgaris* L.) due to difficulty in achieving cell separation in the cotyledons. This study aimed to unveil insights into the molecular mechanism of the HTC defect and optimize a CRISPR-Cas9 knockout system for HTC candidate genes in *P. vulgaris*.

Using RNA sequencing, numerous differentially expressed genes (DEGs) were established between a slow- and a fast-cooking bean, providing a wide gene pool for selection of candidate genes of the HTC defect. Molecular processes relevant to HTC were uncovered, including up-regulated DEGs in the slow-cooking bean within the cell periphery. The cell wall has predominantly featured as a critical contributor to the HTC defect due to the dynamics of pectin adhesion. In this study, some cell-wall modifying enzymes including a pectin methyl-esterase (PME) were differentially expressed. Further investigations of the contribution of PMEs to the HTC defect revealed that the PMEs and their inhibitor proteins belonged to a large multi-gene family in *P. vulgaris*, with low expression in seeds. Notably, two genes encoding a PME and a PME inhibitor (PMEI) were highly expressed in the fast- and slow-cooking beans, respectively. Subsequent analyses revealed that PMEs show increased gene expression and enzymatic activity in fast-cooking beans and may be necessary for promoting the fast-cooking phenotype in *P. vulgaris*, since the action of PME may further encourage the activity of cell wall modifying enzymes which soften the cell wall. We hypothesized that the high expression of the PMEI in the slow-cooking bean could result in the inhibition of the activity of PME, causing the HTC phenotype. Therefore, both the PME and PMEI genes were targeted for knockout using CRISPR-Cas9. However, common bean is highly recalcitrant to transformation and *in vitro* regeneration, with no successful CRISPR studies reported yet. Using a *Rhizobium rhizogenes*-mediated hairy root assay, the efficiency of the designed sgRNAs to induce mutations in the target genes was rapidly determined. Further, successful induction of callus and subsequent transformation using *Agrobacterium tumefaciens* was accomplished. This protocol was successfully used to induce potentially useful mutations in the PME and PMEI sequences in the calli of the fast- and slow-cooking beans respectively. Calli from the HTC beans were able to regenerate into whole plantlets with shoots containing the Cas9-sgRNA constructs.

In conclusion, results from this study unveiled molecular processes effectuating the HTC defect in common beans, including the contribution of pectin methyl-esterase enzyme and its inhibitor protein. An efficient callus induction and transformation protocol for the common bean is provided here, putting the crop in a position to benefit from improved technologies such as CRISPR-Cas9.