

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



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CHIC Newsletter

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

 CRISPRcon, Conversations on Science, Society, and The Future of Gene Editing (Online) Date and Time: Thursday, October 15 from 4:45-5:15pm Eastern time Weekly Theme: Equity, Environment & Agriculture Topic: CHIC Project
Website: https://giperscon.org/_check.out.the.website.for.more.insightful.discussions.a

Website: <u>https://crisprcon.org/</u>, check out the website for more insightful discussions as other CHIC members plan to also lead other exciting webinars

- European Researchers' Night, *Online*, 27 November 2020 roundtable discussion, VR and AI interactive activies, *more details to follow soon*
- European Science Week 2020!, *Online*, 10 November 2020, discussion with high school students, CHIC members, and an array of fun, enjoyable, and interactive activities, *more details to follow soon*

Call for help! We are still looking for more European high schools that might be interested in being a part of the roundtable discussion. If you know anyone, please contact rtipan@idconsortium.es for more information.

- Agrosym 2020, XI International Agriculture Symposium, 8-11 October 2020, Bosnia and Herzegovina, *more details to follow soon*
- Regional Stakeholder Consultation with regulators and CSOs, more details to follow soon



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

Work Package 1 implements the new breeding technologies in chicory. New breeding technologies is a rather general term. In CHIC this means genome editing methods based on CRISPR-Cas (is an NPBT, a tool to change DNA in an intended way). The consortium, together, are developing four different methods for the delivery of CRISPR tools to chicory cells. Following these methods should produce identical genetic outcomes but may only differ in the degree of "DNA invasiveness" (*This would identify how much recombinant DNA technology is used in the process.*). For the regulators and the general public this approach is very important because the less DNA invasiveness is performed the more genome editing might be acceptable to the stakeholders.

In the first two years of the program, the two methods with the lowest degree of DNA invasiveness have been fully implemented, and the Chicory plants have been grown to maturity, which eliminated the genes responsible for root bitter compounds by using genome editing.

Meanwhile, other Work Package1 partners continue to work on alternative genome editing molecules (a better solution 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.

Implementation of NPBT for Dietary Inulin

Inulin is a dietary fiber with health-promoting characteristics targeting human body's gut health. Inulin is extracted from root chicory and used in many food products. The yield and the length of the inulin molecule determines the value and health-beneficial character of this compound. Using NPBT we want to increase both the yield and the length of inulin. The natural production of terpenes in chicory makes the inulin extraction method less efficient and more costly. Therefore, the goal of Work Package 2 is to reduce the production of natural chicory terpenes.

Mutant chicory plants were generated in which the genes encoding the inulin break-down enzymes are blocked using NPBT which resulted in small alterations in the plant DNA. Researchers have analyzed the DNA level on all mutated plants using various methods. The selected mutants have multiplied by root cuttings and will be used for induction experiments of inulin break-down, to show the effect of the targeted mutations on inulin yield and profile.

We plan to show that mutants of terpene biosynthesis generated in Work Package 1 are reduced in the synthesis of bitter compounds, or the total lack of the bitter compounds.



Photo by Ingrid van der Meer The photo shows CRISPRed mutants in inulin biosynthesis.



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Implementation of NPBT in Chicory for Bioactive Terpenes

The latex of chicory plants contains large amounts of sesquiterpene lactones, which interfere with the extraction of inulin. In Work Package 3 the objective is to identify biological activities of interest for these sesquiterpene lactones and to increase their production by using new plant breeding technologies. Bioactivity assays identified one promising sesquiterpene lactone with anti-inflammatory activity and extracts containing anti-microbial activity, for which the responsible substances are being identified. Using transcriptome data and the newly sequenced genome, candidate genes for the biosynthesis of the sesquiterpene lactones and the formation of the laticifers have been identified and are being functionally characterized.



Chicory lines with modified terpene composition (red label) and WT chicory (white label) growing in a growth chamber.

Technical, Risk and Regulatory Assessment of NPBTs

This group assesses the technical, regulatory and safety aspects of the different new plant breeding methods used in the project and the chicory plants and substances produced by those plants. A current RNA guide has been used to generate chicory plants using the conceptional different NPBTs. The plants have already been generated for ribonucleoprotein (RNP) and Plasmid delivery for stable transformation and Flp plants are being regenerated. Additionally, also off-targets for the common RNA guide have been assessed and tested during first screens, which require further validation. In terms of the regulatory issue, ECJ's ruling indicates that the risk assessment of products derived by genome editing is currently the same as GMOs in Europe.

However, further discussions are ongoing. Regulatory regimes, or legislations, all around the world are adopting to genome editing process, or novel legislations are being decreed. In many countries e.g. Russia and India, discussions are still ongoing, and further opinions are being collected for this task. Assessing how safe products derived by genome edited chicory, terpene samples were tested for cytotoxicity using human intestinal epithelial cells (they line intestinal lumen, which performs the primary functions of digestion, water, and nutrient absorption). The group has made good progress so far and further improvement are expected in the near future.

RNP-complexes of SpCas9 and common sgRNA (1:1) coincubated with off-target- and target amplicons in vitro for 2 h and seperated in 2% (w/v) Agarose-TAE





	1a with	1a without	2a with	2a without	2b with	2b without
Defined as GMO due to ruling	No	No	Yes	Yes	Yes	Yes
Breeding	With backcrossing	Without backcrossing	With backcrossing	Without backcrossing	With backcrossing	Without backcrossing
Cultivation	Open field	Open field	Greenhouse	Greenhouse	Open field	Open field
Process optimized for	Combined process	Combined process	Terpene production	Terpene production	Terpene production	Terpene production
Products	Inulin (food) + Terpene (pharmaceutical or nutriceutical*)	Inulin (food) + Terpene (pharmaceutical or nutriceutical*)	Terpene (pharmaceutical or nutriceutical*)	Terpene (pharmaceutical or nutriceutical*)	Terpene (pharmaceutical or nutriceutical*)	Terpene (pharmaceutical or nutriceutical*)
Further characteristics * Application to be deci	ided		Inulin will be produced according to current commercial process			

Adapted concepts for the environmental and socio-economic assessment

Socio-economic and Environmental Impacts on the Whole Value Chain

Work Package 5 evaluates the socio-economic and environmental impacts on the whole value chain of the new developed chicory crops. In addition to these quantitative assessments, a qualitative research on societal issues about the hindering or facilitating of chicory innovation will be applied, this research is closely connected to activities in Work Package 6.

With broader input from all partners, six concepts for commercialization of chicory were defined, but differ in aspects, 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 on the new value chain developed in CHIC, focusing on the effects on GDP, growth, competitiveness, employment, greenhouse gas emissions, primary energy demand, water consumption, and land use.

For the quantitative assessment, data collection from other Work Packages has started to get insight in the CHIC process, models for the socio-economic and environmental assessment have been adapted due to the requirements of the CHIC process, and goal, scope and system boundaries were defined.



Stakeholder Engagement

Work Package 6 engages stakeholders to further align technology innovation with societal needs, which includes identifying possible facilitating and hindering factors and assessing stakeholders' acceptance of genome-edited chicory and derived products (inulin or terpenes) for commercial development.

The main activities were to update our stakeholder engagement, communication and transparency strategy document on our project website, to advance the development and engagement of our permanent Stakeholder Advisory Group (SAG), and the first steps in implementing the modified strategy for stakeholder assessment. As the legal framework for genome edited plants in the EU could very well change in

Timeline							
SHCs	1st (WP4/5)	2nd (WP4/5)	3rd (WP6)	3rd (WP6)			
Geo. focus	3 x national: NL, B, F	1 x regional: NL, B, F	2 x EU: NL, B, F, EU, other MS				
SHs	breeders, farmers, inulin producers, food producers, retailers	environmental groups, consumer groups, regulators	all				
Part.	15-20	20-25	30-50				
Aim 1	Develop	scenarios	Discuss CHIC prelim. results				
Aim 2	Develop "events hinderir	" (~ faciltating and ng factors)	Discuss events ir scenarios	n Discuss management options			
Outcome	More events, revised scenarios	Revised events, refined scenarios	Impact of events on accepability	s Final assessement			

Acronyms: AAI: Acceptability Assessment Instrument, B: Belgium, F: France, Geo.: geographical, MS: Member States, NL: The Netherlands, Part: number of participants, SH: stakeholders, SHCs: stakeholder consultations.

the future, our approach is to investigate different commercialisation scenarios within two different legal contexts (status quo and hypothetical future). The idea of identifying and assessing the possible facilitating and hindering factors is organised as a multistep participative process involving all project partners, the SAG, and external stakeholders via workshops, interviews, and online questionnaires. Several preparatory steps for setting up our methodology have been achieved and preparations for stakeholders' workshops were started.

The stakeholder consultations that were scheduled earlier in the year were affected due to the global Covid-19 crisis. Therefore, one stakeholder workshop was conducted online. Two more were postponed to autumn this year and might also be required to be held online. Check with project leaders for more information in this year's planned events.



The scenario approach agreed in the consortium and with the SAG aims to explore two possible futures –depending on the regulatory status of GE crops – and two possible products (improved inulin and terpenes). The main characteristics of the three scenarios are specified above.



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CHIC brochure explains the products and benefits for consumers of which 2000 copies have been produced and distributed

Exploitation, Dissemination and Communication

During the last 9 months, apart from continuing to share updates about the project through publications on social media networks, news, articles, and videos, the following two activities should be highlighted, Art and Science:

- Anna Dumitru and 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 "AFTER-TASTE," an artistic presentation based on the health of the human olfaction and gustatory systems (sense of smell). The feedbacks and contents between these two systems are based on the primary and secondary compounds found in the chicory root.
- New training activities: A set of games are being developed with the DEI Group from University Carlos III using virtual and augmented reality to educate teenagers about the main CHIC concepts.
- New videos have also been published recently: Work Package 1 - Development of Four Conceptually Different NPBTs and Work Package 3 - Implementation of NPBT in Chicory for Bioactive Terpenes. Please check our YouTube channel <u>here</u> for fun new videos about CHIC! Don't forget to share with others about this amazing project.

Commercial Exploitation of Chicory as a Multipurpose Crop

Work Package 8 aims to develop an exploitation strategy according to two strong business cases for NPBT chicory -based dietary fibre and bioactive terpenes. Improved inulin and terpene fractions are subjected to *in vitro* bioactivity and safety evaluation and, afterward, business cases for both lines will be created. Commercially available inulin was subjected to both upper and lower intestinal gut models. It was also observed that during mouth, gastric and duodenum phases, inulin remains more or less intact, while during lower intestinal phases gas formation shows inverse correlation to inulin DP.

In addition, a slight increase in gut probiotic Lactobacillus group bacteria was observed after inulin digestion. In studying anti-inflammatory effects of chicory terpenes, a physiologically relevant model of the inflamed intestinal mucosa is currently being developed. The inflammation inducers have been tested individually and in cocktail combinations. It was shown that both IL-1 β and TNF- α are a promising individual stimuli but not combined; and these two mediators led to a decrease of the monolayer integrity, which is an expected phenotype upon intestinal inflammation and an increased production and release of IL-8.



CHIC brochure explains the products and benefits for consumers of which 2000 copies have been produced and distributed



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 1 (WP1) ...to develop four different NPBTS to be used in chicory.

Work Package 2 (WP2) ...to develop NPBT-based chicory varieties with improved dietary inulin quality.

Work Package 3 (WP3)

...to explore the potential of laticifers and sesquiterpene lactone biosysthesis for improved or new chicory products.

Work Package 4 (WP4)

...to address the technical assessment, risk assessment and regulatory assessment of NPBTs.

Work Package 5 (WP5)

...to evaluate the socio-economic and environmental impacts on the whole value chain.

Work Package 6 (WP6)

...to engage stakeholders for achieving a more responsible innovation process.

Work Package 7 (WP7)

...to inform stakeholders and broader public in innovative ways about project activities, results and related topics.

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.





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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.



Covid-19 and Gene Editing Ethical and Legal Issues



Point-of-Care Test for Covid-19 Using CRISPR



Grow More Crops With Less Water





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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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