A transient expression test system for the cleavage activity of customizable endonucleases *in planta*

Nagaveni Budhagatapalli, Götz Hensel, Stefan Hiekel, Twan Rutten and Jochen Kumlehn
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben
budhagatapalli@ipk-gatersleben.de

Site-specific genome engineering is a powerful tool for elucidating gene function in plants and transcription activator-like effector nucleases (TALENs) are one of the promising platforms. TALENs comprise customized TALE DNA binding domain and FokI cleavage domain which induces DNA double-strand breaks (DSBs) at predetermined loci on the chromosomes. Subsequently, DSBs trigger DNA repair mechanisms which are either error-prone deletions/insertions via non-homologous end joining (NHEJ) or genome editing via homology-directed repair (HDR) using customized repair template. These repair systems results in targeted mutagenesis or precise genome editing and based on this, we developed a system to readily validate the activity of TALENs transiently. In principle, incorporation of TALEN target sequence into *GFP* coding sequence leads to a nonfunctional protein. Co-bombardment with both TALEN units in barley epidermal leaves reconstitute *GFP* after TALEN-induced DSBs and error-prone repair. Theoretically one-third of the cells transformed with test vector and TALENs should show GFP fluorescence. In order to standardize the procedure plasmid containing constitutive expressed mCherry will be co-transformed. This system can be routinely used to assess the binding and cleavage activity of TALENs before stable plant transformation procedures and also can be exploited to check the activity of RNA-guided endonucleases (RGENs).