

# PlantEd

**Genome editing in plants**

**Cost Action CA18111**

**4<sup>th</sup> PlantEd Conference**

**18-20 September 2023**

**Porto, Portugal**

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## **Book of Abstracts**

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**CRISPR/Cas targeted inactivation of guaianolide oxalate formation in chicory**

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Root chicory (*Cichorium intybus* var. *sativum*), an industrial crop species used for the production of a fructose polymer inulin, has been shown to contain a mixture of bitter tasting sesquiterpene lactones (STLs), that are currently discarded as waste. For several STLs found in plants of the Asteraceae family including chicory, interesting bioactivities have been demonstrated, including potent anti-cancer, anti-malarial, anti-inflammatory, anti-fungal and anti-bacterial activity. This activity is mainly attributed to guaianolide STLs; in chicory the most abundant STLs are lactucin, lactucopicrin, and 8-deoxylactucin, found predominantly in their oxalated forms in the latex of the plant. Several steps in the biosynthetic pathway of these compounds have been unraveled recently. However, the enzymes involved in the formation of STL oxalates, the most abundant form of STLs in chicory, have not yet been identified. Candidate genes for the chicory oxalate-CoA ligase (CiOxL) and chicory STL oxalyl transferases (CiOxT) putatively involved in the STL-oxalate formation were identified. Next, introduction of CRISPR/Cas reagents into chicory by *Agrobacterium tumefaciens*-mediated stable transformation was used to inactivate gene candidates putatively involved in STL-oxalate formation, and several chicory lines with edited genes were successfully regenerated. Detailed genotyping of mutant lines revealed the presence of indels leading to frame-shift predominantly, varying from 1 to 44 base pairs in length. Detailed genotyping also confirmed previous observations that plants transformed via *Agrobacterium* often showed chimerism, and a mixture of different on-target edits in one plant was observed. Leaves of plants carrying mutations in CiOxL or CiOxT were characterized by LC-MS to determine changes in terpene profile. The analysis showed that the production of STLs was reduced or eliminated in leaves of several CiOxL and CiOxT4 plants. Surprisingly, not only the oxalated terpenes were reduced but also the non-oxalated STLs, perhaps due to feedback regulation or toxicity of non-oxalated forms. These results contribute to further elucidation of the STL pathway in chicory and show that *Agrobacterium*-mediated plant transformation with CRISPR/Cas reagents requires detailed genotyping for characterization of genome edited plants.

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