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Explanatory Note

Challenges for the detection of genetically modified food or feed originating from genome editing

EU Reference Laboratory for Genetically Modified Food & Feed (EURL GMFF)

in consultation with the European Network of GMO Laboratories (ENGL)

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Contents

Executive summary

The recent ruling of the European Court of Justice has confirmed that organisms obtained by mutagenesis techniques are genetically modified organisms (GMOs). However, in contrast to organisms originating from conventional mutagenesis techniques, those obtained by new mutagenesis techniques are not exempted from the obligations of the GMO EU regulatory framework. This ruling raises questions about the detectability of the corresponding GM food and feed products.

A case study is used in this document to explain and discuss possibilities and limitations for the detection and quantification of (known and unknown) genetic modifications in plant products derived from new mutagenesis techniques.

Many of the mutations induced by new mutagenesis techniques cannot be unequivocally distinguished from natural mutations because such genome editing technologies are able to create very precise and limited genome changes that mimic the result of potential naturally occurring mutations. Moreover, mutations obtained by genome editing technologies could also not be differentiated from those introduced by conventional mutagenesis techniques which have been incorporated in traditional breeding programs and are often not thoroughly documented.

Products of genome editing could only be detected and identified in imports of commodity products by enforcement laboratories when prior knowledge on the altered genome sequence, a validated detection method with appropriate selectivity and certified reference materials are available, similarly as required for the authorisation of current transgenic GMOs.

However, when the modification involves only a SNP or few nucleotide changes, it would not be possible to identify whether the mutation originated spontaneously or was induced by conventional or new (genome editing) mutagenesis techniques. Moreover, it is unlikely that methods for the quantification of GMO products with small genome modifications in complex food or feed materials provide the level of selectivity needed for the enforcement of legislation, such as the one on labelling.

In the absence of prior knowledge on the genome-edited changes, it is likely that non-authorised genetically modified food and feed products obtained by genome editing would enter the EU market undetected. The EU control system for GMOs and corresponding food and feed products may not function as efficiently for unauthorised genome-edited products compared to transgenic GMOs. In particular, the principle of zero tolerance for unauthorised GMO on the EU market is more difficult to maintain.

1 Scope

The recent ruling of the European Court of Justice stating that organisms obtained by new mutagenesis plant breeding techniques are genetically modified organisms (GMOs) falling under the regulatory framework for GMOs has also raised questions about the detection of such products. This note intends to explain current and envisaged possibilities and limitations of laboratory approaches for the analytical detection of genome-edited crops and their food or feed products.

2 Scenario

A ship arriving at the harbour of Rotterdam is carrying 20,000 t of bulk grain maize from outside the EU. There is no declaration in the accompanying documents that the maize consignment is genetically modified.

The custom laboratory should check if the consignment does not contain 'GM maize'.

The experimental screening for the presence of GMOs, using a detection strategy targeting DNA sequences commonly inserted for transgene modifications, did not indicate the presence of GMOs.

However, since genome editing techniques[1] may have been applied to the grains, the questions to be answered by the laboratory are:

- Do the maize grains originate from 'natural'[2] breeding or from mutagenesis?

- In the latter case, have they been obtained by a 'conventional mutagenesis'[3] technique or by a genome editing technique?

3 Customs controls and EU GMO legislation

The forecasted volume of maize imported into the EU in 2018 is about 13.4 million tonnes[4]. Shipments of thousands of tonnes are thus headed to EU harbours, frequently to Rotterdam, where they await clearance for downloading the commodity.

Bulk grain that arrives in a harbour, and similarly any food or feed product produced from it, is a compound product composed of different source materials, including crop varieties with different genetic backgrounds, cultivated by various farmers in various regions of the world and present in different proportions.

Verification of compliance with the EU food and feed legislation is achieved through a mixed system of document traceability and laboratory testing[5]. According to EU

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1 Genome editing encompasses a set of new breeding techniques, including *e.g.* oligo-directed mutagenesis and CRISPR-Cas, which result in specific targeted alterations in the genome of an organism without introducing foreign DNA.

2 Natural breeding refers to mating or natural recombination between plants within a species. It is the traditional mechanism for altering the genetic material of a plant, resulting in new varieties of plants for horticulture and agriculture.

3 Conventional mutagenesis refers to techniques and methods that alter the genetic material of an organism in a way that does not occur naturally, i.e. through the application of physical or chemical mutagens that induce random mutations in the DNA. Such mutagenesis techniques are considered to have a long history of safe use in plant breeding.

4 Forecast volume of maize imported to the European Union (EU 28) from 2015 to 2025 https://www.statista.com/statistics/614405/maize-import-volume-european-union-28/ (accessed on 31/08/2018).

5 Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Official Journal* L 106, 17.4.2001, p. 1–39; Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Official Journal* L 268, 18.10.2003, p. 1–23; Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

legislation, accompanying documentation is provided with the indication on whether the lot contains GM maize or not. Moreover, custom inspectors collect and prepare a sample for laboratory analyses (controlling for GMOs, mycotoxins, heavy metals, pesticides, etc.) according to the applicable sampling schemes and recommendations[6].

The GMO regulatory system put in place in the EU[5] is mainly focused on the distinction between products resulting from conventional plant breeding techniques, including conventional mutagenesis, and those produced through recombinant DNA technology involving DNA from sexually non-compatible species (transgenic technology). The recent ruling of the European Court of Justice of 25 July 2018 to a request from the French Conseil d'Etat[7] reconfirms that organisms obtained by conventional mutagenesis techniques are exempted from the GMO EU regulatory framework, but not those resulting from the application of more recent site-directed mutagenesis techniques, such as various genome editing technologies[8]. The latter organisms and the food/feed products thereof should be considered as GMOs requiring prior authorisation and control measures for their presence on the EU market.

4 Creating genetic diversity by plant breeding

In natural plant breeding, parent plants with desirable characteristics are crossed in order to incorporate the beneficial features of both parents into future generations. This has been done for more than 100 years world-wide, sometimes involving crosses to wild relatives carrying interesting properties, such as durable disease resistances or even forcing crosses between species. Advances in whole genome sequencing in recent years have revealed that the genome sequences of crop species are extremely diverse and dynamic. For instance, it is estimated for maize that about 50 % of the genome differs between two varieties. A comparison between two maize inbred lines showed that their genomes contained respectively 3,408 and 3,298 unique insertions and deletions (InDels), with an average size of approximately 20 kbp (20,000 base pairs) and a range covering 1 kbp to over 1 Mbp[9].

Spontaneous natural mutations are expected to change the genome at each reproduction cycle. For instance, there is a seven in 1 billion chance in the model plant arabidopsis (*Arabidopsis thaliana*) that any given base pair will mutate in a generation[10], meaning that 175 new single nucleotide polymorphisms (SNPs)[11] would arise per 100 individual plants. This natural mutation rate may be increased as much as 250 fold by *in vitro* culture conditions. In rice, more than 54,000 novel DNA polymorphisms were identified in a line that went through *in vitro* culture (and 8 cycles of self-fertilisation), compared to the wildtype line. The relatively slow rate of natural mutation has also been increased by several orders of magnitude by conventional mutagenesis, such as irradiation or chemical

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6 ISO 24333:2009 Cereals and cereal products – Sampling. https://www.iso.org/standard/42165.html.

7 C-528/16 Judgment of the court (Grand Chamber) on 25 July 2018 http://curia.europa.eu/juris/document/document.jsf?docid=204387&mode=req&pageIndex=1&dir=&occ=first&part=1&text=&doclang=EN&cid=639261.

8 Explanatory Note from the Scientific Advice Mechanism (SAM) High Level Group of Scientific Advisors to the European Commissioner for Research, Science and Innovation on the nature and characteristics of New Breeding Techniques, including genome editing, and how they are similar to, and different from, conventional breeding techniques and established techniques of genetic modification (https://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf#view=fit&pagemode=none).

9 Jiao, Y., Peluso, P., Shi, J., *et al.* (2017) Improved maize reference genome with single-molecule technologies. *Nature* 546: 524-527.

10 Ossowski, S., Schneeberger, K., Lucas-Lledó, J.I., Warthmann, N., Clark, R.M., Shaw, R.G., Weigel, D., Lynch, M. (2010) The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* 327:92-94.

11 Single Nucleotide Polymorphisms are the most frequent natural mutations found in any organism from humans to plants and refer to nucleotide differences at a given position between two genome sequences of the same species.

treatment of seeds or pollen, which have been applied in breeding for several decades[12]. Such mutant plants have been incorporated in traditional breeding programmes and have contributed to the current crop diversity.

The cluttered nature of plant genomes reaffirms the notion that the structure of DNA in crop plants is dynamic. It changes in response to human selection and natural mutation processes. Novel variability is created at every new breeding cycle. This natural playing field constitutes the genomic diversity that forms the basis of our current agricultural crops and derived food products. As a result, the concept of one reference genome per plant species is insufficient to capture the genomic diversity observed in nature and in commercial species. Instead, a pan-genome database encompassing all sequence variations in a species would be required to form the reference basis for describing the genome of every crop. The compilation of such pan-genome databases has only started for a few major crop species such as maize[13], soybean[14], wheat[15] and rice[16]. However, the sequence information is lacking for many other crops, including major food crops commonly cultivated in less developed countries. Other limitations for establishing such a comprehensive database are listed in Section 7.

5 The notion of GM 'event'

The main target for risk assessment and traceability in GMO legislation is the so-called transformation event originating from the unique insertion of a new combination of genetic material at a specific position of the host genome. Therefore, currently validated detection methods are targeting a DNA sequence of 70-150 base pairs across one of the junctions between the transgenic insert and the plant DNA. The selected sequence is unique for the particular genetic modification and thus the corresponding PCR method is 'event-specific'. Such a method allows the detection as well as quantification of the DNA of the transgenic plant in relation to the total amount of DNA of the plant species, e.g. maize.

In the case of genome-edited plants, an 'event' could refer to the mutated sequence at a specific site in the genome. However, in case of a short mutated sequence, this may not be "a new combination of genetic material", as there is a probability that identical sequences may naturally occur elsewhere in the plant genome. This probability is increasing with a decreasing sequence length and an increasing size of the plant genome. Using a genome editing technology, the same mutation may also be introduced independently in different genetic backgrounds, which stretches the notion of a (single) 'event'. Consequently, detecting a short target sequence does not necessarily allow to identify its origin, whether naturally occurring or resulting from genome editing. Moreover, the development of event-specific PCR methods as described above may not lead to sufficiently selective quantification methods for very short modified sequences (one or a few nucleotides long).

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[12] Jankowicz-Cieslak J., Tai T. H., Kumlehn J., Till B.J. (2016) Biotechnologies for Plant Mutation Breeding. SpringerLink ISBN 978-3-319-45019-3; Anderson, J.A., Michno, J.-M., Kono, T.J.Y., Stec, A.O., Campbell, B.J., Curtin, S.J., Stupar, R.M. (2016) Genomic variation and DNA repair associated with soybean transgenesis: a comparison to cultivars and mutagenized plants. *BMC Biotechnology* 16:41.

[13] Hirsch, C.N., Foerster, J.M., Johnson, J.M., Sekhon, R.S., Muttoni, G., Vaillancourt, B., Penagaricano, F. (2014) Insights into the maize pangenome and pan-transcriptome. *Plant Cell Online* 26:121–135; Lu *et al.* (2015) High-resolution genetic mapping of maize pan-genome sequence anchors. *Nature Communications* 6:1-8.

[14] Li, Y.-H., Zhou, G., Ma, J., *et al.* (2014) *De novo* assembly of soybean wild relatives for pan-genome analysis of diversity and agronomic traits. *Nature Biotechnol.* 52:1045-1054.

[15] Alaux, M., Rogers, J., Letellier, T., *et al.* (2018) Linking the International Wheat Genome Sequencing Consortium bread wheat reference genome sequence to wheat genetic and phenomic data. *Genome Biology* 19:1-10.

[16] The 3,000 rice genomes project (2014) *Gigascience* 3:7; Zhao, Q., Feng, Q., Lu, H., *et al.* (2018) Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice. *Nature Genetics* 50:278–284.

6 Current status of GMO detection possibilities

Do the maize grains originate from 'natural' breeding or from mutagenesis?

Many varieties on the market today have been obtained through programs of genetic improvement that have included conventional mutagenesis methods. According to the Joint FAO/IAEA Mutant Variety Database[17], at least 3,281 cultivars in 175 plant species, including all major crops (e.g. maize, rice, wheat, barley, and soybean), have been developed through conventional mutagenesis and are being cultivated worldwide. The genomic changes induced by such techniques, including SNPs, sequence duplications and large sequence deletions, sometimes disrupting or eliminating gene function, may often not be distinguishable anymore from natural mutations that accumulated over breeding generations. There is incidental knowledge in the literature on individual genes that have been mutated through these techniques and the corresponding modified plant phenotypes. However, such information does not cover the whole spectrum of randomly introduced mutations.

Conventional mutagenesis has a long history of safe use and was integrated in breeding programmes from the mid-1930s. Therefore, it is currently not feasible to distinguish plants resulting from chemical or irradiation (conventional) mutagenesis from those to which these techniques have never been applied[12].

In case the maize grains have been obtained by a mutagenesis technique was it one which has conventionally been used ('conventional mutagenesis') or was it a genome editing technique?

As explained above, plants resulting from conventional mutagenesis cannot be readily distinguished from their natural breeding counterpart. Therefore, the focus here is on the detection of genome-edited plants. These cannot be detected with the current GMO screening strategies targeting common sequences used in the development of transgenic GMOs.

For the detection of genome-edited crops, three situations can be distinguished: (A) genome-edited crops with an (ongoing) EU market authorisation request; (B) unauthorised genome-edited crops for which information on the mutation is known or can be retrieved; and (C) unauthorised genome-edited crops for which information is not available.

(A) Genome-edited crops under an authorisation request

- A quantitative PCR (qPCR) or digital PCR (dPCR) method and control material for its validation would be available from the GMO applicant as part of the authorisation dossier, while the reference material would be in the production phase. Once validated, the detection method can be used by Member States laboratories. It is the task of the applicant to ensure that the detection method is specific for the product, i.e. also in case of gene editing.
- For smaller mutations (one or a few nucleotides only) the verification of method specificity and robustness would require more efforts by the EURL GMFF and laboratories participating in the method validation. The GM quantification at decision-relevant levels would be analytically very challenging and does not seem to be applicable for routine testing by enforcement laboratories.
- The absence of the transgenic sequence used in the genome editing process in the GMO and, by consequence, in the marketed food/feed products would have to be demonstrated in the authorisation dossier.

(B) Unauthorised genome-edited crops for which information on the mutation is known or can be retrieved

[17] Joint FAO/IAEA Mutant Variety Database, https://mvd.iaea.org/#!Home (accessed on 31/08/2018).

- Prior knowledge on the genome-edited mutation may have been communicated publicly or could be derived from literature, company information, patents, etc.
- When the mutant DNA sequence can be retrieved from publicly available information or through a research effort, a qPCR or dPCR method could be developed that is able to detect the mutation (and quantify it in case of larger sequence changes). Targeted sequencing-based approaches, for instance using capture probes or sequence enrichment, focusing on likely genome-edited sequences could also be used for more comprehensive high-throughput approaches.
- However, when the modification involves only a SNP or few nucleotide changes, it would not be possible to identify whether the mutation originated spontaneously or was induced by conventional or novel (genome editing) mutagenesis techniques. For other cases involving multiple mutations, the analysis would be very complex and still not necessarily conclusive.
- Evaluation approaches based on the statistical probability of finding a set of linked nucleotide mutations of a certain size in a genome (e.g. > 20 nucleotides) may hint to the presence of induced rather than spontaneous mutations.
- Transgenic DNA derived from the genome editing process that has remained in the final plant product is unlikely to be found in marketed products. Nonetheless, these could be detected using screening methods targeting e.g. conserved CRISPR-Cas sequences. Such detection methods need to be developed and validated.

These approaches could detect those genome-edited products for which intelligence was collected, without a proof of complete coverage. Detection of such DNA alterations, however, does not necessarily allow to unequivocally concluding whether they were the result of intended genome editing experiments or of a spontaneous mutagenic event or conventional mutagenesis processes.

(C) Unauthorised genome-edited crops for which prior knowledge on the mutation is not available

The detection of genome-edited crops for which no information is available is even more challenging than in case B since no PCR method can be developed without prior knowledge of the DNA sequence including information on the mutation.

- Sequence-based screening, i.e. whole genome or exome sequencing[18], will usually not detect such mutants, as the reference basis to compare the sequenced assemblies to is by itself variable.
- Pan-genome databases, encompassing all sequence variations in a species, are just starting to be compiled for just a few crops. The establishment of reliable pan-genome sequence collections for every commercially relevant crop would require a significant input of financial resources in sequencing infrastructure and bioinformatics tools. The necessary human resources to create such a knowledge centre or scientific network in the coming years are large.
- The analysis could be easier for mutations generating new sequence combinations of a larger size. Still, complete gene deletions, duplications and translocations may also occur spontaneously in plant genomes (particularly maize) or as a result of conventional mutagenesis and selective breedings.

[18] Exome sequencing is based on the targeted capture and sequencing of 1–2 % of 'high-value genomic regions' (subset of the genome, including regions coding for proteins) which are enriched for functional variants and harbour a low level of repetitive regions. It is technically easier to analyse than whole genome sequencing.

Even with a huge research effort in plant genome analysis, it seems impossible to resolve all potential disputes on the natural or genome editing origin of mutations identified by the various actors in the field, unless confirmed by the developing company (e.g. replacement of a wildtype maize gene with a natural resistance allele). Enforcement of the GMO legislation for genome-edited crops hence appears quite challenging.

7 Implementation issues

The implementation of methods for the detection of GMOs resulting from genome editing depends strongly on the prior knowledge of the mutation. For products already authorised or under an authorisation request, the detection could be similar as for current transgenic GMOs. The same could be expected for products for which sequence information on the mutation is publicly available and can be proven to be reliable and complete. Real-time PCR-based methods and derived multiple screening approaches are well-established analytical techniques adopted by all EU Official Control Laboratories. Digital PCR is being investigated by many laboratories as a complementary or alternative approach. The validation of new methods for the detection and quantification of genome-edited plants should follow standard practices. However, quantification in case of small changes, such as SNPs and small mutations, would be challenging and difficult to apply to real-market food or feed samples. The compliance to EU requirements of methods for detection and quantification[19] of SNPs by real-time (quantitative) PCR or digital PCR seems also to be difficult in terms of sensitivity and accuracy as indicated by studies conducted in other fields[20,21,22] . Detection of single point mutations generated by these new technologies does not necessarily allow identifying their origin, whether naturally occurring or resulting from genome editing while quantification may not be achievable.

Detection and identification of known genome-edited crops could also be pursued by sequence-targeted Next Generation Sequencing (NGS) approaches. Unfortunately, these approaches rely on the existence of high-quality reference genomes, are time consuming, expensive and require experienced staff. They further demand genome data management services and bioinformatics expertise. All these factors are currently limiting the implementation of these techniques in many official control laboratories. Harmonisation of the experimental set-up and of data analysis and validation – currently under discussion at ENGL level – should be strengthened among the official control laboratories to improve reproducibility and the possibility of using this technology.

Detection and identification of unknown genome-edited crops is currently not achievable under realistic circumstances. It could be advanced in the future by emerging sequencing approaches. However, their application would require the existence of a constantly updated crop database with reference pan-genomes including a comprehensive coverage of sequence variations. Establishing, updating and managing such a database remains hypothetical at this stage.

It needs to be underlined that genome sequencing is less efficient than current approaches based on PCR screening methods for the routine detection of unknown genetic modifications obtained by transgenic technologies because:

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[19] European Network of GMO Laboratories (ENGL) (2015) Definition of minimum performance requirements for analytical methods of GMO testing. http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

[20] Schwarz, G., Bäumler, S., Block, A., Felsenstein, F.G., Wenzel, G. (2004) Determination of detection and quantification limits for SNP allele frequency estimation in DNA pools using real time PCR. *Nucleic Acids Research*. 32:e24. doi:10.1093/nar/gnh020.

[21] Yu, A., Geng, H., Zhou, X. (2006) Quantify single nucleotide polymorphism (SNP) ratio in pooled DNA based on normalized fluorescence real-time PCR. *BMC Genomics*. 7:143-152. doi:10.1186/1471-2164-7-143.

[22] Miotke, L., Lau, B.T., Rumma, R.T., Ji, H.P. (2014) High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR. *Analytical Chemistry*. 86:2618-2624. doi:10.1021/ac403843j.

- It requires more analysis and evaluation time before conclusions are available. This may not be compatible with the restricted time for clearance of a ship at the harbour. Typical sequencing services for complex and unknown structures require currently several weeks. The discovery of a suspect lot may thus come too late to be practically implementable. The number of samples that could be tested for unknown genome-edited changes through application of sequencing technology would therefore be limited to a few dozen per year (compared to a few thousand for current GMOs);
- It has a lower sensitivity compared to current approaches based on PCR screening methods. The identification of a small number of mutant sequences in a compound sample, such as in the scenario proposed here, will be challenging, just as the sequencing technique itself is not error-free[23]. It is necessary to combine different sequencing approaches to overcome the accuracy limitations of each. This would further add to the costs and time required for the analysis;
- Such an approach would be more open to legal disputes. Indeed, plant genomes are dynamic and any newly detected sequence variant could potentially also result from a novel natural mutation or from a breeding line of which the sequence was not yet included in the pan-genome database. Particularly small mutations cannot be attributed with high reliability to a genome editing result.

8 Conclusions

With respect to the challenges for GMO detection laboratories as framed in the scenario above the following can be concluded:

Most of the mutations induced by genome editing technologies cannot be unequivocally distinguished from natural mutations as they may also occur naturally. Plant genomes have inherently a considerable sequence variability which is at present not only insufficiently documented for any crop, but the genomes keep also changing over time. Moreover, mutations obtained by new mutagenesis techniques can currently not be differentiated from those induced by conventional mutagenesis techniques, which have been incorporated in traditional breeding programs and are often not thoroughly documented.

Currently products of genome editing could only be detected and identified in imports of commodity products by enforcement laboratories when prior knowledge on the altered genome sequence, a validated detection method and certified reference materials are available, similarly as required for the authorisation of current transgenic GMOs.

An intelligence-based approach through searches in the literature, in applications for authorisation or notifications outside the EU, patents, company websites, etc. may enlarge the knowledge base on expected genome-edited changes. This would need to be further developed and could increase the possibility of detecting those changes via PCR technologies or targeted sequencing approaches.

However, when the modification involves only a SNP or few nucleotide changes, it would not be possible to identify whether the mutation originated spontaneously or was induced by conventional or new (genome editing) mutagenesis techniques. Moreover, it is unlikely that methods for the quantification of GMO products with small genome modifications in complex food or feed materials provide the level of selectivity needed for the enforcement of legislation, such as the one on labelling.

In the absence of prior knowledge on the potential genome-edited mutations in a crop, detection is not feasible with the current analytical capabilities of enforcement laboratories. Emerging sequencing-based analysis for the detection of unknown products

[23] Xiao, Y., Sriram, P. C., Srinivas, A. (2012) A survey of error-correction methods for next-generation sequencing. *Briefings in Bioinformatics* 14:56-66.

of genome editing would require significantly more time and resources by enforcement laboratories, compared to the currently applied quantitative PCR technologies. This may affect the timely clearance of goods entering the EU market as required for dealing with the scenario envisaged in Section 2 above.

The EU control system for GMOs and corresponding food and feed products may not function as efficiently for unauthorised genome-edited products compared to transgenic GMOs. In particular, the principle of zero tolerance for unauthorised GMO on the EU market is more difficult to maintain. There is a non-negligible probability that products obtained by genome editing may enter the market undetected or will only be detected, by current or future analytical technologies, after their introduction into the EU market. This may result in a higher number of alerts through the RASSF portal[24] and a number of legal disputes on whether a mutated sequence originated from a (potentially novel) natural mutation, conventional mutagenesis techniques or from new mutagenesis techniques.

[24] Rapid Alert System for Food and Feed (RASFF) - https://ec.europa.eu/food/safety/rasff_en.

Glossary

Conventional mutagenesis techniques, as used in this document, are methods that alter the genetic material of an organism in a way that does not occur naturally, namely through irradiation or chemical treatment of plant tissues which induces random mutations in the DNA. Such mutagenesis techniques are considered to have a long history of safe use in plant breeding and do not involve the use of recombinant nucleic acid molecules or genetically modified organisms.

CRISPR-Cas9 is the abbreviation for 'clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9'. It is one of the most popular gene-editing techniques and is derived from bacteria.

Digital PCR (dPCR) is a version of real time PCR which is based on splitting the reaction mixture into thousands of distinct partitions. Counting the number of positive (fluorescent) partitions against the negative ones is used for estimating the number of DNA molecules with the target sequence in the reaction mixture.

DNA polymorphism is any difference in the nucleotide sequence between individuals. These differences can be single nucleotide changes, deletions, insertions, or even changes in the number of copies of a given DNA sequence. SNPs (single nucleotide polymorphisms) are the most common type of DNA polymorphism.

Exome sequencing refers to sequencing only the protein-coding genes in a genome (known as the exome).

ENGL is the European Network of GMO laboratories, a consortium of EU official laboratories aiming at exchanging information and harmonising the enforcement of EU legislation

EURL GMFF is the European Union Reference Laboratory for Genetically Modified Food and Feed, operated by the Joint Research Centre (JRC) of the European Commission.

Event see transformation event.

Genome is the complete genetic material present in a cell or organism.

Genome editing, also called gene editing, is a group of mutation technologies that allow to modify the genetic information by adding, removing, or altering DNA sequences at a specific location in the genome.

GMO is the acronym for Genetically Modified Organism. According to EU legislation, it means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Mutagenesis is a process by which the genetic information of an organism is changed. Conventional mutagenesis techniques are based on using irradiation or chemical treatment of pollen or seeds to generate random mutations. Site-directed mutagenesis techniques, including genome editing, aim at making specific mutations in a targeted manner.

Next Generation Sequencing (NGS) is a common term for several high-throughput sequencing approaches using the concept of massively parallel processing.

Pan-genome is the entire genome set of all variants of a biological species. It includes genome sequences present in all variants (core genome) and sequences present only in some variants of a species (variable or accessory genome).

PCR is the acronym for Polymerase Chain Reaction. Techniques used in molecular biology to exponentially amplify, by in vitro synthesis, a specific segment of DNA to millions of copies, which can be detected and quantified.

Phenotype is the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Real time PCR, also known as quantitative polymerase chain reaction (qPCR), is a PCR technique that allows to monitor the amplification of a targeted DNA fragment during the PCR process, i.e. almost in real-time, and not only at its end, as in conventional PCR.

Reference material is a material which is homogeneous and stable with respect to one or more specified properties and used for calibration or quality control of a measurement process.

Screening in GMO detection is an analytical procedure used to identify the possible presence of GMOs by targeting the most-common transgenic genes or genetic elements.

Single nucleotide polymorphism (SNP) is a variation in a single nucleotide (building blocks of DNA) that occurs at a specific position in the genome to an appreciable degree within a population (e.g., > 1 %).

Specificity is the property of a detection method to respond exclusively to the target of interest.

Transformation event refers to the inserted sequence corresponding to a new combination of genetic material in a specific location in the genome.

Whole genome sequencing is the process of determining the complete DNA sequence of an organism's genome at a single time.

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