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Mapping of plant SynBio developments in the agri-food sector

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Abstract

The European 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.

Key words: Synthetic Biology; Agri-Food; Plant; Commercialization; Deliberate Release

Question number: EFSA-Q-2019-00340

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Summary

Synthetic biology is a rapidly developing research field resulting in new techniques for the design of genetically modified organisms (GMO). In order to get an overview of GMO applications that are in the pipeline for commercialization, EFSA requested JKI to map these developments using a horizon scan. More specifically, this horizon scan focusses on synthetic biology (SynBio) cases describing the use of genetically modified plants (GMP) intended for a deliberate release into the environment and with a possible application as an agri-food product in the near future. This request is to address the Terms of Reference of a European Commission (EC) mandate on this topic published under mandate number M-2018-0205 in the EFSA register of questions.

Relevant SynBio cases were identified using a search strategy including systematic literature review, expert interviews and a collation of companies. Inclusion criteria on type of agri-food product and intended use of a GMP, enabled the listing of relevant SynBio cases. The screening of articles resulted in 60 potential relevant articles, 387 proof of concept studies and 362 potential relevant reviews. After full screening of the text, 27 articles were considered relevant. Seven of the 27 relevant studies optimized oil composition or phytosterole production in plants. Many attempts were made to improve photosynthesis by introducing bacterial genes or by converting the C-fixation pathway from a C3 pathway into a C4 pathway. But only five developments were considered potentially leading to market releases in the near future (~ 5 years). Three publications addressed improved abiotic stress tolerance to salt-, drought- or heat stress or a combination. Improving resistance against biotic stress factors have been the goal in three publications. Medical use of producing special fibre has been reported in one of the retrieved studies. The *de novo* domestication of wild type tomato using genome editing is one of the chosen putative market relevant publication. Another two papers tend to improve the nutritional value of crops. One study each aimed for better nitrogen fixation and phytosensing of invading pathogens. Currently, flax producing medical fibre seems closest to be released and has already been tested under European field conditions.

From the expert consultations it was clear that most applications of synthetic biology in plants are far from application. During the search of SynBio companies, 18 were identified that support the development of SynBio products. Given the definition of SynBio as agreed for this report, it seems most likely that plants will enter the international market in the next 10 years that have been modified by metabolic engineering. Moreover, plant chassis, part libraries or artificial organelles may essentially evolve linked to other approaches like metabolic engineering or modified photosynthesis and are not considered to be released to the environment/field as a stand-alone product.

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1. Introduction

1.1. Background and Terms of References as provided by the requestor

This contract was awarded by EFSA to:

Contractor: Julius Kühn-Institut - Federal Research Centre for Cultivated Plants

Contract title: Mapping of plant SynBio developments in the agri-food sector

Contract number: NP/EFSA/SCER/2018/03

Rapidly evolving developments in synthetic biology may require adaptation of existing risk assessment methodologies. Revisiting and improving these methodologies are suggested to ensure continued safety of future products resulting from applications of synthetic biology1. This was concluded by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER)2 and the Scientific Committee on Consumer Safety (SCCS).

These scientific committees defined synthetic biology (SynBio) as "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms". They recognized six categories of SynBio developments: 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; and 6) Citizen science (Do- It-Yourself biology).

At the end of 2018, the European Commission requested the European Food and Safety Authority (EFSA) for an opinion on genetically modified organisms for agri-food uses and developed through SynBio and their implications for risk assessment methodologies. This request is published under mandate number M-2018-0205 in the EFSA register of questions3.

In preparing this opinion EFSA has asked JKI to perform a horizon scan of SynBio developments with application in the agri-food sector under the contract title 'Mapping of plant SynBio developments in the agri-food sector'. The contract was awarded by EFSA to JKI under the contract number NP/EFSA/SCER/2018/03 and the period of investigation was from 1 February to 30 April 2019. The overall objective of the contract was to gather relevant information on plant SynBio developments moving towards practical applications in the next decade, arising from the previously mentioned SynBio categories, and to report this information in a technical report.

The outcome of this scan supports the Terms of References 1 and 2 of the above mandate and support EFSA to define relevant cases to consider during the drafting of opinion on SynBio developments mandated by the EC. The specific objectives of the contract resulting from the present procurement procedure are as follows:

[1] SCENIHR, SCCS, SCHER (2014) Synthetic Biology I Definition, Opinion, September 2014. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf
SCENIHR, SCCS, SCHER (2015) Synthetic Biology II - Risk assessment methodologies and safety aspects, Opinion, May 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf
SCENIHR, SCCS, SCHER (2015) Synthetic Biology III – Research priorities, Opinion, December 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_050.pdf
[2] SCENIHR and SCHER are merged into the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER)
[3] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?5

1) Considering the SynBio categories defined above, to identify and produce a list of plant SynBio developments with potential practical applications in the agri-food sector4 and their intended use. This task should focus on applications that are in the pipeline for commercialization (deliberate release into the environment for commercial purposes) or in an experimental phase beyond the (theoretical) proof of concept stage.
2) Based on the list produced in the objective above, to prioritise the plant SynBio developments that are most likely to move towards practical applications in the agri-food sector in the next decade. Criteria used to prioritise relevant plant SynBio developments should be made explicit and justified, and be agreed with EFSA.

1.2. Interpretation of the Terms of Reference

To achieve the highest quality of data, within the time and budget constraints of this study, several sources for searching relevant SynBio cases were chosen. A scientific literature review was conducted as described in 2.2. Next to this, expert interviews and a collection of companies active in the field of plant SynBio were recorded.

The actual definition of SynBio as well as "application" with relevance for the EFSA mandate and in the context of (higher) plants was considered being hard to target clearly. This was partly due to the relative open definition of SynBio, and the practical and actual range of "applications" to be considered under the EFSA mandate. Finally, the scope was defined for the purpose of this procurement, as described below.

Plants within the scope of this report are higher plants or macro-algae, model plants except Arabidopsis (other typical model plants, e.g. Nicotiana, might also be used for production and were not excluded from the general scope). From these objectives it is clear that this horizon scan does not include the use of genetically modified (GM) micro-organisms and GM animals.

Methods, technologies and approaches considered in this report comprise the techniques/approaches described in 1.1. including classical transgenesis and genome editing. Nevertheless, "classical" genetically modified plants such as herbicide tolerant or insect resistant plants and such stacked events where excluded. Genome editing of simple traits (e.g. single guides, disjunct knockouts) were also not considered relevant5. Articles/papers/information were considered in case either of the techniques mentioned above was used in order to design a "complex" modification of the plant characteristics, i.e., a metabolic pathway by more than a single "edit" or insertion of a simple transgene casette. Note: while many scientists consider metabolic engineering/design not being synthetic biology, it is included in the definition of SynBio used in this report – and hence in the reported outcome.

"Applications" are considered in case the (higher) plant is not Arabidopsis, in case there is advanced research documented and an active environmental release (cultivation) seems possible (although the current state is still in an experimental phase) or a field release was already performed. Plants modified

[4] With a focus on agri-food/feed products falling within the remit of EFSA. These include: GMOs, food enzymes, novel food, as well as plant protection products.

[5] A systematic map about applications of genome editing in plants has been published by the group at the website of the German Federal Ministry of Food and Agriculture (BMEL): https://www.bmel.de/SharedDocs/Downloads/Landwirtschaft/Pflanze/GrueneGentechnik/NMT_Stand-Regulierung_Anlage4-Aktualisierung.pdf?__blob=publicationFile .The extended map has recently been submitted for publication to Environmental Evidence and is under review. The protocol is already published (open access): https://environmentalevidencejournal.biomedcentral.com/articles/10.1186/s13750-018-0130-6

for the production of therapeutics etc. were not considered as being part of the scope of the EFSA mandate unless they are likely grown in the field.

2. Data and Methodologies

2.1. Data

The extraction of data from relevant articles was performed filling the Appendix A. Besides the bibliographic data, all data concerning the name of the modified plant, the name of the donor organism from which genes have been transferred or modified, the description of the new trait and the envisaged commercial product (if any) have been extracted. Furthermore, the used molecular techniques, the degree of the molecular modification including the involved gene(s), the phase of development, type of release and testing conditions, as well as the geographic region of field trials/releases have been collected from the relevant articles. Eventually, an estimation (based on the authors' expert judgement) whether the respective organism will be grown in the field or might appear as commercial release in the EU in the next 10 years (or not) is given, including remarks considering the degree of SynBio progress. The estimation may or may not be correlated to the official regulatory status of the case.

2.2. Methodologies

2.2.1. General search strategy

The initial investigations (internet; literature searches) revealed that plant related "applications that are in the pipeline for commercialisation (deliberate release into the environment for commercial purposes) or in an experimental phase beyond the (theoretical) proof of concept stage" may be considered limited. Essentially, "(2) minimal cells and designer chassis, (3) protocells and artificial cells, (4) xenobiology" appear not being used in a sufficiently advanced manner in the context of plants (see: search strategies). We also assumed that "(6) citizen science (Do-It-Yourself biology)" using SynBio in plants is not advancing scientific developments.

The search string/strategy was refined as described below for the systematic literature analysis, following the background as provided by EFSA in the specification of the procurement (see section 1.1.). The search covered the period from 1998 till the 28th February 2019. The time frame was chosen since SynBio developments with an anticipated market releases are very unlikely before 1998.

Key issues for the implemented search strategy were:

- the coverage of SynBio in plants
- the reduction of the number of irrelevant hits
- ensuring the coverage of likely relevant papers
- achieving a data set manageable within the given time frame.

The efficiency of the improvement of the search string was repeatedly checked by testing a small number of hits by the different search strings categorizing them in:

- relevant
- potentially relevant proof of concept (POC)
- not relevant
- reviews
- unclear

and optimizing it for reasonable percentage of relevant or potentially relevant publications. To evaluate the thoroughness of the search strategy, the authors handpicked studies from reviews and checked if they were included in the hits from the literature search.

The final search was performed applying the optimized search string in Web of Science core collection including CAB Abstracts (relevant subgroups) and Scopus; in addition, a separate search reconsidered the publications linked to applications of genome editing (see also 2.2.3.). After screening of 6624 hits from the search on a title/abstract basis, the authors preselected 60 publications to screen on full text level. After this screening, the number of relevant articles identified was 27. The information extracted from those full text publications is presented in Appendix A, published together with this report on the EFSA website.

2.2.2. Development of the search string

To screen literature from 1998 for applications of SynBio in crop plants in an optimized manner, the authors progressively tested different search string designs. By doing so, an efficient string design was elaborated maintaining a manageable number of retrieved publications to enable a detailed manual screening for relevance and data extraction. The searches were performed on title and abstract level.

The first search string comprised a collection of terms describing interventions associated with SynBio (e.g., metabolic engineering, pathway design, synthetic nucleotides) and crop plants (Populations/Intervention design (PI) of the search string). The search string was solely tested within the Web of Science Core Collection database and resulted in 5876 hits (search string 1.1; all consecutive search strings are reported in Annex A). The authors checked the amount of hits for every intervention to identify interventions that either led to many hits (e.g., synthetic cell* or Synthetic biology) or none at all (e.g., Xeno nucleic acid). In addition, the authors did not use abbreviations (like e.g., XNA; HNC) since those generated hits by unrelated topics in other contexts. The scientific cultivar names were placed in quotation marks since the authors wanted to find crop plants and not their wild relatives from the same genus. This approach was proven successful in previous literature searches done at JKI. After discussion of the search strategy with EFSA, the authors added commercial herbs and ornamental plants in the list of plant related terms (search string 1.2, Annex A). This addition led to a new outcome of 6701 publications.

The research team used a list of the search results with the PI 1.2 search string transferred to Appendix B (published together with the report on the EFSA website) and randomly picked 25 publications to check their relevance (see Appendix B; Tab Relevance). From those 25 publications none was considered relevant or even potentially relevant. Because of the high number of hits with mostly theoretical work or research performed in bacteria or micro algae, the authors restricted the output to publications containing the terms flower, flowers, herb, herbs, crop, crops, plant, plants, seed, seeds, algae, alga (Population/Population/Intervention (PPI) design) as a first elimination criteria (search string 2.1, Annex A). This precondition (first block of terms used in the search string) limited the search results to the plant-kingdom. By repeating these general terms in the second block of the whole string and combining the blocks by AND it was assured not to miss publications that only refer to "plant*" or "crop*" etc. without mentioning a specific cultivar in the title or abstract. This strategy led to a reduced output of 5876 in the Web of Science Core Collection database. However, most of the gained publications were still out of scope as it was checked by randomly picked 25 publications from the search results (Table1).

Table 1: Level of relevance of 25 randomly picked publications from search string 1.2 and 2.1

	Not relevant	Review	Unclear	Potentially relevant (POC)	Relevant
# Hits search string 1.2 (PI)	22	2	1	0	0
# Hits search string 2.1 (PPI)	16	3	1	4	1

Additionally to the assessment of the previously 25 randomly picked publications, the authors checked the output of the search against 59 publications that were picked from reviews considered to cover the scope (e.g., clearly described cases of high complexity metabolic engineering), but the set of derived publications was not evaluated on abstract level as such. From those 59 publications, the authors found 18 with the PI 1.2 search string and 17 with the PPI 2.1 search string (see Appendix B; Tab "Coverage test"). Thus, the authors concluded that the search design was still inefficient and needed further improvement. The authors screened the abstracts of missing relevant and potentially relevant publications for new keywords and could identify a variety of new terms which were added to develop search string 2.2 (see Annex A). However, adding those further terms resulted in a number of 8858 publications in the Web of Science Core Collection, which were a large number to be screened within the given time frame.

Refining the search string by using the proximity operator "NEAR/"

For further refinement, the authors used the proximity operator "NEAR/" that enables the inclusion of terms within a specified distance of words in any order (e.g.: artificial NEAR/2 (plastid*)). Now the database generates a hit as soon as the word artificial is written next to plastid* within a distance of two words (e.g., artificial plastid; plastid […] artificial; artificial […] plastid; …). In order to maintain the clear arrangement of the search string, the authors separately listed the terms in the third block (a).

a) [("artificial *nucleotide" OR "artificial cell*" OR "artificial gene* network*" OR "artificial nucleic acid*" OR "artificial plant cell*" OR "biobrick*" OR bioengineer* OR "desig* DNA part*" OR "engineered nucleotide sequenc*" OR "engineered sequenc*" OR "genetic circuit*" OR "metabolic design" OR "metabolic engineering" OR "minimal genome" OR "multiplex automated genome engineering" OR "non-canonic* amino acid*" OR "pathway design" OR "design* pathway*" OR "*protein engineering" OR "engineer* *protein*" OR protocell* OR SynBio OR "synthetic *nucleotid*" OR "synthetic bio*" OR "synthetic cell*" OR "synthetic DNA*" OR "synthetic gen*" OR "synthetic gene* cluster" OR "synthetic life" OR "synthetic plant cell*" OR "synthetic promoter" OR "minimal cell*" OR "system* bioengineering" OR "xeno nucleic acid" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "artificial plastid*" OR "synthetic plastid*" OR "plastid* desig*" OR " design* plastid*" OR carboxysom* OR transplastom* OR ((photorespirat*) AND (engineer* OR bypass OR decreas*)) OR (stable AND (transformation OR integration)) "synthetic operon" OR multigene* OR ((gene* OR enzym* OR protein* OR transgen*) NEAR/30 (introduce* OR transfer* OR transform*)))]

Subsequently, the authors rearranged terms with concordant words in different subgroups using the operator "NEAR/" (e.g., the terms "desig* DNA part*", "metabolic design", "design* pathway*", "plastid* desig*" and "design* plastid*" became (desig* NEAR/1 (plastid* OR pathway* OR DNA OR metabol*))).

This resulted in six subgroups, each containing a "NEAR/" operator (b).

b) [((artificial NEAR/1 (plastid* OR *nucleotide OR cell OR cells OR gene* OR nucleic)) OR (desig* NEAR/1 (plastid* OR pathway* OR DNA OR metabol*)) OR (engineer* NEAR/1 (bio* OR sequenc* OR metabol* OR multiplex OR *protein*)) OR (synthetic NEAR/1 (bio* OR nucle* OR cell OR cells OR DNA OR gen* OR plant OR operon OR plastid* OR promot*)) OR synbio OR "minimal cell" OR "minimal cells" OR "minimal genome" OR ((photorespirat*) NEAR/1 (engineer* OR bypass OR decreas*)) OR (stable AND (transformation OR integration)) OR ((gene* OR enzym* OR protein* OR transgen*) NEAR/20 (introduce* OR transfer* OR transform*)) OR "biobrick*" OR "genetic circuit*" OR "non-canonic* amino acid*" OR protocell* OR carboxysom* OR transplastom* OR multigene* OR bioengineer*))]

1. Doing so, the lowest number of hits generated in the Web of Science database search using "NEAR/1" as operator was still 27.156 (search string 3) and still very high number of publications to screen within the given time. Since the objective of the research was to gather relevant information on plant SynBio developments in the agri-food sector moving towards practical application, the authors decided to focus the search string on such "well-developed" SynBio applications.

Therefore, the authors added terms that describe a "comparison" and an "outcome" to the search string structure ("PPI" -> "PPICO").

"Comparison": the modified plant is supposed to show benefits compared to the wild type/parental variety expressed by the terms:

(high OR higher OR increas* OR improve* OR optimi* OR enhance*)

"Outcome": traits and products being highly relevant for the market. Therefore, the authors chose the terms:

(biomass OR productivity OR metabolism OR "plant performance" OR "bio* plastic*" OR bioplastic* OR biofuel* OR "bio* fuel*" OR biodiesel OR "bio* diesel" OR photosynth* OR toleran* OR "*biotic stress" OR ("*biotic stress" NEAR/4 (low OR lower OR decreas* OR less)))

The PPICO structure fully covered the previous test results and reached a total hit number of 4.772 in Web of Science. Thus, the PPICO search satisfied the authors requirements and the literature search was performed.

The PPICO structure satisfied both demands: it narrowed the search results but performed qualitatively better than the other search strategies. It is precisely defined due to its five categories that all have to be met on title/abstract level. Nevertheless, it may have happened that papers were excluded, which discuss synthetic biology approaches in an early stage of development. Even though the authors would not have considered them as relevant on full text level, this could have affected the statistics for POC. In addition to this, the authors cross checked the 59 picked publications used to improve the search string on title/abstract base if they were really of relevance for the Scope and eventually came up with 16 potentially relevant publications. Out of these 16, the authors found 11 using the PICO search string and 15 using the PPICO search string (see Appendix B; Tab "Coverage test"). The single missed one using the PPICO search string was not archived in the Web of Science database and thus could not be found. Considering the strict time frame given to accomplish the literature review, the PPICO search string was chosen as the best alternative to work with.

2.2.3. Final search string

The final search string selected is:

TS=((flower OR flowers OR herb OR herbs OR crop OR crops OR plant OR plants OR seed OR seeds OR algae OR alga)
AND
(plant OR plants OR crop OR crops OR seed OR seeds OR herb OR herbs OR alfalfa OR Medicago OR alga OR algae OR apple OR apples OR malus OR banana OR Musa OR barley OR Hordeum OR basilicum OR basil OR "Ocimum basilicum" OR bean OR beans OR Phaseolus OR beet OR cabbage OR "Camelina sativa" OR canola OR capsicum OR pepper OR carrot OR "Daucus carota" OR celery OR "Apium graveolens" OR cherry OR "Prunus avium" OR Cocoa OR "Theobroma cacao" OR coconut OR "Cocos nucifera" OR coffee OR Coffea OR corn OR maize OR "Zea mays" OR cotton OR "Gossypium hirsutum" OR cucumber OR cucumis OR curcuma OR dandelion OR Taraxacum OR duckweed OR duckweeds OR "lemna minor" OR eggplant OR Solanum OR flax OR "Linum usitatissimum" OR "fodder beet" OR garlic OR ginger OR grape OR grapevine OR vitis OR grapefruit OR hazelnut OR hazel OR "Corylus avellana" OR hemp OR "Cannabis sativa" OR jujube OR "Ziziphus jujuba Meikl" OR kiwi OR "Actinidia deliciosa" OR leek OR lemon OR "Citrus limon" OR lentil OR "Lens culinaris" OR lettuce OR Lactuca OR lime OR "Citrus latifolia" OR lupine OR lupines OR manihot OR "Manihot esculenta" OR melon OR oat OR avena OR olive OR "Olea europaea" OR onion OR allium OR orange OR "Citrus aurantium" OR "Citrus sinensis" OR oregano OR Origanum OR parsley OR "Petroselinum crispum" OR pea OR "Pisum sativum" OR peanut OR peanuts OR "Arachis hypogaea" OR pear OR Pyrus OR peppermint OR "Mentha piperita" OR pineapple OR "Ananas comosus" OR "Piper nigrum" OR poplar OR potato OR pumpkin OR Cucurbita OR radish OR Raphanus OR rapeseed OR oilseed OR Brassica OR "Brassica carinata" OR raspberry OR "Rubus idaeus" OR rhubarb OR "Rheum rhabarbarum" OR rice And Oryza OR rosemary OR Rosmarinus OR rye OR "Secale cereale" OR sesame OR "Sesamum indicum" OR sorghum OR soybean OR soy OR "Glycine max" OR spinach OR "Spinacia oleracea" OR strawberry OR Fragaria OR "sugar beet" OR "sugar-beet" And "Beta vulgaris" OR sugarcane OR "Saccharum officinarum" OR sunflower OR "Helianthus annuus" OR tobacco OR "Nicotiana benthamiana" OR "Nicotiana tabacum" OR tomato OR walnut OR "Juglans regia" OR wheat OR Triticum OR zucchini OR allspice OR "Pimenta dioica" OR anise OR "Pimpinella anisum" OR "bay leaf" OR "Laurus nobilis" OR basil OR "Ocimum basilicum" OR bergamot OR "Monarda species" OR cumin OR Cuminum OR mustard OR pepper OR Piper OR borage OR "Borago officinalis" OR caraway OR "Carum carvi" OR cardamom OR "Elettaria cardamomum" OR chervil OR "Anthriscus cerefolium" OR chives OR "Allium schoenoprasum" OR cicely OR "Myrrhis odorata" OR cinnamon OR Cinnamomum OR coriander OR Coriandrum OR dill OR "Anethum graveolens" OR fennel OR Foeniculum OR fenugreek OR "Trigonella foenum-graecum" OR ginger OR "Zingiber officinale" OR horseradish OR "Armoracia rusticana" OR hyssop OR "Hyssopus officinalis" OR lavender OR Lavendula OR "lemon balm" OR Melissa OR "lemon grass" OR "Cymbopogon citratus" OR licorice OR "Glycyrrhiza glabra" OR lovage OR "Levisticum officinale" OR majoram OR "Origanum majorana" OR nutmeg OR "Myristica fragrans" OR oregano OR Oreganum OR parsley OR "Petroselinum crispum" OR mint OR Mentha OR "poppy seed" OR "Papaver somniferum" OR rosemary OR Rosmarinus OR saffron OR "Crocus sativus" OR sage OR Salvia OR savory OR "Satureja hortensis" OR sesame OR Sesamum OR sorrel OR Rumex OR tarragon OR "Artemisia dracunculus" OR turmeric OR "curcuma longa" OR vanilla OR clove OR "Syzygium aromaticum" OR Thyme OR Thymus OR Celosia OR Chrysant* OR Dianthus OR Carnation OR Gentian OR Geranium OR Kalanchoe OR Lilium OR Lillies OR Lily OR Marigold OR "Tagetes erecta" OR "Morning Glory" OR "Ipomoea purpurea" OR Nerium OR

"Nerium oleander" OR Orchid OR Orchis OR Pansy OR Petunia OR Pelargonium OR Storksbills OR Poinsetta OR "Euphorbia pulcherrima" OR Rosa OR Rose OR Tulip OR tulipa)
AND
("artificial *nucleotide" OR "artificial cell*" OR "artificial gene* network*" OR "artificial nucleic acid*" OR "artificial plant cell*" OR "biobrick*" OR bioengineer* OR "desig* DNA part*" OR "engineered nucleotide sequenc*" OR "engineered sequenc*" OR "genetic circuit*" OR "metabolic design" OR "metabolic engineering" OR "minimal genome" OR "multiplex automated genome engineering" OR "non-canonic* amino acid*" OR "pathway design" OR "design* pathway*" OR "*protein engineering" OR "engineer* *protein*" OR protocell* OR SynBio OR "synthetic *nucleotid*" OR "synthetic bio*" OR "synthetic cell*" OR "synthetic DNA*" OR "synthetic gen*" OR "synthetic gene* cluster" OR "synthetic genom*" OR "synthetic life" OR "synthetic plant cell*" OR "synthetic promoter" OR "minimal cell*" OR "system* bioengineering" OR "xeno nucleic acid" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "artificial plastid*" OR "synthetic plastid*" OR "plastid desig*" OR " design* plastids" OR carboxysom* OR transplantom* OR (photorespira* NEAR/3 (engineer* OR bypass OR decreas*)) OR "synthetic operon" OR "gene transfer" OR ((transgen* OR gen*) NEAR/4 (transform* OR introduc* OR insert*)))
AND
(high OR higher OR increas* OR improve* OR optimi* OR enhance*)
AND
(biomass OR productivity OR metabolism OR "plant performance" OR "bio* plastic*" OR bioplastic* OR biofuel* OR "bio* fuel*" OR biodiesel OR "bio* diesel" OR photosynth* OR toleran* OR "*biotic stress" OR ("*biotic stress" NEAR/4 (low OR lower OR decreas* OR less))))

Hits in "Web of Science Core Collection": 4772

Searching for genome editing applications within the scope of SynBio

Inserting keywords for the genome editing techniques (e.g., CRISPR/Cas or TALEN) in the search string naming further terms for interventions ("OR"), the authors gained too many hits. Connecting such keywords with "AND" as a separated intervention increased the number of hits also too vigorously. Therefore, the authors cross-checked the "ELSA-GEA-project", in which all genome editing applications of the agri-food sector until May 2018 had been reviewed[6]. To cover the period from May 2018 till the end of February 2019, the authors created an extension of the final search string with the addition

of AND ("Genome editing").

The authors checked the output search results for duplicates and added the remaining 54 publications from this specific genome editing search to the publication list for the screening on the basis of title/abstract. Finally, from the 54 publications, two potentially relevant publications were identified and both papers were already contained in the results from the PPICO search strategy.

2.2.4. Used databases

[6] https://www.bmel.de/SharedDocs/Downloads/Landwirtschaft/Pflanze/GrueneGentechnik/NMT_Stand-Regulierung_Anlage4-Aktualisierung.pdf?__blob=publicationFile

Using the final search string in Web of Science Core Collection and in "CABI: CAB Abstracts and Global Health database", resulted in 11358 hits. As this number was very large for the further screening in the given time frame, the authors used CABICODES to select key categories within the CABI database.

The authors selected the following categories: Agriculture (General), Economics (General), Food Policy, Food Security and Food Aid (Discontinued March 2000), Marketing and Distribution, Plant Science (General), Pathogen, Pest and Parasite and Weed Management (General), Soil Science (General), Forestry, Forest Products and Agroforestry (General), Aquatic Sciences (General), Engineering and Equipment (General), Natural Resources (General), Food Science and Food Products (Human), Forage and Feed Products (Non-human), Non-food/Non-feed Agricultural Products (General), Biotechnology, Wastes (General), Other Sciences. The search focused on the mentioned categories and led to a total number of 2143 hits in the "CABI: CAB Abstracts and Global Health" database.

Hence, for the search in the CABI database the following term needed to be added:

AND CCO=(AA000 OR EE100 OR EE500 OR EE700 OR FF000 OR HH000 OR JJ000 OR KK000 OR MM000 OR NN000 OR PP000 OR QQ000 OR RR000 OR SS000 OR WW000 OR XX000 OR ZZ000)

The detailed documentation of the development of the search string is reported in Annex A. The final search string adjusted to each database semantics was run with the core collection and the CABI: CAB Abstracts and Global Health database via Web of Science, Scopus and PubMed. At title/abstract level, the search in PubMed gained no results. Therefore, PubMed was overall excluded (Table. 2). The search results were collated and duplicates were subtracted. The final number of 6624 articles (including the ones from the separate search of genome editing articles; see 2.2.3.) was retained to be screened for relevance at the title/abstract level (Table 2, full documentation in Appendix A).

Table 2: Number of publications found in databases with the "PPICO" search string

	Web of Science	**CABI (selected classes)**	**Scopus**	**PubMed (excluded from search)**	**Total**
# Hits	4772	2143	101	Title/Abstract: 0 All fields: 4071	7016
# Duplicates	0	418	28	4.5	446
# Hits without duplicates	4772	1725	73		6570
Genome editing	54*)				6624

*) Since the fields for the search in SCOPUS and CABI: CAB Abstracts and Global Health database are restricted, we only used the Web of Science Core Collection for the search of genome editing.

2.2.5. Exclusion criteria

Each publication was separately screened on title/abstract level by two reviewers to preselect potentially relevant publications, which were separately reviewed in detail by two team members afterwards. The

team performed a consistency check with all reviewers separately screening 50 papers and rating their relevance by using the following **exclusion criteria**:

- The modified organism/research is out of scope (pure medical applications, nanotechnology, microorganisms, micro algae, yeasts, animals, *Arabidopsis*)
- "classical" GMO, stacked (functionally disjunct) events or simple single traits (herbicide resistance, insect resistance). In contrast, single traits that are **inserted** or **designed** to alter the metabolic pathways of the target organism or leading to intended multiple alterations in the metabolome stay "included".
- no application (basic research, proof of concept not expected relevant).

The team solely defined "exclusion" rather than "inclusion" criteria because the scope of SynBio is somewhat open and thus the definition of what should be "in" is more critical for the outcome if "in" cannot be strictly defined for plant applications in agri-food use (see sections 1.1. and 1.2.).
The team discussed all publications that were sorted differently by the reviewers to agree on a consistent decision making.

2.2.6. Cross-checking relevant reviews

The team randomly picked 10 reviews from the years 2017-2019, screened their reference lists for important publications and identified 22 articles. After screening their abstracts, the authors only considered four of them to be relevant (see Appendix B; Tab "Check Reviews; Check relevant; Check not relevant"). All four relevant articles taken from the reviews could be found within the search results. Therefore, the authors concluded that the given strategy was sufficient to find potentially relevant publications about SynBio applications in plants.

3. Results

3.1. Literature review results

3.1.1. Search statistics

The final search performed in Web of Science core collection including CABI: CAB Abstracts and Global Health (relevant subgroups), Scopus as well as adding the specific search for genome editing applications (see 2.2.3. and 2.2.4.) resulted in 6624 articles (timeframe 1998-February 2019; see Appendix C).

The authors screened the articles based on title/abstract and classified them in four subcategories (i) potentially relevant, (ii) proof of concept studies with potential market relevance (POC), (iii) potentially relevant reviews, which were cross checked and (iv) unclear status on title/abstract (T/A) level or missing abstract.

The screening resulted in 60 (0.9%) potentially relevant articles, 387 (5.8%) proof of concept studies with potential market relevance and 362 (5.5 %) potentially relevant reviews. Three articles remained unclear on T/A level or abstracts were missing (Figure 1).

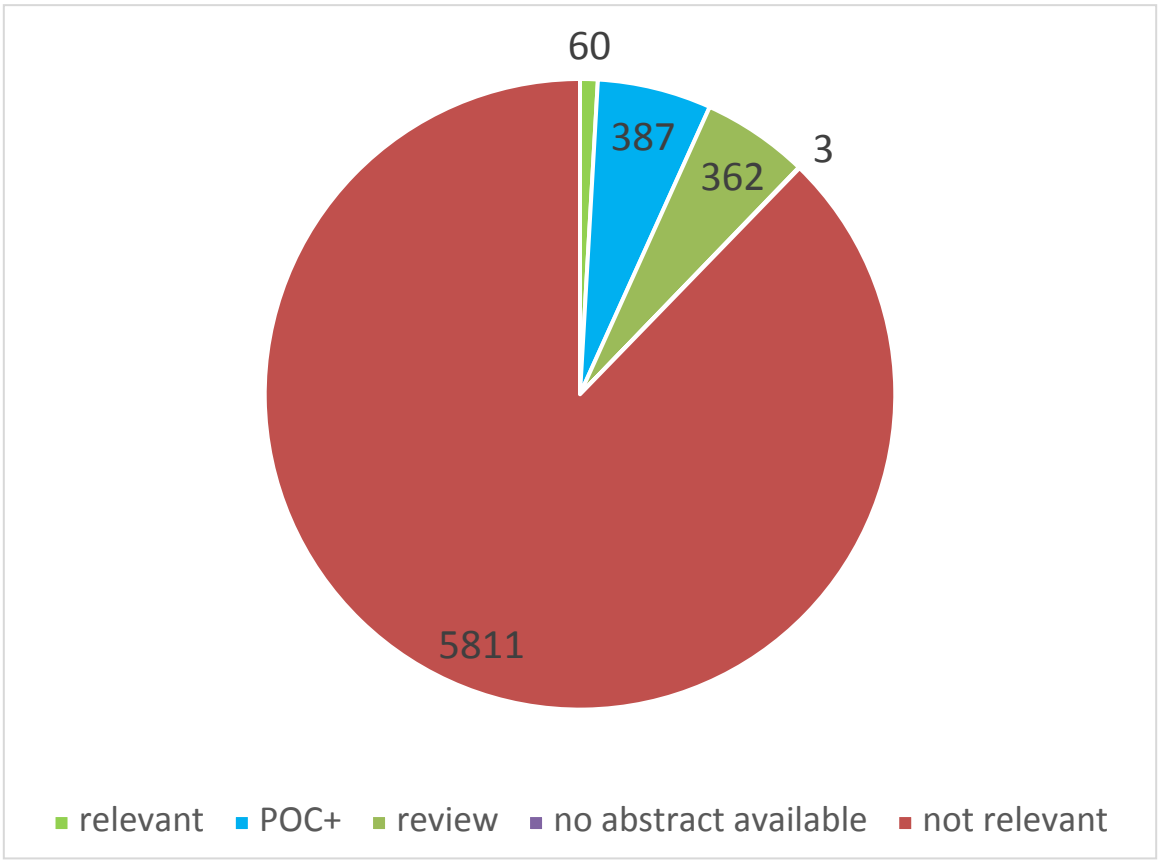

Figure 1: Distribution of the articles in the corresponding subcategories i-iv after screening on title abstract basis

The oldest "relevant publication" identified was published in 2010. From there onwards, the number of relevant publications increased resulting in 19 relevant articles published in 2018 (Table 3). Considerable "proof of principles" were recorded since 1998, but a substantial increase was also documented after 2010.

Table 3: Distribution of the article subcategories according to their time of publication after screening on a title/abstract base

Year	Relevant	POC+	Review	No. abstract	Not relevant
2019*	3	10	5	0	61
2018	19	60	55	2	559
2017	10	63	42	0	521
2016	8	45	35	0	577
2015	6	38	29	0	481
2014	7	29	27	0	406
2013	2	17	33	0	378
2012	1	24	18	0	309
2011	3	22	27	0	288
2010	1	19	14	1	253
2009	0	10	13	0	208

2008	0	7	13	0	192
2007	0	9	5	0	188
2006	0	5	8	0	114
2005	0	5	9	0	130
2004	0	8	6	0	124
2003	0	3	0	0	107
2002	0	3	4	0	135
2001	0	4	5	0	167
2000	0	2	8	0	215
1999	0	3	2	0	211
1998	0	1	4	0	187
Total	60	387	362	3	5812

*Only until February 2019

3.1.2. Data extraction statistics

After screening on title/abstract level, the authors reviewed the full text of the relevant articles and started to extract the data. After full text screening, 27 relevant articles remained (Figure 2).

The team excluded articles on the basis of the criteria as described in section 2.2.5., particularly the following:

- work was only done in *Arabidopsis thaliana* with no foreseeable application in crop plants
- modification was a single trait without pleiotropic effect.

We essentially included articles that refer to

- introduction of whole pathways
- metabolic engineering by introducing foreign genes while down regulating endogenous genes
- more than one gene has been introduced in a coordinated manner
- planned commercialization of the product.

The reasoning for each case was documented in the data extraction sheet (see Appendix A, published together with this report on the EFSA website).

Two publications remained unclear during the assessment. In one of those publications, the authors could not identify what was done exactly. The authors of those publications used a lot of different genes from different pathways in different plants and did not describe the work adequately (Polturak et al., 2017). In the other one, the authors used four different genes from various organisms, but combined them only in pairs of two (Waterer et al., 2010). Both publications defined as unclear seem to be rather excluded than included from the scope. Additionally, two articles were neither accessible for the authors nor for EFSA within the timeframe of the investigation.

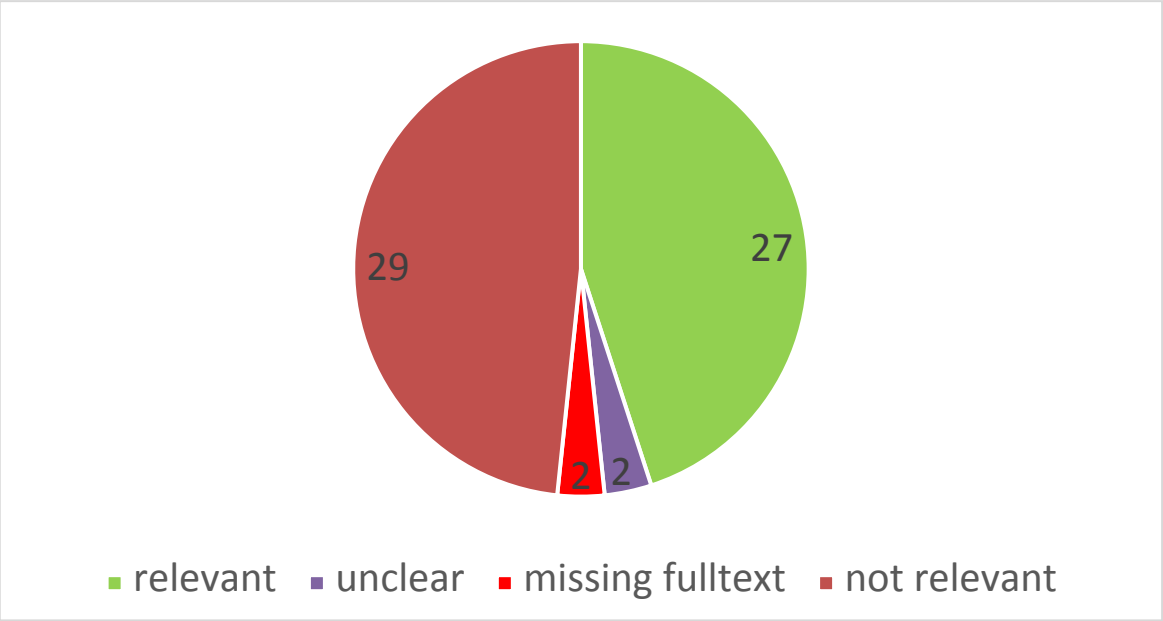

Figure 2. Final distribution of relevant, not relevant, unclear and missing full text of the 60 publications (selected on title/abstract basis) after full text analysis.

Within these 27 relevant articles, the oldest publication with market relevance appeared in 2011. After 2014, the authors identified more studies fitting the used description of SynBio according to sections 1.1. and 1.2. (9 publications in 2018).

Table 4: Distribution of the article subcategories according to their time of publication after screening of full text articles

Year	Relevant	Unclear	Missing full text	Not relevant
2019*	3	0	0	0
2018	9	0	0	10
2017	3	1	1	5
2016	4	0	0	4
2015	2	0	1	3
2014	3	0	0	4
2013	1	0	0	1
2012	1	0	0	0
2011	1	0	0	2
2010	0	1	0	0
2009	0	0	0	0
2008	0	0	0	0
2007	0	0	0	0
2006	0	0	0	0
2005	0	0	0	0
2004	0	0	0	0
2003	0	0	0	0
2002	0	0	0	0
2001	0	0	0	0

2000	0	0	0	0
1999	0	0	0	0
1998	0	0	0	0
Total	27	2	2	29

*Only until February 2019

3.1.3. Overview of the extracted data

Seven of the 27 relevant studies optimized oil composition or phytosterole production in plants. Many attempts were made to improve photosynthesis by introducing bacterial genes or by converting the C-fixation pathway from a C3 pathway into a C4 pathway. But only five developments were considered being potentially released to the market in the near future. Three publications addressed improved abiotic stress tolerance to salt-, drought- or heat stress or a combination. Improving resistance against biotic stress factors have been the goal in three publications. Medical use of producing special fibre has been reported in one of the retrieved studies. The de novo domestication of wild type tomato using genome editing is one of the chosen putative market relevant publication. Another two papers tend to improve the nutritional value of crops. One study each aimed for better nitrogen fixation and phytosensing of invading pathogens (Table 5).

Table 5: Applications addressed in 27 relevant papers with field releases or being considered close to field assessment

Oil and biofuels	7
Photosynthesis	5
Abiotic stress	3
Biotic stress	3
Nutritional improvement	2
De novo domestication	1
Nitrogen fixation	1
Medical product	1
Phytosensing	1
Unclear	3

Most "advanced research" has been performed in *Nicotiana tabacum*, which was modified either to produce Artemesin, or for enhanced photosynthesis, or the production of modified fatty acids and secondary metabolites, or for phytosensing of bacterial infections.

In potato, the focus was on securing and improving its yield by improved stress resistance and photosynthesis.

The *de novo* domestication via CRISPR/Cas was done in tomato by editing four genes, but those tomatoes have only been tested under greenhouse conditions (Zsögön et al., 2018). Just as recent is the work in tomatoes for the production of pyrethrins as an insecticide (Xu et al., 2018). In 2013, the nutritional value of tomato was improved by enhancing its Vitamin E content (Lu et al., 2013).

Improving photosynthesis and biofortification by enhancing its lysine content were the intent of the investigations performed in rice. Both attempts have already been tested under field conditions. While

the study for improved photosynthesis has been published in 2019 (Shen et al., 2019), rice with high lysin content has already been grown in field tests (Yang et al., 2016).

In sugarcane, TALEN was used to knockout a whole gene family to improve its saccharification by a reduced lignin content. It was tested under field conditions and published (Kannan et al., 2018). In 2015, sugarcane was modified for biofuel production, but to our knowledge no field studies have been performed (Zale et al., 2016).

There have been no field trials with modified *Nicotiana benthamiana*; this tobacco was transformed to produce a halogenated precursor of indigo and for having an improved N-fixation (Fräbel et al., 2018).

A banana with an improved tolerance was tested four years under field conditions in a study that has already been published Vishnevetsky et al., 2010.

The field tested Camelina has an optimised omega-3 long chain polyunsaturated fatty acid accumulation.

The abiotic stress tolerance and photosynthesis in cotton was improved by a higher Glycine betaine content.

The amount of polysaturated fatty acids was increased in Crambe by overexpression of transgenes and downregulating of two endogenous genes.

The production of improved medical fibre in flax is already advanced enough for medical trials, which makes it the most advanced application with market relevance (Table 6).

Table 6: Plant species modified and number of occurrences in relevant studies (28) retrieved in 27 publications

Plant	Number of studies	Field releases
Nicotiana tabacum	9	3
Potato	4	1
Tomato	3	-
Rice	2	2
Sugarcane	2	1
Nicotiana benthamiana	2	-
Banana	1	1
Camelina	1	1
Cotton	1	1
Crambe	1	-
Flax	1	1
Rape seed	1	-

In total, 11 out of the 27 publications reportedly tested plants under field conditions. Most of these trials were conducted outside of the EU (Figure 3). A third of all field studies took place in the U.S. (four), followed by China (three). One field trial each took place in Australia, Canada and Japan.

In the EU, the modified canola with changes in its fatty acid composition was tested in the U.K. and the flax producing improved medical fiber was tested under field conditions in Poland.

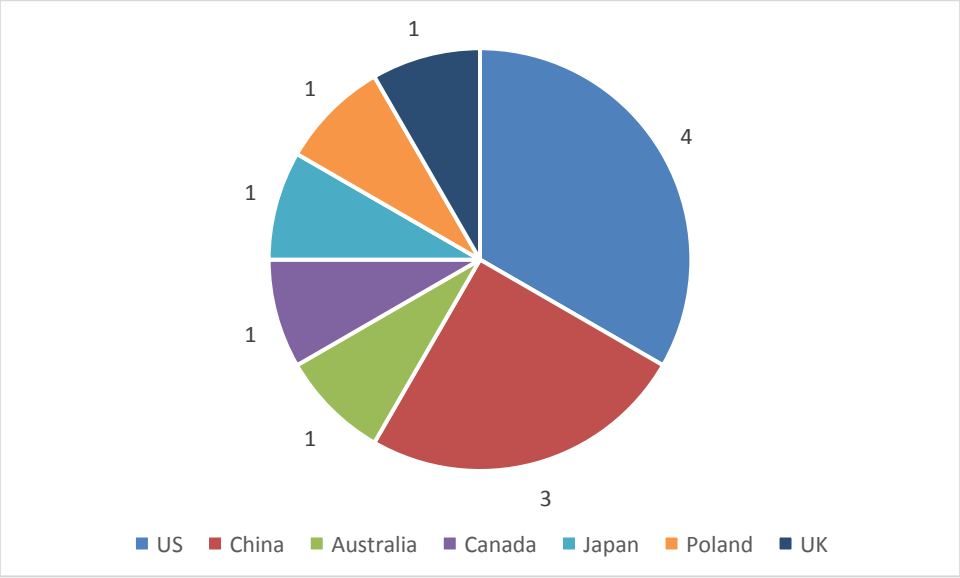

Figure 3: Number of modified "SynBio plants" tested in field trials in various countries

However, for each of the modified plants - the estimated year of their commercial release in the EU is difficult to predict as the information given in scientific articles is not sufficient to estimate how far the progress really is (e.g., the time lag between experiments and publication). Currently, flax producing medical fibre seems closest to be released and has already been tested under European conditions. Nevertheless, the authors would estimate that the majority of plants will not be released commercially within a 10 year time frame as either the work is in an early stage of progress, or it is not intended to release the plant in the EU at all (e.g., sugarcane) (Figure 4).

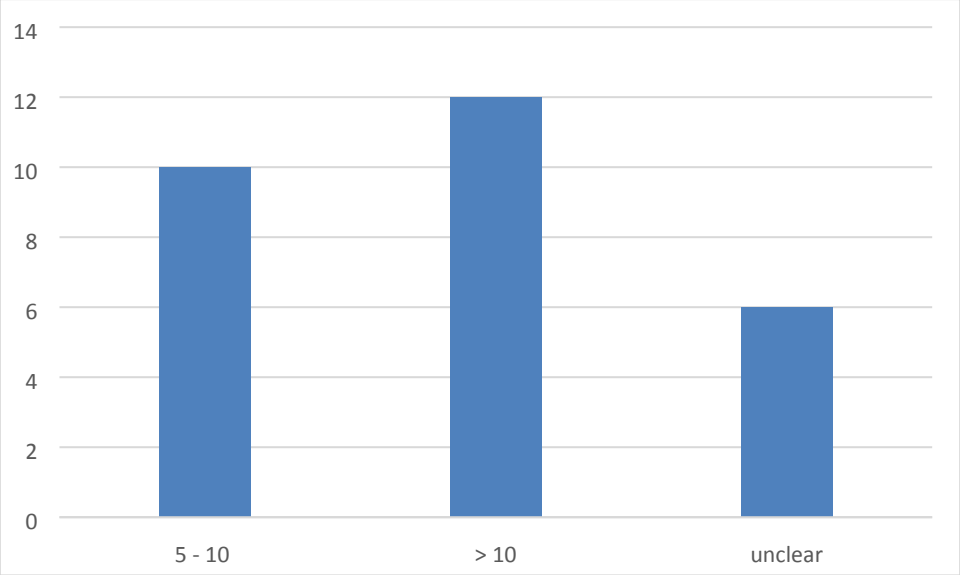

Figure 4: Estimated year of commercial release in the EU

The different levels of complexity of the genetic modification are very broad. The range goes from the insertion of single genes up to introduction of seven genes. Genome editing led to prominent changes by induced mutations in four genes (tomato) or knocking out of whole gene families (107 gene copies

in sugarcane by TALEN). Mostly classical transgenesis was used up to today to introduce foreign genes. Halogenated indigo precursors producing tobacco was achieved by using the modular cloning technique GoldenBraid. The production of artemesin and the replacement of RuBisCo in tobacco was done by transplastomics. In addition in tobacco, the plastid genome was engineered for enhancement of the vitamin E content.

3.1.4. Case studies

Photosynthesis/Photorespiration

(1) Shen et al., 2019 introduced a new chloroplastic photorespiratory bypass in **rice** through multi-gene transformation (OsGLO3, OsOXO3 and OSCATC). The genes originate from the nucleus and were transferred into the chloroplast. They catalyse the reactions from glycolate to oxalate (OsGLO3) and later on to CO2 and H2O2 (OsOXO3), the peroxide is then scavenged by OsCATC. The successful transformation of this bypass into rice chloroplasts resulted in increased photosynthesis efficiency and nitrogen content as well as enhanced biomass yield under greenhouse and field conditions. The field experiment was conducted consecutively in 2015, 2016 and 2017.

(2) South et al., 2019 used a similar system to improve photosynthesis in **tobacco** chloroplasts. They analysed three differently designed photorespiratory pathways. The first one consists of five genes from the *E. coli* glycolate oxidation pathway, the second one uses glycolate oxidase and malate synthase from *Cucurbita maxima* and catalase from *E. coli* and the third one contains the same plant malate synthase and the glycolate dehydrogenase from the green algae *Chlamydomonas reinhardtii*. In conjunction with this, the authorsalso expressed RNAi constructs and thereby down regulated the native chloroplast glycolat-glycerate transporter to limit the flux through the common pathway. The best performing pathway was the third one combined with the down regulation of the native pathway. This combination resulted in 24% biomass increase under greenhouse and more than 40% under field conditions, respectively. For the field trial the three best performing lines of the third pathway were used as replicates in the years 2016 and 2017.

Salt tolerance/Abiotic stress

(3) Song et al., 2018 tested the salt tolerance and yield of transgenic **cotton** lines expressing two genes from *Arthrobacter pascens* for the synthesis of glycine betaine, the most universal osmoprotectant. The two genes glycine sarcosine methyltransferase (ApGSMT2) and dimethylglycine methyltransferase (ApDMT2) were used as a co-expression construct and increased the glycine betaine content 4 to 5 fold. Under salt stress condition the transgenic lines exhibited a stronger photosynthetic capacity and better PSII performance compared to the wild type, resulting in improved growth and approximately 20 to 30% higher yield. The experiment was conducted using a completely randomized block design with five repetitions in the years 2015 and 2016.

(4) Shimazaki et al., 2016 constructed **potato** lines expressing the transcription factor DREB1A from *Arabidopsis thaliana* to improve growth under salt stress conditions. The AtDREB1A was transformed into potato and best performing lines were selected. Later on a salinity test was performed in a netted-house (it is a greenhouse with three main additional features: screens on the windows to exclude incoming insects carrying pollen, an anterior entrance chamber to prevent direct access to the outside, and a central ditch in the floor to collect discharged water). Under normal conditions the transgenic lines produced significantly less tubers than the non-transgenic lines. Salt stress condition (100mM NaCl) caused over all less tuber weight, but the transgenic lines produced about 50% more and larger tubers than the non-transgenic lines.

(5) Wang et al., 2018 used a binary construct containing five stress responding genes (NCED3 = Nine-Cis-Epoxycarotenoid Dioxygenase 3, ABAR = ABA Receptor, magnesium-chelatase subunit chlH, CBF3 = C-repeat Binding Factor 3, LOS5 =molybdenum cofactor sulfurase, ABA3 and ICE1 = interactor of little elongation complex ELL subunit 1) from *Arabidopsis thaliana* to transform **rapeseed**. The experiments have been performed under greenhouse condition, but the propagation of transgenic lines in the field. The multigene transformation led to multiple parameters that were affected but in general enhanced growth and enhanced stress resistance (related to temperature, drought) was reported under greenhouse conditions.

Biofuel

(6) Kannan et al., 2018 used a single TALEN pair to knockout 107 copies of the 109 genes large family of COMT (caffeic acid O-methyltransferase) in **sugarcane**. The field trials were performed in 2015 with a randomized complete block design (RCBD) of single row plots. The COMT mutants grown in the field displayed an improved saccharification efficiency of cell wall-bound sugar up to 44%. The improved saccharification goes along with a reduced lignin content with no difference in biomass production and agronomic performance. Therefore, these plants are well suited for biofuel production.

Nutritional modification

(7) Lu et al., 2013 transferred a pathway of three genes (HPT, TCY and TMT) for the biosynthesis of tocochromanol cloned as a synthetic operon from the cyanobacterium *Synechocystis sp*. PCC6803 into **tomato** chloroplasts of two commercial varieties (Dorothy´s Green and Green Pineapple) and a red fruited one (IPA-6). The tocopherol and tocotrienol level (collectively referred to Vitamin E compounds) was up to tenfold higher in the leaves as well as red and green fruits of the transplastomic plants compared to the wildtype. The authors conclude that using a synthetic operon and transformation of chloroplasts is superior compared to transformation into the nucleus. Interestingly, during chloroplast to chromoplast transition in fruit ripening, the level of tocopherol and tocotrienol was going down in the red fruit variety to only half the level found in the green fruit varieties. The experiments were performed solely under greenhouse conditions so far.

(8) Usher et al., 2017 produced transgenic **camelina** that contains multiple genes from different organisms, Δ6-desaturase gene from *Ostreococcus tauri* (OtΔ634), a Δ6 fatty acid elongase gene from *Physcomitrella patens* (PSE1) a Δ5-desaturase gene from *Thraustochytrium sp.* (TcΔ5), a Δ12-desaturase gene from *Phytophthora sojae* (PsΔ12) and an ω3-desaturase from *Phytophthora infestans* (Pi-w-3; or *Hyaloperonospora parasitica*, (Hp-ω32. All genes were cloned individually and combined in a single transformation vector. In addition, for a seven gene construct the two genes *O. tauri* Δ5 fatty acid elongase gene37, and EhΔ4, a Δ4-desaturase gene from *Emiliania huxleyi*3 were added on this vector. The field trials were performed in 2015 at Rothamstead Research. The fatty acid and oil composition of the mutant lines varied and were in average lower than the WT. As the composition of oil and fatty acid is very complex, no final conclusion could be drawn from this experiment.

Phytosensing

(9) Fethe et al., 2014 performed a two years field study in 2012 and 2013 using transgenic bacterial phytosensing **tobacco** plants. They cloned a synthetic pathogen-inducible promoter containing four copies of SARE, PR1, ERE and JAR elements respectively, placed upstream of a minimal CaMV promoter to drive the expression of an orange fluorescent protein from *Porites porites*. In summary the field

studies exhibited no consistent pattern but differences to previously performed greenhouse studies upon pathogen induction. It was clear that the field conditions strongly affect the perception of pathogens by this promoter construct.

Biotic stress

(10) Vishnevetsky et al., 2010 could show that transgenic Cavendish **banana** cv. Grand Nain exhibited improved tolerance toward the fungal pathogen Sigatoka in a multi year field study from 2004 to 2008. The construct used in this study contained the Then 42 genes from *Trychoderma harzianum* (an endochitinase), the grape stilbene synthase and the superoxide dismutase from tomato. The rating for Black Sigatoka infections were significantly lower in two out of 10 transgenic lines compared to the control. Other agronomic traits were not significantly affected.

Nitrogen fixation

(11) Allen et al., 2017 used in total 16 different nitrogenase (Nif) genes from *Klebsiella pneumonia* for single or combined expression in the mitochondria of ***Nicotiana bethamiana***. A construct containing fusion proteins of four Nif genes (NifB, NifS, NifH and NifY) was stacked into tobacco and showed stable expression albeit lower than the single copy transformants respectively. A clear conclusion of the positive effect from this experiment for agronomic features cannot be drawn.

3.2. Expert consultations

Besides a bibliographic literature search, the authors contacted specialists (in the field of SynBio) and finally conducted interviews with three scientists: currently active in basic research in chloroplasts, plant biotechnology and risk analysis, all familiar with either technologies considered relevant for SynBio.

All pointed out that their understanding of the term "synthetic biology" is stricter than the SynBio definition used in our research, though they admit that even in the scientific community the definition is blurred. In general they agreed that most applications of synthetic biology (in a strict sense) in plants are far from application (> 10 years to market). For plant synthetic biology they claimed a gap between the theoretical considerations if not promises made by some scientists and the reduction to practice. In the broader sense of SynBio as used in this report metabolic design/engineering based on transgenesis (e.g. golden rice; fatty acid composition in several crops like canola, camelina) was seen as a starting point to more complex modifications of pathways in the future. Beyond that the experts stated that considerable research efforts are especially spend on optimized photosynthesis (e.g. transformation from C3 to C4 plants) and nitrogen fixation (in non-legumes). One expert explicitly mentioned that, he could imagine that a prototype plant for modified photosynthesis or nitrogen fixation might be available in five years and closer to market after ten years. Such applications of SynBio would likely make use of part libraries to back their testing of approaches. Since plant cells are considered to be too complex to be successfully redesigned within a considerable time period, artificial/modified organelles may be developed for the use in plants in the next years.

With regards to the broader definition of SynBio the expert also addressed the use of plants as chassis for the production of phytochemicals or vaccines (molecular farming/pharming). Aside tobacco he mentioned the work of Cathie Martin on tomato as a "chassis" to produce candidate metabolites at industrial scale (Li et al., 2018).

3.3. Companies

A search for "SynBio companies" was conducted in the Ventureradar database (https://www.ventureradar.com) and augmented by companies/institutions known to the authors. Data about their activities were crosschecked or retrieved from the companies' web sites.

The strings used in the database Ventureradar (performed on 21 Feb. 2019) and its statistics were listed in table 7; and details are provided in Appendix D, published together with this report on the EFSA website.

Table 7: Search strings to identify SynBio companies in the ventureradar.com database

	SynBio term	Plant term	Hits
1	synthetic AND biology	AND (crop OR plant)	87
2	metabolic AND engineer*		14
3	metabolic AND design*		12
4	cannabi* AND synthetic*		21
5	cannabi* AND bioeng*		0
6	carotin* AND synthetic*		0
7	carotin* AND bioeng*		0
8	terpen* AND synthetic*		3
9	terpen* AND bioeng*		0
10	optim* AND pathway		7

The initial search yields 121 different entries (companies, institutions). The websites of each company or institution were revisited for further detailed information beyond the description provided by ventureradar.com. Eight companies had already been closed down, few companies were renamed or merged. Four companies/consortia were added based on further information found during the screening of the websites as well as based on information known by the authors (NuSeed, Florigene/Suntory and Golden Rice Consortium).

The relationship between the companies' business objective and the plants that are in the scope of this report was heterogeneous. Several companies retrieved in the search use microbial production systems (fermentation) to produce plant based metabolites and were excluded from further considerations.

Based on the information available from the companies, their activities have been evaluated and listed in Appendix D under the table tab "Final review". This includes technologies, plants, traits, pipelines, status. The authors note that detailed descriptions of the technologies used and outcomes were frequently not available, i.e. the results display a general presentation of the activities and highlighted products - if available.

Finally, 18 companies or institutions were identified that have at least the know-how and orientation to produce or directly support the development of SynBio plants in the scope of the report. But it remained unclear in many cases which level of genetical complexity has actually been reached as well as whether they progressively develop SynBio products.

At least 11 companies show breeding or pre-breeding activities. Among these are seemingly six that actively work on "more complex" genetic modification, i.e. designed combinations of two or more genes. Among those companies, three targeted oil/fatty acids composition in canola, soy bean and camelina, which have been or are close to be deregulated in USA, AUS, CAN and others. Seven further companies or university institutes offer services for plant biotechnology, at least. Large companies may be considered as likely partners in cooperations or developments, though a disclosure of such cooperations and activities was not accessed/accessible during this research.

Considering the presentation of the companies/institutions, it seems likely that within the next 5 to 10 years plants with "advanced" modified fatty acid/oil composition (canola, soy bean, camelina) may most likely be released to the markets in the Americas and Australia. The products/plants can be considered being derived through *metabolic engineering* (see note in section 1.2.). There are further crops in the pipelines with improved agronomic traits, stress tolerance or nutritional value. An explicit application of synthetic biology (in a narrow sense) in plants and related to a market perspective in the near future has not been identified. However, this company survey does not describe non-disclosed developments in SynBio plants and therefore a forecast of detailed trends is not possible.

4. Conclusion

Screening the scientific literature, a biotech company database and some expert feedbacks showed that SynBio in plants for agri-food use in Europe are not clearly described to advance as such that they are close to being marketed in the near future. Given the definition of SynBio as agreed for this report, it seems most likely that plants will enter the international market in the next 10 years that have been modified by metabolic engineering. It should be noted that most developments in this direction have been undertaken with classical transgenesis, e.g. Golden Rice, and only in recent years genome editing techniques became efficiently applicable. It may be considered being a continuous process of improvement and progress starting from already existing crops with, e.g., less complex modifications like fatty acid composition either transgenic or genome edited via multiple optimized pathways with several modifications (e.g. Nuseed Canola, transgenic) to complex modifications including redesigned regulation and synthesis (e.g. altered carbon fixation). The comparably advanced work on fatty acids metabolism might be due to policy and regulations as invoked by the Food and Drug Administration ruling from January, 1st, 2016 in which FDA "prohibits claims that a food is high in DHA or EPA" (Food Labeling: Nutrient Content Claims; Alpha-Linolenic Acid, Eicosapentaenoic Acid, and Docosahexaenoic Acid Omega-3 Fatty Acids). Though metabolic engineered crops were considered under SynBio within this report, the authors like to stress that many scientist would not consider this kind of work on metabolic engineering as being synthetic biology.

Aside metabolic engineered crops, a few other products where captured with this search. From European perspective, flax producing medical fibre has already been tested under European conditions and seems closest to be prepared for an application for market release. The authors note that this is an estimate and that the regulatory status and actual market expectations have not been analysed as such.

Although changes in a plant`s fitness can be achieved by altering transcription factors, the complexity and integrity of metabolic circuits present challenges that require intensive exploration before any market relevance can be considered.

The work on optimizing photosynthesis is most advanced in prokaryotes, but introducing artificially improved carbon fixation systems in higher plants are currently at the basic research level. The authors similarly evaluated the status of other developments indicated in table 5.

The team is of the opinion that the practical application of synthetic biology in a narrow sense (see categories 1 to 4 of the EFSA mandate in 1.1.) will likely be introduced to plant systems at the level of organelles, e.g. artificial or modified plastids. With regards to the fact that SynBio in plants is still rare and the commercialization difficult to predict.

To address the aspect of the mandate regarding "In addition, EFSA should also identify newer sectors/advances that should be considered among SynBio developments." the authors conclude that no developments, other than the six identified in the background section, have been identified. Moreover, plant chassis, part libraries or artificial organelles may essentially evolve linked to other approaches like metabolic engineering or modified photosynthesis and are not considered to be released to the environment/field as a stand-alone product.

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Abbreviations

EC	European Commission
EFSA	European Food and Safety Authority
GM	Genetically Modified
GMP	Genetically Modified Plants
GMO	Genetically Modified Organisms
JKI	Julius Kühn-Institut
PI	Populations/Intervention design
POC	Proof of Concept
PPI	Population/Population/Intervention
T/A	Title/Abstract
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SynBio	Synthetic Biology

Appendix A – Bibliography

Appendix B – Search Documentation

Appendix C – Data extraction

Appendix D – SynBio ventures

Annex A – Development of the final search string

Search string 1: Population/Intervention "PI" structure

Search string 1.1: "PI" structure: First version

TS=((crop OR crops OR plant OR plants OR seed OR seeds OR algae OR Tobacco OR "Nicotiana benthamiana" OR hemp OR "Cannabis sativa" OR cereal OR cereals OR corn OR "zea mays" OR maize OR canola OR rapeseed OR oilseed OR Brassica OR wheat OR Triticum OR barley OR hordeum OR oat OR avena OR rye OR "secale cereale" OR rice OR oryza OR soybean OR "Glycine max" OR sorghum OR potato OR Solanum OR "sugar beet" OR "sugar-beet" OR "fodder beet" OR "beta vulgaris" OR alfalfa OR Medicago OR tomato OR cucumber OR cucumis OR carrot OR "Daucus carota" OR pepper OR capsicum OR zucchini OR cucurbita OR "egg plant" OR spinach OR "Spinacia oleracea" OR onion OR allium OR bean OR beans OR phaseolus OR pea OR "Pisum sativum" OR sunflower OR "Helianthus annuus" OR mushroom OR fungi OR beet OR cabbage OR lettuce OR lactuca OR pumpkin OR lentil OR "Lens culinaris" OR leek OR rhubarb OR "Rheum rhabarbarum" OR celery OR "Apium graveolens" OR melon OR lupine OR lupines OR radish OR raphanus OR "fruit tree" OR "fruit trees" OR apple OR apples OR malus OR orange OR "Citrus sinensis" OR banana OR musa OR grape OR grapevine OR vitis OR pear OR Pyrus OR cherry OR "Prunus avium" OR raspberry OR "Rubus idaeus" OR strawberry OR Fragaria OR grapefruit OR "Citrus aurantium" OR lemon OR "Citrus limon" OR lime OR "Citrus latifolia" OR kiwi OR "Actinidia deliciosa" OR garlic OR ginger OR curcuma OR "Piper nigrum" OR

parsley OR "Petroselinum crispum" OR peppermint OR "Mentha piperita" OR "sweet basil" OR basilicum OR oregano OR Origanum OR rosemary OR Rosmarinus OR poplar OR manihot OR "Manihot esculenta" OR sugarcane OR "Saccharum officinarum" OR coffee OR Coffea OR cotton OR "Gossypium hirsutum" OR flax OR "Linum usitatissimum" OR dandelion OR Taraxacum OR Peanut OR Peanuts OR "Arachis hypogaea" OR Olive OR "Olea europaea" OR sesame OR "Sesamum indicum" OR cocoa "Theobroma cacao" OR hazelnut OR "Corylus avellana" OR walnut OR "Juglans regia" OR coconut OR "Cocos nucifera" OR jujube OR "Ziziphus jujuba Meikl")
AND
("metabolic engineering" OR "metabolic design" OR "metabolic-engineering" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "pathway design" OR "pathway-design" OR "protein engineering" OR "protein-engineering" OR "synthetic DNA*" OR "synthetic gen*" OR "System* bioengineering" OR "therose nucleic acid" OR "Xeno nucleic acid" OR bioengineering OR CeNA OR GNA OR TNA OR XNA OR "synthetic *nucleotide" OR "artificial nucleic acid*" OR "artificial gene* network" OR "artificial *nucleotide" OR ("artificial gene* circuit*" AND "biological system") OR "artificial plant cell" OR "synthetic biolog*" OR "synthetic plant cell*" OR "synthetic promoter" OR "synthetic gene* cluster" OR "engineered nucleotide sequences" OR "genetic circuit*" OR "artificial genetic system*" OR "desig* DNA part*" OR "multiplex automated genome engineering" OR "orthogon* biosystem*" OR "non-canonic* amino acid*" OR protocell* OR "synthetic biology" OR synbio OR "synthetic life" OR "synthetic cells" OR "synthetic genom*" OR "minimal genome" OR biobrick* OR "engineered sequenc*"))

Hits in "Web of Science Core Collection": 5876

Search string 1.2: "PI" search string: Second version

TS=((plant OR plants OR crop OR crops OR seed OR seeds OR herb OR herbs OR alfalfa OR Medicago OR alga OR algae OR apple OR apples OR malus OR banana OR Musa OR barley OR Hordeum OR basilicum OR basil OR "Ocimum basilicum" OR bean OR beans OR Phaseolus OR beet OR cabbage OR "Camelina sativa" OR canola OR capsicum OR pepper OR carrot OR "Daucus carota" OR celery OR "Apium graveolens" OR cherry OR "Prunus avium" OR Cocoa OR "Theobroma cacao" OR coconut OR "Cocos nucifera" OR coffee OR Coffea OR corn OR maize OR "Zea mays" OR cotton OR "Gossypium hirsutum" OR cucumber OR cucumis OR curcuma OR dandelion OR Taraxacum OR duckweed OR duckweeds OR "lemna minor" OR eggplant OR Solanum OR flax OR "Linum usitatissimum" OR "fodder beet" OR garlic OR ginger OR grape OR grapevine OR vitis OR grapefruit OR hazelnut OR hazel OR "Corylus avellana" OR hemp OR "Cannabis sativa" OR jujube OR "Ziziphus jujuba Meikl" OR kiwi OR "Actinidia deliciosa" OR leek OR lemon OR "Citrus limon" OR lentil OR "Lens culinaris" OR lettuce OR Lactuca OR lime OR "Citrus latifolia" OR lupine OR lupines OR manihot OR "Manihot esculenta" OR melon OR oat OR avena OR olive OR "Olea europaea" OR onion OR allium OR orange OR "Citrus aurantium" OR "Citrus sinensis" OR oregano OR Origanum OR parsley OR "Petroselinum crispum" OR pea OR "Pisum sativum" OR peanut OR peanuts OR "Arachis hypogaea" OR pear OR Pyrus OR peppermint OR "Mentha piperita" OR pineapple OR "Ananas comosus" OR "Piper nigrum" OR poplar OR potato OR pumpkin OR Cucurbita OR radish OR Raphanus OR rapeseed OR oilseed OR Brassica OR "Brassica carinata" OR raspberry OR "Rubus idaeus" OR rhubarb OR "Rheum rhabarbarum" OR rice And Oryza OR rosemary OR Rosmarinus OR rye OR "Secale cereale" OR sesame OR "Sesamum indicum" OR sorghum OR soybean OR soy OR "Glycine max" OR spinach OR "Spinacia oleracea" OR strawberry OR Fragaria OR "sugar beet" OR "sugar-beet" And "Beta vulgaris" OR sugarcane OR "Saccharum officinarum" OR sunflower OR "Helianthus annuus" OR tobacco OR "Nicotiana benthamiana" OR "Nicotiana tabacum" OR tomato OR walnut OR "Juglans regia" OR wheat OR Triticum OR zucchini OR allspice OR "Pimenta dioica" OR anise OR "Pimpinella anisum" OR "bay leaf" OR "Laurus nobilis" OR basil OR "Ocimum basilicum" OR bergamot OR "Monarda species" OR cumin OR Cuminum OR mustard OR pepper OR Piper OR borage OR "Borago officinalis" OR caraway OR "Carum carvi" OR cardamom OR "Elettaria cardamomum" OR chervil OR

"Anthriscus cerefolium" OR chives OR "Allium schoenoprasum" OR cicely OR "Myrrhis odorata" OR cinnamon OR Cinnamomum OR coriander OR Coriandrum OR dill OR "Anethum graveolens" OR fennel OR Foeniculum OR fenugreek OR "Trigonella foenum-graecum" OR ginger OR "Zingiber officinale" OR horseradish OR "Armoracia rusticana" OR hyssop OR "Hyssopus officinalis" OR lavender OR Lavendula OR "lemon balm" OR Melissa OR "lemon grass" OR "Cymbopogon citratus" OR licorice OR "Glycyrrhiza glabra" OR lovage OR "Levisticum officinale" OR majoram OR "Origanum majorana" OR nutmeg OR "Myristica fragrans" OR oregano OR Oreganum OR parsley OR "Petroselinum crispum" OR mint OR Mentha OR "poppy seed" OR "Papaver somniferum" OR rosemary OR Rosmarinus OR saffron OR "Crocus sativus" OR sage OR Salvia OR savory OR "Satureja hortensis" OR sesame OR Sesamum OR sorrel OR Rumex OR tarragon OR "Artemisia dracunculus" OR turmeric OR "curcuma longa" OR vanilla OR clove OR "Syzygium aromaticum" OR Thyme OR Thymus OR Celosia OR Chrysant* OR Dianthus OR Carnation OR Gentian OR Geranium OR Kalanchoe OR Lilium OR Lillies OR Lily OR Marigold OR "Tagetes erecta" OR "Morning Glory" OR "Ipomoea purpurea" OR Nerium OR "Nerium oleander" OR Orchid OR Orchis OR Pansy OR Petunia OR Pelargonium OR Storksbills OR Poinsetta OR "Euphorbia pulcherrima" OR Rosa OR Rose OR Tulip OR tulipa)
AND
("metabolic engineering" OR "metabolic design" OR "metabolic-engineering" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "pathway design" OR "pathway-design" OR "protein engineering" OR "protein-engineering" OR "synthetic DNA*" OR "synthetic gen*" OR "System* bioengineering" OR "therose nucleic acid" OR "Xeno nucleic acid" OR bioengineering OR CeNA OR GNA OR TNA OR XNA OR "synthetic *nucleotide" OR "artificial nucleic acid*" OR "artificial gene* network" OR "artificial *nucleotide" OR ("artificial gene* circuit*" AND "biological system") OR "artificial plant cell" OR "synthetic biolog*" OR "synthetic plant cell*" OR "synthetic promoter" OR "synthetic gene* cluster" OR "engineered nucleotide sequences" OR "genetic circuit*" OR "artificial genetic system*" OR "desig* DNA part*" OR "multiplex automated genome engineering" OR "orthogon* biosystem*" OR "non-canonic* amino acid*" OR protocell* OR "synthetic biology" OR synbio OR "synthetic life" OR "synthetic cells" OR "synthetic genom*" OR "minimal genome" OR biobrick* OR "engineered sequenc*"))

Hits in "Web of Science Core Collection": 6701

Search string 2: Population/Population/Intervention "PPI" structure

Search string 2.1: "PPI" search string: First Version

TS=((flower OR flowers OR herb OR herbs OR crop OR crops OR plant OR plants OR seed OR seeds OR algae OR alga)
AND
(plant OR plants OR crop OR crops OR seed OR seeds OR herb OR herbs OR alfalfa OR Medicago OR alga OR algae OR apple OR apples OR malus OR banana OR Musa OR barley OR Hordeum OR basilicum OR basil OR "Ocimum basilicum" OR bean OR beans OR Phaseolus OR beet OR cabbage OR "Camelina sativa" OR canola OR capsicum OR pepper OR carrot OR "Daucus carota" OR celery OR "Apium graveolens" OR cherry OR "Prunus avium" OR Cocoa OR "Theobroma cacao" OR coconut OR "Cocos nucifera" OR coffee OR Coffea OR corn OR maize OR "Zea mays" OR cotton OR "Gossypium hirsutum" OR cucumber OR cucumis OR curcuma OR dandelion OR Taraxacum OR duckweed OR duckweeds OR "lemna minor" OR eggplant OR Solanum OR flax OR "Linum usitatissimum" OR "fodder beet" OR garlic OR ginger OR grape OR grapevine OR vitis OR grapefruit OR hazelnut OR hazel OR "Corylus avellana" OR hemp OR "Cannabis sativa" OR jujube OR "Ziziphus jujuba Meikl" OR kiwi OR "Actinidia deliciosa" OR leek OR lemon OR "Citrus limon" OR lentil OR "Lens culinaris" OR lettuce OR Lactuca OR lime OR "Citrus latifolia" OR lupine OR lupines OR manihot OR "Manihot esculenta" OR melon OR oat OR avena OR olive OR "Olea europaea" OR onion OR allium

OR orange OR "Citrus aurantium" OR "Citrus sinensis" OR oregano OR Origanum OR parsley OR "Petroselinum crispum" OR pea OR "Pisum sativum" OR peanut OR peanuts OR "Arachis hypogaea" OR pear OR Pyrus OR peppermint OR "Mentha piperita" OR pineapple OR "Ananas comosus" OR "Piper nigrum" OR poplar OR potato OR pumpkin OR Cucurbita OR radish OR Raphanus OR rapeseed OR oilseed OR Brassica OR "Brassica carinata" OR raspberry OR "Rubus idaeus" OR rhubarb OR "Rheum rhabarbarum" OR rice And Oryza OR rosemary OR Rosmarinus OR rye OR "Secale cereale" OR sesame OR "Sesamum indicum" OR sorghum OR soybean OR soy OR "Glycine max" OR spinach OR "Spinacia oleracea" OR strawberry OR Fragaria OR "sugar beet" OR "sugar-beet" And "Beta vulgaris" OR sugarcane OR "Saccharum officinarum" OR sunflower OR "Helianthus annuus" OR tobacco OR "Nicotiana benthamiana" OR "Nicotiana tabacum" OR tomato OR walnut OR "Juglans regia" OR wheat OR Triticum OR zucchini OR allspice OR "Pimenta dioica" OR anise OR "Pimpinella anisum" OR "bay leaf" OR "Laurus nobilis" OR basil OR "Ocimum basilicum" OR bergamot OR "Monarda species" OR cumin OR Cuminum OR mustard OR pepper OR Piper OR borage OR "Borago officinalis" OR caraway OR "Carum carvi" OR cardamom OR "Elettaria cardamomum" OR chervil OR "Anthriscus cerefolium" OR chives OR "Allium schoenoprasum" OR cicely OR "Myrrhis odorata" OR cinnamon OR Cinnamomum OR coriander OR Coriandrum OR dill OR "Anethum graveolens" OR fennel OR Foeniculum OR fenugreek OR "Trigonella foenum-graecum" OR ginger OR "Zingiber officinale" OR horseradish OR "Armoracia rusticana" OR hyssop OR "Hyssopus officinalis" OR lavender OR Lavendula OR "lemon balm" OR Melissa OR "lemon grass" OR "Cymbopogon citratus" OR licorice OR "Glycyrrhiza glabra" OR lovage OR "Levisticum officinale" OR majoram OR "Origanum majorana" OR nutmeg OR "Myristica fragrans" OR oregano OR Oreganum OR parsley OR "Petroselinum crispum" OR mint OR Mentha OR "poppy seed" OR "Papaver somniferum" OR rosemary OR Rosmarinus OR saffron OR "Crocus sativus" OR sage OR Salvia OR savory OR "Satureja hortensis" OR sesame OR Sesamum OR sorrel OR Rumex OR tarragon OR "Artemisia dracunculus" OR turmeric OR "curcuma longa" OR vanilla OR clove OR "Syzygium aromaticum" OR Thyme OR Thymus OR Celosia OR Chrysant* OR Dianthus OR Carnation OR Gentian OR Geranium OR Kalanchoe OR Lilium OR Lillies OR Lily OR Marigold OR "Tagetes erecta" OR "Morning Glory" OR "Ipomoea purpurea" OR Nerium OR "Nerium oleander" OR Orchid OR Orchis OR Pansy OR Petunia OR Pelargonium OR Storksbills OR Poinsetta OR "Euphorbia pulcherrima" OR Rosa OR Rose OR Tulip OR tulipa)
AND
("artificial *nucleotide" OR "artificial cell*" OR "artificial gene* circuit*" OR "artificial gene* network*" OR "artificial genetic system*" OR "artificial nucleic acid*" OR "artificial plant cell*" OR "biobrick*" OR bioengineer* OR "desig* DNA part*" OR "engineered nucleotide sequenc*" OR "engineered sequenc*" OR "genetic circuit*" OR "metabolic design" OR "metabolic engineering" OR "minimal genome" OR "multiplex automated genome engineering" OR "non-canonic* amino acid*" OR "pathway design" OR "design* pathway*" OR "protein engineering" OR "engineer* protein*" OR protocell* OR SynBio OR "synthetic *nucleotid*" OR "synthetic bio*" OR "synthetic cell*" OR "synthetic DNA*" OR "synthetic gen*" OR "synthetic gene* cluster" OR "synthetic genom*" OR "synthetic life" OR "synthetic plant cell*" OR "synthetic promoter" OR "minimal cell*" OR "system* bioengineering" OR "xeno nucleic acid" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "artificial plastids" OR "synthetic plastids" OR "plastid design" OR " design* plastids" OR carboxysom*))

Hits in "Web of Science Core Collection": 5876

Search string 2.2: "PPI" search string: Second Version

TS=((flower OR flowers OR herb OR herbs OR crop OR crops OR plant OR plants OR seed OR seeds OR algae OR alga)
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AND
("artificial *nucleotide" OR "artificial cell*" OR "artificial gene* network*" OR "artificial nucleic acid*" OR "artificial plant cell*" OR "biobrick*" OR bioengineer* OR "desig* DNA part*" OR "engineered nucleotide sequenc*" OR "engineered sequenc*" OR "genetic circuit*" OR "metabolic design" OR "metabolic engineering" OR "minimal genome" OR "multiplex automated genome engineering" OR "non-canonic* amino acid*" OR "pathway design" OR "design* pathway*" OR "*protein engineering" OR "engineer* *protein*" OR protocell* OR SynBio OR "synthetic *nucleotid*" OR "synthetic bio*" OR "synthetic cell*" OR "synthetic DNA*" OR "synthetic gen*" OR "synthetic gene* cluster" OR "synthetic genom*" OR "synthetic life" OR "synthetic plant cell*" OR "synthetic promoter" OR

"minimal cell*" OR "system* bioengineering" OR "xeno nucleic acid" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "artificial plastid*" OR "synthetic plastid*" OR "plastid desig*" OR "design* plastids" OR carboxysom* OR transplantom* OR (photorespira* NEAR/3 (engineer* OR bypass OR decreas*)) OR "synthetic operon" OR "gene transfer"))

Hits in "Web of Science Core Collection": 8858

Search string 3: Population/Population/Intervention "PPI" structure with "NEAR/"

TS=((flower OR flowers OR herb OR herbs OR crop OR crops OR plant OR plants OR seed OR seeds OR algae OR alga)
AND
(plant OR plants OR crop OR crops OR seed OR seeds OR herb OR herbs OR alfalfa OR Medicago OR alga OR algae OR apple OR apples OR malus OR banana OR Musa OR barley OR Hordeum OR basilicum OR basil OR "Ocimum basilicum" OR bean OR beans OR Phaseolus OR beet OR cabbage OR "Camelina sativa" OR canola OR capsicum OR pepper OR carrot OR "Daucus carota" OR celery OR "Apium graveolens" OR cherry OR "Prunus avium" OR Cocoa OR "Theobroma cacao" OR coconut OR "Cocos nucifera" OR coffee OR Coffea OR corn OR maize OR "Zea mays" OR cotton OR "Gossypium hirsutum" OR cucumber OR cucumis OR curcuma OR dandelion OR Taraxacum OR duckweed OR duckweeds OR "lemna minor" OR eggplant OR Solanum OR flax OR "Linum usitatissimum" OR "fodder beet" OR garlic OR ginger OR grape OR grapevine OR vitis OR grapefruit OR hazelnut OR hazel OR "Corylus avellana" OR hemp OR "Cannabis sativa" OR jujube OR "Ziziphus jujuba Meikl" OR kiwi OR "Actinidia deliciosa" OR leek OR lemon OR "Citrus limon" OR lentil OR "Lens culinaris" OR lettuce OR Lactuca OR lime OR "Citrus latifolia" OR lupine OR lupines OR manihot OR "Manihot esculenta" OR melon OR oat OR avena OR olive OR "Olea europaea" OR onion OR allium OR orange OR "Citrus aurantium" OR "Citrus sinensis" OR oregano OR Origanum OR parsley OR "Petroselinum crispum" OR pea OR "Pisum sativum" OR peanut OR peanuts OR "Arachis hypogaea" OR pear OR Pyrus OR peppermint OR "Mentha piperita" OR pineapple OR "Ananas comosus" OR "Piper nigrum" OR poplar OR potato OR pumpkin OR Cucurbita OR radish OR Raphanus OR rapeseed OR oilseed OR Brassica OR "Brassica carinata" OR raspberry OR "Rubus idaeus" OR rhubarb OR "Rheum rhabarbarum" OR rice And Oryza OR rosemary OR Rosmarinus OR rye OR "Secale cereale" OR sesame OR "Sesamum indicum" OR sorghum OR soybean OR soy OR "Glycine max" OR spinach OR "Spinacia oleracea" OR strawberry OR Fragaria OR "sugar beet" OR "sugar-beet" And "Beta vulgaris" OR sugarcane OR "Saccharum officinarum" OR sunflower OR "Helianthus annuus" OR tobacco OR "Nicotiana benthamiana" OR "Nicotiana tabacum" OR tomato OR walnut OR "Juglans regia" OR wheat OR Triticum OR zucchini OR allspice OR "Pimenta dioica" OR anise OR "Pimpinella anisum" OR "bay leaf" OR "Laurus nobilis" OR basil OR "Ocimum basilicum" OR bergamot OR "Monarda species" OR cumin OR Cuminum OR mustard OR pepper OR Piper OR borage OR "Borago officinalis" OR caraway OR "Carum carvi" OR cardamom OR "Elettaria cardamomum" OR chervil OR "Anthriscus cerefolium" OR chives OR "Allium schoenoprasum" OR cicely OR "Myrrhis odorata" OR cinnamon OR Cinnamomum OR coriander OR Coriandrum OR dill OR "Anethum graveolens" OR fennel OR Foeniculum OR fenugreek OR "Trigonella foenum-graecum" OR ginger OR "Zingiber officinale" OR horseradish OR "Armoracia rusticana" OR hyssop OR "Hyssopus officinalis" OR lavender OR Lavendula OR "lemon balm" OR Melissa OR "lemon grass" OR "Cymbopogon citratus" OR licorice OR "Glycyrrhiza glabra" OR lovage OR "Levisticum officinale" OR majoram OR "Origanum majorana" OR nutmeg OR "Myristica fragrans" OR oregano OR Oreganum OR parsley OR "Petroselinum crispum" OR mint OR Mentha OR "poppy seed" OR "Papaver somniferum" OR rosemary OR Rosmarinus OR saffron OR "Crocus sativus" OR sage OR Salvia OR savory OR "Satureja hortensis" OR sesame OR Sesamum OR sorrel OR Rumex OR tarragon OR "Artemisia dracunculus" OR turmeric OR "curcuma longa" OR vanilla OR clove OR "Syzygium aromaticum" OR Thyme OR Thymus OR Celosia OR Chrysant* OR Dianthus OR Carnation OR Gentian OR Geranium OR Kalanchoe OR Lilium OR Lillies OR Lily OR Marigold OR "Tagetes erecta" OR "Morning Glory" OR "Ipomoea purpurea" OR Nerium OR

"Nerium oleander" OR Orchid OR Orchis OR Pansy OR Petunia OR Pelargonium OR Storksbills OR Poinsetta OR "Euphorbia pulcherrima" OR Rosa OR Rose OR Tulip OR tulipa)
AND
((artificial NEAR/1 (plastid* OR *nucleotide OR cell OR cells OR gene* OR nucleic)) OR (desig* NEAR/1 (plastid* OR pathway* OR DNA OR metabol*)) OR (engineer* NEAR/1 (bio* OR sequenc* OR metabol* OR multiplex OR *protein*)) OR (synthetic NEAR/1 (bio* OR nucle* OR cell OR cells OR DNA OR gen* OR plant OR operon OR plastid* OR promot*)) OR synbio OR "minimal cell" OR "minimal cells" OR "minimal genome" OR ((photorespirat*) NEAR/1 (engineer* OR bypass OR decreas*)) OR (stable AND (transformation OR integration)) OR ((gene* OR enzym* OR protein* OR transgen*) NEAR/1 (introduce* OR transfer* OR transform*)) OR "biobrick*" OR "genetic circuit*" OR "non-canonic* amino acid*" OR protocell* OR carboxysom* OR transplastom* OR multigene* OR bioengineer*))

Hits in "Web of Science Core Collection": 27.156

Search string 4: Population/Population/Intervention/"Comparator"/Outcome (PPICO) structure

TS=((flower OR flowers OR herb OR herbs OR crop OR crops OR plant OR plants OR seed OR seeds OR algae OR alga)
AND
(plant OR plants OR crop OR crops OR seed OR seeds OR herb OR herbs OR alfalfa OR Medicago OR alga OR algae OR apple OR apples OR malus OR banana OR Musa OR barley OR Hordeum OR basilicum OR basil OR "Ocimum basilicum" OR bean OR beans OR Phaseolus OR beet OR cabbage OR "Camelina sativa" OR canola OR capsicum OR pepper OR carrot OR "Daucus carota" OR celery OR "Apium graveolens" OR cherry OR "Prunus avium" OR Cocoa OR "Theobroma cacao" OR coconut OR "Cocos nucifera" OR coffee OR Coffea OR corn OR maize OR "Zea mays" OR cotton OR "Gossypium hirsutum" OR cucumber OR cucumis OR curcuma OR dandelion OR Taraxacum OR duckweed OR duckweeds OR "lemna minor" OR eggplant OR Solanum OR flax OR "Linum usitatissimum" OR "fodder beet" OR garlic OR ginger OR grape OR grapevine OR vitis OR grapefruit OR hazelnut OR hazel OR "Corylus avellana" OR hemp OR "Cannabis sativa" OR jujube OR "Ziziphus jujuba Meikl" OR kiwi OR "Actinidia deliciosa" OR leek OR lemon OR "Citrus limon" OR lentil OR "Lens culinaris" OR lettuce OR Lactuca OR lime OR "Citrus latifolia" OR lupine OR lupines OR manihot OR "Manihot esculenta" OR melon OR oat OR avena OR olive OR "Olea europaea" OR onion OR allium OR orange OR "Citrus aurantium" OR "Citrus sinensis" OR oregano OR Origanum OR parsley OR "Petroselinum crispum" OR pea OR "Pisum sativum" OR peanut OR peanuts OR "Arachis hypogaea" OR pear OR Pyrus OR peppermint OR "Mentha piperita" OR pineapple OR "Ananas comosus" OR "Piper nigrum" OR poplar OR potato OR pumpkin OR Cucurbita OR radish OR Raphanus OR rapeseed OR oilseed OR Brassica OR "Brassica carinata" OR raspberry OR "Rubus idaeus" OR rhubarb OR "Rheum rhabarbarum" OR rice And Oryza OR rosemary OR Rosmarinus OR rye OR "Secale cereale" OR sesame OR "Sesamum indicum" OR sorghum OR soybean OR soy OR "Glycine max" OR spinach OR "Spinacia oleracea" OR strawberry OR Fragaria OR "sugar beet" OR "sugar-beet" And "Beta vulgaris" OR sugarcane OR "Saccharum officinarum" OR sunflower OR "Helianthus annuus" OR tobacco OR "Nicotiana benthamiana" OR "Nicotiana tabacum" OR tomato OR walnut OR "Juglans regia" OR wheat OR Triticum OR zucchini OR allspice OR "Pimenta dioica" OR anise OR "Pimpinella anisum" OR "bay leaf" OR "Laurus nobilis" OR basil OR "Ocimum basilicum" OR bergamot OR "Monarda species" OR cumin OR Cuminum OR mustard OR pepper OR Piper OR borage OR "Borago officinalis" OR caraway OR "Carum carvi" OR cardamom OR "Elettaria cardamomum" OR chervil OR "Anthriscus cerefolium" OR chives OR "Allium schoenoprasum" OR cicely OR "Myrrhis odorata" OR cinnamon OR Cinnamomum OR coriander OR Coriandrum OR dill OR "Anethum graveolens" OR fennel OR Foeniculum OR fenugreek OR "Trigonella foenum-graecum" OR ginger OR "Zingiber officinale" OR horseradish OR "Armoracia rusticana" OR hyssop OR "Hyssopus officinalis" OR lavender OR

Lavendula OR "lemon balm" OR Melissa OR "lemon grass" OR "Cymbopogon citratus" OR licorice OR "Glycyrrhiza glabra" OR lovage OR "Levisticum officinale" OR majoram OR "Origanum majorana" OR nutmeg OR "Myristica fragrans" OR oregano OR Oreganum OR parsley OR "Petroselinum crispum" OR mint OR Mentha OR "poppy seed" OR "Papaver somniferum" OR rosemary OR Rosmarinus OR saffron OR "Crocus sativus" OR sage OR Salvia OR savory OR "Satureja hortensis" OR sesame OR Sesamum OR sorrel OR Rumex OR tarragon OR "Artemisia dracunculus" OR turmeric OR "curcuma longa" OR vanilla OR clove OR "Syzygium aromaticum" OR Thyme OR Thymus OR Celosia OR Chrysant* OR Dianthus OR Carnation OR Gentian OR Geranium OR Kalanchoe OR Lilium OR Lillies OR Lily OR Marigold OR "Tagetes erecta" OR "Morning Glory" OR "Ipomoea purpurea" OR Nerium OR "Nerium oleander" OR Orchid OR Orchis OR Pansy OR Petunia OR Pelargonium OR Storksbills OR Poinsetta OR "Euphorbia pulcherrima" OR Rosa OR Rose OR Tulip OR tulipa)
AND
("artificial *nucleotide" OR "artificial cell*" OR "artificial gene* network*" OR "artificial nucleic acid*" OR "artificial plant cell*" OR "biobrick*" OR bioengineer* OR "desig* DNA part*" OR "engineered nucleotide sequenc*" OR "engineered sequenc*" OR "genetic circuit*" OR "metabolic design" OR "metabolic engineering" OR "minimal genome" OR "multiplex automated genome engineering" OR "non-canonic* amino acid*" OR "pathway design" OR "design* pathway*" OR "*protein engineering" OR "engineer* *protein*" OR protocell* OR SynBio OR "synthetic *nucleotid*" OR "synthetic bio*" OR "synthetic cell*" OR "synthetic DNA*" OR "synthetic gen*" OR "synthetic gene* cluster" OR "synthetic genom*" OR "synthetic life" OR "synthetic plant cell*" OR "synthetic promoter" OR "minimal cell*" OR "system* bioengineering" OR "xeno nucleic acid" OR "cyclohexenyl nucleic acid" OR "glycol nucleic acid" OR "hexose nucleic acid" OR "artificial plastid*" OR "synthetic plastid*" OR "plastid desig*" OR " design* plastids" OR carboxysom* OR transplantom* OR (photorespira* NEAR/3 (engineer* OR bypass OR decreas*)) OR "synthetic operon" OR "gene transfer" OR ((transgen* OR gen*) NEAR/4 (transform* OR introduc* OR insert*)))
AND
(high OR higher OR increas* OR improve* OR optimi* OR enhance*)
AND
(biomass OR productivity OR metabolism OR "plant performance" OR "bio* plastic*" OR bioplastic* OR biofuel* OR "bio* fuel*" OR biodiesel OR "bio* diesel" OR photosynth* OR toleran* OR "*biotic stress" OR ("*biotic stress" NEAR/4 (low OR lower OR decreas* OR less))))

Hits in "Web of Science Core Collection": 4772