DNA-free genome editing in potato

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DNA-free genome editing via CRISPR (clustered, regularly interspaced, short palindromic repeat) /Cas9 (CRISPR associated protein) is a new technique in agronomical important crop improvement. In this research an efficient genome editing method, via potato protoplast transfection using CRISPR/Cas9 ribonucleoproteins (RNPs) was established.

As a target gene for genome editing, the MSH2 gene was selected. The MSH2 gene is implicated in MMR (mismatch repairing system). The MMR system is highly conserved in Eukaryotes and it is involved in correction and reorganization of mispaired nucleotides to prevent homeologous recombination. For DNA free genome editing six gRNAs with predicted lower ``off target`` effect were designed.

To evaluate the efficiency of the gRNAs an in vitro cleavage assay was performed. With the efficient gRNAs the in vivo genome editing in protoplasts using purified Cas9 protein was performed.

The established protocol will be useful to breed resistant potato plants toward pest and diseases and to reduce pesticide consumption. Using pesticides have a negative effect on the environment and it is not cost efficient. The regenerated MMR deficient potato plants could be utilized in breeding programs to achieve resistant potato plants from wild relatives where the resistance genes are still yet not known and the conventional breeding is limited by sexual incompatibility.

The genome edited potato plants have a good prospect to be commercialized because they do not contain foreign DNA.

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