

Introducing a herbicide tolerance into the Russian Dandelion by means of genome editing

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The Russian Dandelion *Taraxacum koksaghyz* (*Tks*) is in the focus of scientists as well as industry being a new source for natural rubber (cis-1,4-polyisoprene). Presently that is almost solely delivered by the rubber tree *Hevea brasiliensis*. Due to a fungal affection threatening that population, the supply for the world's rubber market is endangered. Therefore, alternatives are urgently needed, which are able to provide a rubber with similar chemical and physical characteristics. *Tks* is able to deliver such a rubber.

Tks is a diploid *Asteraceae* that naturally grows under sparse conditions in the Tian Shan Mountains, enabling cultivation on marginal acreages in a broad climatic area. The rubber itself is stored in the roots, facilitating the cultivation of *Tks* as a perennial crop. However, a lot of issues have to be solved to make *Tks* a quantitative source for natural rubber. Besides the problems with germination on the field and the slow early growth, the crop still requires a lot of manual labour because an effective strategy against weed as well as automated equipment for harvesting do not exist yet.

To overcome one of those problems, a herbicide tolerance shall be introduced to enable a better competitiveness of *Tks* on the field. This is done in the framework of the "EVITA" project. The target for this approach is the acetohydroxyacid synthase (AHAS), an enzyme indispensable in the metabolism of essential branched-chain amino acids in plants. By single nucleotide substitutions, *Tks* can become tolerant to AHAS-specific herbicides like sulfonylurea or triazolopyrimidine. The unspecific mutagenesis by ethyl methanesulfonate as well as the site specific nuclease system CRISPR/Cas9 are used therefore. Herbicide treatment of seedlings and regenerates, respectively, is used for selection.

From other plants and *Asteraceae* it is known that they can have up to three AHAS homologues. However, according to our results from RACE (rapid amplification of cDNA ends)-experiments and transcriptome data, *Tks* harbours presumably only one AHAS gene. To finally confirm this, a knock-out approach is running. Here non-viable plants are being expected if a homozygous knock-out of the essential AHAS gene occurs.