European Commission

# 34th ENGL Plenary Meeting

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Joint Research Centre

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# 34th ENGL Plenary Meeting
# 27-28 September 2023

# Report

## 1. Welcome of the Chair

The chair welcomed the participants present in the room and connected online; the agenda (Annex 1) was approved.

## 2. Clarification of the NGT proposal (SANTE)

A representative of DG SANTE presented the Commission proposal on plants obtained by certain new genomic techniques (NGTs), namely targeted mutagenesis and cisgenesis/intragenesis. She gave an overview of the objectives and scope of the proposal, covering the deliberate release, including the placing on the market, of NGT plants, NGT food and feed and other products containing or consisting of NGT plants. The proposal is underpinned by a thorough impact assessment supported by extensive consultations and scientific contributions by several bodies, in particular the European Food Safety Authority (EFSA), the JRC/EURL and the ENGL.

The proposal sets two distinct regulatory pathways for two categories of NGT plants, those equivalent and subject to similar rules to conventionally bred plants (category 1) and others that remain subject to the provisions of the GMO legislation with some adaptation as regards risk assessment and detection method requirements (category 2). The applicable provisions were presented as well as the criteria and the procedure to verify the equivalence to conventionally bred plants for category 1 NGT plants. Information on the prohibition to use NGT plants in organic production and on the programme for monitoring and reporting was also given.

Following the presentation there was a lively scientific discussion among participants, with questions and answers; it was commonly agreed that the proposal is a valuable ground for further advancement and that the role of the ENGL is central for the aspects related to the implementation of the future regulation.

## 3. New Genomic Techniques for a Sustainable EU Food System (JRC Seville)

The JRC takes care of various services across different sites; JRC Seville works on cultural, economic and agricultural aspects through guidelines, trade issues, socio-economic impacts, etc. Four years ago, the new Commissioner brought back the new regulation on NGTs, in relation to the Green Deal, itself covering:

- Climate package
- Sustainability
- Farm to Fork strategy (F2F)

- Food production, distribution and consumption
- Sustainability
- Food waste

The tasks given to the Unit in Seville was to understand the impact of those techniques, showing their potential for sustainability.

The speaker outlined the 2030 targets for sustainable food production:

- 50% less pesticides

- 50% less nutrient loss (soil fertility)

- 50% less antimicrobials (sales for farmed animals)

- 25% of land dedicated to organic farming

There is no clear definition of a sustainable food system, but rather a list of elements to which NGTs can contribute (e.g. reducing dependency on pesticides). The new NGT proposal in July 2023 proposes two categories of NGT plants, on which the case studies are focused.

The work done so far includes:

- First analysis in 2021: market applications of NGTs + public online dashboard
  - The conclusion is that there is a lot of potential around the world, for many traits (modified composition, biotic & abiotic stress tolerance, etc.).
  - 426 applicants for targeted mutagenesis, mostly in early R&D (70%), some in advanced (28%) mostly relying on CRISPR (70%).

- In 2022, an analysis of the literature (EU-SAGE database) showed that the large majority of research traits was based on the SDN-1 approach, confirming that the potential is there.

- Then, they looked for field trials (crops developed by the EU and supporting the sustainability of the F2F strategy). A case study was performed looking at how pesticide reduction could be achieved with the use of NGTs (focussing on potato and apple).

- Late-blight-resistant potato:
  - ✓ Context: using 1-3 stacked resistance genes, the study was based on field trials (2011-2015) data already published. Focus on 5 MS highly cultivating potatoes, and scenarios relying on 50% to 80% fungicide reduction.
  - ✓ Result: the study showed a median cost saving of 3-6% of the potato output value per hectare. This cost margin constitutes the incentive for farmers to cultivate these products; beyond, farmers will no longer economically benefit from them and will therefore not be willing to use them. In effect, consumers will not be aware of the pesticide reduction, thus not adding any premium price.
  - ✓ Conclusion: cisgenic varieties can contribute to pesticide reduction, but targeted mutagenesis can also contribute, by targeting host-susceptibility genes. The challenge is to affect these genes without affecting plants' yields.

- Also:
    - Same disease (early/late blight potato): CRISPR-SDN1 KO of 2-4 genes. 2020-2025 field trials in Sweden. Results will come soon.

Current genome-editing projects include:

- On the same day of the release of the NGT proposal, another proposal required the reduction of food waste by 10% in process/manufacturing and by 30% at retail and consumption. In this context, products are being developed in USA:
    - Non-browning bananas (pre-commercial stage)
    - Non-browning lettuce (field trial stage)

- Social and health dimension. For example, low-gluten wheat for people with celiac disease (0.2-2.4% prevalence, but wheat sensitivity can go up to 13%):
    - Using a KO of alfa-gliadin family developed in Spain and in the Netherlands). For more details, see the report on the socioeconomic impact of low-gluten celiac-safe wheat developed through gene editing.
- Low asparagine, low acrylamide wheat (in UK), field trial level.
- Elimination of pungency in mustard green (Brassica juncea). Developed in the US with CRISPR, field trial stage.

4. Detection of CIBUS canola (Sophia Edelmann, BVL, DE)

In 2020, a publication on the detection and quantification of genome-edited plant (Chhalliyil *et al.*) drew the attention of several non-governmental groups. The ENGL promptly reacted and showed that the publication did not fulfil the MPR criteria. As a result, the authors published a correction and since then, a follow-up was made.

In the correction, the authors stated that Cq-values between 32 and 38 were considered inconclusive and needed to be confirmed by 12 Sanger-sequenced replicates. The ENGL is of the opinion that these results need to be compared with results of positive/reference samples.

The authors also pointed to a possible insufficient purity of DNA extracts. To fulfil MPR criteria, DNA extraction protocols need to be specific for different food/feed matrices, ensuring that method specificity is not affected by extraction procedures

In performing experimental testing, the ENGL used different DNA extraction procedures to dismiss the authors' assumption that "G50 purification could increase DNA extraction specificity".

The conclusion was that the Chhalliyil method was not sufficiently robust.

BVL conducted some activities to develop methods using RT-PCR, digital PCR and sequencing.

The dPCR work focussed on an oilseed rape (OSR) SU-tolerant line (40 K). The primers were designed in close proximity to variants of AHAS I and III. Genomic DNA from wild-type (WT) was used to compare with 40 K showing clear differentiation between negative and positive signals. However, differentiation between 40 K and Clearfield was challenging at asymmetric amounts of 40 K (low) and Clearfield (high).

For the validation of this method, LOD, LOQ, linearity, dynamic range, precision and trueness were assessed and fulfilled the MPR requirements. Regarding specificity, requirements were fulfilled for conventional and modified OSR GMs except for Clearfield-OSR. Therefore, the use of an additional method to identify Clearfield was proposed to address this issue.

5. Working Group DNAex (DNA extraction) – Progress update and key outputs (M. Burns, individual expert, UK)

Food samples consist of complex matrices and different categories of ingredients, for example fat, protein, carbohydrates. The ingredients define which DNA extractions are most appropriate to use. In addition, different ingredients, for example vegetables, cheese, etc. present problems with solubilisation, viscosity, and presence of enzymes.

A need for guidance on DNA extraction was identified prior to 2017. In 2017, a EURL training workshop allowed to share knowledge and collective expertise. Different examples of issues were discussed and solutions and troubleshooting were proposed. At the workshop 14 examples were presented and many shared issues were identified.

DNA extraction can be done with different techniques, kits and methods; kits are useful, easy and quick to use but also present challenges to troubleshoot and further validation in case of change of the kits as companies do not provide all the information. Laboratories apply different approaches and common troubleshooting tips.

DNA extraction methods are difficult to harmonise, but the rationale can be, e.g. providing guidance on their selection and use.

The DNA extraction working group was established in 2018. One of the tasks was to create a web space form to share information and foster harmonisation.

The web space development consists of a database fully searchable where the user can look into specific techniques, issues with methods and protocols.

The working group also developed a guidance document, aiming at reviewing methods, protocols and guidance, including literature to identify most common methods, for example extraction methods according to matrix type; dedicated chapters include information on methods validation and verification, assessment of DNA quantity and quality, Decision Support Systems.

6. **ENGL document "Sequencing strategies for the traceability of GMOs** - methods and **related quality aspects" (X. Zaoui, JRC)**

The work of the working group on sequencing strategies on traceability of the GMOs was reported.

The work was initiated in 2017, the mandate was (i) to assess sequencing data/results quality for GMO detection and (ii) to define minimum performance parameters (MPP), the latter eventually deemed too ambitious, thus translating into guidance for future work.

The work was prompted by the increasing demand and emerging challenges: more GM events on the market, unknown and unauthorized GM events, NGT products that present additional challenges with several mutations in one sample and single nucleotide variations difficult to spot with qPCR.

Different solutions are proposed such as multiplex PCR and development of tailored primers and probes; and different sequencing approaches.

Focus of the report is massive parallel sequencing with two types of outputs: long reads and short reads. The difference (compared to Sanger sequencing) is having millions and billions of fragment sequences simultaneously, better coverage, length beyond 300 Kb with long reads sequencing. The strategy is more tailored to whole genome sequencing (WGS), but very costly, at the moment only microbe genome is targeted for WGS. Other strategy is short reads reading which is highly accurate but at the cost of the length (50-300bp); it is suitable for metagenomics or transcriptomics but still with the limitation of rather high costs.

The report contains an overview of all current techniques and different parameters, e.g. new techniques for massive parallel sequencing, including sample extraction and preparation, template amplification and DNA sequencing; workflow and pipelines, different parameters supported by literature and how to assess the result of sequencing; discussion on storage and computed power; wide range of different strategies and methods which call for harmonisation and data comparability: list of recommendations and suggestions to continue this work for MPR; good practice: data storage and quality of data, for example on DNA prep, library synthesis, sequencing reaction, primary, secondary and tertiary data analysis and confirmation of positives.

The report continues with the explanation of different scenarios (e.g. is there prior information on the kind of sample?), challenges and conclusions and future needs (e.g. harmonisation of performance parameters, develop kits for ease and repeatability, open source pipelines for data analysis)

### 7. Detection methods for checking authorized and unauthorized genetically modified (GM) events in India (M. Singh, Regional GMO network, IN)

In India more than eleven million hectares are dedicated to GM crop cultivation, and four GM events of Bt GM cotton have been approved and imported in the country.

India has a stringent regulatory framework, GMO have to get approval for biosafety issues after a thorough assessment.

The National Bureau of Plant Genomic Resources provides molecular testing services for imported materials that are allowed in the country for conducting research after a technical clearance; the major role of the Bureau is to manage and promote sustainable use of agri-horticulture crops and GM detection technology research.

Labelling in India is regulated by the environment protection act covering GMO and genome-edited plants; every imported consignment of 24 selected crops should be accompanied by a non-GMO certification and the tolerance limit for the presence of GMOs at 1%;

For the import of transgenic plant materials, quarantining and molecular testing are required; tests are conducted for different viruses, fungi, bacteria and other pathogenic organisms, and targeting GM screening elements (e.g. terminators) and more specific transgenic elements.

The GM detection facilities are in compliance with ISO and national regulations and are capable of conducting PCR-based tests to support certifications and labelling.

DNA-based tests are developed and validated; also protein-based methods, mainly lateral flow strips, are used as rapid tests.

Relevant EU guidance is consulted for the implementation of the regulations and to ensure reliability of testing results; collaboration with ENGL partners is active, e.g. for the creation of GMO matrix and decision support systems, implementation of multiplex PCR methods (6-plex and 10-plex) and Loop-mediated isothermal amplification (LAMP) method.

The key challenges identified for the future are the increasing number of GMO to be identified, which requires more efficient detection strategies, the issue of unauthorized GMO, the availability of reference materials.

## 8. Current status and challenges of GMOs detection in Latin America and Caribbean region (RLAC-OGM) (N. C. Castanheira Guimarães, Regional GMO network, BR)

The network of GMO laboratories in Latin America and Caribbean regions encompasses over 42 countries. The network was initially fostered by the ENGL but the pandemic had an impact on the activities and now the network is restarting. A survey in 2017 identified as main needs capacity building, GMO analysis, risk analysis and networking.

Past activities on the RLAC-GMO included participation to a study tour at the JRC Ispra, participation to regional workshops on GMO analysis, participation to trainings organised by the JRC.

Proficiency testing rounds were organised by laboratories in Brazil; the production and stability assessment of materials were performed; the study required testing for identification and quantification of GM events and screening element.

Recently a survey with the network was conducted to verify how the work can be resumed and review the state of the art perspective; the survey was conducted in eight countries.

The main difficulties mentioned by the participants are CRM price and availability, cost of reagents, limited staff, limited budget for maintenance of ISO accreditation, implementation of analysis and proficiency studies for all GMO scopes; NGT products cannot be analysed as labs do not have the necessary knowledge and structure; also the broadening of the scope of the network to food fraud was suggested. A regional meeting will happen during the first trimester of 2024.

## 9. Capacity building initiatives in GM detection in Southern Africa and other challenges (C. Viljoen, UFS, Regional GMO network, ZA)

The network was established in 2009 with laboratories in thirteen countries in southern Africa. A number of issues were identified at the establishment of the network, e.g. lack of a suitable regulatory environment, insufficiently trained human capacity, lack of physical resources/laboratories, lack of access to affordable equipment and consumables.

The objectives of the capacity building activities carried out were qualitative and quantitative analysis of GMOs, quality management, procurement processes etc.; there is a need to reinforce training and develop additional capacities.

The implementation of common approaches is hindered by the fact that no regulatory system is in place in some countries, thus with no requirement for specific information (included sequence

information) to be provided by the technology developer. Risk assessment does not include event-detection methods. GM events are approved in South Africa with no available methods provided, e.g. soybean, GM weed for abiotic stress and herbicide tolerance.

In conclusion, the network is important to help supporting laboratories through engagement and information sharing.

## 10. Malicious uses of genetically modified micro-organisms: identification challenges (N. Roosens, K. Vannes**te, J. D'aes, Sciensano, BE)**

Micro-organisms and pathogens (bacteria, viruses) might be used to deliberately cause harm; they can be categorised (category A, B or C) based on their characteristics (e.g. mortality, morbidity, ease of dissemination) and can also be modified to make them more pathogenic. In this sense, the increasing access to modification technologies, first of all CRISPR-CAS, may pose a risk of GMM used for bioterrorism.

Therefore, there is a need for new tools and risk analysis methods to rapidly identify unnatural epidemics, including methodologies allowing detection of GMM, including gene-edited organisms.

Among the identification technologies, efforts are made to develop and implement approaches like whole genome sequencing of isolates and metagenomics.

Sciensano participated in proficiency tests (PT) activities on high throughput data, organised by the UN Secretary General's Mechanism (UNSGM). The main tasks of the first series of PT on bacterial isolates were species identification, species characterisation, detection of virulence factors, strain characterisation and interpretation; the sequencing data under analysis were produced by Illumina for 36 fictitious bacterial isolates. The results of the study show that while the bioinformatics workflows are easy to use, the bottleneck relies in the availability of suitable databases containing both sequence information and relevant metadata for interpretation. Moreover, the interpretation of the relevant information requires specific expertise on biological threats.

Sciensano also participated in a PT round taking as a model Monkeypox virus (mpox); the scope of the study was the fictitious severe outbreak of monkeypox, suspicion that hostile neighbouring country caused the outbreak deliberately. Data used were partly real, partly metagenomic sequencing data generated in silico; the tasks requested to the participants were identification, metagenomics analysis, genome characterisation, identification of most similar strain, identification and characterisation of irregularities.

While the scope of the study was well defined and the database provided, the PT on mpox was a complex case, with extensive bioinformatics hands-on time required. The study emphasised that even for well-studied organisms, the significance of mutations is often not well understood; the generation of suitable databases to screen for GM organisms is not trivial and requires extensive manual steps and expert knowledge.

## 11. Retrieving sequence of GMOs from public databases to widen screening approaches (M. Colaiacovo, JRC)

M. Colaiacovo presented a work that was carried out at the EURL GMFF, aiming at the identification of unauthorised GMOs in public databases. The availability of GMO sequences is crucial for developing new detection methods and improving screening strategies, but the retrieval of these

sequences from public resources is not a straightforward task. The search of unauthorised GMOs was performed using as a query a 25 bp sequence that is commonly found in GMOs obtained by Agrobacterium transformation. Through a bioinformatics pipeline, 27 GMO sequences were retrieved from the nucleotide (nt) and patent (patnt) databases maintained by NCBI, of which 9 unauthorised GMOs were not included in the internal CCSIS database maintained by the EURL GMFF. The analysis of these sequences revealed groups of GMOs sharing identical sequences surrounding the 25 bp target, which guided an extended search in public patents. This extended search, with the inclusion of sequences found in vector databases, resulted in an additional 7 GMO sequences being retrieved. As a proof of concept, a real-time PCR method targeting one of the GMO groups was tested successfully, showing that the bioinformatics search of public databases can help in the development of new screening strategies for control laboratories.

12. PlantEd COST action (D. Eriksson, SLU, SE)

COST provides networking opportunities for researchers and innovators in order to strengthen Europe's capacity to address scientific, technological and societal challenges.

The PlantEd COST action CA 18111 (https://plantgenomeediting.eu) is dedicated to plant genome editing, with focus on technical platforms, policy and regulations, communication and impact.

The PlantEd network grew in number of participants from 2018 to 2023, reaching up to 612 networking partners sharing experiences and collaborating; various activities were carried out, e.g. training schools for young researchers and short-term scientific missions. There was an active contribution to public consultations launched e.g. by the European Commission and EFSA; particular attention was given to communication (e.g. on YouTube) and to sharing expertise through scientific publications.

The results of a survey among partners of the network revealed that the large majority (> 80 %) felt that through the participation to PlantEd obtained new ideas and knowledge about plant genome editing, new connections and potential collaborations, and that PlantED contributes to the development of plant genome editing in Europe and beyond.

The experience developed with PlantEd will be used to move from networking to research, e.g. with the formation of a large consortium for a new Horizon Europe project starting in 2024.

# Annex 1. Agenda

| | Time | Topic | |
|---|---|---|---|
| 1<br>2<br>3 | 8:45<br>9:00<br>9:45 | ▪ Welcome of the Chair<br>▪ Clarification of the NGT proposal<br>▪ Discussion session: "What does the NGT proposal mean for ENGL guidelines?" | *Session restricted to ENGL members* |
| | *10:30* | *Coffee break* | |
| 4<br>5 | 11:00<br>11:45 | ▪ New Genomic Techniques for a Sustainable EU Food System (E. Rodriguez Cerezo, JRC Seville)<br>▪ Detection of CIBUS canola (S. Edelmann, BVL, DE) | |
| | *12:30* | *Lunch break* | |
| 6<br>7 | 14:00<br>14:45 | ▪ Working Group DNAex (DNA extraction) – Progress update and key outputs (M. Burns, LGC, UK)<br>▪ ENGL document "Sequencing strategies for the traceability of GMOs - methods and related quality aspects" (X. Zaoui, JRC) | |
| | *15:30* | *Coffee Break* | |
| 8<br>9<br>10 | 16:00<br>16:35<br>17.10 | ▪ Detection methods for checking authorized and unauthorized genetically modified (GM) events in India (M. Singh, IN)<br>▪ Current status and challenges of GMOs detection in Latin America and Caribbean region (RLAC-OGM) (N. C. Castanheira Guimarães, BR)<br>▪ Capacity building initiatives in GM detection in Southern Africa and other challenges (C. Viljoen, UFS, ZA) | |
| | *17:45* | *End of day 1* | |

| | Time | Topic | |
|---|---|---|---|
| 11<br><br><br><br>12 | 9:15<br><br><br><br>9:45 | ▪ Malicious uses of genetically modified micro-organisms: identification challenges (N. Roosens, K. Vanneste, J. D'aes, Sciensano, BE)<br><br>▪ Retrieving sequence of GMOs from public databases to widen screening approaches (M. Colaiacovo, JRC) | |
| | *10:30* | *Coffee break* | |
| 13<br><br>14 | 11:00<br><br>11:45 | ▪ PlantEd COST action (D. Eriksson, SLU, SE)<br><br>▪ AOB | |
| | *12:30* | *End of meeting* | |
| | | *Lunch* | |

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