European Commission

Report on the 31st ENGL Meeting

Webex meeting

30 September 2020

The European Commission's science and knowledge service

Joint Research Centre

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(Webex meeting)
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Summary Report

1) Welcome of the Chair

The Chair welcomed the participants and provided some technical instructions for the new online format of the meeting. He noted the impressive number of participants and asked all speakers to leave space for discussions after the presentation. He finally thanked all colleagues providing presentations, in particular external non-ENGL members for sharing their findings.

2) Genetic and genomic tools to develop insect pest control strains (M.F. Schetelig, Justus-Liebig University Giessen, DE)

The speaker presented an overview of classical genetics and transgenic techniques, site-specific recombination, site-specific genome editing and conditional expression systems for developing insect pest control strains. He also presented results of population studies to evaluate introgression of mutant/resistant survivors. He finally discussed some regulatory aspects of genome editing in different countries of the world.

Follow-up discussion

Participants were interested in the gene-drive dilemma of using or not the technology, its risk assessment, the advantages of using genome editing in comparison to chemical mutagenesis and the development of resistance in the modified strains. The speaker remarked that it is very expensive to perform studies on gene drives in Germany because they require safety measures at S3 level. He commented that very few of the approximately 3000 mosquito species are actually responsible for transmitting human diseases and that in his view the extinction of those few species should not cause ecological problems. He remarked that there is no difference between a mutation generated by CRISPR/Cas technology or a chemical agent.

3) An alternative strategy of targeted MinION sequencing of transgene (A.L Boutigny, French Agency for Food, Environmental and Occupational Health & Safety, FR)

The speaker presented an alternative strategy for sequencing transgenes and flanking sequences using only a screening element for the design of a long-range inverse PCR amplification step on circularised DNA fragments. The investigator employed a MinION sequencer for performing long-read sequencing and assembling the final contig sequence of the transgene. A GM petunia was used as a starting DNA material for testing the approach and further developing a detection method.

Follow-up discussion

Several participants requested technical details on the approach or information on different performance parameters. A participant suggested using border sequences of the Ti plasmid for providing a more universal amplification strategy for GMOs generated by *Agrobacterium tumefaciens*-mediated transformation (ATMT). The investigator reported not having determined the sensitivity of the approach given that target amplification strategies are able to amplify very

low amounts of DNA sequences. She remarked that the increased depth of sequencing of the target amplification approach overcame the high error rate of long-read sequencing and provided a high accuracy of the final sequence. Other long-range sequencing approaches should be equally effective.

4) Stress- and chemical induced mutations by endogenous transposable elements (for crop breeding) (E. Bucher, Agroscope, CH)

The speaker presented a strategy for controlled mobilisation of transposable endogenous (TE) elements in plants to generate new phenotypic traits and to avoid using transgenic techniques. They identified a heat responsive TE and obtained its high mobilisation in plant genomes by using stress heat conditions and two drugs blocking the epigenetic silencing of TE retro-transposition. The new mutant plants generated by TE mobilisation were further selected for the desired trait in appropriately designed field trials. The experimental approach was patented and is claimed to offer great potential as a rapid tool for breeding new varieties resistant to high temperature, drought, salt and new pathogens. The two drugs, alpha-amanitin and zebularin, do not have a history of long safe use in agriculture. According to the recent CJEU ruling, the products generated from this approach fall therefore in his understanding under the full application of EU legislation on GMOs. The speaker concluded by discussing the current definition of exemptions to GMO legislation, of mutagens having a history of 'safe use' and of naturally occurring genetic changes.

Follow-up discussion

Various participants supported views of the speaker. A participant expressed his opinion that EU regulations should be science-based and enforceable. He recognised that the distinction between mutations generated by classical mutagenesis or new mutagenesis techniques could be problematic and suggested reconsidering the EU legislation on GMOs. Another participant commented that the drugs described in the presentation were accelerating a naturally occurring process and questioned whether they should be considered as DNA mutagens.

5) PlantEd – COST Action CA18111 – Genome editing in plants (D. Eriksson, Swedish University of Agricultural Sciences, SLU, SE)

The speaker presented the aims, organisation, activities and deliverables of PlantED, a COST action fostering networking, training and communication on genome editing in plants. The research network funded by the EU COST Association includes 354 academic researchers, companies, farmer organisations, public officials and international organisations from 36 COST countries, 5 near-neighbour countries and 8 international partner countries. The speaker remarked that the network could offer informal discussions especially from different perspectives. It has provided input to an open consultation of the Commission on the Green Deal's farm-to-fork strategy and on public consultations of EFSA on the safety assessment of plants developed using site-directed nucleases. The network dedicated substantial resources on capacity building, training schools and support to young researches in less research-intensive countries. They have a website for communicating their activities and deliverables at https://plantgenomeediting.eu/. The network is open to collaboration or joint events and welcomes new proposals.

Follow-up discussion

The speaker specified that applications for participating to the network could be submitted to their website. He further remarked that the network is open also to colleagues not necessarily working on genome editing and that the COST action will be funded until 2023.

During lunch break: Informal room discussion on accreditation of dPCR methods

The Chair noted that a significant number of laboratories has experienced virtual audits by accreditation bodies in 2020. He asked a JRC representative to moderate the discussion on obtaining accreditation for digital PCR methods. The moderator requested the participants to share their experience regarding accreditation bodies and in particularly about the parameters used to assess for accreditation.

A representative from Belgium informed that their laboratory obtained dPCR method accreditation this year. They modified their flexible scope accreditation and extended it to all their PCR-based methods. They had prepared two validation dossiers for absolute quantification of GMOs. They followed the guidance of the ENGL on digital PCR and the advice and knowledge acquired during last EURL GMFF training workshop. They validated dPCR methods derived from qPCR screening methods based on Sybrgreen and decided to test the full spectrum of parameters, in particular the LOD, LOQ and MU. They used CRMs not certified for their target. The validation dossier was well accepted by the auditors. They prepared many quality documents and procedures describing the technical steps for the analysis and interpretation of the results. They used as a template the document distributed at the EURL GMFF training. They mainly use digital PCR methods for the quantification of the number of copies. For the event-specific dPCR methods the laboratory will evaluate the LOD, LOQ and MU parameters but not the specificity, because its validation is considered very labour intensive and not necessary.

A representative from the UK informed that his laboratory has a flexible scope accreditation for digital PCR analysis, not specific for GMOs, but for value assignment and quantification of extracted DNA. He found last year's training workshop and the guidance regarding the volume of droplets very useful. He recommended maintaining a fully trained technician for dPCR, stressed the importance of properly maintained dPCR instrumentation and suggested to use properly produced CRMs, SOPs and control sheets.

The moderator enquired whether the auditors requested the participation to dedicated PTs for dPCR.

The UK representative clarified that at the time of accreditation PTs on dPCR have not been offered. Now, PT providers, for example GeMMa, are becoming more aware on the use of both dPCR and qPCR and are starting to allow the submission of more than one result.

The moderator informed that the EURL GMFF laboratory in Geel, which is accredited for dPCR methods, had participated to a PT with a dPCR method and employed the conversion factor to convert the result into a mass fraction value. Then it used also qPCR and calculated the z-score they would have obtained.

The EURL GMFF laboratory in Ispra informed that the last surveillance audit was also used to extend the scope of accreditation from qPCR to dPCR, but the scope does not cover absolute quantification. The laboratory took advantage of the experience of their colleagues in Geel. They transferred the methods already accredited for qPCR to dPCR, verified the parameters that had to be fulfilled including evaluation of positive and negative droplets, and performed a validation study for a duplex dPCR. The auditor enquired on the CRMs used in the procedure, the number of replicates and the calculations performed. Another question concerned the assessment of the trueness of the measurement results, as CRMs with certified values for DNA copy number ratios are limited.

The moderator explained that the volume of the droplet is an important parameter that needs to be validated. He suggested starting by using a CRM and converting its certified value using the published table of conversion factors or by using a CRM certified for its absolute copy number (e.g. ERM-AD623). Their accreditation body tried to consider the chamber and digital PCR as two very different technologies but the auditees were able to convince the auditors that such a distinction is not necessary in the accreditation scope.

A representative from Austria informed that his laboratory has received an accreditation for dPCR for allergen testing but not for GMOs. They used CRMs provided by a company together

with their self-made reference material containing the allergens. They used the latter as spiking material to test selectivity and were able to get accreditation for quantifying a number of allergens in food. They were not enquired on some parameters mentioned previously such as droplet volume. The auditors were apparently not so familiar with the dPCR technology. They are using dPCR methods for a relative quantification and convert copies numbers/µl into mass using reference materials.

A representative from the EURL GMFF explained the procedure for measuring the volume of droplets. It is complex and not recommended for routine use. The droplet size is a crucial parameter for absolute copy number calculations, but a potential bias is not critical for determining copy number ratios. The variability of the droplet size during experiments under repeatability conditions does not seem to be higher than 2-3%, which is a small value in comparison to the other uncertainty components.

The moderator thanked the participants for their contributions and closed the lunch break session.

6) Strategy to detect GMM in fermentation products: application on the food enzyme market (N. Roosens, Sciensano, BE)

The speaker presented the genetically modified microorganism (GMM) detection strategy developed under the project SPECENZYM, a study of the purity of food enzymes (FE) funded by Belgian authorities. They identified from EFSA dossiers submitted for FE authorisation the bacterial and fungi species most highly represented. The Belgian laboratory developed PCR methods targeting the 16S rRNA bacterial gene and the fungi internal transcribed spacer (ITS) region to identify, by Sanger sequencing and blast analysis, samples that could potentially contain residues of those organisms. To develop first-line screening qPCR methods, they further selected from patent databases target sequences that are naturally not very much widespread (mainly *S. aureus*) and could cover the maximum number of GMM used to produce food enzymes. In addition, PCR nested assays were additionally developed for detecting and sequencing full-length AMR genes. They performed whole genome sequencing analysis on bacterial positive samples isolated from the screening of commercial protease products and DNA walking followed by sequencing from screening of alpha-amylase commercial products to provide analytical tools for GMM identification. Using the generated sequencing data, they developed two construct/event specific methods (for GM protease and GM alpha–amlylase, respectively), which are targeting the junction between the shuttle vector and the alpha-amylase or protease gene. A pilot monitoring study of commercial products revealed a considerable level of GMM contamination on the Belgium and EU food market. The speaker informed that a workshop on the subject will be organised for the 3rd of December.

Follow-up discussion

A JRC representative considered the findings alarming, and asked for a market control system of food enzymes. The speaker remarked that the number of samples was only 51. Therefore, it would be better to perform the analysis on a larger number of samples to confirm that the finding is reflecting a general problem. Another representative from Belgium agreed on the cautious interpretation of the results. The speaker further clarified that the samples were originating from different manufacturers (some have introduced dossiers to EFSA). However, the companies that have produced the enzymes via a GMM are not known. She explained that it is not known, if the multi-copy construct protease/alpha-amylase is present in the bacterial genome or in an autonomously replicating plasmid and it is therefore not possible to determine whether the detection method is event- or construct-specific. She explained that all the methods are publically available (for first-line screening and GM protease) and that the method targeting GM amylase will be submitted for publication soon and is at present available after signing a non-disclosure agreement. It was reminded that from the EU regulatory point of view products containing GMM traces fall under Regulation (EC) No 1829/2003.

7) Discussion on a qPCR method for Cibus® canola

The Chair recalled that an article was published on the 7th of September 2020 in the journal Foods (Chhalliyil et al.)[1] on the detection of a sulfonylurea (SU) herbicide-tolerant oilseed rape (OSR) line from the company Cibus. A press campaign and public debates have immediately accompanied the publication of the results. The publication contains also statements about the ENGL report on the detection of food and feed plant products obtained by new mutagenesis techniques published in March 2019[2]. Therefore, the JRC has added this discussion topic to the agenda of this ENGL Meeting. The EURL GMFF had elaborated a document with first observations, which was sent to all ENGL members in preparation of this agenda point at the plenary meeting. He informed that a colleague of the EURL GMFF had performed a quick bioinformatics analysis on the specificity of the published OSR detection method and requested to present the preliminary results.

The speaker provided some background on the DNA sequences used by the authors for the development of their detection method for the SU OCR line. The EURL GMFF performed blast analyses with the primer sequences and found a perfect alignment of the forward primer oligonucleotide with sequences of other Brassica species, such as *Brassica napus*, *B. oleracea* and *B. carinata.* When using the reverse primer, all alignments except one presented a single mismatch at the last primer base, the SNV targeted by the method. *In silico analysis* of the amplicon sequence presented two mismatches in retrieved *acetolactate synthase 3* gene sequences and a mismatch for the last base pair of the reverse primer except in four cases. Among the conclusions are recommendations that the specificity of the method should be tested experimentally with respect to *R. raphanistrum* and that the presence of the targeted mutation in wild populations of *R. sativus*, *R. raphanistrum* and other weed species with high similarity to the annealing sites of the primers and probe of the published detection method cannot be excluded.

The Chair suggested arranging a joint ENGL statement, also because the article questioned some conclusions of the ENGL report mentioned above. This proposal was strongly supported by many meeting participants. Therefore, the Chair proposed to distribute a first draft to all ENGL members on the following day. The draft would follow the lines highlighted in the preparatory document and would include the comments made during this session. He requested a thorough review of this draft by all ENGL members with a feedback by Friday afternoon. Afterwards the revised draft could be submitted as final ENGL statement. The participants expressed appreciation for the proposed drafting process.

DG SANTE confirmed the importance of such a statement and urged a prompt reaction. They specified that two questions needed to be answered: (a) if the method could be used for enforcement of EU legislation on GMOs and (b) if the article was challenging the published ENGL report on the detection of food and feed plant products obtained by new mutagenesis techniques.

The meeting participants agreed to attach the final ENGL statement to the summary report of this ENGL meeting, thereby publishing it on the publicly accessible ENGL webpage.

8) Rapid response in emergencies (P. Corbisier, JRC)

The Chair welcomed to the meeting the acting Director of JRC's Directorate *Health*, *Consumers and Reference Materials*. The Director expressed his appreciation for the work of the ENGL that continues to be challenging, which is also reflected by the interesting subjects of today's meeting.

[1] Chhalliyil, P.; Ilves, H.; Kazakov, S.A.; Howard, S.J.; Johnston, B.H.; Fagan, J. A Real-Time Quantitative PCR Method Specific for Detection and Quantification of the First Commercialized Genome-Edited Plant. *Foods* **2020**, *9*, 1245. https://www.mdpi.com/2304-8158/9/9/1245

[2] https://gmo-crl.jrc.ec.europa.eu/doc/JRC116289-GE-report-ENGL.pdf

A JRC colleague provided an overview on four main COVID-19 related activities initiated at the JRC during recent months:

1) Development and distribution of a positive control material for COVID-19 PCR testing;
2) Establishment of a status report on the performance of available COVID-19 test methods and of performance criteria for their evaluation;
3) Development of a web database with updated information on COVID-19 test devices, detected analytes and related scientific literature. The application is also offering a dynamic interface for identifying the target region on the virus genome;
4) Evaluation of new detection assays based on CRISPR/Cas technology by using positive and negative clinical samples.

9) Discussion on activities related to SARS-CoV-2

Several participants requested information on mutations in the virus target regions, the specificity, robustness and sensitivity of the CRISPR/Cas assays and a potential contamination of the samples during the analysis. The speaker explained that the specificity had been successfully tested by the method developers on all types of human SARS viruses and that the sensitivity of the method still need to be validated on clinical samples with low copy numbers of SARS-CoV-2. He further remarked that the reagents and target analytes of the assay are well described.

The Chair noted the very impressive list of COVID-19 related initiatives, which have been collected at the EURL GMFF workshop with the NRLs and requested if other ENGL members are planning similar initiatives on this topic. A representative from Slovenia mentioned her involvement in a European project monitoring the virus in wastewaters. The laboratory had also applied for national funding and will then continue the work. A representative from a Belgium laboratory informed about the coordination of three similar activities.

The Chair suggested sharing information and contacts on such activities.

10) AOB

The JRC Director present informed that the JRC is retrieving SARS-CoV-2 sequences to verify if primers and probes of the test methods could still recognise their target region on the virus genome. He remarked that the COVID-19 initiatives are a clear example of the strength and expertise of the ENGL network.

A representative from the UK mentioned the involvement of his institute in the development and validation of COVID-19 RNA and serological tests, the development of vaccines and reference material developments for antibody tests and the organisation of PT schemes. He expressed interest in future collaborations.

Wrap-up

The Chair acknowledged the smooth running of this first virtual ENGL Plenary Meeting and that technical issues did not influence the progress of it. The organisers are encouraged by the overall positive response and by the lively exchange of scientific information and opinions. He reminded that the progress of the WGs was not discussed at the meeting, however the WG deliverables will be in the focus of the following months. The new WG on GMM will now start its activities and various other guidance documents will need to be finalised. He reminded that a draft joint ENGL statement proposal on the OSR detection method would be submitted on the following day and kindly asked to suggest modifications by Friday so that a timely final response could be provided.

The Chair thanked the organisers of the event and the participants for their active contributions. He indicated that it is not possible to predict how the following ENGL meeting would be organised and recommended to remain active and careful.

Annex

Evaluation of the scientific publication *"A Real-Time Quantitative PCR Method Specific for Detection and Quantification of the First Commercialized Genome-Edited Plant"*, P. Chhalliyil *et al.* in: Foods (2020) 9, 1245, by the European Network of GMO Laboratories (ENGL)

Meeting documents available at:
https://englnet.jrc.ec.europa.eu/31stENGLmeeting/default.aspx?InstanceID=1

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