

A European research and innovation project by companies, institutes and universities from 11 European countries and New Zealand



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CHIC Newsletter

Issue 5 - July 2021

About CHIC

CHIC is the Chicory Innovation Consortium. Its objective is to implement New Plant Breeding Techniques (NPBTs) in chicory in order to establish it as a multipurpose crop for the production of health-related products with clear benefits for consumers, and to develop co-innovation pathways with stakeholders for game-changing technologies, such as NPBTs. CHIC will develop four different NPBTs. They will be used to steer bioprocesses in chicory and mobilize its under-explored potential to produce immunomodulatory prebiotics and medicinal terpenes. The conceptually different NPBTs will be assessed with respect to technological potential, risks, regulatory framework and their socio-economic impacts. This will be done in close consultation with a Stakeholder Advisory Group (SAG) composed of relevant stakeholders in industry and society.



Photo by Sensus

Healthier Chicory and Reponsible Innovation

What's New! In this edition, we have updates from each Work Package leader.

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Upcoming Events

AI Game at Schools

DEI Lab at Universidad Carlos III de Madrid showcasing its state-of-the-art augmented reality (AR) and virtual reality (VR) devices school students to learn more about the CHIC project. Expected dates: August 2021

Webinar and In-person event for Farmers by Sensus

Currently in the early stage of planning, Sensus will organize events for farmers. Expected Dates: September 2021 and/or January 2022

• The Art of CRISPR/Cas by WUR NL

PhD students are invited to join this 2-day hybrid event. CHIC researchers and scientists will participate. Expected dates: September 14-15 2021

• European Researchers' Night 2022 -

Details will be forthcoming, expected Date: September 2021

EU-Level Stakeholder Consultation

The future role of new genomic techniques for plant breeding and derived products in the EU organised by the Chicory Innovation Consortium, 6 Oct 2021, 10:00-16:00, online. If interested to participate, please, contact: christian.daye@tugraz.at.



Editorial

The first chicory plants with adaptations in the genome of both the inulin and terpene biosynthesis pathways were confirmed in several partners' laboratories. The plants are now growing in the greenhouses until the stage that the effect of the adaptations on the inulin and terpene accumulation can be studied in the roots. Many more plants are still being produced. Methods for testing safety aspects such as off-target mutations are in development. Bioassays with extracts from roots of wild-type chicory have detected some interesting medicinal activities, including possible leads for novel antibiotics.

The CHIC project is also evaluated on its socio-economic and environmental impact six commercialisation scenarios were defined that differ in aspects such as whether CRISPR edited chicory is regulated as GMO or not, whether it is grown in the open field or greenhouses and what type of products are isolated from them.

The changes at the regulatory field has consequences for the CHIC project. The European Court of Justice ruled that all genome edited plants are regulated as GMOs. However, it was concluded that the planned activities will remain as relevant as before but could have consequences for other parts of the project. Nonetheless, we are moving forward to achieve what we set out to do and share with you our findings soon. Our team has continued to provide you with regular updates and creative contents about the project. A few videos have already been published which you may view anytime via our website: http://chicproject.eu/videos/.

We are looking for further updates and new and upcoming events are on the horizon so keep eye for them!

Dirk Bosch, Project Coordinator

CHIC is an EU Horizon 2020 funded € 7.3 million project (under the grant agreement No 760891) for the development and application of New Plant Breeding Techniques (NPBTs) for chicory. The consortium of 17 participants includes SMEs, an industrial partner, NGOs and research institutes from 11 European countries and one from New Zealand.

The project 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 medical compounds.

CHIC also aims create awareness and facilitate discussion about NPBTs, such as CRISPR, to allow informed decision making by all stakeholders, including the public.



Chicory Blue Flower





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Development of Four Conceptually Different NPBTs (New Plant Breeding Techniques)

This work package is dedicated to implementating the new breeding technologies in chicory. New breeding technologies is a rather general term, but in CHIC, it means genome editing methods based on CRISPR-CAS. We are developing four different methods in parallel for the delivery of CRIS-PR tools to chicory cells. and should have identical genetic outcomes and differ only in the degree of "DNA invasiveness". This is relevant when it comes to the adoption of these new technologies and their products by regulators and the general public. In the first three years, the three methods, with varying degrees of DNA invasiveness, have been fully implemented and plants have been grown to maturity in which the genes responsible for root bitter compounds were eliminated by genome editing. A patent application has been filed on this innovation.

Meanwhile, other partners in the work package continue to work on alternative genome editing molecules (that may work even better in plant cells than CRISPR-CAS) and on a solution for self-incompatibility. This is the feature that chicory plants cannot be fertilized by their own pollen, as can be done in many other plant species, which poses a serious bottleneck in chicory variety improvement by plant breeding. The genes for self-incompatibility in chicory have been found, and solutions to bypass their action are being tested now. Finally, additional work has been performed to improve the chicory genome sequence.

Implementation of NPBT for Dietary Inulin

It is known that inulin is a dietary fiber with gut health benefits. It is extracted from root chicory and used in many food products, as low calorie sugar and as healthbeneficial food fibre. The quality and the length of the inulin molecules determines the value and health-benefits of this compound. We have generated mutant chicory plants in which the genes encoding the inulin break-down enzymes are blocked using NPBT due to the small alterations the CRISPR tools made in the plant DNA. The natural production of terpenes (bitter compounds) in chicory makes the inulin extraction method less efficient and more costly. Therefore, we aimed to reduce the production of natural chicory terpenes, also via CRISPR techniques. All mutated plants were analyzed in great detail on DNA levels using sequencing methods. The mutants that were altered in the terpene biosynthesis genes, showed to have a reduced to almost zero level of the bitter compounds, terpenes.

The selected mutants with altered inulin break-down activity have been multiplied by root cuttings and have been used for induction experiments of inulin breakdown to show the effect of the targeted mutations on inulin quality and profile under autumn/winter conditions, showing that we have generated mutants with highquality inulin and a low level of small sugars and longer inulin molecules (increased mean degree of polymerisation).



Photo by Ingrid van der Meer - Root cuttings of individual chicory mutants.





Implementation of NPBT in Chicory for Bioactive Terpenes

The latex of chicory contains large amounts of sesquiterpene lactones. Bioactivity assays identified one promising sesquiterpene lactone with anti-inflammatory activity and extracts containing antimicrobial activity. Candidate genes, using transcriptome data and the newly sequenced genome, have been identified and are being functionally characterized. The project continues to identify biological activities of interest for these sesquiterpene lactones and to increase their production by using new plant breeding technologies.



Biosynthetic pathway of the major sesquiterpene lactones in C. intybus. Enzyme activities in blue (PS, KS, DBR) have been identified in related species. Enzyme activities in red have not been identified yet. The biosynthesis starts with farnesyl diphosphate (FPP), which is cyclized to germacrene A by germacrene A synthase (GAS). GAO: germacrene A oxidase; CS: costunolide synthase; PS: parthenolide synthase; KS: kaunoilide synthase; HPAc-transferase: hydroxyphenylacetic acid-transferase; DBR: double bond reductase; 15-OH-P: 15-hydroxy-parthenolide. The most abundant STLs in chicory latex are the oxalates of deoxylactucin, lactucin and lactucopicrin.

Technical, Risk and Regulatory Assessment of NPBTs

The assessment of technical, regulatory and safety aspects of the four conceptional different New plant breeding methods have made good progress. A current guide RNA has been used to generate chicory plants which allowed for conceptional different NPBTs.

The work package is divided into five individual tasks:

- Task 4.1 deals with the efficiency analysis of the four conceptional different NPBTs.
- Task 4.2 focuses on the assessment of Off-targets in the genome-edited chicory plants, both tasks contribute to the technical assessment. During the reporting period a current guide RNA has been used to generate chicory plants which each of the conceptional different NPBTs, plants have been generated for all of them (stable, FLP-system, RNP, and Plasmid delivery). Additionally, also Off-targets for the common guide RNA have been assessed and tested in first screens which need further validation.
- Task 4.3 and 4.5 assess the regulatory aspects of NPBTs, due to the ECJ ruling the risk assessment of products derived by genome editing is currently the same as for GMOs in Europe but discussions are ongoing. Regulatory regimes are legislations all around the world that are being adapted to genome editing or novel legislations are being decreed. In many countries e.g. Russia and India, discussions are still ongoing and opinions are being collected in this task the first publication on this has been released recently.
- Task 4.4. deals with the safety assessment of products derived by genome-edited chicory. During the current reporting period, terpene samples were tested for cytotoxicity using human intestinal epithelial cells. The work package has made good progress in the current reporting period which will be further improved in the next period.



PCR amplifications of chicory GAS genes S1-S3 in the lines WPR GAS KO 2, 7, 9, 17, 23, 39, 43, 45,49,52,2b,17b and wildtype WT1, 2,3,4,5, for a preliminary check for mutations.

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Schematic overview of the relation between results of WP2, 3 and 8, the regulatory status, the choice of process and the impact on the consumer. The green blocks in column one represent established results from the respective work packages.

Socio-economic and Environmental Impacts on the Whole Value Chain

The project continues to perform a socio-economic impact and an environmental assessment of NPBTs and the whole value chain. The partners further evaluate whether CRIS-PR edited chicory is regulated as GMO or not.

The impact of different NPBTs on economic and social indicators will be further quantified which include GDP, production volume, growth, competitiveness, and employment. The LCA will give information on e.g. greenhouse gas emissions, primary energy demand, water consumption, land use of the new val-



Schematic illustration of a global, multiregional input output table

ue chain developed in CHIC. With broad input of all partners, concepts were defined that differ in aspects such as whether CRISPR edited chicory is regulated as GMO or not, whether it is grown in the open field or greenhouses, and what type of products are isolated from them. These concepts will be evaluated for their socio-economic and environmental impacts.



Stakeholder Engagement

The CHIC project continues to engage stakeholders about its technology innovation. This includes the identification of possibly facilitating and hindering factors for and the assessment of stakeholder acceptability of commercial development of our genome-edited chicory and derived products (inulin and/or terpenes). The mainline of activity in the reporting period started with the indepth analysis of the Stakeholder Consultations carried out in WPs 4 and 5. The results were then integrated into the research instruments used in WP 6, consisting of the scenarios, T6.3, and the multi-criteria Acceptability Assessment Instrument (AAI), T6.4. The development of both scenar-



CHIC scenarios used in first three Stakeholder Consultations

ios and AAI, is organised as a multi-step participative process involving all project partners, the Stakeholder Advisory Group (SAG), and external stakeholders. Building on this participative process, the next Stakeholder Consultation will take place on 6 October 2021 (online). In preparation, the first round of quantitative data collection via the MCA will be carried out over summer.

A second major line of activity was the further development of and continued interaction with the Stakeholder Advisory Group (SAG, T6.2, D6.4 achieved in month 26).

The COVID-16 pandemic and the ensuing restrictions had an impact on the activities in the reporting period, as the planned stakeholder consultations could not be held as face-to-face workshops. The transformation of these events into virtual-only events required considerable effort but finally worked out well.

Step 1: Define the dimension of acceptability that dominates the individual's answers

Socio-political acceptability, community acceptability, or market acceptability



Step 3: Introduce the scenarios and collect very general reactions on the factors and criteria influencing social acceptability

Step 4: Have respondents assess a series of hypothetical events relevant for all scenarios

Step 5: Have respondents assess a series of more specific hypothetical evens that are relevant only for some scenarios

APART FROM THE UNDERSTANDING OF ACCEPTABILITY, ALL QUESTIONS IN STEPS 3, 4 AND 5 ARE IDENTICAL FOR ALL PARTICIPANTS!

Structure of the Acceptability Assessment Instrument (AAI)



Exploitation, Dissemination and Communication

In the last 18 months, news, articles and videos have continued to be published through the project's social networks.

Art and Science activities:

- Anna Dumitru y Alex May are working with CHIC Consortium members to develop a new sculptural and bio-digital installation entitled "Biotechnology from the Blue Flower" and will be spending time on site with consortium members over the life of the project.
- Jill Scott and Marille Hahne are developing the artistic work "AFTERTASTE" that is based on the health of the human olfaction and gustatory systems and the feedback between these systems and the content is based on the primary and secondary compounds found in the chicory root. They also create a movie entitled "Chicory Unpact"

Advances on training activities:

- Educational tools for school students: A set of games are being developed using virtual, augmented reality, and ICT technology to educate teenagers about the main CHIC concepts.
- Organization of educational activities for "early-stage researchers".
- CHIC partners have organized or have been engaged in events targeted to large and different audiences Interesting stakeholders have been engaged in CHIC project such as teenagers, school teachers, climate change activists.
- Publications in social networks and websites are having a greater impact.

Commercial Exploitation of Chicory as a Multipurpose Crop

We aim to develop an exploitation strategy built on two strong business cases for NPBT chicory-based dietary fibre and bioactive terpenes. Both improved inulin and terpene fractions are subjected to in vitro bioactivity and safety evaluation, and business cases for both lines will be created. Two types of NPBT chicory mutants have been generated and analyzed, one type of mutations targeted to inulin breakdown genes (FEH knock-out, WP2) and the other for germacrene synthase A (CiGAS knock-out, WP1/WP2). Both have been studied for their terpene and inulin quality and quantity compared to commercial chicory.

For studying the anti-inflammatory effects of chicory terpenes, a physiologically relevant model of the inflamed intestinal mucosa was developed. Two particularly interesting chicory extracts were assessed and strong antimicrobial potential was observed - antifungal as well as antibacterial activity towards antibiotic-resistant bacteria. Finally, a new multi-ingredient process was investigated, based on a selective adsorption column for terpenes. A conceptual process design yielding capex/opex details indicated that the sales price for the terpene mixture is in line with specialty ingredient prices with an outlet in food supplements. Currently, three scientific papers are being prepared based on the results obtained partly in WP8.



Social Networks:

- Twitter: Followers: 2021: 14 / Total: 249
- Tweets: 2021: 46 / Total: 204
- Engagement: 2021: 79 / Total: 725 Mentions: 2021: 36 / Total: 201
- Facebook:
- Followers: 2021: 13 / Total: 161
- Page engagement: 2021: 119 / Total: 1.2K Post Likes: 2020: 58 / Total: 480

Post Impressions: 2020: 1.4K / Total: 20K

Post Reach: 2021: 2 7K/ Total: 13K Post Likes: 2021: 174/ Total: 684 Page Impressions: 2020: 2.4K / Total: 24K

Social Networks:

Followers: 891

LinkedIn:

Instagram:

Profile Impressions: 2021: 3.1K/ Total: 14K Netherlands - 5%

Post engagement: 2021: 177/ Total: 692

Post impressions: 2021: 2.9K/ Total: 14K

Post Views: 2021: 6310/Total: 24181

Followers: 2021: 12/Total:191

www.CHICproject.eu: New Users: 2021: 3026 / Total: 13886 Sessions: 2021: 3695 / Total: 18713 Page views: 2021: 8048 / Total: 33135

Countries (2021 Top 5) Countries (Overall Top 5) Indonesia - 18% US = 13% Hong Kong - 10% US - 7% Spain = 6%Germany - 5%

Netherlands = 10% Germany = 5% UK = 4%

Students at a school in Spain had the opportunity to play with the AI CHIC game. Anna and Alex webinar entitled "Biotechnology from the Blue flower" and data analysis of the project's social media platforms.



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Work Packages, Deliverables

The research work packages (WP1-WP8) are organised in four interrelated Research Lines (RL). In order to direct the activities of the project towards the desired impact, each of these Research Lines coincide with Expected Impacts as formulated in the EU call.



Work Package 8 (WP8)

...to develop exploitation strategy as built on two strong business cases for NPBT-based high quality dietary fibre and bioactive terpenes from chicory.



CRISPR and Gene Editing News

Let's see what's happening in the world. Our work is solely focused on new plant breeding techniques. But CRISPR technology is beyond plant science and read what other scientists are doing to improve the lives of others using this innovative technique.



Landmark CRISPR trial shows promise against deadly disease



He Inherited A Devastating Disease. A CRISPR Gene-Editing Breakthrough Stopped It

CHIC

Scientists develop CRISPR/Cas9-based gene drive in plants





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About CHIC Consortium



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For more information, visit our website at chicproject.eu/

Check out our social media platforms and see what's happening at CHIC! (Click on the thumbnails)





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