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28 – 30 SEPTEMBER 2021, Parma

Food Safety Aspects of Integrated Food Systems

Case study-based evaluation of the existing guidelines for the risk assessment of Synthetic Biology applications

efsa European Food Safety Authority

Università di Parma 3SAN School of Advanced Studies on Food and Nutrition

Università di Parma

Università Cattolica del Sacro Cuore

Risk Assessment of GMOs in Europe

EFSA's Scientific risk assessment of regulated products

GMO Applications
Working groups and Panel of Experts

Generic Mandates

- Molecular characterisation (MC)
- Food and feed (FF) safety
- Environmental risk assessment (ERA)

- Scientific Opinions
- Guidance documents
- Technical Notes to guidance

DATA SETS SUBMITTED IN GMO DOSSIERS FOR RISK ASSESSMENT OF GM PLANTS

MC

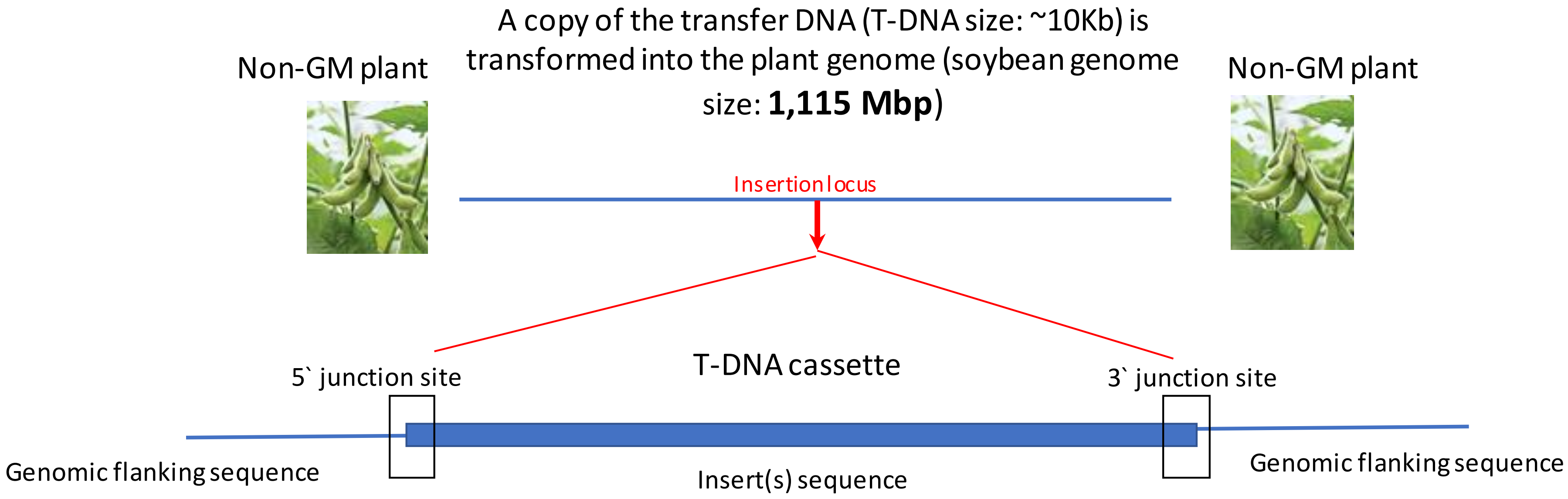
- biochemistry
- molecular biology
- genetics
- plant breeding

FF Safety

- toxicology
- allergenicity/immunology
- nutrition & animal feed
- food chemistry
- biotechnology

ERA

- plant biology
- ecology
- agronomy
- entomology
- biometrics & statistics



GMOs – The beginning

2000s - "conventional" GM crops

- Herbicide tolerant
  Insect resistant plants
- Obtained by "conventional" techniques (transgenesis)
- "Simple" - one/a few new genes/proteins (enzymes or insecticidal)
- Stacks
- Tailored EFSA guidance documents

- Glyphosate tolerant
- Glufosinate tolerant
- Insect resistant (Cry)

GMOs - The evolution

2000 - 2020s

New traits

- Agronomic Performance
- Nutritionally enhanced crops

"Complexity"

- More transgenes, many new proteins
- Transcription factors
- Gene silencing (RNAi)
- Altered plant metabolism
- High-degree stacking

EFSA guidance still applicable, developments needed
eg RNAi, development projects on proteins

Key risk assessment issues

- Safety assessment of new proteins (intractable, toxicity, allergenicity)
- RNAi off targets
- Nutritional assessment
- Comparative analysis
- Complexity, impact on plant metabolism

Advances in biotechnology

GMOS obtained by NGTs, SynBio

New genomic techniques are defined as 'techniques that are able to alter the genetic material of an organism, **developed after the publication of EU Directive 2001/18/EC'**

Synthetic Biology approach

- **Editorial "Advances in genetic engineering: EFSA public consultations in 2020"**
  - EFSA and its GMO Panel proactively assess whether next generation GMOs will challenge the current GMO risk assessment framework, and in which cases further guidance/refinements may be needed
  - More sustainable food production contributing to UN's Sustainable Development Goals and EU's New Green Deal objectives

Synthetic Biology (SynBio)

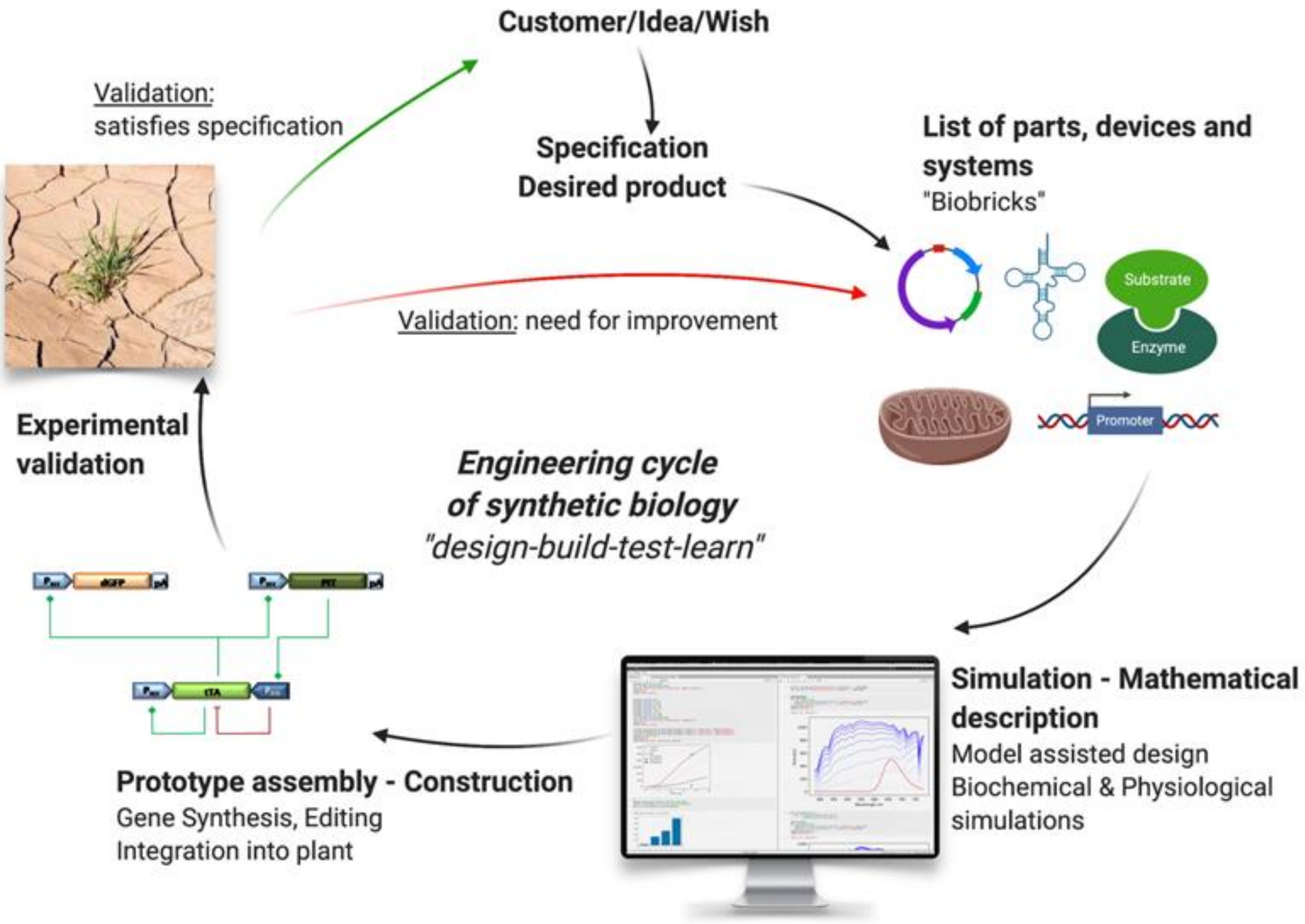
Synthetic biology is an overarching process or strategy comprising theoretical and experimental science, technology and engineering to facilitate the design manufacture and or modification of components, organisms and products

SDN1,2+ODM and similar:
Collection of tools/methods
Give rise to genome modifications targeted to specific sites in the genome (genome editing)

Gene Drive:
System that enables the over-proportionate inheritance of selected genes in a population

In **SynBio, genome editing** tools/methods could be used to achieve a desired product or trait;
Gene drive systems could be used as a functional element approach

The process of SynBio



Six SynBio Categories

1. Genetic part libraries and methods
2. Minimal cells and designer chassis
3. Protocells and artificial cells
4. Xenobiology
5. DNA synthesis and genome editing
6. Citizen science (Do-it-yourself biology)

In 2017 the Scientific Advice Mechanism (SAM) published an explanatory note which included an outline of possible agricultural applications of new techniques in the fields of Synbio

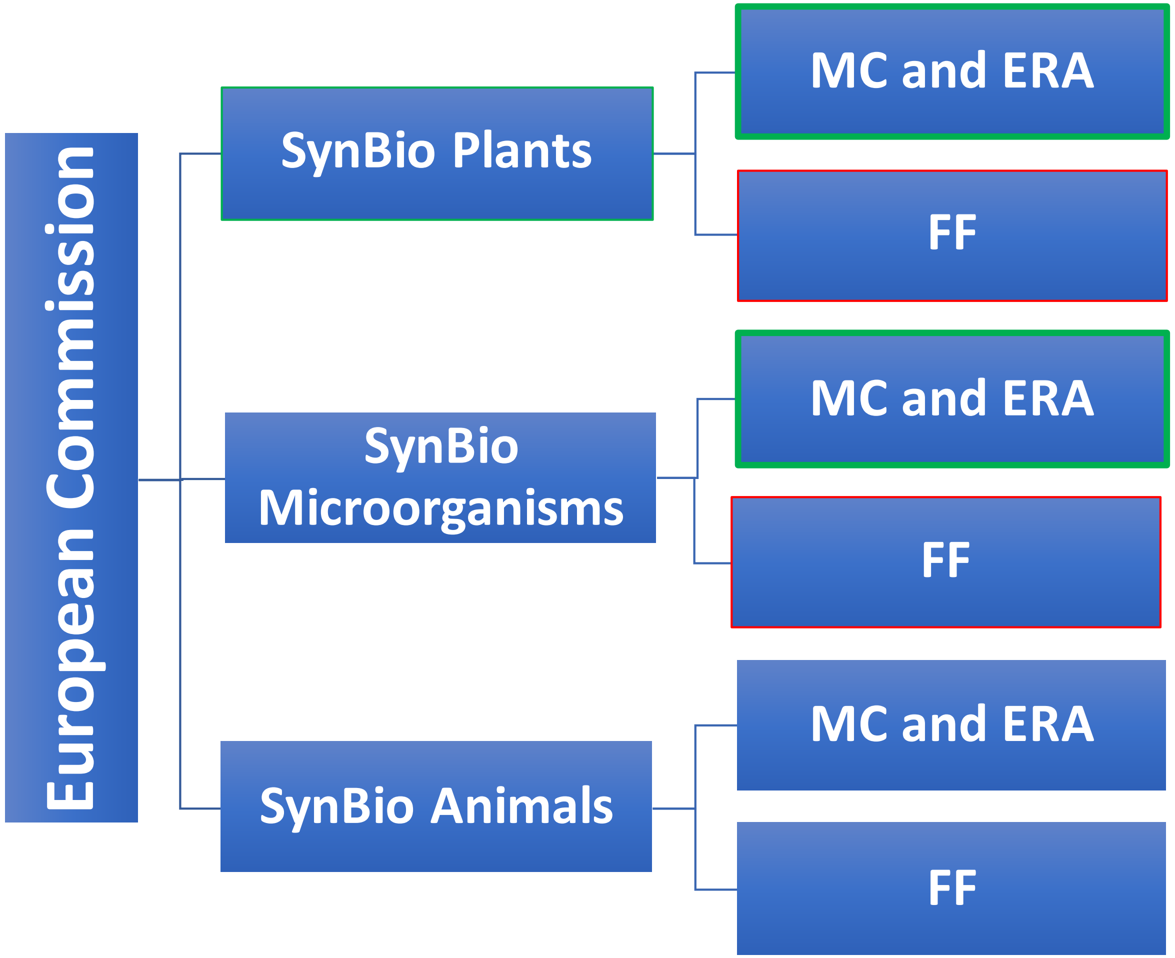
SynBio mandate to EFSA

EC asked EFSA for a scientific opinion on GMOs developed through SynBio and their implications for risk assessment methodologies:

1. To reflect whether and which newer sectors/advances should be considered among SynBio developments;
2. To identify, where possible, potential risks in terms of impact on humans, animals and the environment for current or near future SynBio developments. Identify novel hazards as compared to established GMO techniques;
3. To determine whether the existing guidelines for risk assessment are adequate and sufficient for current and near future SynBio developments or whether there is a need for updated guidance;
4. In case guidances need to be updated, to identify the specific areas where such updated is needed.

Existing Guidelines Documents

Reference	Title
Directive 2001/18/EC	Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms
Commission Directive 2018/350	Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms
EC Regulation No. 503/2013	Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006
EFSA, GMO Panel, 2011	Guidance for risk assessment of food and feed from genetically modified plants
EFSA, GMO Panel, 2010	Guidance on the environmental risk assessment of genetically modified plants



- Viable GMOs
- Agri food and feed uses
- Deliberate release in the environment
- Reaching the market in 10 yr

The scientific opinions inform the EU position in international negotiations on SynBio

Mapping of plant SynBio developments in the agri-food sector

Katharina Unkel, Doerthe Krause, Thorben Sprink, Frank Hartung, Ralf Wilhelm

Julius Kühn-Institut (JKI)- Federal Research Centre for Cultivated Plants
Institute for Biosafety in Plant Biotechnology (SB)

JKI

. an Commission (EC) requested the European Food and Safety Authority (EFSA) for an opinion on genetically modified organisms for agri-food uses and developed through synthetic biology (SynBio) and their implications for risk assessment methodologies. In preparing this opinion EFSA has asked JKI to perform a horizon scan of plant SynBio developments with application in the agri-food sector. Relevant SynBio cases were identified using a search strategy including scientific publications, expert interviews and a collation of companies. The outcome of this scan supports part of the terms of references of the EC mandate and EFSA in defining relevant case studies to consider during the drafting of the scientific opinion.

Plant SynBio developments

- Technology:
  - Transgenes
  - Genome editing
- New trait:
  - Plant food feedstocks for increased nutrition
    - Carotenoids, PUFAs, low gluten
  - Plants with engineered complex traits
    - Yield, tolerance to biotic and abiotic stress
- Number of genes introduced:
    - 1-10, 5-10, Cas9 +sgRNAs introduced (not present in final product), 1-20

Horizon scan of plant SynBio developments

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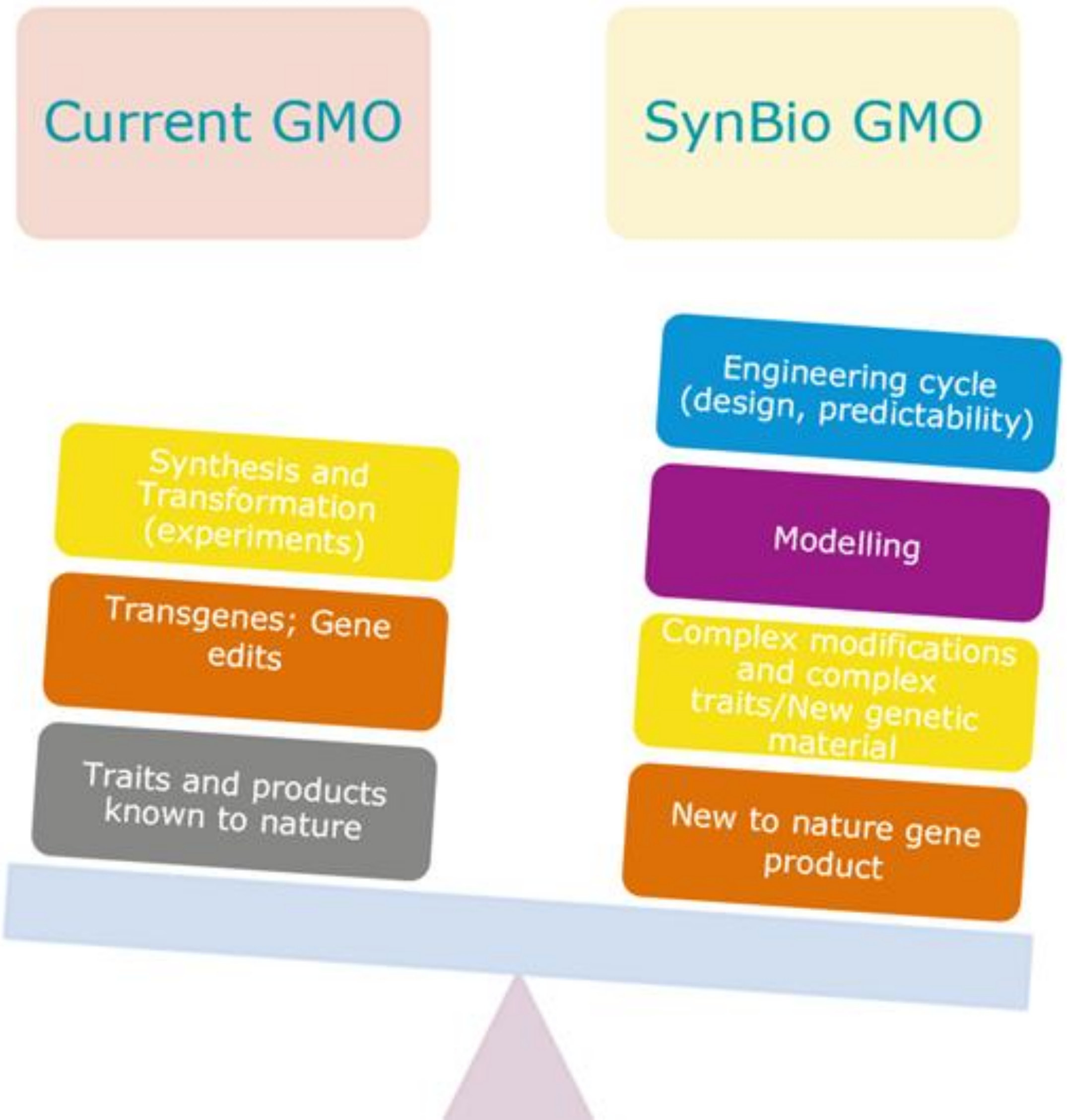
	Description of the new trait	Technological Complexity	Current Status	Example reference
1	Plants or plant cell cultures producing **ingredients for food**, e.g. color.	Low	Proof of concept	Appelhagen et. al., 2018.
2	Plant food or feed engineered for **increased nutrition** – carotenoids, e.g. astaxanthin, PUFAs (polyunsaturated fatty acids), vitamins etc.	Low to medium	Feeding trials	1. Breitenbach et. al., 2016.; 2. Betancor et. al., 2016.
3	**Deletion of undesirable gene products** from crop plants (food or feed), e.g. allergenics, gluten, bitterness etc.	Low to medium	Proof of concept	Sanchez-Leon et. al., 2018.
4	Plants engineered to produce **disease-prevention agents**, e.g. virus-like particles, via oral delivery (animal feed).	Medium to high	Proof-of-concept to product development	1. Kolotilin et. al., 2014 2. Lemire et. al., 2018. 3.http://www.frontlinegenomics.com/news/15211/antibiotic-resistant-bacteria-tackled-new-drug-using-crispr
5	Plants engineered to **improve complex traits** e.g. yield, photosynthetic capacity, tolerance to biotic and abiotic-stress	Medium to high	Proof-of-concept; field-testing in model plant species	1. South et. al., 2019. 2. De la Concepcion et. al., 2019.

Continuum going from conventional GMOs to SynBio

- **No other sectors/advances among the six SynBio categories** which were already identified by the NF-Scientific Committees, were identified by the horizon scan (ToR 1)
- A different approach to genetic engineering: **SynBio strategies apply modelling and analytical approaches to improve the predictability of the engineering** thereby enabling the generation of more **complex traits**.
- Horizon scanning highlights that **plant SynBio products reaching the market in the next 10 years are likely to result from existing technologies** including the insertion of **transgenes and genome editing**. However, the advanced traits being pursued are using engineering principles to achieve them.



Case study 1- Reconstructing a large pathway

Published: 07 October 2012

An enzyme-trap approach allows isolation of intermediates in cobalamin biosynthesis

Evelyne Deery, Susanne Schroeder, Andrew D Lawrence, Samantha L Taylor, Arefeh Seyedarabi, Jitka Waterman, Keith S Wilson, David Brown, Michael A Geeves, Mark J Howard, Richard W Pickersgill & Martin J Warren

Nature Chemical Biology **8**, 933–940(2012) | Cite this article

- Trait: GM **maize engineered** to produce vitamin B12 (cobalamin)
- Tools/Method: **Transgenic** insertion of a molecular stack (multiple engineered genes from one or more bacterial vitamin B12 pathways).
- Approach: requires tight and differential regulation of each of the enzymes in the pathway; potential engineering of each enzyme for optimal function in a plant cell.
- Complexity: likely to integrate **multiple SynBio approaches** including computational modelling of multiple variants of pathway components to select the most promising iterations;
    - optimising the expression level of each gene including the use of conditional switches to maintain the desired levels and patterns of expression within the plant;
    - metabolic modelling of the cell to identify endogenous genes that prevent the accumulation of the end product.

Case study 2- Non transgenic gluten-free wheat

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Plant Biotechnology Journal

Plant Biotechnology Journal (2018) **16**, pp. 902–910 doi: 10.1111/pbi.12837

Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9

Susana Sánchez-León[1,#], Javier Gil-Humanes[2,*,#], Carmen V. Ozuna[1], María J. Giménez[1], Carolina Sousa[3], Daniel F. Voytas[2] and Francisco Barro[1,*]

- Trait: **Low-gluten wheat**, by introduction of targeted mutations of multiple alpha-gliadin genes.
- Tools/Method: using **CRISPR/Cas9 genome editing**. Mutations were achieved using a transgene, and lines that lacked the transgene and only contained the desired mutations were subsequently obtained by segregation, after self-pollination (reported in Sanchez-Leon et al., 2018).
- Approach: likely a targeted approach to remove gluten achieved without the introduction of a transgene and all target genes to be successfully and precisely edited resulting in a gluten-free wheat.
- Complexity: likely to require synbio approaches to correctly identify all gliadins and glutenins in the hexaploid genome of bread wheat and to identify an engineering strategy that introduced mutations of the correct nature and positions in each gene to prevent the accumulation of any peptide fragments associated with initiation of the inflammatory cascade.

Case study 3- Fungus-resistant GM oilseed rape

- Trait: **Fungus-resistant GM oilseed rape**; The combination of strategies will result in plants resistant to a broader range of fungal pathogens.
- Tools/Methods: Obtained by **transgenic insertion** of a plant resistance gene **and genome engineering** of susceptibility genes.
- Approach: This case study is based on the combination of two reported studies:
  - plant engineered to recognise a broader range of pathogens by structure-guided engineering of a nucleotide binding-leucine-rich repeat (NLR) protein (**De la Concepcion et al., 2019**).
  - deletion of additional genes for pathogen susceptibility using genome engineering tools such as CRISPR/Cas9 (**Zaidi et al., 2018**).
- Complexity: The combination of strategies will result in plants resistant to a broader range of fungal pathogens. To achieve this, requires **synthetic biology approaches** including model-guided redesign of the protein from its crystal structure to identify which mutations will enable the identification of new pathogens; and insertion of this redesigned protein into a plant in which endogenous susceptibility genes have been previously identified and edited or deleted.

Case Study based assessment of GMO Guidelines

Based on the three hypothetical case studies plus the outcome of the horizon scanning exercise the GMO Panel concluded:

- **No potential novel hazards compared to established techniques** of genetic modification and no novel potential risks in terms of impact on humans, animals and the environment, were identified (ToR2)

- The evaluation of existing guidelines using the selected case studies, showed that **the current requirements are adequate and sufficient** for the risk assessment of such cases although **not always applicable to all** the three selected case studies (ToR3)

- For Synbio developments in the wider future (> 10 years), **a need may exist to adjust the requirements of the guidelines** to ensure that they are adequate and sufficient for plants engineered with SynBio (ToR4)

Molecular Characterisation

MC RA as currently performed will be sufficient to conclude on the structure and expression of the inserted/modified sequences and on their genetic and phenotypic stability

The specific molecular data requirements for introduced transgenes, are not directly applicable when the modification is obtained using only gene editing approaches

In case gene editing techniques are used, the terminology used in the current guidelines does not always apply; 'genetic modification' may be more suited than 'genetic transformation'

The concepts of event (single and stack) for SynBio plants may need to be reconsidered

Updating of approaches for characterizing and assessing complex traits may be needed

Environmental Risk Assessement

Future SynBio plants-resulting phenotypes may be more complex than those considered in the selected case studies; therefore more challenging for the current RA approaches;

The selection of relevant endpoints to test for their agronomic/phenotypic characterisation may need to be adapted on a case-by-case basis (EFSA, 2015)

The concept of comparator may have to evolve; the implementation and interpretation of the comparison with a non-GM comparator and equivalence with non-GM reference varieties (EFSA, 2015) may have to evolve;

More emphasis may have to be put on investigating potential interactions related to the management of generated SynBio GMPs across the environments in which they could be cultivated.

In particular cases, other risk assessment approaches may be needed that do not rely on history of safe use and the current comparative approach.

Modelling of the characteristics of engineered plants, including their expected behaviour in different environments might aid and improve the comparative analysis and ERA of SynBio GMPs in a given receiving environment.

The 'design' factor in the SynBio GMP offers the possibility to address safety issues already identified during the design-build-test-learn cycle of the SynBio approach ('safe by design')

Need to consider whether possible additional uncertainties related to potential adverse environmental effects of a SynBio GMP could be better assessed through PMEM.

EC biotechnology mandates to EFSA

Reference	Subject	Entry date	Status	Public consultation	Adoption Deadline
EFSA-Q-2018-01000	**Synthetic biology developments** in plants, microorganisms and animals **Project coordinator: Reinhilde Schoonjans**	2018	**2 opinions published;** 2 more in progress	Yes	WP1,3 31/12/2020; WP 2, 4 30/06/2022
EFSA-Q-2018-00619	Genetically modified organisms engineered with gene drives (**gene drive modified organisms**) and their implications for risk assessment methodologies **Project coordinator: Yann Devos**	2018	Published (November 2020)	Yes	31/12/2020
EFSA-Q-2020-00445	In vitro **random mutagenesis** techniques **Project coordinators: Tommaso Raffaelo, Paolo Lenzi**	2020	In progress	Yes	30/09/2021
EFSA-Q-2020-00103	Genetically modified plants developed through **New Genomic Techniques** **Project coordinator:** Konstantinos Paraskevopoulos	2020	Concluded (to be published 30/04/2021)	No	31/10/2020
EFSA-Q-2019-00297	Plants developed using type 1 and type 2 **Site-Directed Nucleases and Oligonucleotide Directed Mutagenesis** **Project coordinator:** Tommaso Raffaello	2019	Published (November 2020)	Yes	31/10/2020
EFSA-Q-2021-00361	Scientific opinion on plants developed through **cisgenesis and intragenesis** **Project Coordinator:** Paolo Lenzi	2021	Started	Yes	30/12/2022

THANK YOU!

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