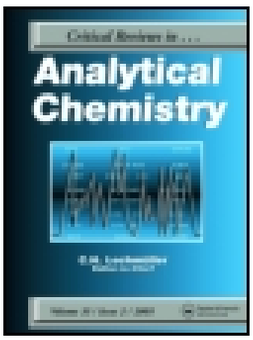

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Biosensors for GMO Testing: Nearly 25 Years of Research

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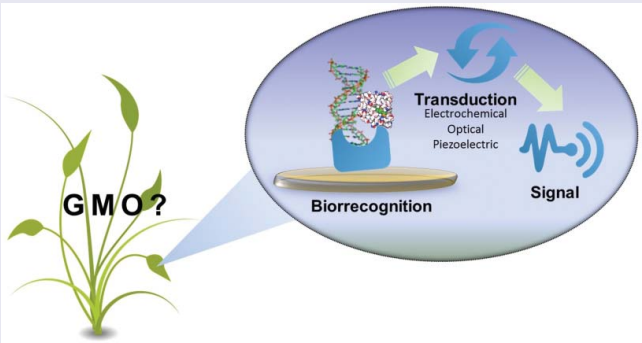
ABSTRACT

In the nearly two decades since genetically modified organisms (GMOs) were first commercialized, genetically engineered crops have gained ground on their conventional counterparts, reaching 185 million hectares worldwide in 2016. The technology has bestowed most of its benefits on enhancing crop productivity with two main traits currently dominating the market: insect-resistant and herbicide-tolerant crops. Despite their rapid and vast adoption by farmers worldwide, GMOs have generated heated debates, especially in European countries (EU), driven mostly by consumers concerned about safety of transgenic foods and about the potential impact on the environment. The need to monitor and to verify the presence and the amount of GMOs in agricultural crops and in food products has generated interest in analytical methods for sensitive, accurate, rapid, and cheap detection of these products. DNA biosensors have been envisioned as a novel DNA-detection technology that would one day substitute current amplification-based methods, providing hand-held, quick, and ultrasensitive gene-level detection. This review summarizes the contributions made in nearly 20 years of research regarding the application of genosensing technology for the qualitative and quantitative determination of transgenic traits.

KEYWORDS

Detection method; DNA biosensor; DNA detection; electrochemical genosensors; genetically modified organism; optical genosensors; piezoelectric genosensors

GRAPHICAL ABSTRACT



Introduction

The advent and application of genetically modified organisms (GMOs) have undoubtedly revolutionized agronomic practices over the past 20 years. GMOs are defined as "organisms, 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."[1] The result is the expression of new, specific protein(s) conferring desirable feature(s) to the – genetically – modified crops, e.g. insect and herbicide resistance. Some benefits of genetic engineering in agriculture include increased crop yields, reduced costs for food production, reduced need for pesticides, enhanced nutrient composition and food quality, resistance to pests and disease, among others. Progress has also been made in developing crops that mature faster and tolerate environmental stressors, allowing plants to grow in conditions where they might not otherwise flourish.[2] All of which is aimed at facing the critical challenge of producing sufficient food for a growing human population living in a changing and unstable climate.[3]

According to International Service for the Acquisition of Agribiotech Applications (ISAAA), 185.1 million hectares of biotech crops have been cultivated in the world until 2016, a year that marked the 21st anniversary of the commercialization of biotech crops.[4]

The rapid adoption of transgenic crops in the United States, Argentina, and Canada stands in strong contrast to the situation in the European countries (EU) where there is a high level of consumer rejection and strict legislation concerning official approval.[5] Opposition to GMOs has been based on concerns about the potential impact of releasing transgenic crops into the environment ranging from gene flow to the development of insect resistance and their impact on non-target organisms.

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Health-related concerns include possible transfer of antibiotic resistant genes to bacteria in the gastrointestinal tract, toxicity (presence of anti-nutrients), and allergenicity of foods derived from GMOs.[5,6]

As a result, the EU has established one of the strictest legal frameworks for the regulation of biotech crops aimed at (i) establishing safety assessment before any GMO is placed on the market, (ii) drawing harmonized procedures for risk assessment and authorization of transgenic events, (iii) setting labeling thresholds for GMOs placed on the market in order to provide freedom of choice to consumers as well as professionals (e.g. farmers and food feed chain operators), and (iv) establishing procedures to ensure the traceability of GMOs in the market. According to European legislation, the presence of GM material in food and feed is governed by Regulation EC 1829/2003 of the European Parliament and the European Council,[7] which insists on a labeling procedure for all products containing GM-based materials. The regulation is supplemented by Regulation EC 1830/2003,[8] which ensures traceability and labeling of GMOs placed on the market. All products containing GM-based materials must be labeled when the content of any authorized GM ingredient exceeds 0.9% of the food/feed ingredients when considered individually. Below this threshold, labeling is not mandatory provided that the presence of GM material is proven to be accidental or technically unavoidable. For non-authorized GM ingredients, the threshold is set at 0.5%, given that the source of the GMO has been pre-evaluated and an appropriate detection method for its presence is available.

This is why the need to monitor and to verify the presence and the amount of GMOs in agricultural crops and in food products has generated interest in analytical methods for sensitive, accurate, rapid, and cheap detection of these products. In this regard, genosensors have been envisioned as a novel DNA-detection technology that would one day substitute current amplification-based methods, providing hand-held, quick, and ultrasensitive gene-level detection. This review summarizes the contributions made in nearly 20 years of research regarding the application of genosensing technology for the qualitative and quantitative determination of transgenic traits.

Genetic modifications of plants

Advances in the field of genetic engineering have allowed for precise control over the genetic changes introduced into an organism. In a broad sense, this is achieved by selecting and extracting genes of interest normally from other organisms, such as bacteria, and inserting the desired DNA fragment into the plant genome.[2] This plant-breeding process is called transgenesis and it refers to the incorporation of foreign/new genes from one species into a completely unrelated species. Genetic transfer is commonly achieved using *Agrobacterium tumefaciens* (biological vector) or biolistic (particle bombardment) technologies.[9] Although both methods have been practiced for more than three decades, contributions in genome editing techniques, e.g. the CRISPR – Cas9 tool, have dramatically enhanced plant genome research and transformation in recent years.[3,10] The use of a biological vector is the most frequent transformation method, which involves the infection of the host plant by Agrobacterium strains leading to genetic transfer from the bacterium and integration into the plant nuclear genome.[11]

Vector constructs for plant transformation contain several genetic elements required for insertion into the plant genome. In addition to the trait gene(s), i.e. sequences that are intended to be inserted into the target organism to confer the desirable trait, a vector construct includes promoter and terminator sequences that enable the plant to express the gene of interest. Promoters are regions of the DNA upstream of a gene's coding region that contains specific sequences recognized by proteins involved in the initiation of transcription.[12] One source of such promoters is CaMV, which is a double-stranded DNA virus affecting plants in the Cruciferae, Resedaceae, and Solanaceae. The 35S promoter of Cauliflower mosaic virus is a functional, well-characterized, and constitutively expressed promoter that enables high levels of gene expression in the host organism. Hence, it has been incorporated into numerous constructs and used to produce many of the genetically engineered crops commercially used today, such as maize, soy, canola, and papaya. PEP carboxylase promoter, which encodes a photosynthetic enzyme, is used less frequently in GMOs.[13] The terminator gene is a DNA sequence that serves as the "stop signal" for gene transcription. The nopaline synthase gene from *Agrobacterium tumefaciens,* namely NOS, serves as a polyadenylation site in most constructs.[14]

GMO detection and quantification

Threshold labeling levels set in different countries vary from 0 to 5%. They are either mandatory (Australia, Brazil, Chile, China, EU, India, Indonesia, Israel, Japan, Philippines, Russia, Saudi Arabia, South Korea, Taiwan, and Thailand) or voluntary (Argentina, Canada, and USA).[15] This fact has driven the need to develop analytical methods able to detect and quantify GMOs at low DNA levels (e.g. tens of genome copies in some cases) in different types of samples, from raw material (agricultural crops) to food and feed commercial samples. The most common detection approach relies on the knowledge that part of the genetic information in GM plants differs from that of the wild-type line. Thus, the genetic modification is by definition detectable at the DNA level. But also, another approach involves the detection of the protein(s) encoded by the inserted trait gene via immunoassays, such as ELISA. However, given their – generally – lesser stability, proteins are often not considered suitable for GMO detection in a wide range of products (e.g. processed food/feed).[16,17]

According to the DNA targets present in GMOs, DNA-based methods can be categorized into different levels of specificity (Figure 1) [16,18]: (i) screening methods, which are the least specific methods because the targets include common DNA elements in GMOs, such as promoters and terminators that are present in many different events, (ii) gene-specific methods, which detect a part of the trait gene associated with the specific genetic modification, (iii) construct-specific methods, which target the junction between two DNA elements, such as the promoter-trait gene or trait gene-terminator, and (iv) event-specific methods, which provide the highest level of specificity because the target is the unique junction, characteristic of each

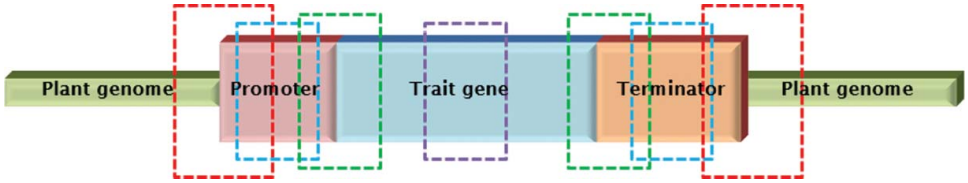

Figure 1. Levels of specificity of GMO methods based on the targeted DNA region: screening (blue-dashed square), gene-specific (purple square), construct-specific (green), and event-specific (red). Adapted from Holst-Jensen et al. [16].

event, found at the integration locus between the inserted DNA construct and the recipient genome.

Qualitative detection methods can be used as an initial screening of food products, i.e. to investigate whether GMO-specific fragments are present. Qualitative analysis could thus be performed on packaged products samples led from the shelves of supermarkets, from stocks at the supply chain, or from raw material. If the qualitative analysis provides an indication of the presence of GMOs, a subsequent quantitative test might give a decisive answer concerning the labeling requirement.[19] In the EU, legislation on GMO labeling drove analysts to harness the initially complex analytical challenge of quantifying GMOs. When implemented, the legal tolerance level did not explicitly specify which measurement units were to be used to calculate the final GMO content in a sample.[20] In 2004, the EU Recommendation 2004/787/EC[21] proposed that this should be done in terms of DNA copy number, i.e. results should be expressed as the ratio of event-specific DNA copy numbers in relation to the target taxon-specific DNA copy numbers, calculated in terms of haploid genomes. This is because the labeling threshold was established for each individual ingredient, so that quantification is based on each GM ingredient in proportion to the global amount of the same ingredient, e.g. GM soybean in proportion to the total amount of soybean. This should to be carried out with event-specific methods.

The GMO analytical procedure can be approached as a modular process starting with sample collection and including all steps performed to determine the presence, identify, and quantify (when necessary) GMOs until finally a measurement result is provided (GMO %).[16] Accordingly, sample preparation, DNA extraction, and detection of individual target sequences can be treated as separate modules that together form a method. A module can therefore be defined as a distinct and limited operation, each of which involves its own input and output material/data. In GMO monitoring, the following modules are usually performed (Figure 2): (1) a sample preparation module where the input material is processed to its homogenized form, e.g. grains to flour; (2) a DNA extraction and purification module where the input material is the homogenized sample and the output material is purified DNA in aqueous solution; (3) an amplification stage in which the specific target region is fragmented and copied exponentially until detectable amounts are obtained (i.e. through the polymerase chain reaction (PCR) or isothermal approaches); (4) a detection module where the input material is purified DNA in aqueous solution and the output material is measurement data, e.g. collection of fluorescence data and translation into a number of target sequence copies; and (5) a data evaluation module, e.g. the number of copies of the taxon-specific and event-specific targets are processed into a final quantitative result.

GMO detection is generally carried out by enzymatic amplification of DNA sequences specific of the transgenic insert by PCR-based methods. Quantitative methods are based in the real-time variant of this technique, which relies on the use of fluorescent molecules to generate real-time data during the different stages of amplification. This allows us to collect fluorescence in the exponential phase, where it is possible to achieve quantification of the amplified DNA fragment (amplicon). Despite the fact that PCR is the reference methodology for DNA detection and quantification, there are still some drawbacks that have motivated researchers into developing alternative methods. These are intended to be less expensive and suitable for decentralized applications and for resource-limited settings. DNA biosensors and sensing platforms have been proposed as low-cost, sensitive, and robust alternatives for DNA sequence-specific detection.

DNA biosensors as platforms for GMO detection

Biosensors are analytical devices that translate a biological event into a measurable signal. When this recognition system involves nucleic acids as receptors and the hybridization reaction as recognition event, the term "DNA biosensor" or "genosensor" is used.[22] The biorecognition event (hybridization reaction) takes place via Watson-Crick base-pairing fundamentally between two complementary sequences, i.e. the support-immobilized synthetic probe and the target sequence. DNA is especially suited to get selective devices because of the high specificity of the base-pairing interaction between complementary sequences, even in the presence of mismatches.

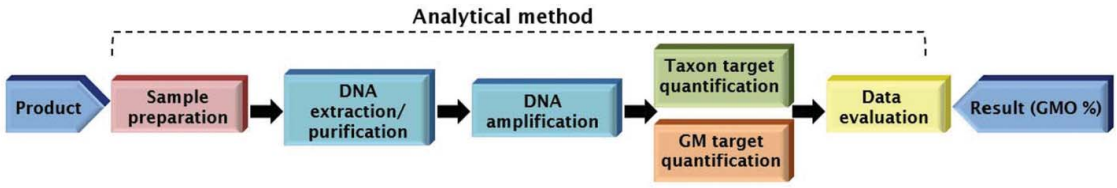

Figure 2. GMO analytical procedure. Adapted from Holst-Jensen et al. [16].

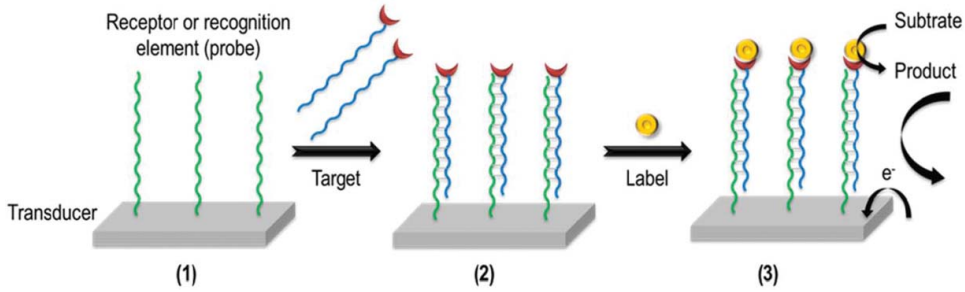

Figure 3. Steps in the design of a genosensor with a labeled target.

The design and construction of a genosensor usually involves the following stages (Figure 3): (a) transducer selection; (b) target-sequence and probe-sequence (complementary to the latter) selection; (c) selection of a convenient probe-immobilization strategy; (d) labeling, although this step will be necessary or not depending on the detection method used; and (e) readout. The optimization of each step is required to improve the overall performance of the device.

Although several transducers have been used in biosensors, as far as we know, only DNA biosensors with optical, piezoelectric, and electrochemical transduction have been successfully developed for the detection of GMO sequences, as was recently reviewed.[23] Figure 4 summarizes the quantity of publications in nearly 20 years of research in DNA biosensors for GMO detection. When the search included the keyword "electrochemical or optical or piezoelectric," the chart indicates that most devices were based on electroanalytical techniques.

In GMO detection, the selection of the target sequence is relatively easy. It depends on the aim of the assay (i.e. screening, qualitative, or quantitative). The nucleic acid sequences of authorized or pre-evaluated transformed plants are well characterized and described in open-access databases (http://www.gmo-compass.org/eng/gmo/db/, http://gmo-crl.jrc.ec.europa.eu/gmomethods/, and http://gmdd.shgmo.org/). The taxon-specific or reference genes have also been described in official PCR methods, which are necessary for the relative quantification of GMOs and for species identification.

Most DNA biosensors have been reported for screening purposes, followed by gene-specific methods. However, only few works have been developed describing the detection of event-specific sequences, which is the highest level of specificity. Of these, all the biosensors proposed are with electrochemical transducer (Figure 5).

The probe immobilization step plays a major role in determining the performance of a DNA biosensor. Control of the surface chemistry and coverage is crucial for the analytical performance of DNA biosensors and sensing schemes. The key features of DNA-modified surfaces are DNA density and hybridization accessibility.[24] Moreover, the immobilization chemistry should be sufficiently specific for probe binding and offer efficient surface blockage in order to avoid unspecific adsorption by other molecular species, e.g. proteins, short oligonucleotides, genomic DNA, etc.

Given the importance of the transducer, we will now describe the different genosensors proposed so far according to the transducer used. We will give a brief overview of each type of biosensor regarding their contributions to the field of GMO testing, with the aim of painting a broad picture of the application of these attractive technologies for food control and legislation compliance.

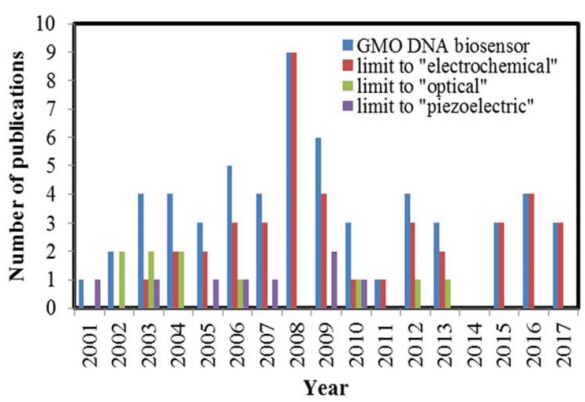

Figure 4. Number of publications reporting GMO DNA sensors since 2001 till 2017. The publications were retrieved from ISI Web of Knowledge and Scopus.

Electrochemical biosensors

Electrochemical DNA biosensors and sensing platforms for GMO testing have been the most popular amongst the other types of transduction schemes. The interest in this field, demonstrated by research groups worldwide, has been encouraged by the simple and relatively low-priced instrumentation, the high selectivity of the base-pairing biorecognition process (hybridization), and the high sensitivity and versatility of electrochemical detection principles through which DNA hybridization can be monitored, e.g. redox enzyme-amplified signaling, surface impedance measurements, electron transfer mediated by DNA binders or intercalators, etc.[13]

A great variety of immobilization strategies has been explored in electrochemical biosensors for GMO detection. The formation of self-assembled monolayers (SAMs) of thiolated probes onto gold electrodes have permitted very low limits of detection, such as picomolar levels for 35S detection [25] or even femtomolar levels for a gene-specific target.[26] Covalent immobilization has been also reported using ethylenediamine as connector.[27,28] Carbon nanotubes are also used due to their high electrical conductivity and long-term stability.[29–33] Hybrid materials based on nanoparticles and polymers have also been reported with the aim of increasing the immobilization surface area and, as a result, the sensitivity.[34–37]

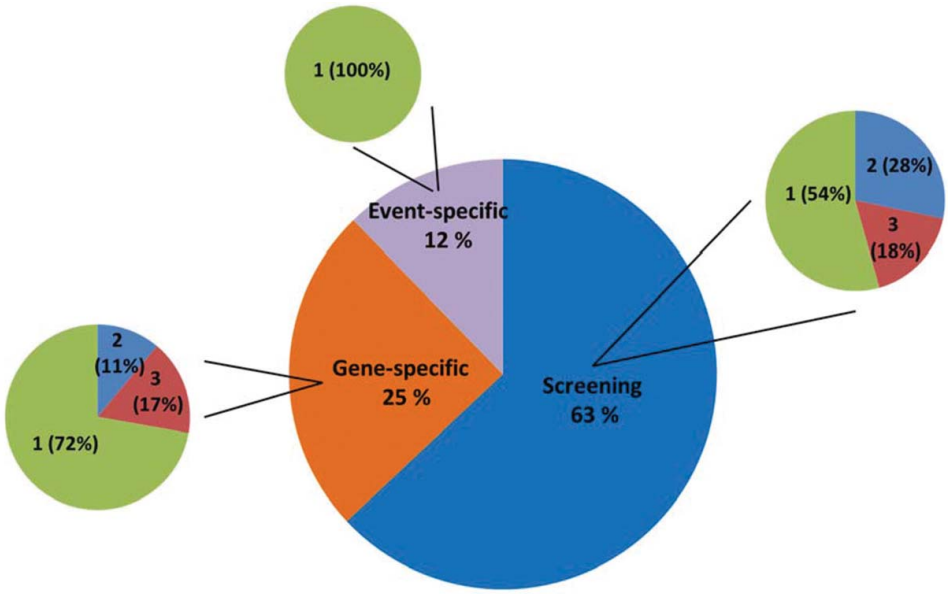

Figure 5. Frequency of reported genosensors based on the specificity level and the transducer used: (1) electrochemical biosensor, (2) optical biosensor, and (3) piezoelectric biosensor.

Most of the described electrochemical biosensors are based on screening methods, the least specific level. Electrochemical biosensors targeting other sequences have also been described, namely PAT (inducing tolerance to glufosinate herbicide), nptII (responsible for antibiotic resistance) genes, CP4 EPSPS (inducing tolerance to glyphosate herbicide), and cry1Ab (inducing insect resistance). All the systems have presented high specificity and sensitivity, highlighting their relevance for GMO analysis. Most genosensors based on gene-specific detection use PAT gene as target, and many authors reported the simultaneous determination of NOS and PAT. Only a few works reported event-specific genosensors, targeting the event of MON810 maize,[38–40] soybean 40–3-2 and A5547–127, maize NK603, T25 and LY038,[41] soybean A270412, [42] and soybean 40–3-2.[43–46] Table 1 summarizes the electrochemical genosensors reported for GMOs.

Although some devices for the simultaneous detection of different GMO sequences have been described with electrochemical devices,[38,39,41] it has not yet been possible to develop a multiplex quantitative sensor with analytical characteristics that compete with the quantitative PCR in real samples. There have been a few reports where quantification has been addressed with real samples, yet overall the contribution of the electrochemical field to GMO testing has not reached that of the PCR-related technologies. Figure 7 shows a timeline of the evolution of electrochemical biosensors and PCR for GMO testing, highlighting the most prominent achievements of each technology throughout the years. More specifically, what has been accomplished so far in terms of simultaneous determination is an electrochemical magneto-assay for the detection of a specific event of transgenic soybean,[43] which comprises the use of streptavidin-coated magnetic beads for the immobilization of biotinylated capture probes (Figure 6).

Optical biosensors

They are based on the transduction of the variation in optical properties of the system because of the interaction of the analyte and the biorecognition layer. Optical biosensors have also been reported for GMO detection due to the high selectivity of the technology that involves the interaction between the selective biomolecule binding layer and the transducer, high sensitivity, and possibility of multiple detection using different wavelengths. Surface plasmon resonance (SPR) is the optical technique that is used more in GMO detection, but electroluminescence and surface-enhanced Raman scattering (SERS) spectroscopy are also applied. SPR responds with high sensitivity to changes in the dielectric constant of the adjacent medium, and consequently, to the refractive index. This feature makes it very suitable for detecting the formation of affinity complexes, such as antigen-antibody or hybridization event between complementary DNA strands.

Different immobilization systems of the capture probe in the gold film have been reported as covalent union,[69,70] biotin–streptavidin union [71–76], and self-assembled monolayers.[77–79]

As in electrochemical biosensors, most of the optical sensors are for GMO screening, detecting 35S promoter, and NOS terminator. Other SPR-based devices detect a part of the train gene associated with the specific genetic modification, called "gene-specific methods." Detection of Cry1Ab genes or CP4 EPSPS has been reported, among others.

Most of the systems address the detection of synthetic oligonucleotides and, in some cases, the use of certified reference materials. Table 2 presents the optical genosensors reported for GMOs detection.

Piezoelectric biosensors

Piezoelectric biosensors are a type of mechanical biosensors that use the piezoelectric effect, i.e. the production of

Table 1. Electrochemical genosensors for the detection of genetically modified organisms.

Target	Electrode	DNA immobilization strategy	Detection method	Sample	LOD (M)	Ref.
35S	SPEAu	SAMs	DPV with enzymatic amplification	Synthetic DNA and bacterial plasmid with PCR amplification	2.5×10^{-10} (synthetic) 1×10^{-9} (amplicons)	[47]
	SPEAu	SAMs	EIS with enzymatic amplification	Synthetic DNA and reference material with PCR amplification	1.2×10^{-12} (synthetic)	[25]
	Au	SAMs	DPV with 4′-aminobenzo-18-crown-6-copper(II) complex as electroactive tag	Synthetic DNA and real sample (DNA extracted with kit)	6×10^{-4} (synthetic)	[48]
	Au interdigitated microelectrodes	Electrocopolymerization with PPy/MWCNTs	EIS (label free)	Synthetic DNA	Not reported	[29]
	SPCE	Covalent attachment to succinimide -functionalized acrylic microspheres onto the AuNPs/SPCE	DPV with anthraquinone-2-sulfonic acid monohydrate sodium salt as indicator	Synthetic DNA and reference material	7.79×10^{-16}	[49]
	SPCE	Exfoliated graphene oxide and gold nano urchin	DPV with hematoxylin as indicator	Synthetic DNA	1.3×10^{-14} (synthetic)	[50]
	GCE	Covalent attachment with ethylenediamine	DPV with MB as indicator	Synthetic DNA	Not reported	[27]
	GCE	Adsorption	DPV with $[Co(NH_3)_6]^{+6}$ as indicator	Synthetic DNA and reference material with asymmetric PCR	Not reported	[51]
	GCE	Adsorption on Pt-nanoparticles	SWV with $[Co(phen)_3]^{+3}$ as indicator	Synthetic DNA and reference material with enzymatic digestion	1×10^{-9} (synthetic)	[52]
	GCE	SAMs onto Au electrodes	DPASV using PbS-nanoparticles onto GCE	Synthetic DNA	4.38×10^{-12}	[53]
	GCE	Adsorption on AuNPs/MWCNT/ graphene oxide nanoribbon	EIS (label free)	Synthetic DNA and real sample (DNA extracted with kit)	3.3×10^{-17} (synthetic)	[54]
	CPE	Adsorption of the DNA in the PbSe/ chitosan composite	DPV with methylene violet as indicator	Synthetic DNA	1.6×10^{-11}	[55]
	Platinum microelectrodes and field-effect transistor	Covalent attachment with SWCNT dispersed in ethylenediamine (platinum electrodes) and electrostatic forces with SWCNT (field-effect transistor)	EIS (label free)	Synthetic DNA and real samples with PCR amplification	1.0×10^{-9} (synthetic)	[56]
	ITO	Adsorption onto reduced graphene oxide/ nanogold/chitosan	Photoelectrochemical detection with ascorbic acid as electron donor	Synthetic DNA and reference material with PCR amplification	1.0×10^{-14} (synthetic)	[57]
35S and NOS	Au	SAMs	SWV with MB as indicator	Real sample (DNA extracted with kit)	Not reported	[58]
NOS	SPCE	Adsorption by controlled potential	SWV with MB as indicator	Synthetic DNA and reference material with PCR amplification	2.4×10^{-6} (synthetic)	[59]
	GCE	SAMs onto Au electrodes	DPASV using CdS-nanoparticles onto GCE	Synthetic DNA	2.75×10^{-12}	[60]
	Au	SAMs	CV with MB as indicator	Synthetic DNA and reference material with PCR amplification	3.6×10^{-8} (synthetic)	[61]
nptII	CPE	Adsorption by controlled potential	SWV with MB as indicator	Synthetic DNA	Not reported	[62]
	Au	SAMs	SWV with enzymatic amplification (aniline polymerization)	Synthetic DNA and plant samples with PCR amplification	1×10^{-10} (synthetic) 2×10^{-10} (amplicons)	[63]
Bar	CPE	Covalent attachment with ethylenediamine	SWV with $Co(bpy)_3$ as indicator	Synthetic DNA	Not reported	[28]
Bar and CP4 EPSPS	CPE	Adsorption onto aluminum films	DPV with MB as indicator	Synthetic DNA (Bar) and real samples with PCR amplification (CP4 EPSPS)	2.25×10^{-8} (Bar) Not reported for amplicons	[64]
Bt	Au	SAMs	Solid state voltammetry using Ag nanoparticles	Synthetic DNA	1×10^{-14}	[26]
	SPE	Covalent attachment with –COOH	EIS with Ag signal amplification	Synthetic DNA	7.2×10^{-14}	[65]

PAT	GCE	Adsorption onto nanogold/nanoPANI–chitosan	EIS (label free)	Synthetic DNA	3.1×10^{-13}	[34]
	Au	Potential-controlled adsorption onto a SiO_2–PATP	EIS (label free)	Synthetic DNA	1.5×10^{-12}	[35]
	GCE	Adsorption onto nanogold/PDC	EIS (label free)	Synthetic DNA	2.4×10^{-11}	[36]
	GCE	Potential-controlled adsorption on ZrO_2/nanogold	DPV with MB as indicator	Synthetic DNA	3.1×10^{-11}	[37]
	GCE	Adsorption on nanoPANI–ZrO_2/Ptyrosine	EIS (label free)	Synthetic DNA	2.68×10^{-14}	[68]
PAT and NOS amplicons	GCE	Adsorption on ZrO_2/SWNTs/PDC	EIS (label free)	Synthetic DNA (PAT) and real samples with PCR amplification (NOS)	1.38×10^{-12} (PAT) Not reported for amplicons	[30]
	GCE	PDDA/PDC–SWNTs films	DPV with MB as indicator	Synthetic DNA (PAT) and real samples with PCR amplification (NOS)	2.6×10^{-12} (PAT) Not reported for amplicons	[31]
	CPE	Potential-controlled adsorption on Poly-lysine/SWNTs	EIS (label free)	Synthetic DNA (PAT) and real samples with PCR amplification (NOS)	3.1×10^{-13} (PAT) Not reported for amplicons	[32]
	CPE	Adsorption on PANI–MWNTs/chitosan	EIS (label free)	Synthetic DNA (PAT) and real samples with PCR amplification (NOS)	2.7×10^{-14} (PAT) Not reported for amplicons	[33]
	CPE	Potential-controlled adsorption on nanogold-CNT/nanoPANI	EIS (label free)	Synthetic DNA (PAT) and real samples with PCR amplification (NOS)	5.6×10^{-13} (PAT) Not reported for amplicons	[67]
PEP	GCE	Adsorption on nano(Au–Pt)-polytyramine	EIS (label free)	Synthetic DNA	3.6×10^{-13}	[66]
SSIIb	Au	SAMs	SWV with $[OsO_4(bipy)]$ as indicator	Synthetic DNA	Not reported	[38]
lvrp	Au	SAMs	SWV with $[OsO_4(bipy)]$ as indicator	Real samples with asymmetric PCR amplification	0.6 %	[39]
Cry1Ab event MON810 35S G1b1 CP4 EPSPS PAT cordapaA lectin SSIIb	Au (array)	SAMs	Chronoamperometry with enzymatic amplification	Synthetic DNA and soybean powder with known GM levels	2.25×10^{-10}	[41]
A2704–12 soybean	CPE	Ionic liquid modified and partially reduced graphene. SAMs	DPV with MB as indicator	Synthetic DNA and real samples with PCR amplification	2.9×10^{-13}	[42]
Event MON 810	3D Au	SAMs	Chronoamperometry with enzymatic amplification	Synthetic DNA and reference material and real samples with PCR amplification	4.8×10^{-10}	[40]
Genome-35S (round-up ready soybean) and Lec	SPCE	Biotin–streptavidin onto magnetic beads	DPV and chronoamperometry with enzymatic amplification	Synthetic DNA	6.5×10^{-13} for event-specific target and 1.9×10^{-13} for taxon-specific target	[43]
Genome-35S (Round-Up Ready Soybean) and Lec	SPCE	Biotin–streptavidin onto magnetic beads	Chronoamperometry with enzymatic amplification	Reference material and soybean flour	53 and 1093 DNA copies for the event-specific and lectin sequences, respectively	[44]
Event round-up ready soybean	FTO	Electrostatic-based attachment onto PAH-terminated layer-by-layer films	EIS	Synthetic DNA	1×10^{-11}	[45]
Genome-35S (round-up ready soybean) and Lec	SPCE	Biotin–streptavidin onto magnetic beads	SWV and chronoamperometry with enzymatic amplification	Real samples with PCR amplification	22 and 243 DNA copies for the event-specific and lectin sequences, respectively	[46]

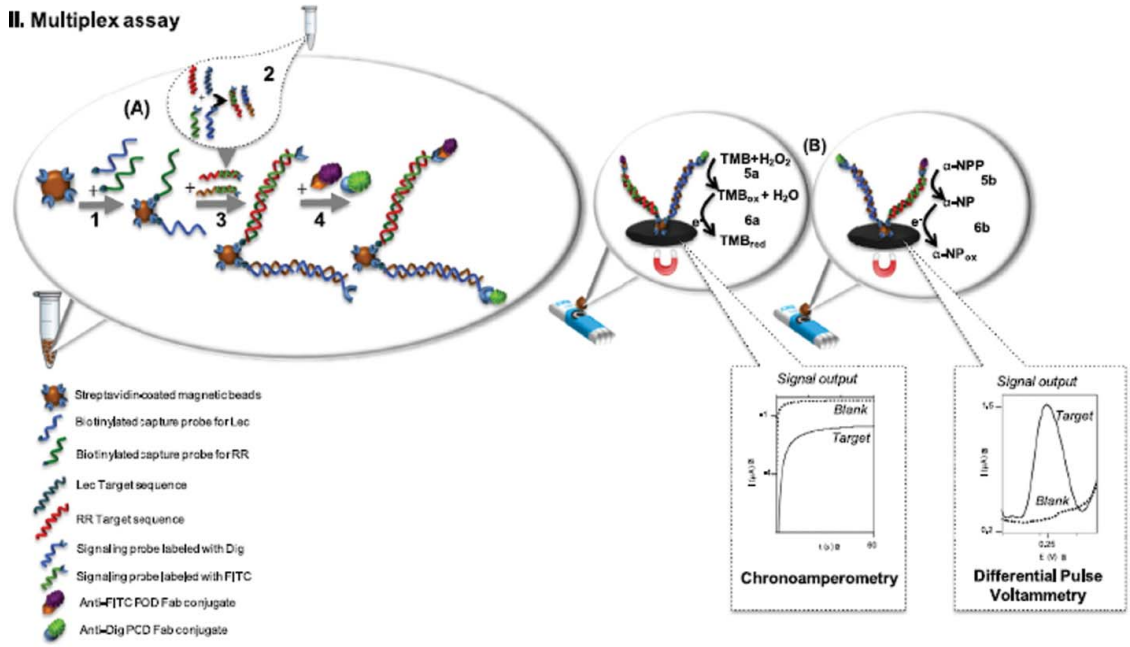

Figure 6. Scheme of the (I) single assays and (II) multiplex assay divided into two steps, recognition (A) and measurement (B): (1) attachment of capture probe(s) to the surface of magnetic beads; (2) homogeneous hybridization between a labeled-probe and target sequence; (3) heterogeneous hybridization with capture probe bound to the beads; (4) addition of the Fab-enzyme conjugate; (5a–6b) enzymatic reactions occurring after adding the enzymatic substrate (TMB/NPP); (6a) chronoamperometric measurement of TMB reduction at the electrode surface; (6b) voltammetric measurement of naphthol oxidation current at the electrode surface. Only showed part II [reproduced from Manzanares-Palenzuela et al. [43], with permission from Elsevier].

electricity/acoustic waves caused by mechanical stress or pressure. These sensors enable the selective and label-free detection of biological events in real time. Quartz crystal microbalances (QCM) are the most commonly devices for biosensing, and are made of a bulk piezoelectric material, i.e. a quartz crystal sandwiched between two metallic electrodes, typically gold. Biorecognition elements are commonly immobilized onto the gold surface. After the hybridization process, an increase in mass and a decrease in the resonance frequency occur. These devices have shown promising results for label-free, real-time, and direct detection of DNA for GMO analysis.

There are several reports involving GMO detection with QCM that not only target screening sequences, most commonly promoter (35S) and terminator (NOS) DNA fragments, but also the Cry1Ab and CP4 EPSPS genes. Different immobilization strategies of DNA probes have been reported, such as biotin–streptavidin union,[82,83] by amino groups [84] and via thiol union.[82,83] Most of the biosensors reported the use of synthetic oligonucleotides for optimization and flours, seeds, or

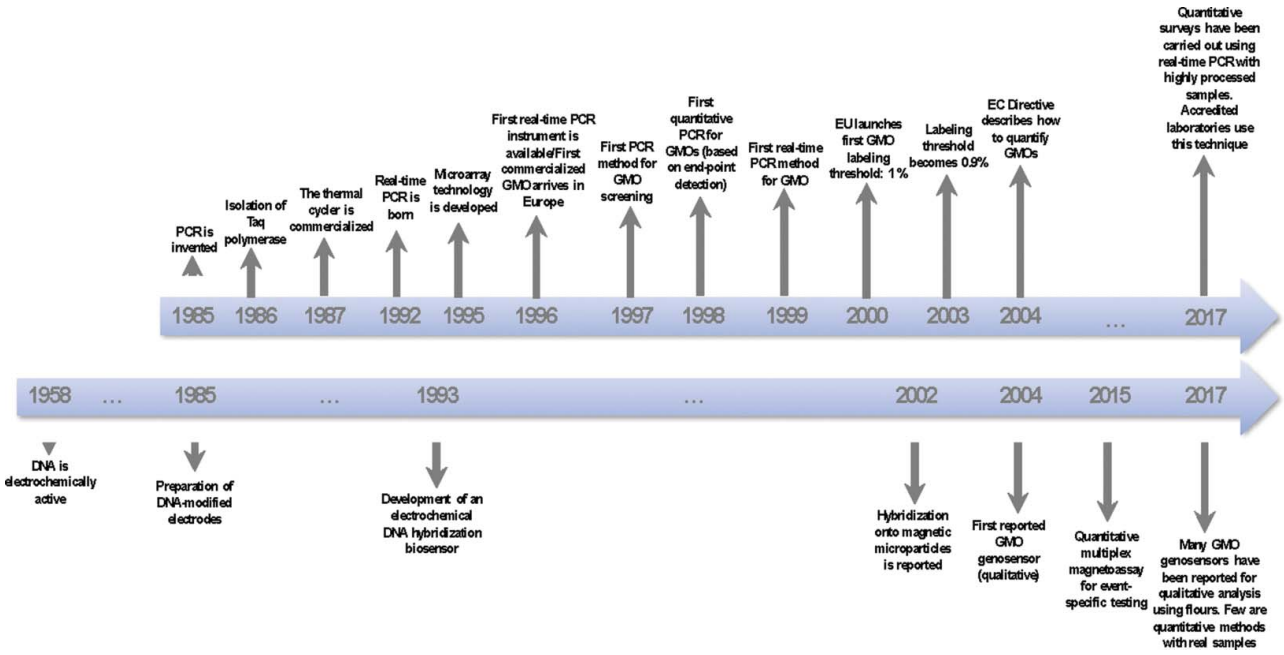

Figure 7. Timeline of the evolution of PCR technologies and electrochemical DNA biosensors and their achievements in GMO testing.

Table 2. Optical genosensors for the detection of genetically modified organisms.

Target	Chip	DNA immobilization strategy	Detection method	Sample	LOD	Ref.
35S	SA sensor chip	Streptavidin–biotin interaction	SPR (BiacoreX)	Synthetic DNA and reference material	—	[75]
	Dextran sensor chip	Streptavidin–biotin interaction	SPR (BiacoreX)	Synthetic DNA, reference material, and real sample with PCR amplification	2.5×10^{-9} M (synthetic)	[76]
	Gold sensor chip	SAM	SPR (BiacoreX)	Synthetic DNA and reference material	2.5×10^{-9} M (synthetic)	[77]
	Gold sensor chip and dextran sensor chip	SAM and streptavidin–biotin interaction	SPR (BiacoreX and SPREETA)	Synthetic DNA and reference material	2.5×10^{-9} M (synthetic with BiacoreX) and 1.0×10^{-8} M (synthetic with SPREETA)	[78]
	Glass slide	SAM	SERS spectroscopy	Synthetic DNA and reference material	1.1×10^{-9} M	[79]
35S and NOS	Gold sensor chip	Streptavidin–biotin interaction	SPR (Biacore)	Synthetic DNA and reference material	1.0×10^{-9} M (synthetic)	[80]
	—	Streptavidin–biotin interaction	Visual	Synthetic DNA and reference material	1.6×10^{-10} M (synthetic), 0.05% (35S), and 0.1% (NOS) (reference material)	[73]
	Platinum	Streptavidin–biotin interaction	Electroluminiscence	Synthetic DNA and real sample with PCR amplification	5.0×10^{-9} M (synthetic)	[74]
35S, NOS; lvr1, PAT, and Cry1Ab	—	Covalent attachment of aldehyde-captured probes onto hydrazine-derivatized silicon biosensor	Visual	Synthetic DNA and real samples	0.1 fmol	[69]
Bt11, Bt176, GA21, MON810, NK603, and T25	—	Covalent attachment of aldehyde-captured probes onto hydrazine-derivatized silicon biosensor	Visual	Reference material and real samples	Not reported	[70]
Bt-176	SA sensor chip	Streptavidin–biotin interaction	SPR (Biacore)	Reference materials	0.5%	[71]
Cry1Ac	SA sensor chip	Streptavidin–biotin interaction	SPR (Biacore)	Synthetic DNA and reference material	Not reported	[72]
Cry1Ab and Cry1Ac	Glass slide	Cross-linking with glutaraldehyde	SERS spectroscopy	Synthetic DNA and reference material	0.1 pg/mL (synthetic)	[81]

Table 3. Piezoelectric genosensors for the detection of genetically modified organisms.

Target	Immobilization surface	DNA immobilization strategy	Detection method	Sample	LOD	Ref.
35 S	Gold surface onto QCM quartz	SAM and biotin–streptavidin interaction	QCM (frequency shift measurements)	Real samples without and with PCR amplification	Not specified	[85]
	Gold surface onto QCM quartz	SAM and covalent attachment with $-NH_2$	QCM (frequency shift measurements)	Synthetic DNA and real samples with PCR amplification	5.0×10^{-7} M	[84]
	Gold surface onto QCM quartz	Adsorption on polypyrrole-MWCNTs	QCM (frequency shift measurements	Synthetic DNA	4×10^{-12} M	[29]
35S and NOS	Gold surface onto QCM quartz	Biotin–streptavidin interaction	QCM (frequency shift measurements)	Plasmid and reference material with PCR amplification	1×10^{-9} M	[83]
	Gold surface onto QCM quartz	SAM and biotin–streptavidin interaction	QCM (frequency shift measurements)	Plasmid, reference material, and real samples with PCR amplification	Not specified	[82]
35S and CP4 EPSPS	Gold surface onto QCM quartz	Biotin–avidin interaction	QCM (frequency shift measurements)	Real samples with PCR amplification	Not specified	[86]
CP4 EPSPS	Gold surface onto QCM quartz	Biotin–avidin interaction	QCM (frequency shift measurements)	Synthetic DNA and real samples without PCR amplification	4.7×10^5 numbers of genome copies	[87]
Cry1Ab	Gold surface onto QCM quartz	Biotin–streptavidin interaction	QCM (frequency shift measurements)	Reference material and real samples with PCR amplification	1%	[88]

processed food as the target raw material. Table 3 summarizes the piezoelectric genosensors reported for GMOs.

A general view and challenges involved in GMO sensing

Compliance with GMO labeling thresholds must be guaranteed throughout the production chain, for which GMO quantification is ultimately required. This is a highly demanding analytical task that is, at present, solely fulfilled by qPCR methods, which are not utterly available in resource-limited environments and are often inadequate for decentralized and on-field analysis. DNA-based biosensors, represent viable alternatives for this end. However, there are still fundamental limitations regarding the performance of these devices that limit them from becoming the gold standard for GMO detection.

Depending on each of the categories addressed in this review, *i.e.* electrochemical, optical and piezoelectric detection systems, we have summarized the limitations based upon the inherent properties of the detection technique, the estimated time for biosensor preparation and level of complexity of such procedures, and probability of reproducibility problems based upon the robustness of the probe immobilization and bioconjugation chemistries.

Regarding the detection techniques, there is a tendency towards electrochemistry over optical and piezoelectric strategies (see Tables 1–3). This trend can be owed to the cost-efficient feature of most electrochemical techniques and their high sensitivity. However, optical and piezoelectric systems can offer an important advantage in the detection of binding reactions, allowing the kinetic evaluation of affinity interactions. SPR, for example, can offer versatility in chip design and simplicity of the measurement in terms of label-free schemes. Yet, despite its advantages, the capabilities in discriminating between specific and non-specific interactions with the sensor surface might be limited. Such statement can be extended to other label-free schemes, such as piezoelectric, which can also be unable to differentiate between target and non-complementary DNA particularly when dealing with real samples.[83,87] As well as EIS, the label-free electrochemical variant, can also present these problems. In addition, label-free strategies often fail to support multiplex analysis. In this sense, label-based approaches provide the highest levels of specificity and multiplex capabilities, but it should be noted that such protocols also provide other limitations, such as time-consuming procedures and often expensive label reagents. Moreover, optical label-based biosensors that rely on visual readout are attractive strategies for quick screening,[69,70] although their ability to quantify DNA sequences can be limited.

As to the biosensor preparation, in many cases nanomaterials and/or polymers, as well as their composites, are incorporated in both label-free and label-based schemes to increase the sensitivity of these devices.[49,54] Whereas the implementation of nanotechnology in biosensors is an exciting field that has been widely explored, the lingered and often difficult-to-reproduce synthesis methods are a major drawback for these biosensors. In addition, these strategies usually involve the electrostatic immobilization of the probe sequence, which may lead to its detachment throughout the preparation steps, and the sensitivity to pH or ionic strength changes, all of which hamper the analytical reproducibility and robustness of the final device.

Regarding the actual applications of these devices, we note that although there are several works that address the determination of GMOs in synthetic samples and in some cases in reference materials, the scarce “real-sample” application and lack of quantitative analysis regarding taxon-specific-to-event-specific ratios stand out as two of the most important gaps of the biosensing technology. Moreover, most of the reported GMO sensors have been focused on screening purposes, while very few attained event-specific detection.

The first aspect to consider is that a GMO genosensor is designed to detect a specific fragment contained within a genomic DNA extract, in which the ratio between the target and interfering species will often be very low. Although some genes are present in high copy numbers in the genome of some species, analysts generally deal with single-copy genes when working with GM plants, making amplification necessary. So far, PCR has been the most frequent amplification process coupled to genosensing. However, other amplification strategies, such as isothermal amplification, could be coupled to biosensors, but this type of amplification is not of widespread use in the field of GMO sensors.[89] An enormous breakthrough in the field would be provided by non-PCR genosensors, meaning that these would surpass the need to incorporate an amplification step prior to readout. Although PCR-free piezoelectric detection of GM DNA from tobacco leaves has been accomplished, this is not the general case for most sensing methods, especially those regarding DNA from foodstuffs.[85] This is a major challenge yet to be conquered in the GMO biosensing field and to achieve this, ultrasensitive devices are required. Methods that combine micro- or nanomaterial-modified surfaces with conductive, area-enhancing materials generally provide the highest sensitivities. However, as previously mentioned, the fabrication of these sensors often appeared to be time-consuming and laborious owed to multiple-step synthesis procedures and lingered electrode modifications. In this sense, the development of simpler and genuinely easy-to-execute protocols remains a challenge. Nonetheless, it is important to highlight that the ultrasensitive genosensors reported so far, which show promising potential for application due to their prominent analytical performances, suffer from a major limitation related to the lack of true quantitative capacity.

Another aspect to be considered is that the GMO content should be determined in relation to a taxon-specific gene and not simply as an absolute estimation of marker sequences. This fact seriously limits the applicability of the methods up to now reported that remain restricted to simple identification or quantitation of transgenic events in an absolute fashion that is not acceptable to establish compliance with legislation, which requires the GMO content to be expressed as the ratio GMO DNA copies/taxon-specific DNA copies, which is a very unbalanced ratio. In order to address this problem, some biosensors have been described for the simultaneous detection of different GMO sequences,[38,39,41] but it has not yet been possible to develop a multiplex quantitative sensor with analytical characteristics comparable to those of quantitative PCR. So far, the closest that has been achieved, in terms of simultaneous determination, is an electrochemical magnetoassay for the detection

of a specific event of transgenic soybean,[43,44] being necessary to perform the amplification and electrochemical measurement separately. One important goal to achieve yet would be to provide a multiplex device enabling simultaneous determination of at least two different DNA sequences (reference gene and inserted gene), in which all phases of the assay, i.e. extraction, amplification and measurement, occur simultaneously. In this sense, the development of DNA biosensors with multiple electroactive markers that allow the detection of different transgenic events is a very promising aspect hardly explored to date.

Other considerations to keep in mind when designing a GMO biosensor for the analysis of highly processed samples are as follows: on one hand, only the DNA biosensors are suitable for this purpose, since the integrity of the proteins are affected by the technological processes and small DNA fragments should be targeted in order to ensure the detection of GMOs. In these cases, the selectivity of the device becomes of crucial importance. On the other hand, DNA extraction, which is a decisive step in the analysis, must result in high quality and in sufficient DNA quantities from a variety of samples. Moreover, the amplification step must also be carefully optimized to obtain close-to-linear responses with the sensor. Overall, all the necessary steps that come before the sensing step must be thoroughly evaluated to obtain accurate results. Ideally, all of these steps would be integrated in a single platform, but this envisioned sensing technology is yet to be accomplished.

The interest in DNA biosensors for GMO detection has been growing due to their possibility for automation and microfabrication based on simple and portable detection systems. Electrochemical microarray-based systems for multiplex DNA detection have become the most interesting kind of approach, given that several construct elements can be co-detected, thus allowing high-throughput GMO diagnostics.[90] Optical platforms based on fast, visual results are also attractive at the consumer level.

Although a remarkable advance in biosensor technology for GMO testing has been reached, there is still much to explore and to achieve in the field, given the increasing interest in enforcing food control regulations and in guarantying transparency regarding food composition to the growingly aware consumers.

References

[1] European Commission. Directive (EC) No 2001/18/EC on the Deliberate Release into the Environment of Genetically Modified Organisms and Repealing Council Directive 90/220/EEC.

[2] Phillips, T. Genetically Modified Organisms (GMOs): Transgenic Crops and Recombinant DNA Technology. *Nat. Educ.* **2008**, *1*, 213.

[3] Altpeter, F.; Springer, N. M.; Bartley, L. E.; Blechl, A. E.; Brutnell, T. P.; Citovsky, V.; Conrad, L. J.; Gelvin, S. B.; Jackson, D. P.; Kausch, A. P.; et al. Advancing Crop Transformation in the Era of Genome Editing. *Plant Cell* **2016**, *28*, 1510–1520. DOI: 10.1105/tpc.16.00196.

[4] Clive, J. *20th Anniversary of the Global Commercialization of Biotech Crops (1996 to 2015) and Biotech Crop Highlights in 2015*. Metro Manila, Philippines, **2015**.

[5] Ponti, L. Transgenic Crops and Sustainable Agriculture in the European Context. *Bull. Sci. Technol. Soc.* **2005**, *25*, 289–305. DOI: 10.1177/0270467605277292.

[6] Dona, A.; Arvanitoyannis, I. S. Health Risks of Genetically Modified Foods. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 164–175. DOI: 10.1080/10408390701855993.

[7] European Commission. Regulation (EC) No 1829/2003 on Genetically Modified Food and Feed.

[8] European Commission. Regulation (EC) No 1830/2003 Concerning the Traceability and Labelling of Genetically Modified Organisms and the Traceability of Food and Feed Products Produced from Genetically Modified Organisms and Amending Directive 2001/18/EC.

[9] Rao, A. Q.; Bakhsh, A.; Kiani, S.; Shahzad, K.; Shahid, A. A.; Husnain, T.; Riazuddin, S. The Myth of Plant Transformation. *Biotechnol. Adv.* **2009**, *27*, 753–763. DOI: 10.1016/j.biotechadv.2009.04.028.

[10] Lombardo, L.; Zelasco, S. Biotech Approaches to Overcome the Limitations of Using Transgenic Plants in Organic Farming. *Sustainability* **2016**, *8*, 497–503. DOI: 10.3390/su8050497.

[11] Gelvin, S. B. Agrobacterium-Mediated Plant Transformation: The Biology Behind the "Gene-Jockeying" Tool. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 16–37. DOI: 10.1128/MMBR.67.1.16-37.2003.

[12] Potenza, C.; Aleman, L.; Sengupta-Gopalan, C. Targeting Transgene Expression in Research, Agricultural, and Environmental Applications: Promoters Used in Plant Transformation. *In Vitro Cell. Dev. Biol. Plant* **2004**, *40*, 1–22. DOI: 10.1079/IVP2003477.

[13] Manzanares-Palenzuela, C. L.; Martín-Fernandez, B.; Sánchez-Paniagua, M.; López-Ruiz, B. Electrochemical Genosensors as Innovative Tools for Detection of Genetically Modified Organisms. *Trends Anal. Chem.* **2015**, *66*, 19–31. DOI: 10.1016/j.trac.2014.10.006.

[14] Holden, M. J.; Levine, M.; Scholdberg, T.; Haynes, R. J.; Jenkins, G. R. The Use of 35S and Tnos Expression Elements in the Measurement of Genetically Engineered Plant Materials. *Anal. Bioanal. Chem.* **2010**, *396*, 2175–2187. DOI: 10.1007/s00216-009-3186-x.

[15] Fraiture, M. A.; Roosens, N. H. C.; Taverniers, I.; De Loose, M.; Deforce, D.; Herman, P. Biotech Rice: Current Developments and Future Detection Challenges in Food and Feed Chain. *Trends Food Sci. Technol.* **2016**, *52*, 66–79. DOI: 10.1016/j.tifs.2016.03.011.

[16] Holst-Jensen, A.; Bertheau, Y.; de Loose, M.; Grohmann, L.; Hamels, S.; Hougs, L.; Morisset, D.; Pecoraro, S.; Pla, M.; den Bulcke, MV.; et al. Detecting Un-Authorized Genetically Modified Organisms (GMOs) and Derived Materials. *Biotechnol. Adv.* **2012**, *30*, 1318–1335. DOI: 10.1016/j.biotechadv.2012.01.024.

[17] Lian, D. S.; Zeng, H. S. Capillary Electrophoresis Based on Nucleic Acid Detection as Used in Food Analysis. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 1281–1295. DOI: 10.1111/1541-4337.12297.

[18] Holst-Jensen, A.; Spilsberg, B.; Arulandhu, A. J.; Kok, E.; Shi, J.; Zel, J. Application of Whole Genome Shotgun Sequencing for Detection and Characterization of Genetically Modified Organisms and Derived Products. *Anal. Bioanal. Chem.* **2016**, *408*, 4595–4614. DOI: 10.1007/s00216-016-9549-1.

[19] Querci, M.; Guy, V.; den, E.; Jermini, M. *Overview, General Introduction on Genetically Modified Organisms (GMOs)*; EU Legislation Joint Research Centre (European Commission):Ispra, Italy, **2006**.

[20] Milavec, M.; Dobnik, D.; Yang, L.; Zhang, D.; Gruden, K.; Zel, J.. GMO Quantification: Valuable Experience and Insights for the Future. *Anal. Bioanal. Chem.* **2014**, *406*, 6485–6497. DOI: 10.1007/s00216-014-8077-0.

[21] European Commision. Recommendation (EC) No 2004/787/EC on Technical Guidance for Sampling and Detection of Genetically Modified Organisms and Material Produced from Genetically Modified Organisms as or in Products in the Context of Regulation (EC) No 1830/2003.

[22] Labuda, J.; Brett, A. M. O.; Evtugyn, G.; Fojta, M.; Mascini, M.; Ozsoz, M.; Palchetti, I.; Paleček, E.; Wang, J. Electrochemical Nucleic Acid-Based Biosensors: Concepts, Terms, and Methodology (IUPAC Technical Report). *Pure Appl. Chem.* **2010**, *82*, 1161–1187. DOI: 10.1351/PAC-REP-09-08-16.

[23] Arugula, M. A.; Simonian, A. L. Biosensors for Detection of Genetically Modified Organisms in Food and Feed. In *Genetically Modified Organisms in Food. Production, Safety, Regulation and Public Health*; Ross Watson, R., Preedy, V. R. Eds.; Elsevier, **2016**; pp. 97–110.

[24] Kasry, A.; Borri, P.; Davies, P. R.; Harwood, A.; Thomas, N.; Lofas, S.; Dale, T. Comparison of Methods for Generating Planar DNA-Modified Surfaces for Hybridization Studies. *ACS Appl. Mater. Interfaces* **2009**, *1*, 1793–1798. DOI: 10.1021/am9003073.

[25] Lucarelli, F.; Marrazza, G.; Mascini, M. Enzyme-Based Impedimetric Detection of PCR Products Using Oligonucleotide-Modified Screen-Printed Gold Electrodes. *Biosens. Bioelectron.* **2005**, *20*, 2001–2009. DOI: 10.1016/j.bios.2004.08.025.

[26] Jiang, X.; Chen, K.; Han, H. Ultrasensitive Electrochemical Detection of *Bacillus thuringiensis* Transgenic Sequence Based on In Situ Ag Nanoparticles Aggregates Induced by Biotin–Streptavidin System. *Biosens. Bioelectron.* **2011**, *28*, 464–468. DOI: 10.1016/j.bios.2011.07.042.

[27] Xu, G.; Jiao, K.; Fan, J.; Sun, W. Electrochemical Detection of Specific Gene Related to CaMV35S Using Methylene Blue and Ethylenediamine Modified Glassy Carbon Electrode. *Acta Chim. Slov.* **2006**, *53*, 486–491.

[28] Ligaj, M.; Jasnowska, J.; Musiał, WG.; Filipiak, M. Covalent Attachment of Single-Stranded DNA to Carbon Paste Electrode Modified by Activated Carboxyl Groups. *Electrochim. Acta* **2006**, *51*, 5193–5198. DOI: 10.1016/j.electacta.2006.03.053.

[29] Lien, T. T. N.; Lam, T. D.; An, V. T. H.; Hoang, T. V.; Quang, D. T.; Khieu, D. Q.; Tsukahara, T.; Lee, Y. H.; Kim, J. S. Multi-Wall Carbon Nanotubes (MWCNTs)-Doped Polypyrrole DNA Biosensor for Label-Free Detection of Genetically Modified Organisms by QCM and EIS. *Talanta* **2010**, *80*, 1164–1169. DOI: 10.1016/j.talanta.2009.09.002.

[30] Yang, J.; Jiao, K.; Yangs, T. A DNA Electrochemical Sensor Prepared by Electrodepositing Zirconia on Composite Films of Single-Walled Carbon Nanotubes and Poly(2,6-Pyridinedicarboxylic Acid), and Its Application to Detection of the PAT Gene Fragment. *Anal. Bioanal. Chem.* **2007**, *389*, 913–921. DOI: 10.1007/s00216-007-1450-5.

[31] Yang, T.; Zhang, W.; Du, M.; Jiao, K. A PDDA/poly(2,6-Pyridinedicarboxylic Acid)-CNTs Composite Film DNA Electrochemical Sensor and Its Application for the Detection of Specific Sequences Related to PAT Gene and NOS Gene. *Talanta* **2008**, *75*, 987–994. DOI: 10.1016/j.talanta.2007.12.049.

[32] Jiang, C.; Yang, T.; Jiao, K.; Gao, H. A DNA Electrochemical Sensor with Poly-l-Lysine/Single-Walled Carbon Nanotubes Films and Its Application for the Highly Sensitive EIS Detection of PAT Gene Fragment and PCR Amplification of NOS Gene. *Electrochim. Acta* **2008**, *53*, 2917–2924. DOI: 10.1016/j.electacta.2007.11.015.

[33] Yang, T.; Zhou, N.; Zhang, Y.; Zhang, W.; Jiao, K.; Li, G.. Synergistically Improved Sensitivity for the Detection of Specific DNA Sequences Using Polyaniline Nanofibers and Multi-Walled Carbon Nanotubes Composites. *Biosens. Bioelectron.* **2009**, *24*, 2165–2170. DOI: 10.1016/j.bios.2008.11.011.

[34] Feng, Y.; Yang, T.; Zhang, W.; Jiang, C.; Jiao, K. Enhanced Sensitivity for Deoxyribonucleic Acid Electrochemical Impedance Sensor: Gold Nanoparticle/Polyaniline Nanotube Membranes. *Anal. Chim. Acta* **2008**, *616*, 144–151. DOI: 10.1016/j.aca.2008.04.022.

[35] Ma, Y.; Jiao, K.; Yang, T.; Sun, D. Sensitive PAT Gene Sequence Detection by Nano-SiO_2/p-Aminothiophenol Self-Assembled Films DNA Electrochemical Biosensor Based on Impedance Measurement. *Sens. Actuator B: Chem.* **2008**, *131*, 565–571. DOI: 10.1016/j.snb.2007.12.046.

[36] Yang, J.; Yang, T.; Feng, Y.; Jiao, K. A DNA Electrochemical Sensor Based on Nanogold-Modified Poly-2,6-pyridinedicarboxylic Acid Film and Detection of PAT Gene Fragment. *Anal. Biochem.* **2007**, *365*, 24–30. DOI: 10.1016/j.ab.2006.12.039.

[37] Zhang, W.; Yang, T.; Jiang, C.; Jiao, K. DNA Hybridization and Phosphinothricin Acetyltransferase Gene Sequence Detection Based on Zirconia/Nanogold Film Modified Electrode. *Appl. Surf. Sci.* **2008**, *254*, 4750–4756. DOI: 10.1016/j.apsusc.2008.01.102.

[38] Duwensee, H.; Mix, M.; Broer, I.; Flechsig, G-U. Electrochemical Detection of Modified Maize Gene Sequences by Multiplexed Labeling with Osmium Tetroxide Bipyridine. *Electrochem. Commun.* **2009**, *11*, 1487–1491. DOI: 10.1016/j.elecom.2009.05.037.

[39] Mix, M.; Rüger, J.; Krüger, S.; Broer, I.; Flechsig, G. U. Electrochemical Detection of 0.6% Genetically Modified Maize MON810 in Real Flour Samples. *Electrochem. Commun.* **2012**, *22*, 137–140. DOI: 10.1016/j.elecom.2012.06.019.

[40] Fátima Barroso, M. F.; Freitas, M.; Oliveira, M. B.; de-los-Santos-Álvarez, N.; Lobo-Castañón, M. J.; Delerue-Matos, C. 3D-Nanostructured Au Electrodes for the Event-Specific Detection of MON810 Transgenic Maize. *Talanta* **2015**, *134*, 158–164. DOI: 10.1016/j.talanta.201..10.017.

[41] Liao, W. C.; Chuang, M. C.; Ho, J. A. Electrochemical Sensor for Multiplex Screening of Genetically Modified DNA: Identification of Biotech Crops by Logic-Based Biomolecular Analysis. *Biosens. Bioelectron.* **2013**, *50*, 414–420. DOI: 10.1016/j.bios.2013.06.044.

[42] Sun, W.; Zhang, Y.; Hu, A.; Lu, Y.; Shi, F.; Lei, B.; Sun, Z. Electrochemical DNA Biosensor Based on Partially Reduced Graphene Oxide Modified Carbon Ionic Liquid Electrode for the Detection of Transgenic Soybean A2704–12 Gene Sequence. *Electroanalytical* **2013**, *25*, 1417–1424. DOI: 10.1002/elan.201300069.

[43] Manzanares-Palenzuela, C. L.; Santos-Alvarez, N.; Lobo-Castañon, MJ.; López-Ruiz, B. Multiplex Electrochemical DNA Platform for Femtomolar-Level Quantification of Genetically Modified Soybean. *Biosens. Bioelectron.* **2015**, *68*, 259–265. DOI: 10.1016/j.bios.2015.01.007.

[44] Manzanares-Palenzuela, C. L.; Mafra, I.; Costa, J.; Barroso, M. F.; de-los-Santos-Álvarez, N.; Delerue-Matos, C.; Oliveira, M. B. Electrochemical Magnetoassay Coupled to PCR as a Quantitative Approach to Detect the Soybean Transgenic Event GTS40–3-2 in Foods. *Sens. Actuator B: Chem.* **2016**, *222*, 1050–1057. DOI: 10.1016/j.snb.2015.09.013.

[45] Manzanares-Palenzuela, C. L.; Fernandes, E. G. R.; Lobo-Castañon, M. J.; López-Ruiz, B.; Zucolotto, V. Impedance Sensing of DNA Hybridization onto Nanostructured Phthalocyanine-Modified Electrodes. *Electrochim. Acta* **2016**, *21*, 86–95. DOI: 10.1016/j.electacta.2016.10.140.

[46] Manzanares-Palenzuela, C. L.; Martín-Clemente, J. P.; Lobo-Castañon, M. J.; López-Ruiz, B. Electrochemical Detection of Magnetically-Entrapped DNA Sequences from Complex Samples by Multiplexed Enzymatic Labelling: Application to a Transgenic Food/Feed Quantitative Survey. *Talanta* **2017**, *164*, 261–267. DOI: 10.1016/j.talanta.2016.11.040.

[47] Carpini, G.; Lucarelli, F.; Marrazza, G.; Mascini, M. Oligonucleotide-Modified Screen-Printed Gold Electrodes for Enzyme-Amplified Sensing of Nucleic Acids. *Biosens. Bioelectron.* **2004**, *20*, 167–175. DOI: 10.1016/j.bios.2004.02.021.

[48] Zhan, F.; Liao, X.; Gao, F.; Qiu, W.; Wang, Q. Electroactive Crown Ester-Cu^{2+} Complex with In-Situ Modification at Molecular Beacon Probe Serving as a Facile Electrochemical DNA Biosensor for the Detection of CaMV 35s. *Biosens. Bioelectron.* **2017**, *92*, 589–595. DOI: 10.1016/j.bios.2016.10.055.

[49] Ulianas, A.; Heng, L. Y.; Ahmad, M.; Lau, H. Y.; Ishak, Z.; Ling, T. L. A Regenerable Screen-Printed DNA Biosensor Based on Acrylic Microsphere–Gold Nanoparticle Composite for Genetically Modified Soybean Determination. *Sens. Actuator B: Chem.* **2014**, *190*, 694–701. DOI: 10.1016/j.snb.2013.09.040.

[50] Aghili, Z.; Nasirizadeh, N.; Divsalar, A.; Shoeibi, S.; Yaghmaei, P. A Nanobiosensor Composed of Exfoliated Graphene Oxide and Gold Nano-Urchins, for Detection of GMO Products. *Biosens. Bioelectron.* **2017**, *95*, 72–80. DOI: 10.1016/j.bios.2017.02.054.

[51] Kerman, K.; Vestergaard, M.; Nagatani, N.; Takamura, Y.; Tamiya, E. Electrochemical Genosensor Based on Peptide Nucleic Acid-Mediated PCR and Asymmetric PCR Techniques: Electrostatic Interactions with a Metal Cation. *Anal. Chem.* **2006**, *78*, 2182–2189. DOI: 10.1021/ac051526a.

[52] Mao-Qing, W.; Xiao-Yan, D.; Li-Yan, L.; Sun, Q.; Jiang, X. C. DNA Biosensor Prepared by Electrodeposited Pt-nanoparticles for the Detection of Specific Deoxyribonucleic Acid Sequence in Genetically Modified Soybean. *Chinese J. Anal. Chem.* **2008**, *36*, 890–894.

[53] Sun, W.; Zhong, J.; Qin, P.; Jiao, K. Electrochemical Biosensor for the Detection of Cauliflower Mosaic Virus 35 S Gene Sequences Using Lead Sulfide Nanoparticles as Oligonucleotide Labels. *Anal. Biochem.* **2008**, *377*, 115–119. DOI: 10.1016/j.ab.2008.03.027.

[54] Wang, S.; Liu, Q.; Li, H.; Li, Y.; Hao, N.Q.J; Zhu, W.; Wang, K. Fabrication of Label-Free Electrochemical Impedimetric DNA Biosensor

for Detection of Genetically Modified Soybean by Recognizing CaMV 35S Promoter. *J. Electroanal. Chem.* **2016**, *782*, 19–25. DOI: 10.1016/j.jelechem.2016.09.05.

[55] Xie, J. K.; Jiao, K.; Liu, H.; Wang, Q. X.; Liu, S. F.; Fu, X. DNA Electrochemical Sensor Based on PbSe Nanoparticle for the Sensitive Detection of CaMV35S Gene Sequence. *Chinese J. Anal. Chem.* **2008**, *36*, 874–878.

[56] Dinh Tam, P. Genetically Modified Organism (GMO) Detection by Biosensor Based on SWCNT Material. *Curr. Appl. Phys.* **2015**, *15*, 397–401. DOI: 10.1016/j.cap.2015.01.017.

[57] Li, Y.; Sun, L.; Liu, Q.; Han, E.; Hao, N.; Zhang, L.; Wang, S.; Cai, J.; Wang, K. Photoelectrochemical CaMV35S Biosensor for Discriminating Transgenic from Non-Transgenic Soybean Based on SiO_2@CdTe Quantum Dots Core-Shell Nanoparticles as Signal Indicators. *Talanta* **2016**, *161*, 211–218. DOI: 10.1016/j.talanta.2016.08.047.

[58] Tichoniuk, M.; Ligaj, M.; Filipiak, M. Application of DNA Hybridization Biosensor as a Screening Method for the Detection of Genetically Modified Food Components. *Sensors* **2008**, *8*, 2118–2135. DOI: 10.3390/s8042118.

[59] Meric, B.; Kerman, K.; Marrazza, G.; Palchetti, I.; Mascini, M.; Ozsoz, M. Disposable Genosensor, A New Tool for the Detection of NOS-Terminator, A Genetic Element Present in GMOs. *Food Control* **2004**, *15*, 621–626. DOI: 10.1016/j.foodcont.2003.10.004.

[60] Sun, W.; Zhong, J.; Zhang, B.; Jiao, K. Application of Cadmium Sulfide Nanoparticles as Oligonucleotide Labels for the Electrochemical Detection of NOS Terminator Gene Sequences. *Anal. Bioanal. Chem.* **2007**, *389*, 2179–2184. DOI: 10.1007/s00216-007-1661-9.

[61] Zhu, L.; Zhao, R.; Wang, K.; Xiang, H.; Shang, Z.; Sun, W. Electrochemical Behaviors of Methylene Blue on DNA Modified Electrode and Its Application to the Detection of PCR Product from NOS Sequence. *Sensors* **2008**, *8*, 5649–5660. DOI: 10.3390/s8095649.

[62] Ligaj, M.; Oczkowski, T.; Jasnowska1, J.; Musiał, G. W.; Filipiak, M. Electrochemical Genosensors for Detection of Monocytogenes and Genetically-Modified Components in Food and Genetically Modified Components in Food. *Pol. J. Food Nutr. Sci.* **2003**, *12*, 61–63.

[63] Wang, J.; Qinling, S.; Tian, N.; Chen, L.; Ziqin, X.; Zheng, J. Electrochemical Detection of the Neomycin Phosphotransferase Gene (NPT-II) in Transgenic Plants with a Novel DNA Biosensor. *J. Appl. Electrochem.* **2009**, *39*, 935–945. DOI: 10.1007/s10800-008-9775-0.

[64] Ren, Y.; Jiao, K.; Xu, G.; Sun, W.; Gao, H. An Electrochemical DNA Sensor Based on Electrodepositing Aluminum Ion Films on Stearic Acid-Modified Carbon Paste Electrode and Its Application for the Detection of Specific Sequences Related to Bar Gene and CP4 Epsps Gene. *Electroanalytical* **2005**, *17*, 2182–2189. DOI: 10.1002/elan.200503355.

[65] Bonanni, A.; Esplandiu, M.; Del Valle, M. Impedimetric Genosensors Employing COOH-Modified Carbon Nanotube Screen-Printed Electrodes. *Biosens. Bioelectron.* **2009**, *24*, 2885–2891. DOI: 10.1016/j.bios.2009.02.023-.

[66] Yang, T.; Zhou, N.; Li, Q.; Guan, Q.; Zhang, Q.; Jiao, K. Highly Sensitive Electrochemical Impedance Sensing of PEP Gene Based on Integrated Au–Pt Alloy Nanoparticles and Polytyramine. *Colloids Surf. B Biointerfaces* **2012**, *97*, 150–154. DOI: 10.1016/j.colsurfb.2012.04.007.

[67] Zhou, N.; Yang, T.; Jiang, C.; Du, M.; Jiao, K. Highly Sensitive Electrochemical Impedance Spectroscopic Detection of DNA Hybridization Based on Au(nano)-CNT/PAN(nano) Films. *Talanta* **2009**, *77*, 1021–1026. DOI: 10.1016/j.talanta.2008.07.058.

[68] Yang, J.; Wang, X.; Shi, H. An Electrochemical DNA Biosensor for Highly Sensitive Detection of Phosphinothricin Acetyltransferase Gene Sequence Based on Polyaniline-(Mesoporous Nanozirconia)/Poly-Tyrosine Film. *Sens. Actuator B: Chem.* **2012**, *162*, 178–183. DOI: 10.1016/j.snb.2011.12.064.

[69] Bai, S. L.; Zhong, X.; Ma, L.; Zheng, W.; Fan, L. M.; Wei, N.; Deng, X. W. A Simple and Reliable Assay for Detecting Specific Nucleotide Sequences in Plants Using Optical Thin-Film Biosensor Chips. *Plant J.* **2007**, *49*, 354–366. DOI: 10.1111/j.1365-313X.2006.02951.x.

[70] Bai, S.; Zhang, J.; Li, S.; Chen, H.; Terzaghi, W.; Zhang, X.; Chi, X.; Tian, J.; Luo, H.; Huang, W.; Chen, Y.; Zhang, Y. Detection of Six Genetically Modified Maize Lines Using Optical Thin-Film Biosensor Chips. *J. Agric. Food Chem.* **2010**, *58*, 8490–8494. DOI: 10.1021/jf100598k.

[71] Feriotto, G.; Gardenghi, S.; Bianchi, N.; Gambari, R. Quantitation of Bt-176 Maize Genomic Sequences by Surface Plasmon Resonance-Based Biospecific Interaction Analysis of Multiplex Polymerase Chain Reaction (PCR). *J. Agric. Food Chem.* **2003**, *51*, 4640–4646. DOI: 10.1021/jf0341013.

[72] Zhao, Z.; Chen, Y.; Xu, W.; Ma, M. Surface Plasmon Resonance Detection of Transgenic Cry1Ac Cotton (Gossypium spp.). *J. Agric. Food Chem.* **2013**, *61*, 2964–2969. DOI: 10.1021/jf3050439.

[73] Kalogianni, D. P.; Koraki, T.; Christopoulos, T. K.; Ioannou, P. C. Nanoparticle-Based DNA Biosensor for Visual Detection of Genetically Modified Organisms. *Biosens. Bioelectron.* **2006**, *21*, 1069–1076. DOI: 10.1016/j.bios.2005.04.016.

[74] Zhu, D.; Liu, J.; Tang, Y.; Xing, D. A Reusable DNA Biosensor for the Detection of Genetically Modified Organism Using Magnetic Bead-Based Electrochemiluminescence. *Sens. Actuator B: Chem.* **2010**, *149*, 221–225. DOI: 10.1016/j.snb.2010.05.047.

[75] Feriotto, G.; Borgatti, M.; Mischiati, C.; Bianchi, N.; Gambari, R. Biosensor Technology and Surface Plasmon Resonance for Real-Time Detection of Genetically Modified Roundup Ready Soybean Gene Sequences. *J. Agric. Food Chem.* **2002**, *50*, 955–962. DOI: 10.1021/jf0109773.

[76] Giakoumaki, E.; Minunni, M.; Tombelli, S.; Tothill, I. E.; Mascini, M.; Bogani, P.; Buiatti, M. Combination of Amplification and Post-Amplification Strategies to Improve Optical DNA Sensing. *Biosens. Bioelectron.* **2003**, *19*, 337–344. DOI: 10.1016/S0956-5663(03)00193-3.

[77] Wang, R.; Minunni, M.; Tombelli, S.; Mascini, M. A New Approach for the Detection of DNA Sequences in Amplified Nucleic Acids by a Surface Plasmon Resonance Biosensor. *Biosens. Bioelectron.* **2004**, *20*, 598–605. DOI: 10.1016/j.bios.2004.03.013.

[78] Wang, R.; Tombelli, S.; Minunni, M.; Spiriti, M. M.; Mascini, M. Immobilisation of DNA Probes for the Development of SPR-Based Sensing. *Biosens. Bioelectron.* **2004**, *20*, 967–974. DOI: 10.1016/j.bios.2004.06.013.

[79] Guven, B.; Hakk Boyac, I.; Tamer, U.; Calik, P. A Rapid Method for Detection of Genetically Modified Organisms Based on Magnetic Separation and Surface-Enhanced Raman Scattering. *Analyst* **2012**, *137*, 202–208. DOI: 10.1039/c1an15629b.

[80] Mariotti, E.; Minunni, M.; Mascini, M. Surface Plasmon Resonance Biosensor for Genetically Modified Organisms Detection. *Anal. Chim. Acta* **2002**, *453*, 165–172.

[81] Chen, K.; Han, H.; Luo, Z.; Wang, Y.; Wang, X. A Practicable Detection System for Genetically Modified Rice by SERS-Barcoded Nanosensors. *Biosens. Bioelectron.* **2012**, *34*, 118–124. DOI: 10.1016/j.bios.2012.01.029.

[82] Mannelli, I.; Minunni, M.; Tombelli, S.; Mascini, M. Quartz Crystal Microbalance (QCM) Affinity Biosensor for Genetically Modified Organisms (GMOs) Detection. *Biosens. Bioelectron.* **2003**, *18*, 129–140.

[83] Minunni, M.; Tombelli, S.; Pratesi, S.; Mascini, M.; Piati, P.; Bogani, P.; Buiatti, M. A Piezoelectric Affinity Biosensor for Genetically Modified Organisms (GMOs) Detection. *Anal. Lett.* **2001**, *34*, 825–840. DOI: 10.1081/AL-100103595.

[84] Karamollaoglu, I.; Avni-Öktem, H.; Mutlu, M. QCM-Based DNA Biosensor for Detection of Genetically Modified Organisms (GMOs). *Biochem. Eng. J.* **2009**, *44*, 142–150. DOI: 10.1016/j.bej.2008.11.011.

[85] Minunni, M.; Tombelli, S.; Fonti, J.; Spiriti, M. M.; Mascini, M.; Bogani, P.; Buiatti, M. Detection of Fragmented Genomic DNA by PCR-Free Piezoelectric Sensing Using a Denaturation Approach. *J. Am. Chem. Soc.* **2005**, *127*, 7966–7967. DOI: 10.1021/ja051345q.

[86] Boganni, P.; Minunni, M.; Spiriti, M. M.; Zavaglia, M.; Tombelli, S.; Buiatti, M.; Mascini, M. Transgenes Monitoring in an Industrial

Soybean Processing Chain by DNA-Based Conventional Approached and Biosensors. *Food Chem.* **2009**, *113*, 658–664. DOI: 10.1016/j.foodchem.2008.07.056.

[87] Stobiecka, M.; Cieśla, J. M.; Janowska, B.; Tudek, B.; Radecka, H. Piezoelectric Sensor for Determination of Genetically Modified Soybean Roundup Ready (R) in Samples not Amplified by PCR. *Sensors* **2007**, *7*, 1462–1479. DOI: 10.3390/s7081462.

[88] Passamano, M.; Pighini, M. QCM DNA-Sensor for GMOs Detection. *Sens. Actuator B: Chem.* **2006**, *118*, 177–181. DOI: 10.1016/j.snb.2006.04.012.

[89] Ahmed, M. U.; Saito, M.; Hossain, M. M.; Ramachandara Rao, S.; Furui, S.; Hino, A.; Takamura, Y.; Takagi, M.; Tamiya, E. Electrochemical Genosensor for the Rapid Detection of GMO Using Loop-Mediated Isothermal Amplification. *Analyst* **2009**, *134*, 966–972. DOI: 10.1039/b812569d.

[90] Michelini, E.; Simoni, P.; Cevenini, L.; Mezzanotte, L.; Roda, A. New Trends in Bioanalytical Tools for the Detection of Genetically Modified Organisms: An Update. *Anal. Bioanal. Chem.* **2008**, *392*, 355–367. DOI: 10.1007/s00216-008-2193-7.