Subgroup on New Genomic Techniques (NGTs)
Minutes of the 2nd meeting

14 May 2025
09:00-13:00
Minutes agreed on 27 June 2025

Location: Online

Attendees:

- Network Participants:

Country	Member State Organisation
Austria	Environment Agency Austria
	AGES - Austrian Agency for Health and Food Safety
Belgium	Sciensano
Croatia	Faculty for Natural Science and Mathematics, University of Zagreb
Czech Republic	Charles University
Denmark	DTU Food
Finland	Finnish Food Authority
France	ANSES
Germany	Federal Agency for Nature Conservation
	Federal Office of Consumer Protection and Food Safety (BVL)
Hungary	Ministry of Agriculture
Ireland	Food Safety Authority of Ireland
Italy	Istituto Superiore di Sanità (ISS)
	INAIL
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	LLC Caszyme, Chief Scientific Officer
Netherlands	Wageningen Food Safety Research (WFSR)
Romania	University of Life Sciences "King Mihai I" Timisoara
	University of Agronomic Sciences and Veterinary Medicine of Bucharest
Spain	Polytechnic University of Valencia
	Ministry for Ecological Transition and the Demographic Challenge
Sweden	The Swedish Board of Agriculture

- Hearing Experts:
  Sara Zenoni (University of Verona); Vittoria Francesca Brambilla (University of Milan).
- European Commission: DG-SANTE

- EFSA:
  NIF Unit: Ana Afonso, Martina Bonatti, Antonio Fernandez Dumont, Andrea Gennaro, Sara Jacchia, Dafni Maria Kagkli, Silvija Kolosevska, Aleksandra Lewandowska, Franco Maria Neri, Nikoletta Papadopoulou, Claudia Parisi, Pietro Piffanelli, Tommaso Raffaello, Marta Rodrigues, Elena Sanchez Brunete, Reinhilde Schoonjans.

1. Welcome and apologies for absence

The Chair welcomed the participants.

2. Adoption of agenda

The agenda was adopted without changes.

3. Agreement of the minutes of the 1st meeting of the subgroup on New Genomic Techniques (NGTs) held on 29 May 2024 in Brussels, Belgium.

The minutes of the 1st Subgroup on NGTs Network meeting had been previously agreed by written procedure on 05 July 2024 and published on the EFSA website.

4. Introduction to the meeting

The Chair provided an overview of the agenda's items. The second meeting of the Subgroup on NGTs is a continuation of the discussion initiated in May 2024.

Recognizing the importance of early dialogue, two case studies were presented to stimulate discussion: one on DNA-free genome edited (GE) grapevine presented by the University of Verona, and the second one on Italy's first field trial with GE rice by the University of Milan.

The agenda included an update from the European Commission (EC) on the current status of the legislative proposal on the regulation of plants obtained by NGTs, which is under review with amendments from the EC, EU Parliament, and EU Council.

Following these items, Germany (BVL) shared insights on their work and reflections on the proportionate and science-based risk assessment of plants obtained by NGTs. The last item in the agenda was an open discussion among the participants.

5. DNA-free genome editing confers disease resistance in grapevine

Abstract

Grapevine (*Vitis Vinifera* L.), is one of the oldest and most cultivated perennial non-climacteric fruit crops in the world. It is also a scientifically attractive crop for several aspects such as the high quality of its reference genome sequence and the presence of many valuable resources such as detailed transcriptomic datasets. However, it is generally recalcitrant to transformation and regeneration, and the application of the New Breeding Techniques is highly impaired by this limitation. We developed an

efficient protocol for the induction of embryogenic callus, the isolation of protoplasts (i.e. cells without wall), and the regeneration of whole grapevine plants. This protocol has been applied to different Italian and international grapevine cultivars demonstrating a clear varietal-specific recalcitrance to *in vitro* regeneration. Moreover, we demonstrated the possibility to obtain a transgene-free edited grapevine by the direct delivery of Cas9/sgRNA RNP complexes into protoplasts and subsequent regeneration.

It is well known that the vineyard system faces strong pest and disease pressures. Downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*) and botrytis (*Botrytis cinerea*) can cause major damage impacting the qualitative and quantitative characteristics of grapevine production. The *V. vinifera* cultivars grown in Europe in the 19th century were all susceptible to these pathogens and receive about the 40% of the fungicides used in the EU, despite covering only ~3% of the agricultural area.

To reduce susceptibility to pathogen attack and confer resistance to pests, we applied the DNA-free genome editing approach to induce targeted mutations in susceptibility genes, which biological activity seems to help pathogens to infect plants. We produced Chardonnay plants edited in *DMR6.1*, a downy mildew susceptibility gene, which showed a significant decrease of pathogen development after *in vitro* inoculation. We started the open field trials for Chardonnay edited in DMR6.1 at the end of September 2024, after receiving the authorization from MASE. Now we are evaluating the performance of edited plants in terms of resistance and productivity.

The success of DNA-free CRISPR/Cas9 application for the improvement of target traits establishes a foundation for promoting viticulture sustainability yet preserving the identity of the grapevine cultivars. The ongoing approval process of the New Genomic Techniques (NGT) legislation at the European level is a pivotal development. If approved, it would provide a clearer and more enabling regulatory framework for the use of transgene-free edited plants in agriculture. This anticipated shift could unlock new opportunities for innovation in grapevine breeding, supporting a more resilient, sustainable, and competitive European viticulture.

Literature

Bertini E, D'Incà E, Zattoni S, Lissandrini S, Cattaneo L, Ciffolillo C, Amato A, Fasoli M, Zenoni S. Transgene-free Genome Editing in Grapevine. Bio Protoc. 2025 Feb 20;15(4):e5190. doi: 10.21769/BioProtoc.5190. PMID: 40028030; PMCID: PMC11865842.

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Nerva L, Dalla Costa L, Ciacciulli A, Sabbadini S, Pavese V, Dondini L, Vendramin E, Caboni E, Perrone I, Moglia A, Zenoni S, Michelotti V, Micali S, La Malfa S, Gentile A, Tartarini S, Mezzetti B, Botta R, Verde I, Velasco R, Malnoy MA, Licciardello C. The Role of Italy in the Use of Advanced Plant Genomic Techniques on Fruit Trees: State of the Art and Future Perspectives. Int J Mol Sci. 2023 Jan 4;24(2):977. doi: 10.3390/ijms24020977. PMID: 36674493; PMCID: PMC9861864.

Bertini, E., Tornielli, G.B., Pezzotti, M. et al. Regeneration of plants from embryogenic callus-derived protoplasts of Garganega and Sangiovese grapevine (Vitis vinifera L.) cultivars. Plant Cell Tiss Organ Cult 138, 239–246 (2019). Https://doi.org/10.1007/s11240-019-01619-1

Discussion

Finland (Finnish Food Authority) asked whether the process could be considered haploid breeding if the starting material was pollen, and whether the NGT grapevine differs from the parental lines beyond the edited loci. The presenter clarified that the transformation was performed using protoplasts—not pollen—derived from the same genetic background as the original variety. Denmark (DTU) asked whether off-target mutations were assessed only at predicted target sites or across the entire genome. The presenter explained that the whole genome sequence of the edited plants was used as a reference to identify off-targets at predicted sites. Additionally, mutations elsewhere in the genome, potentially caused by *in vitro* regeneration or field growth, are under investigation, although data are not yet available. Ireland (Food Safety Authority) asked how the desired trait was selected in the transformants. The presenter clarified that no selection marker was used. Instead, protoplasts transfected with the Cas9 complex were screened after 48 hours to identify those carrying the desired edit, from which plants were subsequently regenerated. Spain (Polytechnic University of Valencia) shared that their research group had targeted the same gene in tomato, observing a similar increase in salicylic acid in the edited plants. Spain then asked whether any pleiotropic effects were observed. The presenter confirmed that no pleiotropic effects were detected in either the DMR6.1 edited plants or the double mutants in greenhouse conditions. However, further investigations will be conducted during field trials in the vegetative growth phase.

6. Ris8imo: The first NGT field trial in Italy

Abstract

Our research group at the University of Milan investigates the developmental biology of rice. With the advent of CRISPR/Cas9 technology, we began generating targeted mutants to address key biological questions. Given the molecular characteristics of the mutations generated by CRISPR/Cas9, we questioned whether such genome edited plants could be cultivated in open fields, similar to those plants produced via random mutagenesis. In 2016, we sought clarification from the Italian Ministry of the Environment, which deferred to the European Court of Justice. The 2018 ruling clarified that CRISPR-edited plants fall under the EU GMO regulation under Directive 2001/18/EC.

In 2023, a gradual shift in public perception toward genome-edited crops—referred to in Italy as TEA (Tecnologie di Evoluzione Assistita)—combined with the impact of recent severe weather on agriculture, led to the authorization of TEA field trials under Article 9-bis of the so-called Drought Decree.

Our research group requetsed authorization for a field trial involving CRISPR/Cas9-edited Arborio-type rice lines with deletions in three putative susceptibility genes to increase resistance to rice blast disease. The application, reviewed by ISPRA, was approved in March 2024, and the field was planted in May 2024. However, the trial was compromised by vandalism in June 2024. Despite this, sufficient seed was recovered to continue the research. In 2025, we secured authorization for three new field trials in Lombardy and Piedmont, now supported by enhanced security measures. This work represents a significant step toward the practical evaluation of genome-edited crops under real-world conditions in Italy.

Discussion

The participants highlighted the shared challenges in setting up field trials and emphasized the importance of protecting scientific work and investments from acts of vandalisms. The presenter agreed that more should be done to safeguard these efforts. The speaker also emphasized the effect of the public disclosure of the coordinates of the field trials which makes them very vulnerable to act of vandalisms. Denmark (DTU) informed that field trials with genome edited potatoes have been authorized but the coordinates of these field trials were not made public. Belgium (Sciensano) also confirmed that disclosing the field trials' location is not mandatory in Belgium and asked whether the Italian regulatory requirements for field trials can be shared after the meeting. The speaker agreed to share the information following the meeting, while noting that the regulatory requirements for field trials apply to GMOs in general and are not specifically tailored to NGT plants. In addition, Belgium, Denmark and Spain expressed their willingness to share their experience with field trials authorization following the meeting. The Netherlands (Wageningen Food Safety Research) asked what type of experiments are planned with the available harvested seeds from the previous trials. The speaker explained that the new filed trials are meant to reproducing the previous ones, with the aim of measuring the lesions' length in leaves caused by the fungal infection. Essentially, the experiments will aim at assessing the resistance of the edited plants to fungal infection compared to control. Additional parameters will be also evaluated to assess the overall impact of the edited loci on the plant physiology.

7. Commission NGT legislative proposal: state of play

Abstract

EC provided an update on the EC proposal for a new regulation of plants obtained by NGTs. The Council of the European Union adopted a negotiation mandate on March 14, 2025, while the European Parliament adopted its position on April 24, 2024, and later confirmed by the newly elected one. EC gave an overview of the main amendments introduced by the Council and the Parliament.

EC also informed that the trilogue negotiations, which involve the Council, Parliament, and Commission, have recently begun. A political trilogue took place in Strasbourg, establishing a commitment from both legislative bodies to work constructively. This was followed by technical trilogues, where experts began reviewing the legislative text article by article to identify areas of agreement.

Discussion

The Netherlands (Wageningen Food Safety Research) raised a question about whether safety and risk assessment are still central to the debate on NGTs, given the current focus on issues like patents and labelling.

EC confirmed that safety remains a key part of the legislative process. This is reflected in Annex II of the Commission's proposal, which outlines criteria for adapted risk assessment. This annex builds on existing EU legislation, Directive 2001/18/EC and Regulation 1829/2003, and incorporates EFSA's scientific work. It does not change the core principles but clarifies how assessments can be adapted based on plant traits, environmental context, and other factors.

Ireland (Food Safety Authority) asked why the Council wants to exclude herbicide-tolerant plants from Category 1 under the proposed NGT legislation. EC explained that the Council believes these plants require risk assessment and monitoring, thus placing them under Category 2.

EFSA asked what the next steps would be in case the legislative proposal moves forward. EC reminded the crucial role that EFSA and the scientific community have played in shaping the proposal, particularly in providing the scientific foundation for its safety and risk assessment framework. If the final legislation remains close to the current version, the next phase will involve developing implementing acts and guidance documents. EC stressed that this upcoming work requires the continued support of the scientific community. EC encouraged the EFSA subgroup on NGTs to stay engaged and contribute their knowledge to help shape the practical application of the new rules.

Czech Republic (Charles University) expressed concerned on the fact that Annex I of the proposed legislation was not discussed with the Network experts. This would have been important since Category 1 NGT plants would not undergo risk assessments, therefore the definition of this category becomes critical. In addition, Czech Republic asked whether the current criteria, which allow for long insertions or substitutions, truly ensure equivalence with conventional breeding methods and provide sufficient safety. In response, EFSA clarified that regulatory decisions are made by risk managers, and that the role of the scientific group is to support the implementation of decisions, such as ensuring verification procedures are robust and fit-for-purpose. EC added that while scientists were not directly involved in drafting Annex I, the criteria were based on scientific literature, EFSA's work, and JRC reports.

The Netherlands (Wageningen Food Safety Research) asked about the potential for similar legislative initiatives on animals and microorganisms, beyond plants. EC noted that EFSA is engaged in mandates covering both animals and microorganisms developed by NGTs. EC also mentioned the Biotech Act (link provided below), a broader Commission initiative currently under development. A study is underway to assess biotechnology applications across sectors, including agri-food, and to identify regulatory challenges. However, the final content of the Biotech Act will depend on the study's findings and further data collection.

LINK to EC Biotech Act: **https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/14627-Biotech-Act_en**

8. Considerations for developing a proportionate and science-based risk assessment of NGT plants in the light of the Commission's proposal

Abstract

The objective of the Commission proposal is to establish an appropriate and proportionate risk assessment for products produced using new genomic techniques. Existing resources, including existing guidelines for genetically modified plants, are not suitable for this purpose. This is because they assume a fixed hazard, namely that recombinant DNA is integrated into the genome. This results in various pathways to harm, with corresponding test hypotheses that have been incorporated into the existing requirements. It is evident that the majority of these are not applicable to

the NGT plants. Therefore, a divergent approach must be adopted given the hazards associated with the specification of these plants and the modifications introduced. The Commission's draft already provides a framework in which the risk assessment should take place. The fundamental structure within which the risk assessment is to be conducted is delineated in Annex II. It defines mandatory and optional data requirements, whereby a plausible test hypothesis, based on identified hazards, must be available as a prerequisite for requesting further optional data. In order to facilitate the navigation of the risk assessment process, the Annex I could be utilized to derive hazards from the criteria for NGT plants Category 1 and to define certain "risk categories", as these criteria essentially express concern about the risks that might be associated with certain modifications. On this basis, it is possible to derive a flow chart for a case-specific risk assessment of NGT plants of Category 2, whereby the principal elements of Annex I and II are reflected. The fundamental element is mandatory specific molecular characterization, which is adapted to "risk categories" derived from the criteria set out in Annex I. Decisions regarding data requirements and the results of this characterization are fundamental to the subsequent risk assessment, as they establish a plausible risk hypothesis in the event that a hazard is identified.

Discussion

The Netherlands (Wageningen Food Safety Research) asked whether molecular characterization in the proposed risk assessment framework includes bioinformatics screening for similarities to toxins and allergens, suggesting this could be an early step before *in vitro* or *in vivo* testing. The speaker confirmed that such analysis depends on the type of genetic modification. For example, if a protein is knocked out and no new protein is expressed, such data may not be necessary. However, if a new or modified protein is expressed, then sequence comparisons with toxin/allergen databases would be required.

Belgium (Sciensano) agreed that applying the regulatory framework designed for transgenic plants to gene-edited ones is problematic, as the two differ significantly at the molecular level. This mismatch has already caused challenges in evaluating field trials notifications with NGT plants, where existing guidance for transgenic plants has proven to be inadequate. Belgium emphasized that the EFSA’s problem formulation approach, already embedded in the Commission’s proposal, offers a flexible and science-based method for assessing risks. Belgium questioned the necessity of developing detailed risk profiles, especially given the existence of a flexible (problem formulation) approach and the low likelihood of receiving many Category 2 NGT plants applications. Further, Belgium noted that the purpose of Annex I, is to set criteria to determine molecular similarity of a NGT plant with a conventionally bred or naturally occurring plant, not to express concern about risks associated with certain modifications. However, as Annex I combines molecular criteria with safety criteria, the purpose of this Annex is confusing and unclear.

France (ANSES) noted that Annex I and its equivalence criteria were not discussed with the GMO Network experts in the Subgroup on New Genomic Techniques, even though they are central to defining Category 1 NGT plants, which will be exempt from risk assessment; such a discussion would have been useful to inform the debates that are still taking place in the trilogue. Regarding the proposal to build on Annex I criteria to develop a proportionate risk assessment method for NGT2 plants, France agreed with Belgium on the confusion in the goal of Annex I criteria: equivalence or

safety. Commenting on a remark from the Commission that the criteria were thought to be "conservative", France questioned the assumption that the number of modifications, or the size of insertions/substitutions, correlates with the level of risk, irrespective of the nature of the modifications. In that respect, France cited EFSA's 2022 opinion emphasizing that the assessment should focus on the resulting sequence of the modified allele, its function, the trait that is associated with it, the history of safe use and be on a case-by-case basis. France reminded the group the ANSES's 2024 opinion, which proposed a comprehensive risk assessment framework for plants developed through targeted mutagenesis using CRISPR-Cas9. Although cisgenic plants were not addressed as such, the framework applied broadly in a graded approach, regardless of the plant category defined in the Commission's proposal. Key elements were the use of prior knowledge, including history of safe use, trait-specific risks, and considerations on the species. France noted that gene editing allows for a broader range of species to be modified compared to transgenic methods.

The presenter expressed agreement with the points raised, particularly on the ambiguity of Annex I and whether it serves as a management or safety tool. They noted that if a plant doesn't meet Annex I criteria, it must undergo risk assessment, highlighting the safety implications. The BVL team is working to translate Annex I criteria into modification categories to guide data requirements. The presenter emphasized the importance of the problem formulation approach, which is well integrated into the proposed risk assessment framework. The presenter also hoped that Annex II remains unchanged in upcoming negotiations, as it currently provides a solid foundation for risk assessment.

9. Open discussion

Denmark (DTU) raised a question about the process for verifying that an NGT plant qualifies as Category 1. They emphasized the importance of applicants providing solid evidence to support this classification and asked whether EFSA plans to develop specific guidance for applicants. Such guidance would help both applicants and risk assessors understand what data is needed. Denmark mentioned the enzyme sector, where clear application guidelines exist, and suggested that similar structured guidance for Category 1 NGT plants would be valuable for ensuring consistency and clarity in the risk assessment process. EFSA explained that if a new regulatory framework is introduced, implementing acts will be needed to define specific requirements. These acts are typically supported by EFSA guidance to help interpret and apply the rules effectively. EFSA also reminded that a key lesson from IR 503/2013 is that when guidance documents are integrated into regulations, this can limit flexibility which is needed for scientific risk assessments, especially in rapidly evolving fields. Unlike regulations, EFSA guidance can be updated with new scientific knowledge, making it more adaptable and effective over time. Denmark (DTU) asked whether the Subgroup on NGTs would be involved in this activity. EFSA clarified that guidances are EFSA or panels outputs, collaboration with the Subgroup on NGTs is important.

The Netherlands (Wageningen Food Safety Research) reflected on the history of safe use and off-target effects in gene editing. It was emphasized that while molecular data is crucial, phenotypic traits remain essential in evaluating plant safety. In plant breeding, off-target effects are often removed during trait developments. However,

this process is less straightforward in animals and microorganisms. The Netherlands also stressed that breeders play a key role in identifying unintended effects, making phenotypic assessment an inherent part of plant risk evaluation.

EFSA reminded about the ongoing mandate to conduct a scientific literature review on new scientific data on NGTs relevant for risk assessment. A protocol has been developed and is now open for public consultation. The aim is to gather feedback, test the protocol through a pilot run, and eventually publish two reports per year based on the relevant scientific publications retrieved by the literature search. The review process includes identifying high-quality studies and assessing whether they provide new evidence that might affect the conclusions of previous EFSA opinions. EFSA encouraged contributions from the group; further updates will be communicated at the next meeting.

France (ANSES) asked clarification on the next steps of the literature review process, whether the assessment of selected articles would be conducted solely by GMO Panel experts or if there would be a call for volunteers to assist in the evaluation. EFSA clarified that the work will be done by EFSA supported by GMO Panels and its working groups if needed, with a possibility to recruit additional experts with relevant expertise for possible cross-cutting WGs. It involves a formal procedure, including evaluating scientific expertise and potential conflicts of interest, with final approval by the head of the NIF unit. The immediate focus is on finalizing the protocol and gathering feedback through public consultation. Pilot runs will help determine the relevance and volume of the literature retrieved. The protocol outlines detailed inclusion criteria, such as language, publication date and publication types, and feedback on it is strongly encouraged.

The Netherlands (Wageningen Food Safety Research) asked for clarification on the scope of the literature review, specifically whether it includes genetically modified or gene-edited cell cultures used in food production (e.g., cultivated meat, cocoa, or coffee cells) and microorganisms like gene-edited bacteria or endophytes that interact with plants, such as nitrogen-fixing root colonizers. EFSA clarified that the literature review will focus only on topics already covered in existing EFSA opinions. For example, in case of GM animals, only organisms within the defined scope of previous assessments will be considered. The aim is not to expand beyond the original mandates.

10. End of the meeting

The Chair thanked the participants for their active participation and the fruitful discussion. The Chair informed the participants that the next meeting may be organized in EFSA in Autumn. The meeting ended at 13:00.