Government Chemist Conference 2023

Safe food for tomorrow's world - food security in challenging global conditions

Malcolm Burns, Head of GMO analytical unit and Principal Scientist (LGC, UK)

Traceability of genome edited products in the food supply chain - Status and challenges

Malcolm Burns, Head of GMO analytical unit and Principal Scientist (LGC, UK)

Content of presentation

- Background and context
  - Official roles and involvement in GMO analysis and advice on genome edited organisms
- Scope and application of genome editing
  - Examples of genome edited crops
- Detection of genome edited organisms/products
  - Status and challenges
  - Comparisons/parallels with conventional GMOs
- Conclusions

Background and context to presentation

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- LGC: Life sciences measurement, testing and research institute
  - 180th Anniversary (2022)
- Government Chemist
  - Statutory function (UK legislation)
  - Provision of impartial/independent referee analysis on a (food) sample as part of official controls, in cases of dispute between a trader/manufacturer and local authority
- National Measurement Laboratory
  - UK's designated institute for chemical and bio-measurement
  - Work globally to harmonise measurement science
- UK National Reference Laboratory (NRL) for GMOs in food and feed
  - Pursuant to (retained) regulation (EU) 2017/625
- GMO authorisations in Great Britain
  - Provision of scientific method validation services as part of the official authorisation process
- Last two positions funded by the FSA

Scope and application of genome editing

Examples of genome edited crops

Introduction

- Genome editing can introduce small changes in the DNA sequences of specific genes known to affect traits of interest, to achieve the desired genetic outcome of traditional processes (e.g., breeding techniques) more accurately and efficiently
- Scientists
  - Production of a range of foods with health, environmental and commercial benefits
  - Developed more quickly than traditional breeding methods
- Policy makers
  - Global food security
  - Climate change
  - Human health challenges

Why produce genome edited organisms?

- Benefits (both potential and realised):
  - Better use of natural resources
  - Tolerance/resistance to biotic stresses (e.g., resistance to plant diseases)
  - Tolerance/resistance to abiotic stresses (e.g., environmental conditions)
  - Better composition (e.g., higher nutritional value)
  - Herbicide tolerance
  - Modified colour/flavour
  - Yield or other agronomic characteristics (e.g., fruit/seed size & weight)
  - Better storage performance (e.g., shelf life)
  - Others (e.g., early flowering, flowering time)

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Examples of commercially available GE products

- “Calyno” High Oleic Soybean Oil (USA)
  - Reduced saturated fat
  - First ever genome edited food product to successfully undergo review by the USDA and FDA and commercialized in the US
- “Waxy maize” (USA)
  - Different starch profile from other corn - used for food starch and some industrial products
  - Commercialisation in the US (clearance for use in Canada, Argentina, Brazil and Chile)
- Enhanced GABA “Sicilian rogue” tomato (Japan)
  - Up to 5 x amount of amino acid GABA (gamma-aminobutyric acid) than regular tomato
  - Helps to reduce blood pressure and is believed to aid in relaxation

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Detection of genome edited organisms / products

Status and challenges

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What is genome editing?

- **Genome editing** (ISO DIS 5058-1) : a group of new targeted mutagenesis techniques that facilitate addition, removal, or alteration of DNA sequences at a specific location in the genome
  - New Genomic Techniques, synthetic biology
  - Example techniques: CRISPR-CAS nucleases, Meganucleases, Zinc finger nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs)

- Generate different kinds of alterations in the genome:
  - Ranging from single nucleotide variations (SNVs), to deletions and insertions of many base pairs

Distinction from GMOs

- Genetically Modified Organisms (GMOs)
- Based on (retained) EU legislation (Directive 2001/18/EC):
  - "*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*"
- Conventional GMOs:
  - Organisms produced using recombinant DNA technology, typically containing DNA sequences randomly introduced from the same or other species, prior to the adoption of Directive 2001/18/EC
    - EC-JRC Technical Report, Wim Broothaerts, et al.,"New Genomic Techniques: State-of-the-Art Review" (2021)
  - Created via transfection/transformation, incorporating larger pieces of foreign DNA into the host genome, often at random sites, frequently along with easily identifiable markers (e.g., promoters and terminator cassettes)

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Key developments (status)

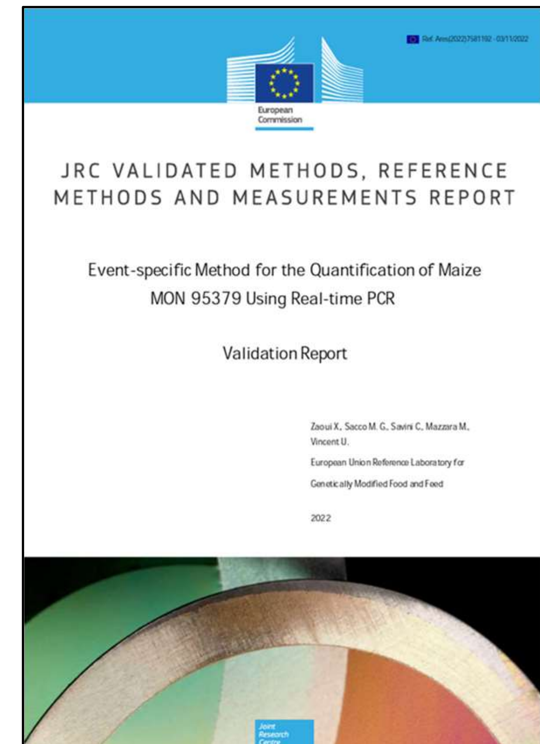
- EU: July 2018: (Case C-528/16) European Court of Justice ruled that products of genome editing (synthetic biology) were regarded as GMOs and fall under the pre-existing legislation for GMOs

- UK: Genetic Technology (Precision Breeding) Act (March 2023):
  - Primary legislation amended the regulatory definition of a GMO to exclude organisms that have genetic changes that could have been achieved through traditional processes
    - Norwegian Committee on Gene Technology: recent publication on recommendations on new legislation for genome editing techniques
  - A sub-set of genome edited organisms may be regarded as Precision Bred Organisms
  - Precision Bred Organism (PBO): an organism which possess any feature of its genome as a result of the application of modern biotechnology, which could have arisen as a result of traditional processes
  - A future analytical challenge will be to determine if any DNA sequence variation exhibited in a food/feed product could have arisen through traditional processes

GMO analysis

* https://gmo-crl.jrc.ec.europa.eu

- Largest common denominator in global framework of GMO analysis: DNA based methods
  - Ubiquitous; resistant to degradation; choice of targets; specificity; stable (qualitative/quantitative)
  - Referred to in the legislative texts
- Quantitative real-time PCR (qPCR)-based analysis is the current preferred DNA-based technique for routine GMO analysis
- EURL-GMFF validated methods for event specific detection of GM varieties provide unequivocal target identification *
  - Collaborative international inter-lab validation


European Commission

JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS REPORT

Event-specific Method for the Quantification of Maize MON 95379 Using Real-time PCR

Validation Report

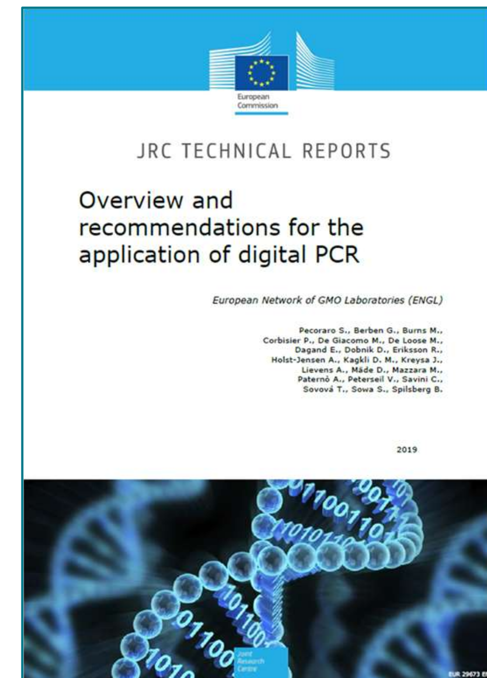
Zaoui X., Sacco M. G., Savini C., Mazzara M., Vincent U.

European Union Reference Laboratory for Genetically Modified Food and Feed

2022

Techniques for GMO analysis

- Real-time PCR (qPCR)
  - Excellent specificity and quantitative capabilities
  - Practicability – most analytical laboratories have these embedded into their infrastructure
- Digital PCR (dPCR) (JRC Technical Report, Pecoraro *et al*., 2019)
  - Less prone to inhibition, sensitive, precise and quantitative in nature
  - Gaining increasing traction, but still less common that qPCR
- Massively parallel sequencing
  - Next Generation Sequencing (NGS) / Whole Genome Sequencing
  - Cost of supporting infrastructure and skillset



Key challenges for detecting genome edited products

- Key challenges: in context of a food sample being referred to the GC function (referee case or advisory capacity) to determine if a sample contains a genome edited product
- Government Chemist role: safeguard the quality of public science and ensure accurate analytical measurement

Statutory function

"an <u>independent and impartial referee analyst</u>, authorised analyst and analyst by reference to or pursuant to certain legislation"

"Using sound analytical science in support of policy and regulation"

Dr Julian Braybrook DSc, CChem FRSC
Government Chemist

Government Chemist

Key challenges for detecting genome edited products

- **Key challenge #1: Technical detection of small sequence alterations**
- Genome editing can introduce small targeted DNA sequence alterations
- Technically challenging, but entirely feasible, to detect small genomic alterations (e.g., SNVs)
  - Given *a priori* information on the sequence(s) of interest and associated flanking regions
- Modern molecular biology techniques (qPCR, dPCR and NGS) offer the best potential for detecting genetic changes in an organism's genome
- Should sufficient information be known regarding a sequence alteration, and confidence can be attributed to that sequence alteration being specific to a GMO or genome edited line, then detection, identification and potentially quantitation can be achieved (Lutz Grohman, *et al*., 2019)

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Key challenges for detecting genome edited products

- **Key challenge #2: Establishing the source of a genetic variation**
- General agreement that modern molecular biology techniques (qPCR, dPCR and NGS) offer the best potential for detecting genetic changes in an organism's genome
- Modern molecular biology methods, used in isolation and targeting single small mutations, are unlikely to provide unequivocal information on the source of the mutation
- Genome editing – umbrella term, incorporating plethora of techniques which can differ in mode of action and resultant mutation
  - Would be wrong to generalise on efficacy of every molecular biology approach across all genome edited organisms
- In specific instances, detection may be possible, if enough additional information is available in order to prove that a DNA sequence or sequences are unique to a specific genome edited line
  - e.g. following some types of SDN-3 activity

Additional challenges for detecting genome edited products

- Terminology
- "Genome editing" – umbrella term, encompasses a range of diverse techniques
- A range of diverse terminology to describe the processes and the products
  - e.g., Established Genomic Techniques, New Genomic Techniques, Targeted mutagenesis, Cisgenesis, Intragenesis
- Useful to define/agree terminology to promote a harmonised approach and greater understanding
  - Key texts:
  - Defra's ACRE and EFSA
  - FSA project (FS900243) "*Literature review on analytical methods for the detection of precision bred products*" (In press)

- Quantitative estimation
- Require validated methods for detection and identification first

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Additional challenges for detecting genome edited products

- <u>Screening</u>
- GMO analysis: capitalise upon common GM control elements/markers
  - e.g. p35S, tNOS, Cry1Ab/Ac, ctpt2/cp4 epsps, bar, p35S-pat, pFMV, pNOS, etc.,
  - Cross referencing results from an informative panel of screening markers to known occurrences in GM lines
- Genome edited organisms: challenging, due to lack of inserted elements as a result of genome editing activity

- <u>Reference materials and comparators</u>
- Potential transient nature and segregation of multiple on- and off-target mutations
- Databases – ideally as pan-genomic per taxon, but practical challenges in establishing

Research into detecting genome edited products

- Weight of evidence approaches and minimum qualifying information
- Concept of collective information gathering to build up a unique “signature” of the genome edited organism
  - Site of interest
  - Flanking regions
  - Genetic background
  - (Linked) off-target mutations
  - Epigenetic and epitranscriptomic changes
  - Documentary evidence: supplier, origin, pedigree, etc.
  - Digital traceability: Blockchain

- Continued requirement to provide evidenced based experimental work to further support or refute use of weight of evidence approaches

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Further reading

- Recent publication (w/c 12/06/2023)
  - EC-JRC Technical Report
  - European Network of GMO Laboratories (ENGL)
  - Analytical detection of plant products developed through New Genomic Techniques
  - Published on ENGL webpages
  - https://gmo-crl.jrc.ec.europa.eu/ENGLabs#inline-nav-engl-reports

Conclusions and the road ahead

Conclusions and the road ahead

- Significant challenges for detecting genome edited products
- But not necessarily insurmountable . . .
  - Advent of conventional GMOs in early 2000's: historical challenges
  - EURL-GMFF and ENGL: Measurement unit; measurement uncertainty; method validation; minimum performance criteria
- Continued contribution to the solution:
  - Evidence lead research and development
  - Method validation and measurement uncertainty estimation
  - Traceability
  - Reference materials, databases
  - New techniques and technologies (e.g. dPCR, NGS)
  - Engagement at (Inter)national level to share best measurement practice guidance
  - Sound analytical science (metrology)

ENGL inauguration ceremony
4th December 2002

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- European Network of GMO Laboratories

Thank you for listening

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