GMO DETECTION AND SURVEILLANCE IN NIGERIA

By
Sylvia Uzochukwu, Ph.D
Professor of Food Science and Biotechnology
Federal University, Oye-Ekiti, Nigeria

A paper presented at the first Biosafety Conference, Godfrey Okoye University, Enugu. 26 - 27 November 2015
**NEW PLANT BREEDING**

- In order to improve agricultural practices and nutritional quality, plant breeding techniques have been developed to produce genetically modified (GM) crops.
- They express interesting traits such as herbicide tolerance, insect resistance, and abiotic stress resistance.
- New combinations of their genetic material are created through the use of modern biotechnology.
RIGHT TO KNOW

- The first genetically modified organism (GMO) approved for commercialization was Flavr-Savr tomato in 1994.
- From that time, 181.5 million hectares of planted GM plants in 28 countries were reported in 2014.
- With this came agitations for the consumers’ “Right to know” what contains GMO and what does not.
As a result, many countries have established genetically modified organisms (GMO) legislations to guarantee the traceability of food/feed products on the market, which is a key factor in the implementation of these regulations, and to protect the consumer’s freedom of choice.
IMPLEMENTING REGULATIONS

- GMO labeling policies have been established in several countries around the world with thresholds of tolerance varying between 0 and 5%.
- Post release monitoring is also part of the GMO legislation in some countries.
- The presence of GMO in the food/feed chain, and monitoring of approved GMOs is controlled by the competent authorities.
- Therefore, several GMO detection strategies, mainly based on DNA, have been developed to implement these legislations.
SURVEILLANCE AND GMO DETECTION

- Surveillance means oversight and control.
- To achieve surveillance, the overseeing authorities must carry out analysis, detection and control.
- Section 23(3) of the National Biosafety Management Agency Act of 2015 requires that the party applying for approval of a GMO must provide information on monitoring techniques for the GMO as part of his Risk Assessment – that is,
  - Methods for tracing the GMO and monitoring its effects
  - Methods for identifying the GMO
  - Techniques for detecting transfer of the donated genetic material to other organisms
  - Methods for detecting aberrant gene expression
- These methods basically involve detection of the heterologous or foreign DNA sequence.
Detection of Genetically Modified Crops/foods

- Current analytical methods are mainly carried out by
  - detecting the transgenic DNA
  - or the foreign protein(s) produced
PROTEIN METHODS

- For protein detection, the enzyme-linked immunosorbent assay (ELISA) is the method of choice.
- ELISA is an antibody-based detection method
- ELISA is also a quantitative detection method.
- Antibody-based dip sticks can also be used in the field, for rapid detection of GMOs
Detection of the Cry1Ab protein (GM corn event MON810) by way of an ELISA. (Guertler et al. 2009, Paul et al. 2008)
OTHER PROTEIN METHODS

- As an alternative, the immuno-PCR method can be used to identify GMO protein-based methods also include the use of the mass spectrometry-based technology as a tool allowing characterization of GM crops – reference data base probably required
DISADVANTAGES OF PROTEIN METHODS

- Though protein methods are simple and rapid, they depend on the expression level of the protein, which varies from tissue to tissue, and with development status.
- The protein methods also only indicate the expression of a certain protein. It does not distinguish between events.
- It is for these reasons that GMO detection is mainly accomplished at the DNA level.
- Protein based methods are however the method of choice when trait detection is the objective, eg, detecting the Bt toxin.
First of all, amplifiable DNA needs to be isolated from sample material. If processing has destroyed DNA and/or protein, GMO detection cannot be accomplished using DNA or protein based methods.

Specific sequences on the DNA are amplified by the Polymerase Chain Reaction (PCR) to indicate presence or absence of trans genes or/and associated sequences.
DIAGNOSTIC SEQUENCES

- PCR based GMO detection methods can be designed to detect any or all of these relevant transgenic sequences based on the specific information desired.

- **Broad-spectrum (Screening) GMO tests:** The same viral and bacterial genetic elements are often incorporated in transgenes to regulate expression of the trait gene in the plant. These DNA sequences are targeted and used as broad-spectrum (screening) GMO tests.
  - They are usually the 35S promoter of the cauliflower mosaic virus (CaMV), and the Nopaline synthetase (NOS) terminator of *Agrobacterium tumefaciens*.

- **Construct specific GMO tests:** These assays target specific traits such as the Bt gene.

- **Event-specific GMO tests:** Event-specific tests are used to identify specific GM events.
GMO detection methods based on genetic analysis have many advantages.
Detection cascade for the analysis of genetically modified crops
**Quantification**

- For GMO enforcement of labeling requirements, real-time quantitative PCR (RT-qPCR) is the method of choice.
- qPCR is very similar to the conventional PCR.
- Except that DNA can be quantified, which is mainly achieved by using one of two methods:
  - Adding a DNA binding dye like SYBR Green to the PCR reaction.
  - Adding a sequence-specific hydrolysis probe to the PCR reaction.

- In order to quantify the content of GMO in a sample, a plant specific DNA fragment is quantified next to a GMO specific DNA fragment, and the ratio calculated in %.
Quantitative real time PCR (qRT-PCR) using SYBR green dye. SYBR Green binds unspecifically to double-stranded DNA, emitting green light; the more DNA is amplified, the more SYBR Green binds to double-stranded DNA and the more green light is emitted.
Scheme of a probe-based quantitative real-time PCR assay – TaqMan florogenic probe:
Primers and probe bind specifically to the respective DNA fragment, The quencher absorbs the fluorescence signal of the reporter dye, DNA polymerase extends the primers, DNA polymerase hydrolyses the probe, by probe hydrolysis, the proximity of reporter dye and quencher dye is broken up, reporter dye emits the fluorescence signal. The more DNA is amplified, the more fluorescence signal can be detected.
Q PCR IS THE ROUTINE METHOD

- Due to its numerous advantages, the quantitative PCR (qPCR) is the method of choice for the enforcement laboratories in GMO routine analysis.
- To address its challenges (CRM, inhibitors etc) there are
  - Alternative GMO detection methods for
    - Faster detections of single GM target (e.g., loop-mediated isothermal amplification),
    - Simultaneous detections of multiple GM targets (e.g., PCR and then capillary gel electrophoresis, microarray, and Luminex),
    - More accurate quantification of GM targets (e.g., digital PCR),
    - Characterization of partially known (e.g., DNA walking and Next Generation Sequencing (NGS))
    - Or unknown (e.g., NGS) GMO.
Figure 1: Suitable application of GMO detection approaches regarding the adopted strategy as well as the available information about the sequences of tested GMO.
MULTITASKING

- By combining several taxon-specific, event-specific, and construct-specific TaqMan markers in a 96-well pre-spotted plate, a real-time PCR-based, ready-to-use multi-target analytical system has been developed to allow the simultaneous identification of thirty-nine GM events.

- With multiplex PCR-based methods, several DNA targets can be detected in a single reaction. It presents the advantage to decrease the number of reactions necessary to test the potential presence of GMO in a sample.
INTERPRETING RESULTS

- All individual GMO detection methods must be validated and approved. Only validated methods should be used.
- To interpret qPCR results, databases such as the GMOseek and GMOfinder databases are available.
- They contain reliable information on GMO.
- The JRC-GMO-Matrix platform, combines the GMOMETHODS database and the Central Core DNA Sequences Information System help interpret results.
- The JRCGMO-Matrix platform is also strengthened by the JRCGMO Amplicons database which contains publically available puta-tive GMO-related sequences.
ALTERNATIVE MULTIPLEX STRATEGIES

Still with the aim of going further in the development of multiplex assays, several methods not based on qPCR have been also developed using notably

- **CGE** – florescently labelled primers allow the discrimination between amplicons of the same size
- **Microarray** – Amplicons are hybridized on the array, allowing the simultaneous detection of more than 250 000 targets in one assay
- **Luminex technologies.** Biotinylated targets amplified by single or multiplex PCR assays could be analyzed with the Luminex technology,

Two main steps are generally followed.

- PCR
- Analysis using the three platforms above
LIMITATIONS OF DNA METHODS

- Information on DNA sequence and
- Availability of certified reference materials 
  are essential for DNA methods
- For most of the authorised GM crops, reference material is available.
- However, this is not the case for non-authorised GMO.
- Availability of certified reference materials (CRM) is necessary for quantification
PARTIALLY known GMOs

- When the observed signals do not correspond to known GMO, the presence of unknown GMO, containing at least one known element, could be only suspected.

- The only way to indubitably confirm the presence of GMO is provided by the characterization of sequences from the junctions between the transgenic cassette and the plant genome as well as the unnatural associations of transgenic elements.
**Unknown GMOs**

- When no information about the transgenic cassette is available, the insert and its transgene-flanking regions are identified by the analysis of all inferred contigs derived from reads that partially matched or unmatched with the endogenous plant-species reference genome.

- With this sequencing strategy, the entire DNA library, consisting of sheared genomic DNA, ligated to adaptors, is sequenced. The generated reads are then treated with bioinformatics tools based on prior knowledge of tested GMO.
Challenges to GMO surveillance

- Sequence information and reference materials are missing for non-approved and unknown GMOs and events.
- This complicates the development of new detection methods.
- GMOs with stacked events are produced by cross-breeding of two or more gm crops, and have all traits of the original events.
- One cannot distinguish between two single events on one hand, and stacked events on the other, in complex matrices like food products.
- Stacked events need to be authorized separately from the approval of the single events.
- Stacked events will have a multiplier effect on quantification.
In the European Union (EU), just five countries grew GM crops in 2012: Spain, Portugal, Slovakia, Romania and the Czech Republic.

Currently, 68 GM crops are authorised as food / feed in the EU (as of April 24 2015).

Products containing a GM crop above a GM content of 0.9 % must be labeled.

Non-approved GM crops may not be placed on the EU market (zero tolerance policy).

Surveillance authorities like Health and Food Safety Authorities are responsible for labeling, control, and feed / seed / food analysis -
In Canada and the USA

- In these countries, GMO determined to be safe to human health and environment are not required to be labeled.
- Some states in the USA are passing individual labeling laws.
- Their Federal government is trying to stop this.
- Applicants provide all scientific information and approval is not given till the scientific evaluators deem these scientifically valid.
- A GMO once declared safe, is not further monitored, post release, except there is a specific problem.
GMO CULTIVATION – AFRICAN SITUATION

- Only Burkina Faso, Egypt, South Africa and Sudan grow genetically modified crops, commercially.
- Cameroon, Ghana, Kenya, Malawi, Nigeria, and Uganda have Confined field Trials and Biosafety laws.
- Uganda has CFTs but no Biosafety laws yet.
- Ethiopia, Malawi, Mali, Mozambique, Senegal, Tanzania, Togo, Tunisia, Zambia and Zimbabwe.
- In South Africa, food containing 5% or more of GMOs must be labeled as containing GMO.
THE NIGERIAN SITUATION

- With the Biosafety law passed, Nigeria can cultivate commercially, import, and develop for domestic use and export, GMOs.
- Nigeria has no commercial products yet
- The Biosafety law stipulates that GM products be labelled
- No lower limit of content is given in the law
- And there must be post release monitoring for
  - 150 years for trees
  - 30 – 50 years for other plants
  - 30 years for animals and microorganisms
- Are the monitoring periods realistic?
- This makes surveillance necessary and GMO detection is at the heart of surveillance
GMO THRESHOLDS

- Our biosafety law requires food that contains any level of GMO to be labeled as containing GMO.
- It also requires any food that MAY contain GMO to also be so labeled.
- Which foods will survive the labelling?
- Is the zero tolerance labeling realistic?, Is it fair?
- We need a threshold for labelling.
- International commerce makes zero GMO content now, virtually impossible.
- Also, how will Gari, beans, corn, rice and elubo from yam be labelled?
GMO PRESENCE IN NIGERIA

- A survey of the Southern states of Nigeria, when there was no law, has shown that some processed foods such as corn flakes and baby weaning food in Nigeria contain GM corn.
- The study also showed that some corn consignments imported into the country contain GM corn.
- Inappropriate equipment prevented the quantification of these results.
- This would have indicated whether the presence was intentional or fortuitous.
- At the time though, those concerned broke no law, as there was no law.
- Now that there is a law, the enforcing authority may wish to support studies like these, to achieve proper GMO surveillance.
Also,

- Universities should be supported to set up GMO detection laboratories to act as arbitration laboratories for applicants dissatisfied with results from government laboratories.

- For the avoidance of doubt, GM foods are the key to food security in Nigeria and Africa.
Thank You!