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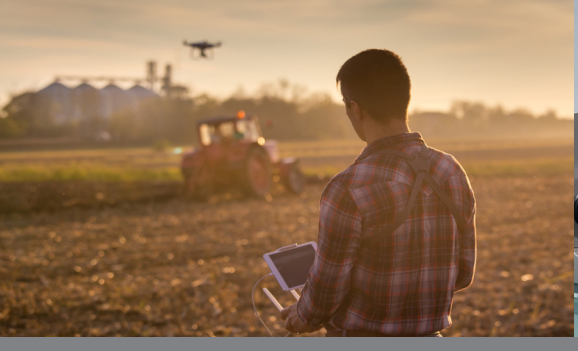

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Advances in identifying GM plants: current frame of the detection of transgenic GMOs

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1 Summary

The discovery in the 1980s of the pathogenic mechanisms of *Agrobacterium tumefaciens* led to transgenesis, a technique for increasing the diversity of traits that could be used in plant breeding. Various other means of plant transformation were then implemented. This new technique was introduced when *in vitro* mutagenesis was stalled due to the lack of screening systems for mutations, until the description in 2000 of the Targeting Induced Local Lesions in Genomes (TILLING) technique, which was then followed by various developments in plant breeding.

Consumer acceptance of the genetically modified organism (GMO) products resulting from these artefactual transformations differed between countries. In the European countries, with a long culinary tradition and numerous products under official quality labels, the precautionary principle, which had previously prevailed in third countries, was introduced in the face of these new techniques, which at the time had lacked any long history of safe use. From then on, these GMOs were only produced and marketed after a risk assessment. In addition, labelling and traceability, according to the farm-to-fork approach, are required with specific and general post-market environmental monitoring. This chapter describes the scientific, technical and regulatory framework of

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this European traceability system, which allows all European consumers to make informed choices about their food. Moreover, this traceability approach enables the coexistence of genetically modified (GM), non-GM and GM-free supply chains and should thus make it possible to avoid mixing food products with those for pharmaceutical, functional food or industrial use (GAO, 2016b).

The framework we describe in this chapter must be used to deal with the traceability of 'new' GMOs and 'hidden' GMOs. GMOs resulting from the *in vitro* mutagenesis of isolated cells and new breeding techniques (NBT), so named by the non-governmental organisations (NGOs) and the farmers' union that brought the dispute before the French Conseil d'État (FCE) in 2015, engendered a conflict that led to the European Court of Justice (ECJ) recalling the 2001/18 directive's definition of GMOs in 2018. The feasibility of this traceability of the 'hidden' and 'new' GMOs is discussed in the next chapter.

1.1 Introduction

Unlike the many targets considered for the detection or identification of molecules (e.g. aflatoxin) or pathogens, GMOs are a highly political–technical–scientific issue. Thus, when they are discussed or explored in contemporary literature and media, the state-of-the-art techniques and scientific knowledge involved are only partially taken into account (Bertheau, 2019; Davison and Bertheau, 2007, 2009). Despite the passage of 30 years, the definition of GMO is still imprecise and contested. Since 2007, the European Commission, when asked for a legal opinion on the GMO or non-GMO status of NBT products as defined by the Dutch Commission on Genetic Modification (COGEM), was unable to explain them – even during and after the decision in 2018 by the ECJ (European Court of Justice, 2018; Schaart and Visser, 2009). While the US had shown a robust precautionary mindset until the Reagan presidency, the European Union (EU), which was lagging in this domain, began to apply the precautionary principle only after the 1987 conference on protecting the North Sea (Wiener and Rogers, 2002). This consideration of a superior principle (the precautionary principle) soon became apparent in European legislative texts, for instance, about GMOs.

Directive 1990/220, replaced by Directive 2001/18 and then consolidated in Directive 2018/350 (Breyer et al., 2009), was the first legislation regulating the release of GMOs in Europe. It defined a GMO as 'an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. This directive was supplemented by Regulation 1829/2003, which regulates GMOs intended for non-crop feed and food, according to the principle of 'one key, one lock' – a way to facilitate authorisations for imported GMOs. Mandatory traceability was implemented by Regulation 1830/2003. As has often been pointed out by various authors, these

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regulations are based on techniques implemented as part of the precautionary principle governing risk assessments. These regulations are in contrast to the approach of third countries that consider only the trait and the presumed related inherent risks. These considerations within European regulations are in line with the European 'White Paper' on health security (European Commission, 2001; Garcia, 2006; Spranger, 2015) and the Cartagena Protocol, where the EU and its Member States (MS) are signatories.

Some terms in the Directive 2001/18/EC, such as 'traditional crossing techniques', are confusing and cause significant technical and legal digression. The *minima* definition, adopted by the European Commission (Commission of the European Communities, 1992), specifies that: '"Traditional breeding" means practices which use one or more of a number of methods (e.g. physical and/or chemical means, control of physiological processes), which can lead to successful crosses between plants of the same botanical family'. This definition, therefore, lays the foundation for the interpretation of 'traditional breeding' in the context of GMOs, which is in line with the recent decisions of the ECJ (July 2018) and of the French Conseil d'État (Conseil d'État, 2020). Another ambiguity surrounds the generic term 'genetics'. The rational interpretation would be that, given the progress of scientific knowledge over the past 30 years, this term also includes epigenetics and epitranscriptomics. However, up to now, interpretation based on sound science has had no significant repercussions in the European risk assessment guidelines and procedures.

Epigenetics (DNA methylation and 'histone code') and epitranscriptomics (RNA modifications), among others such as organelles' genomes, are indeed not taken into account in the current European Food Safety Authority (EFSA) risk assessments. In contrast, the transmission of those changes to offspring – a significant risk assessment element in biology and a crucial point in European directives – has long been proven. A recent EFSA colloquium on epigenetics, a term defined in 1942 by Conrad Waddington, recorded that little knowledge was available (EFSA et al., 2016). Epigenetics is the study of how genes and their products influence the expression of the phenotype in a given organism, free from any activity that involves genetic alteration. This definition evolved to describe epigenetics as being 'primarily concerned with the mechanisms through which cells become committed to a particular form or function and through which that functional or structural state is then transmitted in cell lineages' (Jablonka and Lamb, 2002, p82). The lack of consideration of these transmission mechanisms to descendants is clearly in contradiction with the European precautionary principle. Therefore, the European directives 1990/220, 2001/18, and 2018/350 do not address the transmissibility of artefactual genetic, epigenetic and epitranscriptomic and organelles' modifications arising from techniques which are still currently under development. The regulatory situation in other countries for GMOs and 'new

GMOs', whether or not they are signatories to the Cartagena Protocol that we discuss here, seems even more complicated (Friedrichs et al., 2019a,b; GAO, 2016b; Van Eenennaam et al., 2019).

1.1.1 The Cartagena Protocol on Biosafety

This 2000 International Protocol (CPB), which came into force in 2003, was implemented in the EU by Regulation 1946/2003. It uses a GMO definition slightly different from the European one but covers all *in vitro* mutagenetic (production of genetic mutations on isolated plant cells) techniques. The protocol is restricted to the living modified organism (LMO), i.e. its capacity for dissemination and reproduction and therefore, fundamentally, that which could impact ecosystems. Thus, the Europeans, signatories to the protocol, accepted this LMO concept where the definition corresponded well to the European directives (Husby, 2007).

From 2010, the 'Nagoya–Kuala Lumpur Additional Protocol' implements Article 27 of the CPB. Under this treaty, supply chain operators (stakeholders placing products on the market, producers, exporters, importers and transporters) will be held responsible, including financially, for the movement of LMOs between the MS and any potential damage caused. The EU, a signatory to this protocol ratified by the MS, must therefore be able to identify the LMOs produced or in transit through its territory and must inform the importing third countries (1946/2003, 65/2004). European companies are thus responsible if there is an insufficient qualification of their living products in this respect, such as seeds.

1.1.2 The 'proven safety' of GMOs

Directives 2001/18 and 2018/350 specify: 'This Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record'. The appreciation of a long history of proven safety can only be judged on the history of the techniques used and their mass production. The 2018/350 Directive offers an explicit reference to what cannot be considered 'a technique traditionally used for various applications and whose safety has long been proven'. Transgenesis is included in its Annex 1A, among the new techniques producing GMOs, offering a valuable indication of the legislator's intention when deciding not to consider the process as traditional and of proven safety. Therefore, the timeframe for transgenic plants were 1983 for the proof of concept, 1986 for the first field experiments, 1990 for the first commercial genetically modified (GM) tobacco, 1994 for the first commercial GM vegetable, tomato, and 1996 for the first mass-marketed soybean. Thus

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2001, the year of the Directive's release, can be considered the beginning of the safety monitoring of GMOs.

Conversely, what were the techniques developed during the previous decades from 2001 that could have resulted in commercial products that would be of proven safety, *a fortiori* in 2020? The participants of an international symposium in London in 2016 concluded that the set of *in vitro* techniques, that is, the use of isolated plant cells, used in transgenesis and genome editing techniques, had changed very little over these decades (Altpeter et al., 2016; Hiei et al., 2014; Ikeuchi et al., 2019; Ledford, 2016; Maher et al., 2020; Que et al., 2014).

Apart from the first transgenic GMOs within the meaning of Directive 2001/18 and the *in vivo* mutagenesis identified by the Food and Agriculture Organization (FAO), no variety derived from *in vitro* cultures of isolated cells was listed before the 2000s (National Academies of Sciences Engineering and Medicine, 2004; National Research Council, 2009). Therefore, the *in vitro* cultures of isolated cells had no proven safety record.

Finally, the genotype–phenotype relationship of mutations transmissible to offspring remains an overall unmet challenge. Despite the progress in sequencing, the somaclonal variation, random mutations and epimutations remain challenging to exploit, except for rare traits that are easily selectable, such as pesticide tolerances. It was only at the beginning of the twenty-first century that the TILLING technique enabled their exploitation through costly 'high-throughput' platforms, capable of screening thousands of mutants and making it possible to exploit, by marker-assisted selection, some of these random mutations and epimutations (Anderson et al., 2016; Irshad et al., 2020; Manzanares et al., 2016; McCallum et al., 2000a; McCallum et al., 2000b; Unterseer et al., 2014). The difficulties in the adaptation of TILLING to various species and the costly installation of platforms that were able to screen the numerous mutants, which took several years, meant a delay in delivering practical outcomes despite some recent 'speed breeding' (Kurowska et al., 2011; Pratap et al., 2018, Watson et al. 2018).

Indeed, with the current state of the available scientific and legal data and the absence of traceability and post-marketing surveillance systems, it is estimated that the minimum necessary duration to enable confidence in the demonstration of a long-proven safety could be well above 30 years.

1.1.3 'Hidden GMOs' and new techniques for modifying genomes and epigenomes

We have previously mentioned the ECJ and the FCE decisions. This challenge is currently decisive in the political definition of GMOs that should be traced. In 2007, the Dutch COGEM had drawn up a heterogeneous list of various techniques (the NBTs) for which the European Commission was questioned as to the GMO

status, or not, of the resulting products (Schaart and Visser, 2009). The lack of a legal response from the Commission allowed the various stakeholders to ensure that the resultant products were not GMOs as envisaged by some third countries.

This absence of a response from the EC proved to be a loophole into which companies rushed. The marketing of varieties which were tolerant to herbicides (VrTH in French), with unknown status and for which the breeding processes were not available at the time of registration in the national catalogues of cultivars, was the catalyst to close this loophole. In 2015, in response to the herbicide-tolerant varieties, such as Clearfield rapeseed, a consortium of nine French environmental associations and agricultural unions filed an action before the FCE to annul an article of the Environmental Code which excluded organisms obtained by 'mutagenesis' from the scope of French GMO regulations. In 2016, the FCE considered it necessary to seek the opinion of the ECJ on four questions of interpretation of the European law, while adding requests on the status of products resulting from new techniques for modifying genomes and epigenomes. In 2018, the ECJ reminded[1] that organisms obtained by mutagenesis (thus including *in vitro*, i.e. with a step of isolated cell cultures) are indeed GMOs within the meaning of Directive 2001/18, with a few ones being exempted. The Annex I.B.'s exemption applies only to mutagenic techniques developed mainly before its adoption and whose products would have demonstrated a proven safety (implied by prolonged commercial use).

This ECJ decision conformed with the definition of traditional breeding found in Directive 90/220 and the above recalled 1992 CEC's definition. Therefore, the GMO Directive was determined to cover organisms derived from *in vitro* cultures of isolated cells and NBTs (as new techniques mainly developed after 2001). As a result, the Conseil d'État enjoined the French government in February 2020 to (i) amend the Environmental Code and to (ii) establish a restrictive list of mutagenesis techniques or methods traditionally used for safety purposes that have long been proven, allowing their exemption. The French Government was finally asked to identify, within the Official Catalogue of Varieties, varieties obtained by mutagenesis techniques that were mainly developed after the adoption of Directive 2001/18. Those cultivars should have been subject to the obligations applicable to GMOs, in terms of risk assessment, prior authorisation and traceability and labelling in particular (GAIN USDA, 2021), and, thus, should be withdrawn from the market. The draft decree of the French authorities triggered a community barrage of questions and concerns.

In November 2019, The Council of the EU requested[2] the Commission (Council Decision (EU) 2019/1904) to submit, by 30 April 2021, 'a study in light of the Court of Justice's judgment in Case C-528/16 regarding the

1 https://eur-lex.europa.eu/legal-content/FR/TXT/?uri=CELEX:62016CJ0528
2 https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en

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status of novel genomic techniques[3] under Union law' (Directives 2001/18/EC and 2018/350, Regulations (EC) 1829/2003, Regulation (EC) 1830/2003 and Directive 2009/41/EC). This EC request for April 2021 did not prevent the Commission from delivering in advance its conclusion to the French authorities in 2020 about the project of the French decree. An anticipated reply that still militates in favour of political decisions of principle instead of legal and scientific considerations.

The decision of the ECJ is binding and cannot be appealed against. Therefore, in Europe, GMOs' traceability must target any organism that has gone through a phase of isolated cells in *in vitro* culture, including those obtained by adding new techniques.

1.1.4 The mutagenesis exemption

The 2001/18 and 2018/350 directives are based on the precautionary principle, by defining GMOs as human-mutated–artefactual–organisms. They are exempt from the requirements of risk assessment and labelling for the products of techniques with numerous applications and a long safety record at the 2001/18 adoption time. As previously stated, they were no clear indications about which products were to be considered, but the techniques' history made it clear. Several stakeholders, MS and EC executives took the *in vivo* mutagenesis techniques started in the 1930s as an example of exempted methods. For these actors, the *in vivo* International Atomic Energy Association (IAEA) mutants rather massively developed since the 1950s are adequate proof of any random mutagenesis in the requested long history of safety. The stakeholders and executives had then argued that *in vitro* mutagenesis was an in-depth technique developed before 2001 in continuity with the *in vivo* IAEA-listed mutants. Thus, according to them, *in vitro* mutagenesis should be considered as the continuity of the *in vivo* one, and *in vitro*-derived mutants should thus be exempted. However, as pointed out by Thorpe (2012):

'During the 1990s, continued expansion in the application of *in vitro* technologies to an increasing number of plant species was observed. However, only limited success has been achieved in exploiting somaclonal variation (Karp, 1994) or in the regeneration of useful plantlets from mutant cells (Dix, 1994); also, the early promise of protoplast technology remains largely unfulfilled (Feher & Dudits, 1994)'.

There would thus be an apparent drastic technical and historical discontinuity between *in vivo* and *in vitro* techniques. The reasoning is also supported by the effective use of *in vitro* mutants only after the publication of the TILLING technique in 2000 (McCallum et al., 2000a; McCallum et al., 2000b). Indeed, the

3 NGT (Novel Genomic Techniques) is the new name of NBT.

induction of DNA-damaging mechanisms might differ between the *in vivo* and *in vitro* techniques (Brash and Hart, 1978; Krishna et al., 1987). A certain stochasticity of 'rebel' cells will impede a direct complete 3D regeneration from a totipotent isolated cell. A totipotent cell is a single cell that can give rise to a new organism. Thus, several external impulses are necessary to induce the development of organisms (Mojtahedi et al., 2016; Richard et al., 2016). Drug discovery and cancer studies are some of the areas of advanced biology which require the 3D organisation of cells, for example, by creating organoids (3D tissue cultures) when the whole organism is not suitable. Indeed, the varying cell types, spatiotemporal organisation, diffusion gradients and the molecular contents in the interior and the immediate environment; that is, the interactions between the cells and matrix(s), in an organoid, are more representative of life than isolated cell cultures (Edmondson et al., 2014). Epimutations can be observed after *in vitro* fecundation (fertilisation) but not in *in vivo*-conceived children (Peters et al., 2015; Song et al., 2015). Similarly, the somaclonal variations caused in plant cell cultures vary according to the species' reproductive biology, the cultivar and the number of individuals used, and the culture protocol, which does not promise any *in vivo–in vitro* continuity (Martínez, 2018; Skirvin et al., 1994).

The cells and tissues sense their environment and communicate to coordinate themselves through diverse molecules, including nucleic acids whose natural epigenomic 'decoration' (patterns) is essential for non-self-identification (Hammarlund et al., 2020; Monticolo et al., 2020; Sablowski, 2016). This essential epigenetic profile is generally lost during *in vitro* manipulations. Exocytosis is an *in vivo* phenomenon of how the cell transports molecules so that they can be released from the cell without a corresponding model *in vitro*, while polyploid plants exhibit more significant variability in plants regenerated *in vitro* (Gavazzi et al., 1987; Ruiz et al., 2020; Skirvin et al., 1994; van den Bulk et al., 1990; Žárský et al., 2009). Multicellular organisms evolve by selecting, *in vivo*, the germinal and meristematic cells 'resistant' to mutations and epimutations, the opposite of *in vitro* somaclonal variation (Balestrazzi et al., 2020; Burian et al., 2016; Morrow, 1975). This idea of *in vivo* selected cells protected from the effect of mutagens is found in other somatic mutation studies (Franco et al., 2019). Indeed, some species seem to have evolved by selecting low rates of somatic mutations and epimutations, which do not correspond to the variations observed *in vitro* (Hofmeister et al., 2020; Orr et al., 2020). Thus, the *in vivo* and *in vitro* stages and mutageneses look dramatically different, and these legal and biological traits interact with GMO traceability issues.

1.1.5 Summary

The remainder of this chapter, and the next one, will consider the various techniques used to generate GMOs within the ECJ and FCE context. After a

section dedicated to the current European frame in place for GMO traceability, this chapter will also explain the elements that are considered to be scars and signatures which could likely detect and identify new GMOs, and even suggest the technique used at their origin. 'Scars' are the unintended genetic and epigenetic modifications due to techniques related to isolated cells in *in vitro* cultures. 'Signatures' are both the unintended changes and the molecular patterns necessary for a mutagenesis technique to be performed, such as gene editing.

Due to the EC and MS' inability to launch research programmes on detecting and identifying 'hidden'[4] and new GM varieties and prove their artefactual character, since February 2020, many stakeholders and some policymakers and scientists have expressed that the ECJ's decision cannot be implemented (Haut Conseil des Biotechnologies (HCB), 2017; Scientific Advice Mechanism (SAM), 2017; Vain, 2007; van der Meer et al., 2021). Indeed, compared to transgenic GMOs with the random insertion of foreign DNA providing a univocal molecular signature, the new challenge is that with the similarity of the essential components of organisms such as nucleotides and DNA, cut repair systems are impeding us from distinguishing, easily in one step, some targeted modifications that may be natural or artefactual, such as SNV and indels of SDN1[5] and SDN2 (Rostoks, 2021). This is a way of saying that a sand dune and a skyscraper are indistinguishable because they are both made from sand.

However, the European Network of GMO Laboratories (ENGL) recognised that analytical traceability was possible to facilitate the enforcement of procedures, if enabled by the classification of products as GMOs (European Network of GMO Laboratories (ENGL), 2019). The information gathered from notifiers could allow laboratories to develop identification methods by considering several converging elements, such as molecular markers, in a matrix approach. These elements, that would make these products from NBT *in vitro* cultures detectable and identifiable, and the applicable European GMO regulations, differentiating them from spontaneously mutated products, are now considered. The transgenesis-derived GMOs can serve as a framework and model for the detection of these 'new GMOs'. The lack of engagement,[6] created by the European Commission's refusal in 2017[7] to let ENGL work on the detectability of products derived from NBTs, should not be repeated. Indeed, all techniques and targets exist for the classical detection of these new GMOs. The detection techniques required have been developed in recent years by the ENGL network of laboratories (Holst-Jensen et al., 2012, 2013).

4 The French VrTH (herbicide-tolerant varieties such as Clearfield rapeseed)
5 Sequence-Directed Nuclease Mutagenesis
6 https://www.infogm.org/6646-ue-experts-se-penchent-methodes-de-detection
7 https://www.infogm.org/6678-nouveaux-ogm-commission-europe-veut-elle-tracabilite https://www.infogm.org/6679-new-gmos-does-european-commission-want-them-traceable

2 The technical framework of the traceability of GMOs

2.1 Introduction

Preliminary studies with stakeholders have made it possible to define a threshold for exemption from GMO labelling in a situation of adventitious (accidental) or technically unavoidable presence (Bertheau, 2002; Bertheau et al., 2002; Davison and Bertheau, 2007; Regulations 258/1997 then 2015/2283, 49/2000, 50/2000, 1830/2003). The unit was not defined in these scenarios, but the reasoning and data were based on polymerase chain reaction (PCR)-targeted DNA techniques, which were later formally endorsed by the ENGL network. The European General Food Law (Regulations 178/2002, 2019/1381) made traceability a mandatory 'one step forward and one step back' for all food products, including GMOs. It established the EFSA and the European Union Reference Laboratories (CRL-GMFF renamed EURL-GMFF), installed for the first time in a European authority (JRC, Ispra, Italy). Regulation 1830/2003 established an administrative traceability system for easily placing genetically modified (GM) food/feed on the EU market. It further complemented Article 21 of Directive 2001/18/EC by exempting labelling products below a 0.9% threshold of adventitious (accidental) and technically unavoidable presence of GMOs. A European network of State Control laboratories and research laboratories was informally set up in Ispra (Italy) in 1999, based on the Belgian and French network models. This network was officially opened by the European Commissioner for Research, Philippe Busquin, in December 2002. This ENGL network for GMO detection has since integrated the National Reference Laboratories (NRL), established by Regulation 1981/2006.

The various regulations and directives oblige the GMO producers who wish to market their products in Europe to provide a detection/identification/quantification method and the control material for interlaboratory validation of the procedure provided. In addition, a commercial source of reference material must be made available to private and enforcement laboratories. Originally prepared from seeds certified at the JRC IRMM[8] of the European Commission, the production of these reference materials, at this time, originated from seeds as powder and then in DNA form, but was gradually transferred by the petitioning companies to a private actor. The quality of this new reference material was recently judged to be flawed by the enforcement laboratories. The ENGL and the NRL had to re-specify the rules and criteria[9] of production in the face of these producers' laxity (EURL-GMFF, 2019b).

8 Institute for Reference Materials and Measurements of the Joint Research Centre.
9 https://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

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Most of the ENGL research laboratories participated in the FP4–FP7[10] European programmes. Those projects focused on GMO traceability strategies (QPCRGMOFood and GMOChips), on cultures' co-existence (SIGMEA) and finally the traceability and co-existence in the whole supply chains (Co-Extra, the largest of those European research programmes) (Bertheau, 2013a; European Commission, 2010).

As in other areas of food safety, these control laboratories are seeking ISO 17025 accreditation. Therefore, the laboratories participate in private, national and EURL-GMFF[11] proficiency tests (Broothaerts et al., 2020; International Standard Organisation (ISO), 2017; Ribarits et al., 2021; Trapmann et al., 2014; Žel et al., 2006). The ENGL laboratories also participate in the interlaboratory validations of the petitioners' methods. Therefore, the ENGL laboratory network is the technical, regulatory and scientific backbone of a complex detection domain whose goals, functionalities and rules are still evolving. The European Commission strongly supervises/verifies that there should be this world reference in GMO traceability.

Before any scientific consideration, the definition and controls of GMOs depend fundamentally on political negotiations as much as its measurement unit, and this frames the technical, scientific and the recent legal upheavals.

2.2 Traceability in the GMO context

Traceability is a vital requirement for consumers, European consumers in particular. Traceability is the ability to trace the history, application, use and location of an item or its characteristics through recorded identification data. As stated by the Codex Alimentarius, 'Traceability is the ability to track the movements of a food among the specific stages of production, processing and distribution', a statement following the EU's 'Hygiene Package' (Dwinger et al., 2007). Document-based traceability is the first requirement along food-processing chains. It is a prerequisite for compliance with the international specific quality management standards (ISO 9000:2015, ISO 9001:2015) and is linked with food safety standards (ISO 22000:2018 and ISO 22005:2007). Traceability is mandatory in the EU since the General Food Law (178/2002 regulation) of the 'Hygiene Package'. The information must be available 'one step forward and one step back', and traceability documents must be retained for GMOs for a minimum of five years. This regulation avoids any distortion of competition and additional costs in the EU. Indeed, the GMOs' documentary traceability requirements do not differ from the other EU-mandatory traceability obligations. This device was implemented to satisfy the precautionary principle

10 https://ec.europa.eu/eurostat/cros/content/research-projects-under-framework-programmes-0_en
11 https://gmo-crl.jrc.ec.europa.eu/Proficiency-tests.html

introduced by the community 'White Paper' of 2001 (Aerni, 2019). The complete integration of the general procedures for traceability and alerts in the EU has enabled the development of a reliable traceability system for GMOs, included in the RASFF[12] alert system, without additional cost (Demortain, 2011; Parisi et al., 2016; Price and Cotter, 2014). However, despite these regulatory controls, fraud, adulteration and accidental contamination, whether or not due to human error, are still possible, although control systems such as the blockchain, may reduce the products' falsifiability (Demestichas et al., 2020).

The history of GMOs is indeed a long process of unexpected contamination of seeds and food chains, due to human errors in particular, for example, by unauthorised products such as StarLink® (intended for the industry) or Prodigene (intended for pig vaccination) corn varieties (Ellstrand, 2003; Price and Cotter, 2014). This inability to trace GMOs in some areas of the world was exacerbated after September 2001 when the US authorities' concern increased about their failure to protect themselves from malicious GMOs, especially if they were possible weapons of bioterrorism/biowarfare. The US Government Accountability Office (GAO) recommended strengthening the cooperation between the US agencies concerning traceability, which could have led to a convergence of operations between the regions of the world (Davison and Bertheau, 2008; GAO, 2008, 2016a, b). The fight against bioterrorism spurred the development of PCR devices or other detection systems, miniaturised and usable in the field or storage silos, together with techniques and strategies applicable for the detection of unknown biological elements and unknown GMOs such as NBT products (Hedman et al., 2018; Holst-Jensen et al., 2011; Minogue et al., 2019; Parida et al., 2020; Sugumar and Kong, 2008).

Transgenic GMOs provide unambiguous signatures by their random insertions and sometimes rearrangements of the insert, but the need to control analytical costs led to the development of the 'matrix approach'. The primary goal of this approach was to determine the elements common to several GMOs to establish screening strategies, followed by the identification–quantification for authorised GMOs. Thus, the databases made it possible to identify the most authorised GMOs' unequivocal profiles (patterns). Any discrepancy with these profiles allowed a suspicion of the presence of unauthorised GMO(s). Any information (sequence, geographical origin, nature of the processed product or whistle-blower alerts) can also be used. This matrix approach was adopted in all control laboratories. Such multi-parametric multi-step methods are used in many detection/identification fields with workflow systems, such as metabarcoding (Liu et al., 2020a) or other decision-making tools (Ruttink et al., 2010; Bellocchi et al., 2010; Bohanec et al., 2013). The detection of unknown GMOs is also possible, through a variant of the matrix approach

12 Rapid Alert System for Food and Feed https://ec.europa.eu/food/safety/rasff_en

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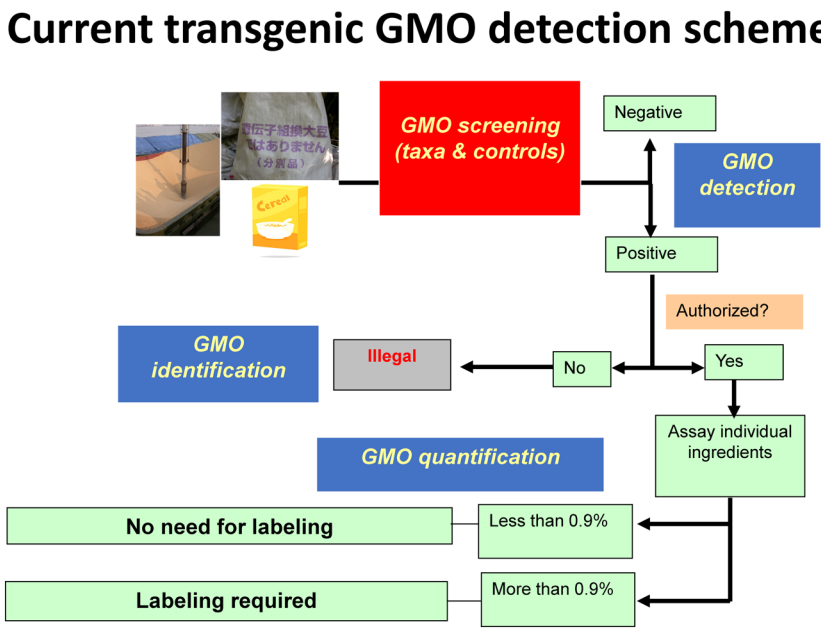

Figure 1 Current two-step procedure for the detection of transgenic GMOs. The matrix approach can be implemented more in depth, when necessary, e.g. for unknown GMOs.

– the differential quantitative PCR – which works by quantifying the sequences common or not to several GMOs (Cankar et al., 2008; Gruden et al., 2013). Detecting and univocally identifying one or more GMOs, even the unknown ones, with the matrix approach, is more straightforward than identifying the taxa of an ecosystem (Ruppert et al., 2019).

Due to the strong consumer rejection of these new products, the detection of GMOs from transgenesis developed rapidly (Regulation 258/1997 then 2015/2283) (Levidow and Marris, 2001; Gaskell et al., 2004; Kantar, 2019). The multiplication of GMOs was accompanied by a census of their characteristics in tables, and then in databases such as GMOMETHODS[13], GMDD[14] and EUGenius[15] (Hemmer, 1997; Dong et al., 2008; Bonfini et al., 2016). These databases made it possible to organise the cost-effective analyses by successive steps, to determine one or several GMOs – from the screening step, using elements common to several GMOs, to a unique GMO identification, through its patterns or its unequivocal signature, followed by quantification. Various private or public tools were subsequently developed on the web,[16] or

13 https://gmo-crl.jrc.ec.europa.eu/gmomethods/
14 http://gmdd.sjtu.edu.cn/
15 https://euginius.eu/euginius/pages/home.jsf
16 https://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/

as the JRC pre-hybridised and implementable probe plates (Angers-Loustau et al., 2014). This type of methodological approach (see Fig. 1) provides a standardised basis (CEN/TS 16707, 2014) that can be extended to other targets, as discussed later in this and the next chapter. Varieties, like the Elite varieties of commercial cultivars, pangenomes and structural variants are also identifiable by such approaches (Alonge et al., 2020; Li et al., 2018; Sohn et al., 2017), for example, by detecting lesions in non-replicating DNA (Moreno et al., 2016). This polymorphic, multi-target screening step is capable of targeting from point variant to chromosomal rearrangement. It uses simplex or multiplex PCRs in e.g. SNPLex or DNA chips (Hougs et al., 2017; Wilkesa et al., 2016). Figure 1 presents a very simplified scheme of data analysis for identifying the GMOs of an agri-food product. Some determination criteria – such as epigenetic and epitranscriptomic modifications – have not been incorporated, for ease of reading. However, the principle remains the same: after organising the data, the analyses by dichotomous choices classically used in the detection of transgenic GMOs will allow, with the help of decision support tools (tables, decision support systems (DSS), artificial intelligence) to detect any new GMO, such as NBT products, and to verify that the traits used in the detection and quantification are not natural because they are integrated into an artefactual context, the unequivocal signature of the GMOs. The convergent flow of evidence, given as an example in Fig. 1 of the next chapter, is particularly illustrated in the case of an SDN1 or SDN2 modification; an SDN3 change would be identified by the univocal signature of the insert's edge fragments (these modifications are discussed further in the next chapter).

The analytical methods in this context must meet several objectives at the lowest cost, to identify and quantify a target and allow a multi-target approach to detect unknown GMOs. Various alternative methods have been developed, such as the bioassay of plantlets (e.g. herbicide-tolerant soybean seedlings) or infrared detection, to avoid the destruction of valuable material or large quantities of material (Anklam et al., 2002; Burgos, 2015). This last method might lead to a suspicion of the presence of unknown GMOs (Bonfini et al., 2001; Michelini et al., 2008; Fumiere et al., 2009). These methods have not experienced any commercial or enforcement developments.

Some immunological methods continue to demonstrate that they are of interest in some specific situations. An immediate benefit of the development of GMO detection methods in the early 2000s was the standardised development of methods and procedures used later on in other detection sectors; however, the associated societal benefit, positive externalities and an essential return on investment are difficult to quantify (Davison and Bertheau, 2007, 2009; Bertheau and Davison, 2013).

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2.3 The DNA target molecule in detection

DNA was chosen as the preferred target for this approach to analytical traceability, as the methodology is usable (i) from the field to the final consumer, with (ii) a continuity of detection available along the supply chains, and (iii) the versatility of the approaches it provides from the screening of sequences common to several GMOs to the quantification of univocal sequences, and (iv) agility from the sequencing of short, amplified fragments to e.g. the chromosome walking for the identification of unknown GMOs. DNA preserves well. It can be found in ancient, mummified and fossilised samples and products after many industrial processes, even if the size of the extractable fragments has decreased. The decrease in size is not too much of a problem, since quantification in quantitative real-time PCR (QRT-PCR) and other derived techniques are more reliable with relatively small pieces. Only highly refined products, such as certain industrial sugars and oils, contain such small amounts of DNA that the analytical cost becomes prohibitive.

The DNA unit proposed by the ENGL network was accepted by the European Commission (2004/787). The method for calculating the GMO content (GMO%) is based on the haploid genome unit:

$$\text{GMO\%} = \frac{\textit{Copy Number of the GMO} - \textit{specific sequence}}{\textit{Copy Number of the Taxon} - \textit{specific sequence}} \times 100$$

The PCR, qualitative or quantitative real-time, is the dominant target amplification technique for (i) its sensitivity, (ii) its specificity at several levels (from point mutation to structural mutant) and (iii) its versatility (detection, sequencing, nested-PCR to improve specificity and sensitivity and a combination of techniques such as SNPLex and amplification for hybridisation on DNA chips). However, some limitations in multiplexing, especially the amplification errors, are often forgotten, for example, in preparing sequencing libraries. The development of small amplification devices, driven, for example, by biodefense's needs, allows their use from the seed to the field, from the grain silo to the transformed product through the cargo. Signal amplification techniques have not been successful, particularly following QRT-PCR development with a calibrator (Miao et al., 2015; Vora et al., 2008). Some other target amplification techniques continue to be used or even take precedence over PCR in some situations. Of particular note is the current trend towards isothermal nucleic acid amplification techniques such as LAMP, RPA and NASBA (Bodulev and Sakharov, 2020; Kumar et al., 2018), with less sophisticated and cheaper devices and generally much shorter amplification time. The lower need for extraction and purification of target DNA due to lower sensitivity to inhibitors gives these techniques another advantage.

Moreover, they can be easily used for DNA and RNA amplification at different points of need, such as fields (Zou et al., 2020), with more understandable readings when coupled with qualitative detection towards a dipstick (Lateral Flow Device) or flocculation (Singh et al., 2019b, Mason and Botella, 2019). On the other hand, the more complex methodological developments, such as the need to define four or six primers or accentuated constraints in multiplexing, without taking into account the effect of various inhibitory reagents, hamper their use, which has not yet been integrated into the standards and methods proposed by the notifiers (Parida et al., 2008; Lobato and O'Sullivan, 2018; Zou et al., 2020). Ligase Chain Reaction (LCR) is a technique able to detect point mutations and is used in multiplex techniques such as SNPLex (Chaouachi et al., 2005). New techniques which do not use enzymes, such as the Host Cell Reactivation (HCR) Assay, and could be used on-site (point-of-need), are not yet in the GMO methodological pipelines (Ikbal et al., 2015; Ouyang and Han, 2019).

The use of digital PCR (ddPCR) is another discernible technological trend. This application of limit dilution sub-sampling techniques has the advantage of reducing the effect of PCR inhibitors (Quan et al., 2018) and their dependence on reference materials of fluctuating quality and availability.

Indeed, some GMOs withdrawn from the market can take years to disappear from supply chains, but remain quantifiable (Regulation 619/2011). This regulation also established a 0.1% tolerance threshold for unauthorised GMOs in feed to ease the flow of international trade, provided the EFSA had studied the dossier. Such a threshold does not apply to food or seeds.

These simplex or multiplex techniques have been completed by high-detection density techniques such as biosensors, microarrays and DNA chips, for which the amplification of the signal and the target can increase the detection sensitivity (Chen et al., 2018; Vora et al., 2008). These types of multi-target biosensors, previously developed by the European GMOChips programme, are particularly well suited for screening and the matrix approach (Chaouachi et al., 2005; Gruden et al., 2013; Holst-Jensen et al., 2013; von Gotz, 2010). Tiling chips could also be adapted for GMO detection purposes (Gregory et al., 2008).

2.4 Other GMO detection targets

Inexpensive and rapid, the immunological tests developed are still used by some operators but have not been integrated into the routine of state controls (Fraiture et al., 2015; International Organization for Standardization (ISO), 2004; Stave, 2002). Some proteins may be present in several GMOs, thus preventing the differentiation between authorised and unauthorised transformation events, while the protein production varies significantly

between tissues and varieties (Grothaus et al., 2006; SeedQuest, 2011; Van den Bulcke et al., 2007). In some cultivars, the protein content may be even lower than the quantification limit. Nevertheless, the immunological tests have proven their usefulness for operators, particularly in some particular cases such as Cry9C, the only reported case of a protein specific to a transformation event. Indeed, immunological tests were used to remove StarLink®, a GM corn approved for industrial use and animal feed, from the food market, which would have cost nearly US$1 billion[17] (Bratspies, 2003; Diaz et al., 2002; Price and Cotter, 2014; Schmitz et al., 2005). StarLink® was the first example of a long and controversial series of contaminations due to insufficient supply chain segregation. These tests can be used mainly in the field or at harvest and transport to silos, and are still used in production quality control in the seed field as for RoundUp Ready® soybeans, that is, mainly with self-pollinated species. Nevertheless, these immunological tests (Agdia, SDIX, EnviroLogix, Creative Diagnostics) are still used for dispersion studies (Strain et al., 2014), or as versatile screening means, for example, in 'GMO-free' supply chains (Identity Preservation, Eurofins).

Epigenetic and epitranscriptomic targets have so far not been considered in the case of transgenic GMOs. These targets are nevertheless detectable but do not have the critical feedback from the DNA target detection (Bey et al., 2016; Fojtová and Fajkus, 2020; Mozgová et al., 2015). Apart from the reverse transcription usable for RNA detection with PCR (RT-PCR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), recombinase polymerase amplification (RPA) and various other methods will be applicable in GMO traceability (Zhao et al., 2020).

2.5 DNA extraction

The matrix effect is often underestimated, as for PCR for sequencing libraries (Pasquali et al., 2019; Robin et al., 2016). From the beginning of GMO detection, it was necessary to choose between a general lysis-extraction process followed by a purification step and specific processes adapted to each matrix. The latter choice would have increased the accreditation efforts and training of personnel to different methods. The current consensus is to use a general technique, most often CTAB-based, followed by purification to reduce the amount of inhibitors present (International Organisation of Standardization (ISO), 2005a). The phenol–chloroform process remains the best on a diagnostic lab scale but can be discarded for its risks (Bonfini et al., 2001; Piskata et al., 2019; Sajali et al., 2018). The dramatic difficulties faced with DNA extraction explain the absence

17 https://en.wikipedia.org/wiki/StarLink_corn_recall https://www.ncbiotech.org/news/lessons-aventis-starlink-corn-recall

of an ISO standard. This difficult extraction step partly explains the growing success of alternative amplification methods to PCR and ddPCR, which are less sensitive to PCR inhibitors (Walker and Hsieh, 2019).

Specific matrices and highly refined products are always subject to peculiar treatments (Corrado, 2016; Demeke and Jenkins, 2010; Gryson et al., 2002; Waiblinger et al., 2007). Documentary traceability, with the conservation of samples before processing, is then essential. Ideally, the sample preparation should allow several analyses, including screening – which is a subset of the matrix approach – followed by identifying the GMOs, all with sufficient replications to assess the measurements' variability (Block et al., 2013; Gerdes et al., 2012a).

2.6 GMO quantification

The QRT-PCR assay remains the method of choice for its versatility, especially the standardisation that has accompanied it, despite alternative quantification techniques (Holst-Jensen et al., 2006; International Organization for Standardisation (ISO), 2005c, d, 2006). The availability of reliable reference materials is currently necessary for this technique. The matrix approach and the detection of epigenetic and epitranscriptomic changes also require reference materials that are as complete as possible, such as crushed seeds. Indeed, the current trend to develop alternative certified reference materials, such as plasmids, or suppress them in front of the ddPCR, poses a risk for current and future applications of, for example, the matrix approach or chromosome walking (Trapmann et al., 2010; Caprioara-Buda et al., 2012; Deprez et al., 2016).

Qualitative immunological or PCR methods can be used with subsampling strategies, of which ddPCR is only an application, to determine the GMO content according to a previously defined threshold (Bertheau and Kobilinsky, 2004; Kobilinsky and Bertheau, 2005; International Organization for Standardization (ISO), 2005b, 2020; Macarthur and von Holst, 2012). A cost function has even been integrated into the International Seed Testing Association (ISTA) and the EU-acknowledged methods for seed quality determination (Gregoire et al., 2002; International Organization for Standardisation (ISO), 2020; Laffont et al., 2005; Regulatory Committee of Directive 2001/18, 2020; Remund et al., 2001).

A great deal of work has been done to reduce the measurement uncertainties, and with them, litigation. However, the recent regulations aimed at reducing the GMO content by requiring the mass conversion of measurements made into haploid genome equivalents may increase these uncertainties at several levels and increase litigation (Corbisier et al., 2017; Corbisier and Emons, 2019; EURL-GMFF, 2019a; Rose et al., 2011; Žel et al., 2012).

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The vast uncertainties due to sampling are not considered in laboratory practices (Gy, 2004a,b).

2.7 Standardisation of GMO detection methods

The analytical traceability of GMOs was the first systematic application of PCR and its quantitative variations, together with the necessary international guidelines and standards (ISO 21569, 21570, 21571, 24276, 21098). This standardisation work is based on performance criteria that are still relevant, both routinely and for petitioners' supplying methods (Bertheau et al., 2002; European Network of GMO Laboratories (ENGL), 2015; International Organization for Standardisation (ISO), 2006 – ISO 24276). The ISO working group on GMOs, initiated by France, has now extended its activities to identifying varieties by the matrix approach of biomarkers (fingerprinting) (ISO/TC 34/SC 16 Horizontal methods for molecular biomarker analysis). The mutual recognition of the different variants of techniques developed by the firms is in progress through standardisation at the International Union for the Protection of New Varieties of Plants (UPOV)[18] and ISO; International Organization for Standardization (ISO/TC 34/SC 16), 2015; International Organization for Standardization (ISO), 2019a,b,c).

Variety identification by fingerprinting is an ancient practice whose principles are usable for all GMO traceability (Kwon et al., 2005; Bhargava and Sharma, 2013; Korir et al., 2013 Matsaunyane and Dubery, 2018; Singh et al., 2019a). This set of techniques addressing the nuclear genome and epigenome complements the use of organelle (chloroplast) genomes, for species and variety/cultivar identification and for marker-assisted breeding (Daniell et al., 2016; Żmieńko et al., 2011). These techniques, already implemented by the ISTA[19] laboratories, involve multi-parametric analyses of numerous molecular markers for the unambiguous identification of new GMOs. Tiling DNA chips used in plant breeding will facilitate these identifications and classifications (Anderson et al., 2016; Liu, 2007). The increasingly used techniques such as NASBA and LAMP are not the priority of standardisation, which remains a long and expensive procedure.

This lack of consideration for such techniques can also be explained by the significant changes they would entail for analytical laboratories in training, purchasing equipment, or evaluating the equivalence between PCR and these methods.

However, the accuracy of all these laboratory techniques depends on the representativity of the samples. We thus have to consider the aspects of sampling all along the supply chains.

18 Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular, https://www.upov.int/meetings/en/topic.jsp?group_id=261
19 International Seed Testing Association

3 Sampling and supply chains coexistence issues

3.1 Introduction

GMO sampling strategies are not fundamentally different from those used in other areas of quality control. They are conditioned by the size, homogeneity of the products' distribution, and the distribution of the element(s) to be detected. They also vary according to the position in the supply chains (Onori et al., 2013). The detection of small quantities of GMOs, known as LLP,[20] that is, a GMO which is currently unauthorised but in imported feed or withdrawn from the market, remains a significant issue (Demeke and Perry, 2014; Demeke et al., 2014; Grohmann et al., 2014).

Homogeneous products such as seeds or products processed in large quantities have standardised sampling plans, including cost functions (Allnutt, 2006; Hochegger et al., 2016; Kobilinsky and Bertheau, 2005; Laffont et al., 2005; Remund et al., 2001). On the other hand, sampling in the field, in the framework of coexistence, or cargoes, imposes complicated and expensive sampling strategies that introduce substantial measurement uncertainties (Bancal et al., 2014; Bellocchi et al., 2009; Esbensen et al., 2012; Eurachem and CITAC, 2007; Kay and Paoletti, 2001; Minkkinen et al., 2012; Paoletti et al., 2003). Some estimates indicate sampling uncertainties in the final measurements, which are at least 100 times greater than those of the analytical ones (Gy, 1998).

The heterogeneous distribution of the elements to be detected, as for mycotoxins, increases the uncertainties, complicates sampling plans and increases costs (Armitage, 2003; Miraglia et al., 2009; Onori et al., 2013). Several commitments such as high confidence limits, low detection thresholds, and the management of withdrawn GMOs also increase costs (EURL-GMFF, 2011; European Commission, 2011a). Accordingly, some authors propose using simplified mycotoxin-specific sampling designs instead of the new complex ones.

Field sampling, especially for cross-pollinated species, is undoubtedly one of the most challenging and contentious steps (Darmency et al., 2009),. It has been the subject of much work in the SIGMEA and Co-Extra research programmes because of the long-distance dissemination for open-pollinated plants (Brunet et al., 2013; Messéan et al., 2006; Sustar-Vozlic et al., 2010). Some viable pollens can spread 3 km for corn and more than 20 km for *Agrostis*, while many contamination points outside the fields and regrowth in agricultural sites make it challenging to manage pollen and kernel flows. Finally, GMO production areas shall be kept outside of regions with wildtype relatives, such as *Beta maritima*, genetic resources, or the production of hybrid varieties (Aono

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et al., 2006; Bailleul et al., 2016; Busi and Powles, 2016; Emberlin et al., 1999; Rousseau et al., 2006; Snow, 2012). There are no reliable sampling plans for such particularities. Accordingly, some authors recommend using simplified sampling plans and postponing the GMO content assessment to the storage at the exit of the field. All these difficulties, complicated agricultural practices and crop rotations, with the absence of register and coordination organisms, have led to the recommendation to restrict GM crops in areas dedicated to the opposite of flexible coexistence, based on farming neighbours' negotiations (Bertheau, 2012, 2013; Czarnak-Kłos and Rodríguez-Cerezo, 2010; Rizov and Rodriguez-Cerezo, 2015; Rizov and Rodriguez Cerezo, 2014).

A land-sharing *vs* land-sparing option is similarly openly debated about biodiversity preservation, for instance, in natural parks.

3.2 Sampling techniques

A best practices-based sample preparation is as essential for GMOs as it is for other testing in traceability systems. It must routinely allow for practical limits of detection (LoD) and quantification (LoQ) to be reached, without increasing measurement uncertainties (European Network of GMO Laboratories (ENGL), 2011). The reliability of any analysis is based on the representativeness of the portion of the sample that is tested (Pitard, 2019). The reduction of the bulk samples via homogenisation and then to the test portion is a laborious, often contaminating procedure, and a source of measurement uncertainties, for which guidelines have been defined (Berben et al., 2014). The laboratories initially faced many practical problems regarding volumes and masses that could be processed quickly with easily cleanable mixing and grinding equipment. In any case, the extraction of fast, cheap and free DNA of the maximum amount of amplification inhibitors remains a challenge, both in terms of the reliability of the results and the cost involved.

The cost and unreliability of field sampling may lead to recommendations for simplified plans that are cheaper, even if they are less reliable, which means that sampling may be postponed until harvest and move towards the silos (Bailleul et al., 2016; Bannert, 2006; Miraglia et al., 2009). Long-distance dissemination of viable pollen (more than 20 km for some) is probably more important than initially estimated (Brunet et al., 2013; Heuberger et al., 2010; Rousseau et al., 2006). Such field sampling difficulties are exacerbated by the statistical power and suspected contamination that could be reached. For example, the sizes of required samples for a 95% confidence limit of detection would be:

- 100 plants at 3% contamination level.
- 200 plants at 1.5% contamination level.

- 300 plants at 1% contamination level.
- 3000 plants at 0.1% contamination level. Such a practical threshold is generally targeted for avoiding labelling of technically unavoidable or fortuitous presence at the 0.9% GMO legal threshold.

Even if sample-pooling strategies make it possible to reduce the number of samples tested in the first instance, the limits of laboratories and collectors are very quickly reached. These difficulties explain the many precautions taken in seed production, such as exclusion zones for the production of maize or sugar beet hybrid seeds, and the numerous GMO seed contaminations observed over the years.

Due to the difficulties in the field sampling of open-pollinated crops, the arrival of materials at the storage silo can be the first opportunity to carry out testing for the presence of GMOs. The samples taken by probes are typically used to analyse the incoming material. Specific procedures are required to analyse large products, such as beets, for pooled analyses. While immunoassays are still used for their relative cost, they lose ground to PCR and alternative methods, for instance, the detection of several GMOs with mobile PCR instruments and direct quantification. Isothermal techniques can replace PCR, mostly when deep-stick or flocculation formats have been adapted to facilitate their use. The storage in silos often allows a reduction in GMO contents in a given batch by illegal mixing of the contents. Leaving the storage facilities is probably the most critical step for export and industrial processing stages. It is the object of the most significant precautions to prevent cross-contamination.

Only a few tests are carried out downstream, because most firms rely on hazard analysis critical control point (HACCP) approaches to avoid contamination. For several decades, governments have favoured organisations' self-monitoring to disengage themselves, while the governments' enforcement services, facing their increasingly reduced means, have to endorse these sources of adulteration with documentary traceability, unless there is suspicion of contamination or presence (Davison and Bertheau, 2008; Dwinger et al., 2007). The underlying reasoning of such self-monitoring is that a firm's reputation takes a long time to establish, whereas the mistrust induced by fraud takes a long time to redress. Therefore, analytical checks are only carried out in severe doubt or emergency cases such as those determined by the MS' RASFF[21] network. It is likely that after an initial challenge phase in the detection of NBT products, where operators are testing the willingness of MS to implement the decision of the ECJ, the same documentary traceability-based enforcement strategy will prevail for NBT products.

21 Rapid Alert System for Food and Feed https://ec.europa.eu/food/safety/rasff_en

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4 Conclusion

This chapter has considered the technical advances developed to identify transgenic GMOs and assure the traceability of GMOs in food supply chains. The use of different targets and signal amplification of variants, with or without hybridisation, makes available a range of qualitative and quantitative methods (QRT-PCR, LAMP, NASBA, LCR, SNPLex…) that can be chosen, depending on the target. These methodological approaches can be applied from the field to the laboratory, from point mutation to the junction sequence between rearranged elements and from single to multiple targets (Bertheau, 2013; Bertheau, 2019; Chhalliyil et al., 2020). Associated with various decision support tools, they allow, via the matrix approach, the identification of any GMO. Most of the methods and guidelines necessary for the analytical traceability of GMOs in the supply chains are available on the EURL-GMFF website.[22]

The implementation of GMO traceability systems is a techno-political decision, subject to many pressures. Changing the definition of GMOs or the measurement unit in the future will only reinforce citizens' mistrust in politicians, administrations, and researchers interested in these techniques. As noted in the recent ENGL report (ENGL, 2019), the current EU regulations, with their mandatory requirement for the supply of reference materials and biomarkers such as those used in varietal identification, would, with some probable adjustments, mostly suffice to ensure their traceability, whatever the method of obtaining them.

Techniques to detect all kinds of molecular targets, like 'hidden' and 'new' GMOs, are available. The issues of detection and identification, such as defining targets and routine methods for those GMOs, are considered in more depth in the next chapter.

5 Where to look for further information

The book resulting from the Co-Extra European research program gives an overview of the problems and solutions concerning the traceability and coexistence of GMO and non-GMO supply chains (https://onlinelibrary.wiley.com/doi/book/10.1002/9781118373781).

The site of the European GMO reference laboratory and the ENGL network provides the majority of references necessary for any analytical practice in GMO detection (https://gmo-crl.jrc.ec.europa.eu/).

Louis Pasteur (1822–1895) professed: "Have the cult of the critical spirit. Reduced to itself, it is neither an awakener of ideas, nor a stimulant of great things. Without it, everything is null and void", so in the face of the assertions of the economy of the promise of firms, please consider carefully sites like this one: https://www.gmwatch.org/en/.

22 https://gmo-crl.jrc.ec.europa.eu/default.htm

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