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TOWARDS THE METABOLIC ENGINEERING OF TRITERPENE SAPONIN BIOSYNTHESIS IN *MEDICAGO TRUNCATULA* BY THE CRISPR/CAS9 SYSTEM

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CRISPR/Cas9, genome editing, Medicago truncatula, triterpene saponins

Saponins are a large family of specialized secondary metabolites produced in many plant species which display several biological activities. They are commonly considered to play an important role in plant defense. In addition, saponins have also been shown to be hypocholesterolemic, hypoglycemic, immunostimulatory, antioxidative, anti-inflammatory and cytotoxic compounds. The aim of this work is to evaluate the possibility to manipulate the triterpenoid saponin biosynthesis in barrel medic (*Medicago truncatula* Gaertn.) through precise genome editing techniques based on the CRISPR/Cas9 technology. The application of CRISPR/Cas9 system for knocking out some genes involved in saponin biosynthesis will allow us to increase our knowledge in relation to the production of these compounds and their involvement in plant growth.

A CRISPR/Cas9 toolkit was developed within the GoldenBraid (GB) cloning standard, allowing simple and practical assembly of constructs for plant gene editing. Two genes which are involved in sapogenin biosynthesis were selected as target genes, and for each target gene we designed two different single guides RNAs (sgRNAs) by use of the CRISPR-P 2.0 design web tool. Using the modular GB cloning system we generated CRISPR/Cas9 gene knockout vectors which contain the neomycin phosphotransferase II (*NPTII*) selectable marker gene that confers resistance to kanamycin, a human codon-optimized Cas9 driven by the CaMV 35S promoter, and two sgRNAs under control of the *Arabidopsis* U6 polymerase promoter. The CRISPR/Cas9 constructs were introduced into EHA105 disarmed *A. tumefaciens* strain for plant transformation. Experiments of *A. tumefaciens*-mediated transformation in *M. truncatula* are currently under way.