

DNA-free genome editing

Janina Metje-Sprink, Thorben Sprink and Frank Hartung

Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg

E-mail of corresponding author: janina.metje@julius-kuehn.de

New breeding technologies like CRISPR/Cas systems have become fast, easy and widely used genome editing tools and were entitled „breakthrough of the year 2015“ by Science journal. Typically, RNA-guided endonucleases (RGENs) are delivered into plant cells by transfection with plasmids or by *Agrobacterium tumefaciens* mediated T-DNA transfer. These methods induce stable expression of the CRISPR system in the host, which increases the chance of unwanted off-target effects. Furthermore, the system goes along with a possible integration of recombinant DNA and therefore the existence of transgenic plants [as intermediates]. Removal of that foreign DNA is not always possible e.g. in plants that reproduce asexually. Therefore, new

genome editing methods are needed without the introduction of foreign DNA. In a DNA-free genome editing system preassembled Cas9 protein-guide RNA ribonucleoproteins (RNPs) are directly delivered to the plant cell in a vector-free manner. RGEN RNPs targeted mutagenesis is highly efficient. RNPs are demonstrated to act immediately upon delivery and are degraded rapidly in the cells. The short activity period greatly decreased chance for off-target effects. Mutants obtained by this method are completely transgene free and are indistinguishable from naturally occurring genetic variations. DNA-free Genome Editing promise a safer and more precise application of Genome Editing.