



Current status and trends in the analysis of GMO and new genomic techniques

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Abstract

The detection of genetically modified organisms (GMO) is crucial for regulatory compliance and market control. Therefore, detection methods are constantly being developed and improved. New genomic techniques (NGT) present unique challenges for detection due to the minimal genetic changes they introduce. The ‘International Conference on GMO Analysis and New Genomic Techniques’ held in March 2023 in Berlin, explored advances in detection methods, such as Next-Generation Sequencing and digital PCR, and highlighted the need for global cooperation and capacity-building initiatives. The conference emphasized international collaboration and addressed the challenges of establishing robust methods for identifying NGT-derived organisms.

Keywords Genetically modified organisms · New genomic techniques · Detection · Identification · Network · Capacity-building

1 Introduction

Since the first International Conference on GMO Analysis in 2008 (Van den Eede and Speroni 2008), major advancements in analysis techniques, such as Next-Generation Sequencing (NGS) and digital PCR (dPCR), have significantly improved the ability to detect and identify genetically modified organisms (GMO). Simultaneously, new genomic techniques (NGT), including CRISPR-Cas9 and other gene-editing technologies (Broothaerts et al. 2021; Liu et al. 2022), have rapidly developed. Considering the potential of NGT for genetic modifications, an effective market control

requires adapted analysis methodologies for successful detection and identification.

The ‘International Conference on GMO Analysis and New Genomic Techniques’ held in Berlin in March 2023¹, provided an essential forum for discussing the progress on detection and identification for both “classical” GMOs and NGT-derived products. Attended by nearly 500 participants from over 50 countries, the conference also offered a platform for international networking and global information sharing.

2 Conference overview

The conference covered 7 sessions over 3 days, focusing on technical challenges and innovative tools for detecting and identifying GMOs and organisms produced through NGTs². These sessions also addressed evolving methods for GMO traceability. During the conference, a particular emphasis was put on an international cooperation and capacity-building, which was supported by the German government

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¹ <https://www.bfr-akademie.de/gmo2023>.

² <https://www.bfr-akademie.de/english/programme-gmo2023>.

together with the Secretariat of the Convention on Biological Diversity.

The first session highlighted the global increase in authorised GMO products. Control laboratories face substantial challenges in developing reliable routine testing protocols, especially as more products enter the market. In addition, challenges related to the technology used and the availability of reference materials were presented.

Subsequent sessions explored advancements in the detection and identification technologies of NGT-derived organisms, including PCR and NGS-based methods. For example, a new PCR method was presented to detect a CRISPR-Cas9 waxy maize. The detection of single nucleotide modifications might require “multi-target approaches”, combining multiple detection techniques to enhance sensitivity and accuracy.

Sessions on “classical” GMO detection discussed improvements in existing methods, such as needing up to 23 different screening elements to detect most GMO potentially on the market, reflecting the complexity of modifications which exist today. Discussions also focused on the limitations of current technology, emphasising the potential for NGS in conjunction with bioinformatics to analyse the high amount of data produced to close these gaps.

In addition, other aspects such as laboratory accreditation, sampling strategies, and DNA extraction must be taken into account to achieve reliable results. Examples of regional networks in China, South Africa and Mexico, as well as the virtual Network of Laboratories for the Detection and Identification of Living Modified Organisms, were presented in the session on “Regional networks: Experiences from different regions”. In all cases, the benefits of information exchange and cooperation among scientists pursuing the same goal were emphasised for knowledge-sharing and capacity-building.

Problems associated with the availability of funding, the reliable supply of analytical consumables, and access to information were also addressed. In particular, the availability of information on methods and strategies to apply a minimum of screening to detect a maximum of potential GMO in a sample was presented in the session on “Global information exchange”. The Joint Research Centre of the European Commission (JRC), the Biosafety Clearing-House (BCH), the information-sharing platform of the Cartagena Protocol; the European Initiative for a Unified Database System (EUGenius), and company representatives presented platforms that are publicly available. Some of these information platforms provide e-learning materials and technical resources, as well as opportunities to apply for training sessions.

The last session “Alternative approaches to GMO traceability” was dedicated to digital solutions for traceability

in the future. A novel blockchain approach was presented, which might be suitable for applications where the availability of information is required to provide assurance to partners and consumers. Finally, it was pointed out that more transparency in global agricultural value chains is needed to achieve the goal of providing consumers with reliable labelling information. Lessons could be gleaned from other traceability systems already in use, such as those for minerals or timber. The complete programme, together with the presentations, is accessible online³.

3 Discussion

3.1 GMO analytics

Significant advances have been made in molecular techniques for GMO analysis since the first global conference in 2008. PCR-based methods, particularly real-time PCR, remain the preferred method for routine analysis. NGS is currently being established in an increasing number of laboratories. Unlike PCR-based methods, which determine the presence or absence of a particular DNA sequence, targeted NGS-based methods are capable of revealing the exact sequence information, potentially allowing for further characterisation of a GMO. Examples of targeted approaches using NGS are based on the enrichment of specific DNA segments prior to sequencing. The major advantage of such approaches is an increased analytical sensitivity. Both, PCR-based and targeted sequencing methodologies require prior knowledge of the GMO for its detection and potential identification. Using whole genome sequencing (WGS) detection may, in some cases, be possible even without prior knowledge of the GMO under investigation. Nevertheless, either the availability of reference DNA sequences or exact information of the modification are a prerequisite for GMO detection to facilitate the correct interpretation of the obtained sequence data. Untargeted NGS and WGS analyses are more cost- and time-intensive than PCR analyses, and currently insufficiently standardised. Further development and harmonisation are needed for a broad and convenient application in routine GMO analysis.

In control laboratories, dPCR is also gaining relevance, offering a more sensitive alternative to conventional PCR. Further novel approaches, including isothermal amplification, artificial intelligence, and blockchain technology for traceability, are being explored. It is important to note that their applicability is still in a developmental stage. The number of NGT applications in plants, animals and microorganisms is growing rapidly, with the majority of NGT-derived

³ <https://www.bfr-akademie.de/english/archive/2023/gmo2023.html>.

organisms being engineered using CRISPR-Cas9 technology to produce small edits, such as single nucleotide variations (SNV) or short insertions/deletions (InDel) for gene knock-outs. The general challenges for the development of methods for the detection of organisms derived from NGT include (i) obtaining accurate information on the specific organisms and the introduced sequence alterations and (ii) the availability of positive or reference material. Prior information (e.g., modified sequence, genomic location) is a prerequisite to implement amplification-based targeted methods. Innovative methods and new approaches are being explored but are still in a stage of research and development and not yet fit for the purpose for routine application in regulatory testing.

A major challenge in NGT detection are the small-scale genetic changes, such as SNVs, which are in principle indistinguishable from natural mutations. While it is technically feasible to detect such minimal genetic alterations, the results generally do not allow for drawing reliable conclusions about an unambiguous causal link to the use of NGT. This is because these types of modifications could also occur naturally through spontaneous mutations (e.g., under herbicide selection pressure), or can be induced by physical or chemical mutagenesis techniques, which are exempt from legislative requirements. This situation may change in the case of multiple base pair alterations, particularly when such edits occur in combination or together with other genetically linked markers (e.g., other spontaneous mutations associated with the intended nucleotide alterations). In this case, the probability that the exact same combination of alterations has arisen spontaneously decreases, leading to an increased potential of a reliable identification of an NGT product (Guertler et al. 2023). Further challenges arise from applying detection methodologies to bulk grain and large seed samples. While NGS is appropriate for application to single plants or small numbers of plants or seeds (Shillito et al. 2021), it fails as being impracticable when applied to commercial situations like international trade in commodities.

3.2 Regulatory considerations

Countries worldwide like Argentina, Brazil, Colombia, India, Japan, Kenya, Nigeria, the Philippines and others are revising their regulatory frameworks (Sprink and Wilhelm 2024) to accommodate NGT products. The revised regulations often foresee different regulatory requirements for organisms that were produced using specific techniques and/or have defined molecular characteristics. As a result, these countries have taken the approach to not regulate all organisms produced using NGT the same way as “classical” GMO. In other jurisdictions, such as New Zealand,

NGT-produced organisms would be considered to be GMOs and regulated in the same manner (Fritsche et al. 2018). NGT-derived organisms may resemble “classical” GMOs or be indistinguishable from conventionally bred organisms, creating challenges for labelling, trade, and enforcement.

The EU has been discussing a complement to its GMO regulation, which has basically been developed in the late 1980s. In 2018, the European Court of Justice has ruled that all organisms produced using NGT are to be classified as GMO. Soon after, the European Commission has started working on a proposal for a complementary regulation in an inclusive process, also involving European Food Safety Authority (EFSA), the JRC, the European Network of GMO laboratories (ENGL), various stakeholders, and the general public. The Commission’s proposal of 2023 for a new regulation on plants obtained by certain NGT aims to strengthen sustainability goals across a wide range of plant species, enable an environment for research and innovation, and maintain a high level of protection of health and the environment. This proposal was generally confirmed by the European Parliament.⁴

3.3 Networks and cooperation

Global cooperation is critical for building capacities in GMO analysis. Multiple national and regional networks enable the exchange of information and experience among scientists. Face-to-face workshops and training sessions foster personal connections and technical expertise, contributing to capacity-building. This supports countries in establishing and maintaining reliable GMO detection and identification facilities and systems, thereby enabling regulatory enforcement. Under the United Nations, the Network of Laboratories for the Detection and Identification of Living Modified Organisms, coordinated by the Secretariat of the Convention on Biological Diversity (CBD) and hosted by its BCH, provides an electronic information exchange platform for experts in the field.

Capacity-building initiatives, such as the EU’s ‘Better Training for Safer Food’ (BTSF) program, are essential for ensuring international harmonization and effective regulatory enforcement.

Participants of the conference highlighted the effectiveness of supportive networks, the importance of continued knowledge-sharing and practical training to strengthen global GMO analysis capacities. They emphasised the need for more face-to-face workshops and training sessions to foster personal connections and technical expertise.

Particularly some Asian, African, and South and Central American countries suffer a lack of technical equipment and

⁴ https://food.ec.europa.eu/plants/genetically-modified-organisms/new-techniques-biotechnology_en.

infrastructure for the implementation of currently established methods for detection and identification. Access to consumables and chemicals is often not reliable. The combination of high costs and a lower priority of GMO detection is a major challenge in many regions. Frequent difficulties include maintaining the cold-chain transportation and specific requirements due to customs demands. Mutual support networks could be one way to mitigate these challenges at the working level.

4 Conclusions

PCR-based methods remain the gold standard for routine GMO detection, while dPCR and NGS offer promising future applications. Access to information on genetic modifications is an essential prerequisite for a successful method development. Detecting small mutations introduced by NGT remains a challenge, requiring further development of detection methods, information and reference materials. There is currently no technical solution to identify whether an unknown organism—or a product thereof—contains a small mutation derived from NGT, whether it occurred naturally, or resulted from classical breeding techniques. Regulatory frameworks are evolving, international harmonisation would reduce obstacles in trade and enforcement. Enhanced global cooperation through networks and capacity-building initiatives is crucial for ensuring the reliable detection and identification of GMOs and NGT products in the future.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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