Transfection of *Taraxacum koksaghyz* protoplasts with CRISPR/Cas9

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Russian Dandelion (*Taraxacum koksaghyz)* is an upcoming new crop. It is able to produce a cis-1,4-polyisoprene (natural rubber) which is equivalent to the material conventionally obtained from the rubber tree (*Hevea brasiliensis)*. The focus of the BMBF funded "EVITA" project is to introduce a herbicide resistance into *T. koksaghyz* for better weed management during cultivation.

Classical as well as new breeding technologies have been used in order to change the DNA sequence of the gene encoding for the acetohydroxyacid synthase (AHAS). The AHAS enzyme possesses an essential function in amino acid synthesis and is therefore a target for weed control in crops.

EMS mutagenesis of *T. koksaghyz* seeds did result in tolerant plants, though the plants did not carry tolerance conferring mutations in *AHAS1*.

For direct mutation of specific target sites in *AHAS1*, different CRISPR/Cas9 approaches have been set up. *Agrobacterium tumefaciens* mediated transformation of *T. koksaghyz* explants with plasmids encoding for the Cas9 enzyme as well as the single guide RNA (sgRNA) have been performed, but did not result in plants with favored mutations, so far. An *in vitro* cleavage assay to test the cleavage capability by using the specific sgRNAs and Cas9 was successful by showing induction of double strand breaks in *AHAS1*.

As *Agrobacterium* mediated transformations did not lead to transformants up to now, transfection of protoplasts was done. Regeneration of plants from protoplasts is difficult and was therefore not the primary goal of this approach. The objective is to proof the CRISPR/Cas9 system being able to work not only *in vitro* but also *in vivo*.

Therefore, two days after transfection of protoplasts with purified *Streptococcus pyogenes* Cas9 and sgRNA, protoplasts have been harvested and genomic DNA was isolated. Out of the DNA, *AHAS1* was amplified, cleaved *in vitro* (cleavage assay) and sequenced subsequently.
Sequencing results will show, if and to what extent the CRISPR/Cas9 system did perform *in vivo* in *T. koksaghyz* protoplasts.