Chicory as a multipurpose crop for dietary fibre and medicinal terpenes

Grant Agreement Nº 760891

This Project has received funding from the EU Horizon 2020 research & innovation programme under grant agreement N.760891

The 7th International Horticulture Research Conference

DEVELOPMENT OF 4 CONCEPTUALLY DIFFERENT NPBTs IN *C. INTYBUS*

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What is CHIC about? What are the main objectives?

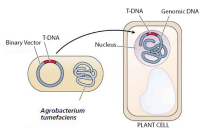
1. CHIC is an innovation project aimed at implementing New Plant Breeding Techniques (NPBTs) in chicory, in order to establish it as a multipurpose crop for sustainable molecular farming of products with consumer benefits.
2. CHIC aims at developing chicory varieties as a crop to increase the diversity and sustainability of agricultural production while serving consumer needs. These varieties shall produce improved dietary fibres and medicinal compounds. CHIC also aims to facilitate a transparent discussion and create awareness about New Plant Breeding Techniques such as CRISPR.

The strategies

Stable delivery in plant

1. Integration of T-DNA

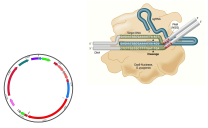
2. Integration of T-DNA + excision



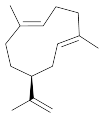
Transient delivery in protoplasts

3. Transfection of plasmid DNA

4. RNPs (DNA-free)

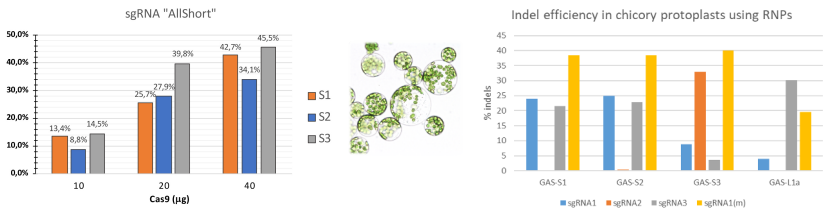


The genes



Germacrene A and its derivates are responsible for chicory's bitter taste and they are also an obstacle for the purification of inulin from this crop. We thus chose to knock-out these genes using different NPBTs.

Protoplasts editing of CiGAS using RNPs



Transient assays on protoplasts were used to test several sgRNAs, finding the optimal Cas9 quantity and testing for Cas9 activity in the presence of few mismatches in the target sequence.

Regeneration of plants from edited protoplasts

CiGAS-S1

(-CTACTCCTTACATAAGGGATAGAGTACCAGA-)	wt
--CTACTCCTTA----------GAGTACCAGA--	-11
--CTACTCCTTA----------GAGTACCAGA-	-11

CiGAS-S2

(-CTACTCCTTACATAAGGGATAGAGTACCAGA-)	wt
--CTACTCCTTAC----GGGATAGAGTACCAGA--	-4
--CTACTCCTTACAATAAGGGATAGAGTACCAGA-	+1

CiGAS-S3

(-CTACTCCTTACATAAGGGATAGAGTACCAGA-)	wt
--CTACTCCTTACAATAAGGGATAGAGTACCAGA--	+1
--CTACTCCTTACAATAAGGGATAGAGTACCAGA--	+1

A sgRNA targeting a common region of CiGAS-S1/S2/S3 was used to generate a **triple biallelic knock-out mutant** from protoplast transformation with RNPs. Eventually, different sgRNAs were used to obtain all the possible combinations of knockouts for the genes.

Ongoing: We obtained a complete knockout of CiGAS genes transiently delivering CRISPR/Cas9 machinery into protoplasts. Our efforts are now focused to achieve the same result using a stable delivery approach, followed by the elimination of the T-DNA either through the segregation of the transgene or through an excision mechanism mediated by a heat-shock inducible recombinase. Furthermore, an off-target analysis has already started, so in the end it will be possible to compare all the different NPBTs in terms of efficiency and precision of editing. In the next step, we will phenotype the plants for their level of terpenes and Germacrene A derivates.

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