NUTRITION & FOOD INNOVATION UNIT

Network on Risk Assessment of GMOs

Minutes of the 13th meeting (TELE)

Held on 7th April 2022, WEB-conference

(Agreed on 6 May 2022)

Participants

- **Network Representatives of Member States (including EFTA Countries):**

Country	**Name**
Austria	Marion Dolezel, Markus Wögerbauer
Belgium	Adinda De Schrijver
Bulgaria	Dimitar Djilianov, Tzveta Georgieva, Krasimira Zaharieva
Cyprus	Antri Tello Varnava
Croatia	Sanja Miloš, Renata Hanzer
Czech Republic	Zuzana Doubková, Miloslava Navrátilová, Jaroslava Ovesná
Denmark	Jan Pedersen, Morten Tune Strandberg
Estonia	
Finland	Kirsi Tormakangas, Annikki Welling
France	Youssef El-Ouadrhiri
Germany	Anastasia Matthies, Wolfram Reichenbecher,
Greece	Argyrios Boulis, Margarita Karavangeli
Hungary	Rita Andorkó
Ireland	Cristina Arroyo, Bernadette Murray
Italy	Marzia De Giacomo, Elena Sturchio
Latvia	Lelde Grantina-Ievina

Lithuania	Zygimantas Janeliunas, Odeta Pivorienė
Luxembourg	Luc Schuler
Malta	
Netherlands	Gijs A. Kleter, Marco Gielkens
Poland	Slawomir Sowa
Portugal	
Romania	Luminita Raluca Mihalachioiu
Slovakia	Zuzana Kozovska, Zuzana Ševčíková
Slovenia	Martin Batič
Spain	Carmen Cuadrado, Gema Pérez Farinós, Félix Ortego
Sweden	
Iceland	
Liechtenstein	
Norway	Ville Erling Sipinen

- **Observers:**

Armin Čolaković (Bosnia and Herzegovina), Nur Koyuncu (Turkey), Martin Schrott (Switzerland)

- **European Commission:**

Alexandre Huchelmann (DG SANTE), Juliette-Marie Margueritte (DG SANTE), Olga Orlova (DG SANTE)

- **Hearing experts and EFSA GMO Panel members:**

None

- **EFSA:**

Chief Scientist Office (CSO): Yann Devos

NIF Unit: Ana Afonso (chair), Yustina Anna Olshevska Grigorov, Michele Ardizzone, Giuseppe Condorelli, Giacomo De Sanctis, Silvia Federici, Antonio Fernandez Dumont, Andrea Gennaro, Tilemachos Goumperis, Paschalina Grammatikou, Anna Lanzoni, Paolo Lenzi, Aleksandra Lewandowska, Dafni Maria Kagkli, Franco Maria Neri, Nikoletta Papadopoulou, Pietro Piffanelli, Tommaso Raffaello, Reinhilde Schoonjans, Franz Streissl

IT support: Giulia Frattini

1. Welcome and apologies for absence

The Chair welcomed the participants.

Due to current COVID-19 restrictions, this meeting was conducted fully remotely.

2. Adoption of agenda

The agenda was adopted without changes.

3. Agreement of the minutes of the 12th meeting of the Network on risk assessment of GMOs held on 10th June 2021 (TELE).

The minutes were agreed by written procedure on 28 June 2021 and published on the EFSA website[1].

4. Topics for discussion

AGENDA ITEM 4: EFSA Reorganization - brief introduction

Ana Afonso (Chair) shared with the participants an update on EFSA's reorganization. On 27 March 2021 the transparency regulation entered into force sparking a reflection on the best way to develop EFSA's manner of working. A series of high-level steps were integrated into the new way of working regarding a) mandates & dossier intake; b) preliminary activities to risk assessment; c) risk assessment and d) output publication and discussion.
Considerations on changes to be implemented led to a new EFSA organization which now includes two departments for science, namely Risk assessment production (ASSESS) and Risk assessment service (ENABLE). Moreover, some improvements were introduced to emphasise the importance of partnership leading to the creation of the Communication and Partnership (ENGAGE) department.
Regarding the Risk assessment production (ASSESS) department, the GMO unit was merged with the Nutrition (NUTRI) unit, leading to a new unit called Nutrition and food innovation (NIF), which provides support to the work of two panels, the NDA and GMO panel. All scientific and administrative support are now managed by the Risk assessment services (ENABLE) department.
The chair shared the principal NIF contact points:

- Head of Unit: Ana.AFONSO@efsa.europa.eu
- Functional mailbox NIF@efsa.europa.eu
- GMO MS Network coordinator: Tommaso.RAFFAELLO@efsa.europa.eu
- General questions should be addressed through ASK EFSA: Ask a Question (europa.eu)

Finally, the chair shared some additional information on GMO applications, below:

- On Jan 21st, all ongoing questions were migrated to the new interactive portal - Open EFSA.
- Risk Assessment Workflow (RAW) Questions Archive: All EFSA Questions which are completed are accessible through a static report and all supporting files will be available upon request.[2] Further information can be requested via Ask EFSA.[3]
- There is a useful application toolkit which contains information in the area of GMO applications: tools, data procedures, legislations, etc.[4]

[1] Available at https://www.efsa.europa.eu/sites/default/files/2021-06/12th-meeting-gmo-network-minutes.pdf
[2] https://www.efsa.europa.eu/it/register-of-questions
[3] https://connect.efsa.europa.eu/RM/s/askefsa
[4] https://www.efsa.europa.eu/en/applications/toolkit

- GMO Network Members can have access to the biweekly newsletter with some generic info on the status of applications (clock or stop of the clock) and information on webinars or public consultations. However, confidential information on applications will only be available to the Member States for the purpose of targeted consultation as per regulatory requirements.

AGENDA ITEM 5: Confidentiality assessment Directive 2001/18/EC Part C

The item was not discussed due to unforeseen unavailability of the speaker and the item will be presented at a next network meeting.

AGENDA ITEM 6: Statement complementing the EFSA Scientific Opinion on the assessment of genetically modified oilseed rape MS11 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2016-138) - OpenEFSA Link

Abstract

MS11 oilseed rape is a GM plant developed to express a male sterile and a herbicide-tolerant trait. MS11 as single event is not meant for commercialisation since it is only part of a breeding system to produce the hybrid stack product MS11 × RF3 which is intended for commercialisation. MS11 is assessed in application EFSA-GMO-BE-2016-138. The EFSA opinion on this application was inconclusive because a complete compositional dataset could not be provided. At the previous GMO network meeting (June 2021) questions were raised on EFSA-GMO-BE-2016-138 by Denmark and Belgium on the scientific approach taken and on what would be the best way forward for MS11 and similar type of applications.

In June 2021, EFSA received a mandate from the European Commission with the request to assess additional information on MS11 received from the applicant. The GMO Panel considered the new information on the stack MS11 × RF3 not adequate for the assessment of single event MS11. Therefore, the GMO Panel identified the need to generate new data for compositional analysis and requested to the applicant to perform ad hoc field trials for that purpose. However, the applicant did not provide the new field trials and did not propose reasonable alternatives. The GMO Panel statement is inconclusive because there are no new experimental data on the compositional analysis of MS11, the comparative analysis dataset remained incomplete and the food/feed risk assessment inconclusive.

Discussion

Kleter Gijs (the Netherlands) proposed that a more pragmatic approach could be followed based on realistic scenarios and on the fact that no safety concerns were identified in the stack. Franco Maria Neri (EFSA) replied that it was not possible for the EFSA GMO Panel to consider such an approach because the scope of EFSA-GMO-BE-2016-138 was for food and feed uses, import and processing of MS11.

Adinda De Schrijver (Belgium) asked clarification on the text of the EFSA GMO Panel statement. Specifically, the GMO Panel had stated that the comparative analysis of the stack MS11 × RF3 would not be adequate for the assessment of MS11, because it would not be possible to distinguish the effects linked only to MS11 from those derived from the interaction MS11 × RF3. Adinda De Schrijver (Belgium) asked whether there were compositional differences identified for MS11 × RF3, whether those were biologically relevant and whether potential unintended effects were linked to MS11, RF3 or interactions (for example, considering data on RF3 as well). Franco Maria Neri (EFSA) replied that the assessment of the

compositional differences identified for the stack was not completed (because the single was inconclusive). He also remarked that it is extremely difficult in general to understand the origin of the differences found in a stack. Belgium noted that looking into these data could be a feasible route to end the deadlock in this dossier.

Adinda De Schrijver (Belgium) pointed out that the deadline for the public consultation on the EFSA GMO Panel statement is 11 April 2022; however, the data submitted for the mandate will only be made available to Belgium after the deadline, on 12 April 2022. She asked whether it would be possible to change the deadline to improve the feedback of the public consultation. Ana Afonso (EFSA, Head of NIF) took note on this and will verify whether this is possible.

Adinda De Schrijver (Belgium) commented that the experience with EFSA-GMO-BE-2016-138 should be used as an example to avoid similar situations in the future, with a lengthy assessment process and no final conclusion. Franco Maria Neri (EFSA) replied that this request is welcome and the implementation of transparency EFSA regulation should hopefully help avoiding similar issues in the future.

AGENDA ITEM 7: Request for placing on the market of Soy Leghemoglobin produced from genetically modified *Pichia pastoris* (EFSA-GMO-NL-2019-162)- OpenEFSA Link

Abstract

EFSA received two applications submitted to gain authorization for the use of soy leghemoglobin (the liquid preparation is referred to as "LegH Prep") produced from genetically modified *Pichia pastoris* (*P. pastoris*) as a flavoring ("meaty taste") in meat analogue products that will be marketed in the European Union (EU). One application is received under Regulation 1829/2003 in October 2019 and one application is received under Regulation 1331/2008 in January 2022. The GMO Network was informed about the status of these applications, the scientific content of the Reg. 1829/2003 dossier and how the risk assessment work is being organized in EFSA.

Discussion

Gijs Kleter (the Netherlands) commented that a number of *P. pastoris* proteins were assessed indicating that soy leghemoglobin doesn't show any similarity to allergens. However, he stated that many endogenous proteins from *P. pastoris* show multiple alignments with different type of allergens. This is an interesting case that could serve as an example on how to improve allergenicity assessment.

Adinda De Schrijver (Belgium) requested clarifications on how EFSA is organising the assessment, in particular on the involvement of other EFSA panels. She also noted that the phrase in the EFSA guidelines on genetically modified micro-organisms (GMMs) of 2011 that the GM food and feed regulation [(EC) No 1829/2003] applies only because of the presence of DNA in the final product, is subject to ongoing discussions (as mentioned by EFSA). The reason for AP162 to be risk assessed would rather lie in the presence of proteins from the GMM in the product (up to 35% protein impurities may be present). She also asked about the assessment of Horizontal Gene Transfer (HGT). According to GMO legislation and Directive 2001/18, an ERA only needs to be done in case of import of viable material, which does not appear to be in this case.

Reinhilde Schoonjans (EFSA) replied that EFSA recruited additional expertise in the standing GMO WGS to cover for scientific RA expertise with microorganisms.

Regarding the applicability of the GM food and feed Regulation on this type of product, EFSA is aware that discussions are ongoing on Risk Managers level. A recent paper presenting a legal overview on this matter ("rDNA Traces in Fermentation Products Using GMMs") will be shared with the GMO Network. Regarding HGT, she stated that it would be very useful to receive such comment during the targeted MS consultation that was re-opened for this case.

According to the dossier, the applicant will currently not produce Soy Leghemoglobin in the EU. Should it be produced in the EU in the future, Adinda De Schrijver (Belgium) recalled (as is also written in the dossier) that such production would fall under the contained use regulation. Thus, Adinda De Schrijver, deduced that also in the (hypothetical) case of production in the EU, the product that will enter the EU environment will not contain viable material and an environmental risk assessment would not be necessary.

Cristina Arroyo (Ireland) asked why this application falls under the GMO Regulation (in addition to the additives/flavourings) and the differentiation between "produced FROM" and "produced BY". She referred to the presentation mentioning that "the GMM *Pichia pastoris* is used as a processing aid[5]". She quoted the 2006 Report from the EC (page 24) stating that "*When the GM micro-organism is used as a processing aid, the food and the feed resulting from such production process are not to be considered as falling under the scope of the Regulation.*" Based on her interpretation, this ingredient is produced '*BY*' a GMM which acts as a processing aid, rather than produced '*FROM*' a GMM. In this case, she would consider this application falling under the Novel Food Regulation, not the GMO Regulation.

Reinhilde Schoonjans (EFSA) replied that the legal pathway of the product and the interpretation of the law is outside EFSA's remit. At the time of validation of the GMO dossier, EFSA addressed the discrepancy between the actual use and conditions to place the product on the market and how the scope was defined in the mandate. The latter was meanwhile rectified by referring to Art. 3.1.c. She also invited the participants to read a suggested paper which includes considerations on the legal aspect for these types of products (Title: *rDNA Traces in Fermentation Products Using Genetically Modified Microorganisms* , available at https://stoffr.lexxion.eu/article/STOFFR/2021/3/6).

Ana Afonso (Chair, EFSA) added that the process for the EFSA mandate is different for the two regulatory frameworks: one application under the food additive legislation comes as an EC mandate and the other one under the GMO legislation comes from a member state. EFSA will ensure coordination between the work of the GMO and FAF panel.

AGENDA ITEM 8: EFSA Approach to the risk assessment of double transformants

Abstract

At the GMO Network meeting, EFSA presented a relatively new methodology, already deployed in a few applications, named 'Site Specific Integration'. The technique allows the insertion of the desired transgenic sequence in a specific site of the genome, avoiding the negative aspects related to random integration. The method requires two sequential transformation steps: i) the first step introduces

[5] In the dossier this is mentioned as "used as production microorganism"

a break in the host genome in a desired location, which facilitates the insertion of a sequence, generally named as 'landing pad'. The landing pad integrates via homologous recombination and functions as a scaffold to accommodate the desired sequences delivered with the second transformation. ii) in the second step, the exogenous DNA recombines with elements of the landing pad, for a precise insertion. The system could be used for other applications, such as the removal of undesired sequences, previously included in the landing pad.

EFSA described the technique and used an application as a case study, presenting the steps used by the applicant to insert the desired transgenic sequence within the landing pad. EFSA presented a few important considerations about the RA, obtained following communication with EC. In particular, it was mentioned that these kinds of events, with two insertions occurring in the same locus, are assessed as singles, rather than stacks; that this procedure does not necessarily reduce the RA process, since all the necessary information, including elements involved in the first transformation step, would still be required by EFSA.

A brief discussion followed the presentation, with comments related to the COMPERA area of RA.

Discussion

Gijs Kleter (the Netherlands) asked about the need to perform an extensive compositional analysis and phenotypic agronomic assays in these types of applications where a landing pad is used to insert a new event.

Paolo Lenzi (EFSA) replied that the landing pad approach may facilitate the risk assessment. However, EFSA also needs to make sure that the first transformant is also fully characterized. In case a stand-alone dossier on the first transformant is not available, EFSA still needs to assess that the final event does not raise safety concerns, taking into consideration different aspects such as the interruption of endogenous genes, newly created ORFs, etc.

AGENDA ITEM 9: **Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants (GMPs) obtained through synthetic biology - OpenEFSA Link**

Abstract

Anna Lanzoni (EFSA) presented the key elements of the draft scientific opinion endorsed by the Panel in December 2021, and a preliminary overview of the outcome of the public consultation (19 January-20 March 2022). It was reinforced that this scientific opinion falls in the context of a mandate covering Synbio developments in various areas (GM microorganisms, plants and animals); specifically, this is a follow-up of the previous opinions on Synbio GMPs (on MC and ERA, EFSA GMO Panel, 2021). It was also remarked that the scope of this opinion is to evaluate the adequacy of existing guidelines for the food and feed risk assessment of Synbio GMPs, and not to provide a new guidance. The draft opinion concluded that the current guidelines are in principle adequate, and an update may be needed as regards the safety assessment of new proteins and comparative/compositional analysis (choice of the comparator, multiple comparators, comparative analysis protocol, statistical analysis and potential integration of alternative RA approaches for the safety and nutritional assessment of Synbio GMPs and derived FF, e.g. as for other novel foods). Eight entities participated in the public consultation, and around 150 comments were received. Many of these related to the terminology used (e.g. "engineered organisms",

"established techniques", definition of "synthetic biology"), on the appropriateness of the case-studies identified, and on the conclusions. The comments will be duly taken into consideration by the Working Group in the finalisation of the draft scientific opinion. The adoption of the scientific opinion by the GMO Panel is foreseen in June 2022.

Discussion

Wolfram Reichenbecher (Germany) asked whether the comments received in the public consultation will be published. Anna Lanzoni (EFSA) replied that the comments will be published in a specific annex of the scientific opinion. Ana Afonso (Chair, EFSA) added that it is already possible to have access to such comments using the following link: https://open.efsa.europa.eu/consultation/a0c7U000000IBr0QAG.

Wolfram Reichenbecher (Germany) commented on the selected case-studies included in the opinion stating that part of the scientific community would not agree with their use as cases of synthetic biology due to the fact that genome editing techniques have been applied. He also commented on the use of the term "conventional GMO or GMP" that should not be used since it could be misunderstood, and it could cause confusion from the legal point of view.

Anna Lanzoni (EFSA) replied that synthetic biology is considered an approach and not a technique (various techniques can be indeed combined together). She also remarked the importance to distinguish these two concepts when considering the selected case-studies. The case-studies represent a situation where a model is created and validated. For instance, case-study #1 required a significant effort to select which plant pathways to be modelled in order to obtain the desired phenotype.

Anna Lanzoni (EFSA) also informed that consistency must be maintained with previous work where the term "conventional GMO" was used. However, consistency in terminology will be assured in the scientific opinion.

Reinhilde Schoonjans (EFSA) informed that the case-studies were carefully selected considering that synthetic biology is not a clearly defined area and different opinions exist about its definition.

Nikoletta Papadoupoulou (EFSA) reminded that there is no application that has reached the market and that would achieve such a large number of mutations as case-study #2 (i.e. gluten-free wheat). All of these case-studies were selected by the panel without already having a published proof of the engineering steps taken. Case-study #2 could require the synthetic biology approach to correctly identify the number of gliadins deleted in the genome.

AGENDA ITEM 10: Mandate on scientific assessment of teosinte - OpenEFSA Link

Abstract:

Yann Devos (EFSA) presented the recently published EFSA Statement[6] updating the environmental risk assessment (ERA) conclusions and risk management (RM) recommendations of EFSA's 2016 Technical Report[7] on EU teosinte. Teosinte, a

[6] https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7228
[7] https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-1094

group of wild species related to maize originating from Mexico and Central America, has emerged as a new weed in maize fields in two European countries, France (FR) and Spain (ES). In these regions, teosinte is considered a noxious agricultural weed that is subject to control and/or eradication measures and monitoring. In EFSA (2016), the available scientific information on teosinte was assessed for its relevance for the ERA and RM of genetically modified (GM) maize MON810, Bt11, 1507 and GA21 for cultivation. Since the publication of EFSA (2016), new scientific evidence on teosinte that is relevant for the ERA and RM of maize MON810, Bt11, 1507 and GA21 has become available. Following a request of the European Commission, EFSA evaluated whether the ERA conclusions and RM recommendations of EFSA (2016) remain applicable or require revision in light of new scientific evidence on teosinte. A protocol was developed to clarify the interpretation of the terms of reference of the mandate and make them operational. The assessment relied on evidence retrieved via an extensive literature search and from reports of the Competent Authorities of France and Spain, and on (hearing) expert testimonies. A limited collection of 18 publications of varying relevance and quality was retrieved and assessed. It was noted that the overall environmental exposure to GM teosinte hybrid plants, bearing either the insect resistance or herbicide tolerance trait or both, will remain low compared to exposure to GM maize, provided that measures continue to be employed to monitor, control and/or eradicate EU teosinte in infested agricultural areas. Therefore, in line with EFSA (2016) and if the measures employed to monitor, control and/or eradicate teosinte in infested agricultural areas remain in place, it is assumed that the impact of insect resistance and/or herbicide tolerance in GM teosinte hybrid progeny (potentially acquired through hybridisation between GM maize and teosinte) on target and non-target organisms, the abiotic environment and biogeochemical cycles will be very low under EU conditions. Overall, the 2022 EFSA Statement concludes that the ERA conclusions and RM recommendations of EFSA (2016) remain applicable, except those pertaining to the use of glyphosate-based herbicides on maize GA21 which should be considered under Regulation (EC) No 1107/2009.

Discussion

Adinda De Schrijver (Belgium) informed participants that the 2016 EFSA Technical Report on teosinte has been a useful reference in the frame of deliberate releases of GM maize events into the environment for experimental purposes.

Slawomir Sowa (Poland) asked whether EFSA considered risks related to the cross between conventional maize varieties and teosinte. He indicated that there are more than 4000 maize varieties in the common catalogue with different traits that may impact the persistence and invasiveness potential of teosinte. He also asked whether the presence of teosinte in the ecosystem could represent a risk. Yann Devos (EFSA) replied that in the frame of the ERA of GM plants, the focus is on assessing the consequences of the acquisition of transgenic traits by teosinte through vertical gene flow However, he noted that vertical gene flow from conventional maize to teosinte represents important baseline data on the hybridization potential between maize and teosinte, which is considered explicitly in the ERA of GM plants. In this respect, reference was made to the evidence reported by Le Corre et al. (2020).

Gijs Kleter (the Netherlands) mentioned that teosinte occurred on a list of ornamental garden plants about 20 years ago, presumably as a kind of plume

grass, and wondered whether this scenario should be considered in environmental risk assessments of GM maize events for cultivation.

AGENDA ITEM 11: Literature reviews for the risk assessment of GMO application

Abstract

ANSES informed the GMO Network that since the 1st January 2022 the high council of biotechnologies (HCB) is no longer in charge of the risk assessment of GMOs concerning the environmental aspects, and that ANSES is now in charge of all GMO risk assessment aspects for applications handed in under the 1829/2003 Regulation.

A new Biotechnology unit was created in this context and attached to risk assessment department of ANSES.

Concerning the literature review, the observations of ANSES' Biotechnology working group are about the Article 6 of implementing regulation 503/2013 concerning the additional information related to the risk assessment of GMO food or feed. In many cases, the Anses' WG considers that these requirements are not met.

ANSES' WG considers that for a new event, the systematic reviews are very limited considering that this crop, and food and feed have not yet been widely used. For a renewal request, ANSES WG considers that the systematic reviews must be in accordance with EFSA's guidance documents.

ANSES' WG has some proposals:

- distinction of articles retained and not retained;
- exclusion criteria should be less selective;
- inclusion criteria must take into account terms associated with risks aspects;
- complete information concerning the reviewers must be available;
- analysis must be performed double bind and completely presented.

ANSES' WG recommends that the validation of admissibility of submissions must be in accordance with EFSA guidance documents relating to the systematic review and to have a distinction between new applications and renewal requests.

Discussion

Gijs Kleter (the Netherlands) reminded that the applicant should make available all information for that specific GM. He also explained that a very specific question must be addressed by the literature searches performed in the context of GMO applications, and information not addressing that specific question would not be included. He also wondered whether evidence mapping or alternative ways for performing literature reviews could be included to fulfill the requirements.

Paolo Lenzi (EFSA) commented that 1) the inclusion/exclusion table in the EFSA Note is not fully exhaustive and represents just an example, and 2) EFSA agrees with the presenter regarding the description of the reviewers. EFSA acknowledges that sometimes the description of the reviewers was not fully adequate, and clarifications were asked to the applicants on this issue.

While there is always room for improvement, Yann Devos (EFSA) indicated that applicants have made significant efforts to comply with EFSA's Explanatory Notes

on literature searching, and that the quality of searches and their reporting has improved accordingly.

Slawomir Sowa (Poland) commented that nowadays we assess "simple GMOs" in "complex environments". However, in the future we will have to assess "complex GMOs" obtained also by synthetic biology in "complex environments". Artificial intelligence could provide help in performing high quality systematic searches in the near future.

Ana Afonso (Chair, EFSA) replied that there are ongoing EFSA initiatives to use artificial intelligence for systematic reviews, mainly at the early stages of the literature search. These tools may assist and complement the assessment done by information specialists.

AGENDA ITEM 12: Activity on NGT – round table - Risk assessment activities in relation to NGT

Abstract

Tommaso Raffaello (EFSA) introduced a brief recap on the new genetic techniques (NGTs) report published in 2021 by EFSA as a background for the discussion. He reported that, on November 2019, the Council of the European Union requested the European Commission (EC), in light of the Court of Justice's judgment in Case C-528/16, to submit a study regarding the status of NGTs under Union law. The EC mandated EFSA, to provide an overview on the risk assessment of plants developed through NGTs by taking into account EFSA's previous scientific opinions, EFSA ongoing work on the topic, as well as opinions published by competent authorities and national institutions since 2012.

The baseline set was based on the Joint Research Centre (JRC) document on new plant breeding techniques (JRC, 2011), the EC-SAM report on new techniques in agricultural biotechnology (EC-SAM, 2017), and a total of sixteen scientific opinions from Member States (MS). In addition, three EFSA scientific opinions on cisgenesis (EFSA GMO Panel 2012), SDN-3 (EFSA GMO Panel 2012) and SDN-1, 2 & ODM (EFSA GMO Panel 2020) were included. The MS scientific opinions contained information on site-directed nucleases (SDN) type 1, 2 and 3 technologies, oligonucleotide-directed mutagenesis (ODM), cis/intra-genesis, RNA-dependent methylation (RdDM), grafting, reverse breeding and agro-infiltration.

EFSA asked the GMO Network participants whether:

- any update on the information summarized in EFSA report was available;
- any new activity/report/publication was available on NGT since the publication of the EFSA report (April 2021);
- any activity for NGTs carried out at MS level in microorganisms was available.

Discussion and round table

PART 1: The update on the member states activity on New Genomic Techniques (NGTs) as provided by the participants during the 13th GMO Network meeting (7 April 2022) or via an update after the meeting can be found in Annex 1.

The comments and updates provided by the participants is summarized in the table below.

Member State	Update/Comments provided by the participant(s)
Germany	Wolfram Reichenbecher (Germany) provided an update on activities and outputs of the BfN on NGTs on risk assessment, on detection and traceability and on legal aspects (see Annex 1 – Germany update).
The Netherlands	Gijs Kleter asked to Wolfram Reichenbecher (Germany) whether bacteriophages were considered in the project/publication mentioned by Germany in their summary (see **Annex 1** – Germany update). Wolfram Reichenbecher (Germany) replied that although he has no information immediately available about that, he could retrieve such information from the colleagues who are running the project on GM viruses (see **Annex 1** – Germany update). Marco Gielkens provided an update on a big project founded by the government and focused on new technologies (e.g., NGTs) and their impact on safety assessment. He informed that additional information on these projects can be presented at the next GMO network meeting.
France	Youssef El Ouadrhiri informed that France received a mandate to provide scientific support to the French authority on the description of NGTs. Dedicated working groups were created for analysing NGT applications and guidance. This mandate focused on CRISPR-Cas technology for the most useful crops (e.g., maize and rapeseed).
Norway	Ville Erling Sipinen shared the link of a report published on 29 October 2021 (available at https://vkm.no/english/riskassessments/allpublications/crisprandothergenomeeditingtechniquesimplicationsforriskassessment.4.581a91ee16d1a06e872a6bca.html). In this publication the current EFSA guidelines for risk assessment of GMO were reviewed in order to assess if they were suitable for RA of some case examples of gene edited (GE) organisms (plants, animals and micro-organisms). A second report is also in preparation which focuses on risks and benefits of specific GE crops and implications with the agriculture in Norway. Ana Afonso (Chair, EFSA) asked whether these investigated benefits were linked to sustainability aspects. Ville Erling Sipinen (Norway) replied that the sustainability aspects were not part of the mandate/request.
Finland	Kirsi Törmäkangas informed that no NGT risk assessment project is currently ongoing. However, she informed that the Finnish Government published in May 2021 the results of a research project on the potential of NGTs for enabling sustainable growth (available at https://vnk.fi/-/tutkimus-

	mahdollistavatko-uudet-genominmuokkaustekniikat-kestavaa-kasvua-suomessa-ja-euroopassa-?languageId=en_US).
Austria	Markus Wögerbauer provided an update on the most recent activities on NGTs in Austria. He reported four research projects (three of them were also mentioned by Germany in their update). He also introduced an ongoing project which focuses on patents of important crops produced in Austria with GE technologies. The project is the result of the collaboration between the Agency for Health and Food Safety (AGES) and Environmental Agencies and funded by ministry for transport, innovation and technology. However, the output is not available yet.
Belgium	Adinda De Schrijver informed about three advices of the Belgian Biosafety Advisory Council on field trials with maize modified by CRISPR-Cas (SDN-1 type of modifications) which have recently been published (available at https://www.bio-council.be/en/advices). She also shared the link of a recent publication (Sturme et al. (2022). Occurrence and Nature of Off-Target Modifications by CRISPR-Cas Genome Editing in Plants. *ACS Agricultural Science & Technology.* *https://doi.org/10.1021/acsagscitech.1c00270*).
Italy	Marzia De Giacomo provided some updates on the research activity on genetic improvement and NGTs in Italy. The details about the updates can be found in **Annex 1**.

PART 2: EFSA discussed with the audience the following points: a) What would you consider "proportionate" risk assessment for plants generated via NGT? b) Which criteria would you consider for a "proportionate risk assessment?". Two polls were launched to the participants.

POLL N°1: Do you consider 'proportionate risk assessment' as synonymous of 'case-by-case' approach?

Results:

Yes 50%

No 50%

Discussion

Some MS considered the need to have flexibility in the risk assessment depending on the product and new genomic technique under assessment very important. In addition, problem formulation would be very relevant for the risk assessment of plants obtained by new genomic techniques. The concept of 'proportionate' needs to consider that the risk assessment for crops obtained by NGTs does not differ from the risk assessment for plants with similar traits obtained using other non-regulated technologies.

Some MS considered that 'proportionate risk assessment' and 'case-by-case' are not synonymous, although they are linked. The concept of GMO groups with equal

or similar risk profiles could be introduced and the proportionality principles in the risk assessment could also be emphasized.

However, it was debated that in the case no risks are identified for certain crops, it could be difficult to classify them according to risk profiles. One of the MS replied that the concept of GMO groups is already in use in legal frameworks and the GMO categorisation is clearly defined. Indeed, it is possible to say that the outcome of all the individual risk assessments of the GMOs belonging to a specific category could be identical or very similar in that respect, and you can also apply general measures for mitigating risks for those special type of categories.

Other MS also considered that 'proportionate risk assessment' and 'case-by-case' are not synonymous. Moreover, creating categories of risk, especially with NGTs, would not be optimal because there is a wide range of plant issues and traits to be considered as well as different possibilities of intervention. A 'case-by-case' approach would be preferrable.

Other MS proposed that the 'case-by-case' approach should be proportionate to the risk of the product. Although 'proportionate risk assessment' and 'case-by-case' are not synonymous, these two notions point to the same direction.

POLL N°2: Which criteria would you consider for 'proportionate risk assessment'?

Results:

Plant species being edited 11%

Expressed trait 21%

Technique used for editing 15%

Presence/Absence foreign DNA 19%

Presence/Absence newly expressed proteins(s) 16%

Scope of the product 11%

Others 3%

Discussion

Ana Afonso (Chair, EFSA) explained that the criteria proposed in the poll were used by other regulatory organisations across for developing regulatory frameworks of NGT products.

It was asked whether these criteria listed in the Poll 2 would also be useful to distinguish such risk profiles or such risk categories as discussed previously.

One MS stated that all the items listed in the poll may be used as criteria to define 'proportionate risk assessment'. However, one or more aspects may be considered at the same time when defining a specific risk category.

Regarding the 'Other' criteria listed in Pool 2, it was mentioned that the 'comparability' with the same changes in the same risks in conventional and traditional GM crops should be considered when taking a decision about what is appropriate for the risk assessment of a specific NGT product. It was also mentioned that the receiving environment should be listed as an important criterion.

Marion Dolezel (Austria) reminded that there is another policy element in the European Commission inception impact assessment related to the sustainability

dimension of the product derived from NGT. Ana Afonso (EFSA) clarified that the aspects related to sustainability is not in the current EFSA workplan. Moreover, it is not clear whether the aspects related to sustainability are within EFSA's remit.

AGENDA ITEM 13: Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology - OpenEFSA Link

Abstract

Antonio Fernandez (EFSA) presented the main highlights of the scientific opinion on development needs for allergenicity assessment. Briefly, although the Codex Alimentarius and EFSA guidance documents successfully addressed allergenicity assessments of single/stacked event GM applications, experience gained and new developments in the field call for a modernisation of some key elements of the risk assessment. Furthermore, more complex future products will likely challenge the overall practical implementation of current guidelines mainly targeted to assess a few newly expressed proteins. Therefore, it is timely to review and clarify the main purpose of the allergenicity risk assessment and the vital role it plays in protecting consumers' health. EFSA also highlighted the importance of shaping together new ways of collaboration with Member States, in particular for complex scientific topics such as allergenicity. To this end, a series of questions were presented aiming to stimulate the dialog with Member States.

Discussion

Gijs Kleter (the Netherlands) asked whether the project will include input from the European Horizon' conferences or from internal research activities. Antonio Fernandez Dumont (EFSA) replied that both inputs are taken in consideration. From the EFSA side, a procurement was launched last year to start an activity for the ranking of allergens. But this will not be sufficient and more research and further efforts at a larger scale will needed. In addition, Ana Afonso (Chair, EFSA) added that the GMO Panel is advertising this project both inside and outside EFSA. The outcome was already shared in scientific meetings and to the EFSA panel members and, additionally, will be used for external collaborations. Moreover, Ana Afonso (Chair, EFSA) invited the GMO network to scientifically support this activity considering the limited budget of Horizon Europe. Overall, it was considered important to continue the discussion on the topic and specific Member States will be contacted to address the questions raised at the meeting. The outcome of the consultation will be presented in a subsequent Network meeting.

AGENDA ITEM 14: Animal dietary exposure in GMOs

Abstract

Michele Ardizzone (EFSA) informed the GMO Network on the ongoing activities related to the GMO Panel statement on animal dietary exposure for feed derived from GM plants, with reflections on i) weaknesses acknowledged on current animal dietary exposure approaches, ii) the aim of the GMO Panel statement, iii) a related project ongoing on the topic of feed classification and consumption databases, and iv) the timelines foreseen for the ongoing activities and involvement of interested parties.

Discussion

Gijs Kleter (the Netherlands) expressed his view on the substantial difference on the methodology used to derive food consumption data for Human Dietary Exposure (HDE) and the one that should be put in place to implement further the feed consumption data collection for Animal Dietary Exposure (ADE), e.g. consumer surveys may be considered difficult to be put in practice for animals.

Michele Ardizzone (EFSA) agreed with that consideration, already taken in consideration with the contractor. Furthermore, other aspects have also been considered making the approach for ADE focusing not only on several species but also on the physiological state of the animal when the diet changes. He also emphasized two aspects: 1) the work is still ongoing, and 2) the available information should be harmonized to be included in document endorsed by the GMO Panel.

Adinda De Schrijver (Belgium) asked whether the statement of the GMO Panel will only be shared with interested stakeholders in June/July. Michele Ardizzone (EFSA) replied that the GMO Panel will endorse the final document and stakeholders (industry or general networks) will be involved in an early stage to capture early on possible contributions before the GMO Panel adoption. Ana Afonso (Chair, EFSA) clarified that a targeted consultation can be launched. The outcome of the consultation could be shared in a dedicated meeting or by written procedure (probably in Q3 2022). Michele Ardizzone (EFSA) informed that the information on this project will be presented at the next applicant meeting. After that, stakeholders may have the possibility to read the draft document and make comments to incorporate new feedback in the document before the adoption.

Closure of the meeting

The Chair thanked the GMO Network members for their active participation and the fruitful discussion.

The draft minutes will be shared with the participants and published on the EFSA website together with the presentations within 15 working days. The meeting was closed at 16.00.

ANNEX 1: Update on the Member States activities on New Genomic Techniques (NGTs) as provided by the participants during the 13th GMO Network meeting (7 April 2022) or via an update after the meeting.

Germany update:

Federal Agency for Nature Conservation

I 2.6 GMO – Regulation – Biosafety 7. April 2022

BfN Activities on New Genetic Techniques

I. On Risk Assessment

1) Biosafety Considerations of NGT crops

a) Finished project, Environment Agency Austria

Overview about recent developments of NGTs for crop improvement and identification of possible biosafety issues.

Eckerstorfer et al. (2019) An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). doi: 10.3389/fbioe.2019.00031

A case-specific premarket RA is required for all NGT plants, including several proposed steps to identify unintended changes and/or confirm absence of unwanted transgenic sequences.

b) Issue of RA of GE plants further discussed within IG GMO (Interest Group within EPA/ENCA network):

Eckerstorfer et al. (2021) Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. doi.org/10.3390/biotech10030010

Because of a wide range of species, GE methods and traits, risks associated with individual GE applications will vary a lot. There is no safety by default for whole groups of GE applications (risk profiles). Instead, a focused case-specific approach should be followed and further guidance developed on trait and method related considerations.

c) Conribution to the discussion about the regulation of NGTs

BfN Viewpoint (2021) New developments and regulatory issues in plant genetic engineering. https://www.bfn.de/sites/default/files/2021-10/Viewpoint-plant-genetic-engeneering_1.pdf

The paper examines the context and the assumptions of the Commission's Inception Impact Assessment on NGTs. It deals with possible policies and discusses criteria against which future legislative proposals should be measured.

2) Horizon Scanning Biotechnology

Ongoing project, FGU (Project Genetic Engineering and the Environment)

To identify via horizon scanning of new biotechnologies early signs of potential risks and opportunities and support the discourse on a national, european and international level. Selected publications:

Kawall (2019) New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. doi: 10.3389/fpls.2019.00525

The outcomes of GE can produce organisms with new genetic combinations that would not occur naturally. It falls short to compare all genomic alterations or allelic combinations generated by CRISPR/Cas generally as identical to natural variations.

Kawall (2021) The Generic Risks and the Potential of SDN-1 Applications in Crop Plants. doi: 10.3390/plants10112259

It highlights the need for a case-specific RA of crop plants derived from SDN-1 applications considering characteristics of the product *and* the process to ensure a high level of protection of human and animal health and the environment.

https://fachstelle-gentechnik-umwelt.de/en/home/

3) Molecular Characterisation

Ongoing project, NORCE/Genök, Norway

Project aims to further develop methods for molecular characterisation of classic and GE GMO considering technical development of analytics and databases.

Two publications planed.

4) GM Viruses

a) Ongoing project, Environment Agency Austria

WP 1: Horizon scanning and overview
WP 2: Adequacy of existing guidelines (ERA and monitoring)

b) BfN, co-authorship publication

Lentzos et al. (2022). Eroding norms over release of self-spreading viruses. *https://www.science.org/doi/10.1126/science.abj5593*

The paper deals with the best practice of horizon scanning and highlights that the unsolved challenges during the release of GM viruses are valid as before, so well-established norms must be kept.

5) GM Microorganisms, Algae and Animals

Prepared project

WP 1: Horizon scanning and overview
WP 2: Adequacy of existing guidelines (ERA and monitoring)
WP 3: Technology assessment

II. On Detection and Traceability

1) Analysis of detection methods for GE and classic GM crops

Finished project, Austrian Agency for Health and Food Safety and Environment Agency Austria

Ribarits et al. (2021) Genome-Edited Plants: Opportunities and Challenges for an Anticipatory Detection and Identification Framework. *https://doi.org/10.3390/foods10020430*

Ribarits et al. (2021) Detection Methods Fit-for-Purpose in Enforcement Control of Genetically Modified Plants Produced with Novel Genomic Techniques (NGTs). *https://doi.org/10.3390/agronomy11010061*

Project concludes that a database should be established that (a) lists if possible all classical and GE crops potentially on the global market and (b) collects sufficient information for their identification by laboratory-based methods.

2) Detection and Traceability of GM Products

Prepared project

The project will investigate the traceability of commodity flows to complement detection methods in the field of GMOs, and theoretical concepts will be further developed.

III. On Legal Aspects

BfN commissioned several legal opinions on NGTs and the impact of the ECJ's (2018) judgement, case C-528-16. Selected publications:

1) *Spranger (2017) In-depth **analysis of various European directives and regulations** with regard to their potential to regulate environmental effects of New Technologies besides Genetic Engineering Law Summary* https://www.bfn.de/sites/default/files/2021-10/NT_Auffangrechte_RGutachten_Spranger_en.pdf

For various and different reasons none of them is suitable to provide adequate legal and control standards for the ERA of new technologies.

2) *Spranger (2019) Memorandum on the question of the **applicability** of the statements of the European Court of Justice in case C-528/16 to the area of regulation of **Directive 2009/41/EC on the contained use** of genetically modified micro-organisms* https://www.bfn.de/sites/default/files/BfN/recht/Dokumente/spranger_dir_2009_41_ec_contained_use_c_528_16.pdf

The ECJ judgement applies to the Directive on contained use as well.

3) *Spranger (2019) Memorandum on the **international trade law implications** of the judgment of the European Court of Justice in case C-528/16* https://www.bfn.de/sites/default/files/2021-10/memorandum-international-trade-law-implications-of-the-judgment-of-the-european-court-of-justice-in-case-c528-16.pdf

The ECJ judgement does not violate international trade law. Moreover, the world trade law explicitely allows for different, even trade restrictive regulations, as long as they are based on scientific and consistent considerations.

4) *Spranger (2021) An Innovation Principle in Gene Technology Law? In: Biotechnology Law Report 40 (6), S. 389–392. https://www.liebertpub.com/doi/epdf/10.1089/blr.2021.29251.tms*

The innovation principle is a purely political concept, but not a legally based or binding principle; it is not suitable to restrict or eliminate the precautionary principle.

5) Spranger (2022) **Challenges for the traceability of NGT** from a legal perspective. *In preparation*

For further publications in English or German, search with 'Spranger' at www.bfn.de

Austria update:

Austrian – Activities, Reports, Publications (2021 -2022)

Research projects:

1. Nachweismethoden für genomeditierte und klassische GV-Pflanzen („Detection methods for genome edited and conventional genetically modified plants").

Principal Investigator:	Collaboration **AGES/UBA** (Austrian Agency for Health and Food Safety / Federal Environment Agency)
Period:	**2019-2021**
Funding:	**BfN** (Germany) Federal Agency for Nature Conservation
Output:	1x final report; 2x peer-reviewed publications
Final report:	Ribarits A, Stepanek W, Hochegger R, Narendja F, Prat N, Wögerbauer M. 2022. **Analyse von Nachweismethoden für genomeditierte und klassische GV-Pflanzen.** BfN Schriften 622
Publications:	1. Ribarits A, Eckerstorfer M, Simon S, Stepanek W. 2021. **Genome-Edited Plants: Opportunities and Challenges for an Anticipatory Detection and Identification Framework**. Foods 10:430. 2. Ribarits A, Narendja F, Stepanek W, Hochegger R. 2021. **Detection Methods Fit-for-Purpose in Enforcement Control of Genetically Modified Plants Produced with Novel Genomic Techniques (NGTs)**. Agronomy 11:61.
Conclusions:	Please see Germany update (section II.1.)

2. Risk assessment of plants developed by New Techniques - Potential biosafety issues associated with current applications.

PI:	**UBA** (Federal Environment Agency Austria)
Period:	**2019-2020**
Funding:	**BfN** (Germany) Federal Agency for Nature Conservation
Output:	2x final reports; 2x peer-reviewed publications

Final report:	1. Eckerstorfer M, Dolezel M, Greiter A, Miklau M, Heissenberger A, Steinbrecher R. 2020. **Biosafety Considerations for Plants developed by Genome Editing and other new Genetic Modification Techniques (nGMs) and Considerations for their Regulation**. BfN Skripten 592 Final Report. 2. Eckerstorfer M, Greiter A, Heissenberger A. 2020. **Comparison of existing Regulation Frameworks in non-EU Countries with a Focus on the respective Requirements for Risk Assessment**. BfN Skripten 598 Final Report.
Publications:	1. Eckerstorfer M, Heissenberger A, Reichenbecher W, Steinbrecher R, Waßmann F. 2019. **An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs).** Front. Bioeng. Biotechnol. 7:31. doi: 10.3389/fbioe.2019.00031**.** 2. Eckerstorfer, M. F., Engelhard, M., Heissenberger, A., Simon, S., Teichmann, H. (2019). **Plants developed by new genetic modification techniques - comparison of existing regulatory frameworks in the EU and non-EU countries**. *Front. Bioeng. Biotechnol.* 7:26. doi:10.3389/fbioe.2019.00026
Conclusions:	Please see Germany update (section I.1.)

3. Relevanz von Patenten für mit Genome Editing erzeugte und für die landwirtschaftliche Anwendung in Österreich bedeutsame Nutzpflanzen im Hinblick auf das Patentrecht und die Arbeit des Biopatent-Monitoring-Komitees ("Relevance of patents on important crops produced by genome editing for Austrian agriculture").

PI:	Collaboration **AGES/UBA** (Austrian Agency for Health and Food Safety / Federal Environment Agency)
Period:	2020 - 2021
Funding:	BMVIT (Federal Ministry for Traffic, Innovation and Technology)
Topic:	a) Discussion of the relevance of patent law concerning "biopatents" on genome edited crops of importance for cultivation in Austria b) Investigation if "bio patents" for crops important for Austria contain information relevant for the molecular characterization of such plants
Output:	not available yet

4. Biosafety of Genome Editing Applications in Plant Breeding (UBA/BAFU Switzerland; IG GMO (Interest Group within EPA/ENCA network))

PI:	**UBA** (Federal Environment Agency Austria)
Period:	2021
Funder:	BAFU (Schweiz) Swiss Federal Office for the Environment (FOEN/BAFU)
Output:	1x peer-reviewed publication
Publications:	1. Eckerstorfer, M.F.; Grabowski, M.; Lener, M.; Engelhard, M.; Simon, S.; Dolezel, M.; Heissenberger, A.; Lüthi, C. **Biosafety of Genome Editing**

	Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. BioTech 2021, 10, 10. https://doi.org/10.3390/biotech10030010
Conclusions:	Please see Germany update (section I.1.)

Italy updates:

Research activities on genetic improvement and NBTs in Italy:

1) To support the assessment of NBTs' potential for Italy, Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF) approved in 2018 **a sustainable agriculture research plan 'BIOTECH'** coordinated by the Italian Council for Agricultural Research and the Analysis of Agrarian Economy **(CREA)** in collaboration with several **Italian universities**, The National Research Council (**CNR)** and the **Mach Fondation**, that will end in August 2022. The aim was to apply cisgenesis and genome editing in crop plants to acquire knowledge on gene functions, to develop new genotypes and to promote the diffusion of new breeding techniques in the Italian scientific community.

   The main lines of research, with results already obtained in the laboratory, include:

   - Seedless aubergines, obtained by the Experimental Institute for Horticulture, tomato plants that are able to inhibit the germination of certain weeds and kiwi seedlings that contain mutations on genes involved in controlling bacterial susceptibility.
   - University of Milan has developed some tomato lines resistant to water stress by partial silencing with gene editing of a gene that regulates the opening of stomata. And it is working with the same aim on grapevine.

   Production of cisgenic/edited plants aimed at improving biotic stress resistance (Oidium and Peronospora in grapevine, fire blight in apple and pear, bacterial canker in kiwifruit, Peronospora in sweet basil), quality (accumulation of secondary metabolites in tomato and sweet orange, reduction of seed size in sweet orange and grapefruit, reduction of browning in fresh-cut eggplant) or agronomic traits (seed size in durum wheat, self-compatibility in pear, early flowering in sweet orange, re-flowering in strawberry) is underway.

2) Collaboration between Marche Polytechnic University **(UNI MARCHE)** and University of Bologna **(UNIBO)** (in the MIPAAf project, but also in other projects) has produced vine plants resistant to fungal diseases (botrytis and downy mildew). They have also produced stone fruit plants resistant to sharka with the approach of modifying only the rootstocks, so that the aerial part is protected but not genetically modified.
   The same group is working on strawberry with interfering RNA for fungal resistance and has started a programme for resistance to Drosophila suzukii. They are also working with cisgenesis to speed up the obtainment of re-flowering strawberry varieties.

3) University of Milan **(UNIMI)**, has been using CRISPR/CAS on rice for eight years for disease resistance and growth efficiency traits while waiting for field trials.

4) Italian national Agency for New technologies, Energy, and Sustainable Development **(ENEA)** has been involved in the genetic improvement of species of agricultural interest since the late 1950s. Currently different research groupscontinue to do so using innovative biotechnology:
   - Transgene-free tetraploid potato plants (cv. Desiree) have been generated in which the eIF4E-1 gene, responsible for interaction with the potyvirus VpG protein, has been inactivated by Cas9. Currently in collaboration with CREA, have highlighted how CRISPR-Cas9 targeting of the eIF4E-1 gene extends the PVY resistance spectrum of the Solanum tuberosum L. cv. Desirée .
   - At a preliminary stage the development of tomato lines with reduced allergen content.

5) The Research and Innovation Center of the Foundation Edmund Mach **(FEM),** a research Institute, carries out research in the field of plant biotechnologies. In particular, FEM is applying new breeding technologies to counteract the major biotic and abiotic stresses affecting grapevine and apple and to study gene function. Currently, the main research lines in grapevine are: (i) the study of the genetic basis of stomatal density, which is a key trait for plant response to drought; (ii) the study of lipoxygenase-mediated resistance to fungal pathogens; (iii) the editing of genes of susceptibility to powdery mildew and downy mildew, to obtain clones of commercial varieties more tolerant to these diseases. In apple, a project is in progress which applies cisgenesis to introduce resistance genes for apple scab into commercial varieties. In addition, efforts are ongoing to develop more efficient and exogenous DNA-free gene editing protocols.

Most recent publications:

"The Arabidopsis pattern recognition receptor EFR enhances fire blight resistance in apple". Piazza S., Campa M., Pompili V., Dalla Costa L., Salvagnin U. e Nekrasov V. bioRxiv preprint, https://doi.org/10.1101/2021.01.22.427734.

XIth International Symposium on Grapevine Physiology and Biotechnology 2021 31Oct-5Nov Stellenbosch, Sud Africa:

- **Edited grapevine knocked-out for VvEPFL9-1 showed reduced stomatal density**. Oral presentation. Molly Clemens, Michele Faralli, Claudio Varotto, Mickael Malnoy, Walter Oechel, Lorenza Dalla Costa.
- **Generation of non-transgenic mildew-resistant grapevine clones via gene-editing: potentials and hurdles. Oral presentation** Lisa Giacomelli*, Simone Scintilla, Umberto Salvagnin, Tieme Zeilmaker, Lorenza Dalla Costa, Mickael Malnoy, Jeroen Rouppe van der Voort, Claudio Moser. Fondazione Edmund Mach, Italy
- **Functional Study of Lipoxygenase-mediated Resistance against Erysiphe necator in Grapevine. Poster.** Mikias Damtew Guche , Lorenza Dalla Costa , Francesco Trenti , Graziano Guella , Mickael Malnoy , Claudio Moser , Stefania Pilati Centro Agricoltura

Alimenti Ambiente, Via Edmund Mach, Department of Physics, University of Trento, Italy

Several Italian research groups (Universities, ENEA, The National Research Council (CNR) actively participated in the COST action iPlanta program: modifying plants to produce interfering RNA. The final conference of the project took place on 25 March 2021.

University of Ancona have been working on interfering RNA in fruit-culture for disease control. - CNR of Turin have been working on the use of RNAi for virus control. - The University of Bologna have been working on disease control in fruit species using biotechnology, including RNAi. - ENEA is mainly involved in biosafety of biotechnologies. They have performed RNAi bioassays on beneficial insects (i.e. Chrysoperla carnea and honeybees). They have also synthesized new dsRNAs to be used against Mediterranean pests.

Most recent publications:

"Biotechnological Approaches: Gene Overexpression, Gene Silencing, and Genome Editing to Control Fungal and Oomycete Diseases in Grapevine". Capriotti, L., Baraldi, E., Mezzetti, B., Limera, C., & Sabbadini, S. (2020). International Journal of Molecular Sciences, 21(16), 5701.

"Biosafety of GM crop plants expressing dsRNA: data requirements and EU regulatory considerations". S. Arpaia, O. Christiaens, K. Giddings, H. Jones, B. Mezzetti, F. Moronta-Barrios, J.N. Perry, J.B. Sweet, C.N.T. Taning, G. Smagghe, A. Dietz-Pfeilstetter (2020). Frontiers in Plant Science 11:940.

"Biosafety of bee pollinators in genetically modified agro-ecosystems: Current approach and further development in the EU". S. Arpaia, G. Smagghe, J.B. Sweet. (2021) Pest Management Science, DOI: 10.1002/(ISSN)1526-4998.