Abstract:

Since the studies of plant gravitropism by Charles Darwin, the identity of specific sensors of gravity in plants has been uncertain. To this date, statoliths – starch granules in the root tips – are considered to play a key role in gravity sensing. The role of statoliths as organelles that mediate the gravity sensing ability of plant roots is based on research that uses plants which have severely impaired ability to synthesize starch in general or have their cells that contain statoliths removed or damaged. This represents methodical imperfections that give rise to alternative explanations, like disturbed auxin flow due to heavy damage to the root tip or unknown involvement of starch from other parts of the plant in gravity perception. Thanks to advances in the field of CRISPR/Cas9 technology, we are now able to produce tissue-specific mutants that might help with clarification of whether starch granules in the root tip are involved in sensing gravity and if so, how significant is this involvement. This diploma thesis aimed to answer these questions by adapting the tissue-specific CRISPR/Cas9 system and using it for the creation of mutants that are starchless specifically in the columella cells. Using this approach, we generated one tissue non-specific mutant line and three tissue-specific mutant lines, two of which have targets in genes responsible for starch synthesis. The observations made by fluorescence imaging and genotyping proved that this adapted gene-knockout system works both for non-specific and tissue-specific applications. However, the generated tissue-specific starchless mutants proved to have varying degrees of root tip starch content and differing response degrees to the vector of gravity. Due to this variability, we did not achieve any significant results. We anticipate that achieving plants that are homozygous from the perspective of our insert and selecting them according to the strength of mCherry protein fluorescence might help answer the question of how big of a role the root tip starch granules play in gravity perception. Furthermore, optimization of the adapted CRISPR/Cas9 system could aid the research of tissue-specific mutants.