

Insights into molecular breeding of Russian Dandelion

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In the framework of the BMBF funded project “EVITA”, investigations on the Russian Dandelion *Taraxacum koksaghyz* as a new crop have been conducted. Despite the low rubber yield and unimproved cropping strategies, the plant arouse interest as an alternative source for natural rubber. The projects focus was set on weed management in particular by developing herbicide tolerant plants.

The enzyme acetohydroxyacid synthase (AHAS; old: ALS) is one of the most commonly used and well described targets for herbicide control. Certain amino acid substitutions can confer tolerance against several herbicides of the “ALS inhibitor class”. By using unspecific and specific mutagenesis, the *T. koksaghyz* AHAS gene sequence was changed.

The self-incompatible character of *T. koksaghyz* impeded the classical EMS mutagenesis strategy. Therefore, directly after mutagenesis tolerant plants were selected. The survivors were supposed to produce progeny but reproduction was

hampered by side effects of the EMS application. Finally, less than twenty plants with herbicide tolerance conferring mutations were obtained, whose propagation systems were not affected, allowing crossing and production of tolerant seeds.

Site specific mutagenesis was performed by using CRISPR/Cas9. Via *Agrobacterium tumefaciens*, plasmid constructs encoding for the single guide RNA and the Cas9 nuclease were transformed into explants of *T. koksaghyz*. Stable transformed plants were regenerated and for some individuals changes in the AHAS gene sequence could be detected. But CRISPR/Cas9 is a dynamic system and only progeny with manifested sequence changes and not carrying the transgene anymore are of interest. Therefore, crossings with *T. koksaghyz* wild type are performed. Sequencing will show what InDels have been passed to the progeny and herbicide trials will reveal if the changes can confer herbicide tolerance, too.