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Detection Methods of Genetically Modified Organisms (GMOS): Review

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Abstract

A living organism is considered a genetically modified organism (GMO) when a new foreign DNA segment or transgene is inserted into it to create a new trait. The field of biotechnology is currently developing at a rapid pace, with more traits and applications emerging every day. Due to concerns about the environment and living organisms, societies have not yet accepted this technology. Countries adhere to a strict biosafety protocol to reduce their fear of this issue and detect DNA and GMO protein molecules using a variety of mechanisms to ensure biotechnology products are free of foreign material or contain it at a level below the threshold, if it is present. Based on the quantity and quality of DNA and protein in these samples, these detections are made. Quantitative detection is crucial for determining the GMO threshold for each sample. The DNA-based detection of GMOs using various PCRs, either qualitatively or quantitatively is one of these detection techniques. The second most popular technique for determining how much a protein is expressed in a side organism is protein-based detection. DNA microarray, biosensors, chromatography, and DNA sequencing can all be used to find GMOs. The availability of accurate and sensitive GMO detection techniques allows us to control the presence of GMOs in crops, foods, and ingredient sources.

Keywords: Detection; DNA; GMO; PCR; Protein; Quantification

Abbreviations: GMO: Genetically Modified Organism; EU: European Union; PCR: Polymerase Chain Reaction; QC: Quantitative Competitive; ELISA: Enzyme-linked Immunosorbent Assay; FND: Ferrocenyl Naphthalene Diimide; HPLC: High Performance Liquid Chromatography; APCI-MS: Atmospheric Pressure Chemical Ionization Mass Spectrometry; GC: Gas Chromatography; WGS: Whole Genome Sequencing.

Introduction

What are Genetically Modified Organisms?

A genetically modified organism (GMO) is a living thing that has had its genetic makeup altered through the use of gene technology. It can be a plant, an animal, or a microorganism. In order to combine several smaller pieces of DNA into the genome of the organism to be modified, the genetic modification typically entails inserting a piece of rDNA into the host [1]. A modified (GM) crop is one that has had one or more genes artificially inserted into it, as opposed to the plant naturally acquiring them through crossbreeding or natural recombination. The transgene, or inserted gene sequence, may come from the same species, another species within the same kingdom, or even a different kingdom (genetically modified Bt corn, which produces the natural insecticide, contains a gene from a bacterium). The field of biotechnology is developing quickly, more traits are appearing, and more genetically altered crops are being planted on more acres than ever before. The biotechnology

sector is spending billions on mergers and acquisitions to ensure access to these quickly expanding markets, as well as additional billions on R&D.

GMOs and their derived products have been available on the market for more than ten years. The amount and variety of GMOs (both in terms of taxonomy and the genetic makeup of inserted sequences) are constantly increasing, and detection is becoming progressively more difficult. Today, GMO is more commonly used on plants to enhance their genetic makeup in order to increase nutritional content, make them environmentally friendly (to make them resistant to harsh environments), and make them resistant to biological things (pests that can eliminate or decrease productivity by any means). In recent years, agricultural enterprises in the United States, Canada, and the European Union (EU) have developed new plant varieties by utilizing modern biotechnology, including genetic transformation. At least 40% of corn, 50% of cotton, and 45% of soybean acres planted in 1999 were genetically modified, and at least 60% of food products sold in US supermarkets contain genetically modified organisms (GMOs). GMOs can also be used in food, cosmetics, drugs, and other consumable and non-consumable items to obtain desired traits and more active ingredients. The majority of Western countries and America have adopted GM food for feeding. However, consumers' skepticism stemmed from previous food and environmental concerns as well as mistrust in government and scientific communities, all of which fueled debates about the environmental and public health safety issues of introduced genes. Most people worried about GMOs when it released to the environment and especially if it is used in food materials they afraid those they may not consume freely. To minimize this, researchers can check the product, which comes from companies' weather it has GMO or not using different detection methods. GMO detection is accomplished by detecting a molecule (DNA or protein) that is specifically associated with or derived from the genetic modification of interest. The majority of methods for detecting GMOs have been improved, while only a few methods for detecting proteins or RNA have been developed. The United States government works to ensure that new agricultural biotechnology products are safe for animals, human health, and the environment, avoiding public fear [2]. The primary goal of this review is to evaluate various GMO detection methods and determine which method is best practice.

DNA Based Methods For GMOs Detection

GMO detection methods can be qualitative or quantitative. Some of the qualitative methods include DNA and protein detection to determine whether the target DNA is present or absent. There are many types of PCR for detection, either qualitatively or quantitatively, to understand the gene that is present within the organism, or how much it expresses in number. Although hybridization can detect specific DNA sequences, PCR in its various formats (qualitative PCR, end-point quantitative PCR, and quantitative real-time PCR) has been widely accepted by regulatory authorities [3]. DNA-based methods primarily rely on PCR techniques to multiply specific DNA. Most current detection methods rely on PCR amplification of transgene sequence(s) or immunological methods (primarily ELISA, the enzyme-linked immunosorbent assay) to bind to a transgene gene product (s). All PCR assays require the presence of a number of target DNA sequences in the template, as well as some sequence of the target DNA known to design a specific primer for amplification.

Qualitative PCR GMO Detection: Because of its high specificity and sensitivity, polymerase chain reaction (PCR) is the most commonly used DNA-based technique in most laboratories for GMO detection. Two short pieces of synthetic DNA (primers) complementary to one end of the DNA to be multiplied are required. After annealing these synthetic primers, the DNA polymerase enzyme joined them to create new strands that are complementary to the template DNA strand. The result is determined by gel electrophoresis in the form of a band. The bases for identifying a heterologous, transgenic sequence using the Polymerase Chain Reaction (PCR), which relies on the DNA and RNA sequences of the gene itself, as well as markers required to transform an organism, During transformation, a DNA sequence contains the target sequence, the regulatory sequence, and markers that span the regulatory gene and the gene or target sequence and are used to validate the transformation [4].

Molecular markers have proven to be extremely useful in determining whether genes from another species have been introduced into a species [5]. A foreign gene can enter the plant in two ways: naturally (via gene flow) or through the act of the researcher to modify the plant for a specific purpose. The qualitative PCR was mostly used to determine the 35S promoter and NOS terminator from GM soybeans, maize, and other processed samples. In order to prevent contamination and DNA degradation by specific enzymes, it is especially important to extract and purify DNA from the sample in a cost-effective, simple, and high-yield manner [6].

Quantitative PCR GMO Detection: Because product concentration is a poor indicator of initial template amount, end-point PCR, which is primarily qualitative, cannot be used for GMO quantification. EC 2001/18 states that the recommendation 2004/787/EC, which specifies the copy number of the GM event DNA and an endogenous (species-specific) reference target, describes how to quantify the presence of GMOs. Food products or other environmental survivors may contain GMOs. If it's a food product, we must

pay close attention to the health of the people who can be affected by how much of the gene will be expressed in that modified organism, as the number of genes will determine how quickly the product will take effect on the consumer.

In principle, PCR-based quantization can be done either during or immediately after the PCR (end-point analysis) (real-time analysis). The competition between the amplification of internal control DNA and target DNA during quantitative-competitive PCR typically results in a reduction in detection sensitivity. The products of the PCR reaction can be measured by PCR at the reaction profile's end point. The final amount of amplified DNA from two DNA targets—the one to be quantified and a competitor—is compared in end-point analyses (an artificially constructed DNA that is added in a small and known quantity prior to the PCR amplification and that is co-amplified with the target, which is to be quantified). Similar binding sites for similar primer pairs are present in the rival, but it differs in size. With competitive quantitative PCR, both DNA targets are amplified equally effectively. There are four steps in QC-PCR: The first three steps are coamplification of standard and target DNA in the same reaction tube; separation of the products using the proper technique—such as agarose gel electrophoresis and ethidium bromide staining; densitometric analysis of the gel; and estimation of the relative amounts of target and standard DNA by regression analysis—are the first three steps. The DNA that will be examined is diluted several times, and the competitor is then added. The techniques for competitive PCR are semi-quantitative. According to the theory, using fluorescence makes it possible to precisely calculate the number of cycles required to produce a specific volume of PCR product. This amount equates to the amount producing a fluorescence signal that can be measured before the plateau effect becomes an issue and can be easily distinguished from the background signal. Real-time PCR is faster, automated, and more specific than competitive PCR, but it also requires more expensive and sophisticated equipment.

There are many different technologies available, each with varying degrees of specificity, quantitation potential (or lack thereof), multiplexing, cost-effectiveness, and pee. Real-time PCR quantification relies on a system that continuously monitors PCR output. Because the amount of product produced by competitive quantitative PCR must be a linear function of the initial amount, two DNA targets must be amplified with equal efficiency. Quantitation is an important part of the analysis of GMOs in food because it forms the basis for labeling because the maximum allowed levels of GMOs in food are what are used. Consequently, we require more quantitative PCR methods. PCR is quantitative if the target DNA is amplified alongside an internal DNA standard. Since the amplification of both internal standard and target DNA is simultaneously impacted in systems using the quantitative competitive (QC)-PCR method, the presence of PCR inhibitors will be detected right away. The less-advanced QC-PCR has been replaced by the more complex RT-PCR due to drawbacks like the challenge of standardization and extensive pipetting [7]. Cazzola and Petruccelli [8] conducted a semi-quantitative analysis of the modified maize and soya beans that are sold as food.

Real Time PCR

Among the techniques currently in use, RT-PCR is regarded as the most effective technology for the identification and measurement of GMOs. It is quicker, more automated, and more precise than competitive PCR, but it also requires more expensive and sophisticated equipment. The most effective tool currently available for the detection and quantification of GM crops and products is real-time PCR. It allows for real-time monitoring of the amplification reaction and data analysis when the amplification efficiency is constant (exponential phase or logarithmic phase of the reaction). The various benefits that RT-PCR has over traditional PCR account for its broad applicability in the agro-food production chain. This method does not require gel electrophoresis, which lowers the risk of contamination and saves time. It is also applicable to highly processed foods thanks to the amplification of very short DNA fragments.

Finally, it has high specificity because it uses target-specific probes, which eliminates the need for additional confirmation assays like hybridization, restriction analysis, or sequencing. In addition, multiplex PCR has the benefit of preventing setup errors and saving reagents, even though a slight decrease in sensitivity in terms of detection limit can be anticipated. Multiplex PCR allows for the simultaneous quantification of a reference gene and a transgene. Numerous quantitative methods have been developed recently as a result of the technique's broad applicability and the potential for high levels of specificity and sensitivity. The increase in fluorescent signal produced by particular fluorescent DNA probes or a particular DNA-binding fluorescent dye present in the amplification reaction is monitored and converted into quantitative estimates by software to determine the PCR product increment. By simply interpolating the threshold cycle (Ct), which is defined as the number of cycles required generating; it is possible to determine the DNA content of a sample due to the direct correlation between the amount of PCR product generated and the initial amount of target template.

Multiplex PCR-based Detection Method

Multiple target DNA sequences can be screened and found using the multiplex PCR-based method in a single reaction. The benefit of multiplex methods is that fewer reactions are

required to check a sample for the potential presence of DNA derived from GMOs. Testing and validation must be done meticulously when developing multiplex assays. Further analysis of the pool of amplified fragments produced by PCR is required to differentiate between the various amplicons [9]. Multiplex PCR enables the simultaneous detection of numerous target sequences by incorporating multiple primer pairs into the PCR. Such systems have been created for a number of construct-specific targets [10].

Southern Blotting Techniques

It is a detection technique that can locate the location of a specific sequence and determine whether or not an organism contains a transgene. The Southern blot test method depends on the complementary specificity of the two strands that make up the double helix of double-stranded DNA or hybridize in a sequence-specific manner, and this specificity is used in the detection process [11].

Protein Based Detection Methods

The current method for identifying and measuring novel (foreign) proteins introduced through plant genetic modification is immunoassay. Due to the availability of antibodies with high specificity and affinities, immunoassays are based on the specific binding between an antigen and an antibody. Additionally, immunoassays can be applied qualitatively or quantitatively across a broad spectrum of concentrations. The most popular techniques for identifying protein expression in GMOs are Western blotting and enzyme-linked immunosorbent assay (ELISA).

ELISA (Enzyme Linked Immunosorbent Assay)

In ELISA, a solid phase is used for the antigen-antibody reaction (plates with wells). When an antibody and an antigen interact, a stable complex is created that can be seen by adding a second antibody that is attached to an enzyme. Because they exhibit uniform affinity and specificity against a single epitope or antigenic determinant and can be produced in large quantities, monoclonal antibodies have some advantages over polyclonal antibodies. A color that can be seen or recognized by the naked eye is formed when a substrate for that enzyme is added. This happens as a result of the enzyme's ability to bind to antigens and antibodies [12]. It is crucial to understand that because ELISA is trait-specific, it cannot detect a GMO when several varieties may contain the same trait. As a result, immunoassays in general can be used as screening techniques. There must be a certain proportion of GM materials that can be detected under ideal circumstances for this detection method to be effective. The main disadvantage of using immunological systems to identify the gene's protein product is that the transgenic proteins might not be expressed (or might only be expressed weakly) in the part of the plant used for food production, making it difficult to identify them.

Western Blotting Techniques

For detection following PCR sample amplification and running on gel electrophoresis, Western blotting is primarily used for detection. When determining whether a sample contains the target protein below or above a predetermined threshold level, the western blot is a highly specific method that yields qualitative results [13]. Due to the fact that the protein is separated via electrophoresis under conditions that cause denaturation, any issues with solubilization are avoided, and the target protein does not aggregate or precipitate with unrelated proteins [14].

The Western blot detection limits range from 0.1 to 1%. Sensitivity depends on the level of protein expression in the plant as well as affinity level [15]. However, it is believed that this approach is better suited for research applications than for everyday testing. Sodium dodecyl sulfate SDS PAGE is used to separate the assayed samples after they have been solubilized with detergents and reducing agents. These elements can be transferred to a nitrocellulose membrane or a solid support. Antibodies (high-titer polyclonal antiserum or a combination of monoclonal antibodies raised against the denatured antigenic epitopes) are used to probe the specific sites. Finally, due to their reactions, the bound antibodies are stained with Ponceau, silver nitrate, or Coomassie blue. It has its own limitations because it works best when a protein is newly expressed, even though the level of expression varies between plants.

Other Methods of GMO Detection Methods

Microarray DNA Chip-Technology

In recent years, the technology known as microarrays (also known as DNA chips) has been developed for automated, quick screening of the gene expression and sequence variation of numerous samples. The fundamental difference between microarray technology and conventional DNA hybridization is the number of distinct probes that are attached to a solid surface. Micro-arrays, high-density oligonucleotide arrays (gene chips or DNA chips), and micro-electronic arrays are some examples of different formats. As opposed to DNA arrays, which use PCR products that are deposited onto solid glass slides, DNA chips use short oligonucleotides that are synthesized onto a solid support (microarray). Microelectronic arrays are made of groups of electrodes that can produce current and are covered in a thin layer of agarose bound to an affinity moiety. These techniques are developing rapidly and have many

advantages, but also some limitations. Since the techniques are very sensitive and still under development, they are limited to expert laboratories. Microarrays are also known as DNA chips or biochips. It is a cutting-edge technology for the high-throughput detection of GMOs. This technology allows for the simultaneous detection of a large number of genetic components from complex DNA samples in a single assay. Being a highly advanced technique, it can advance alongside the rising number of newly created GMOs in the food and feed markets. The main benefits of this technology are its high sensitivity and screening [16]. These characteristics enable not only sample analysis for the detection of transgene or control genetic elements, but also an increase in the number of probes analyzed in a single hybridization study [17]. The basic concept is that a large number of designated probes are bound to a solid surface in an array of spots, with each spot having many duplicates of the probe. The desired samples' isolated DNA is then fluorescently marked before being hybridized with an array. The marked DNA segment is still combined with spotted probes built on the opposing DNA sequences during the hybridization stage. Following the hybridization phase, the remaining free-marked sequences and the sequences that are poorly bound to the probes are removed. The array is then scanned to measure the intensity of the individual fluorescence of each spot.

By using multiplex PCR techniques, DNA chip technology and multiplex PCR can be used to distinguish various transgenic events from GMOs [10]. DNA chip technology to qPCR, which yields better results with a higher throughput but a little less sensitivity [18]. Multiplex PCR and nucleic acid arrays have been used successfully to identify a variety of events in GM crops like corn and cotton [19,20]. Using the MQ DNA-PCR (multiplex quantitative DNA array based) method, transgenic events from GM corn were also identified. The PCR primer used in this technology is specific to a particular gene. The primer contained a common tail that enables its reuse in subsequent PCR reactions. After the PCR process is complete, the signal is then seen after the amplified products are hybridized with probes that are fluorescently marked on the DNA array [20]. Researchers have reported using the PPLMD (padlock probe ligation microarray detection) system to identify GM events in maize, cotton, and soybean [21]. Additionally, a study has demonstrated the potential for detecting GM events in corn using the NAIMA (nucleic acid sequence based amplification implemented microarray) system, another detection technology.

This method used tailed primers, which enable multiplex DNA template synthesis in a primer extension reaction and subsequently transcription-based extension using regular primers [22]. The dual chip GMO system was proposed as a solution to the potential issue with the use of fluorescent labels. With this method, PCR amplification with biotinylated target specific primers allows for the simultaneous detection of GM events in maize, soybeans, and rapeseed [23]. A multiplex extension on a microarray with data on an oligo microarray (MACRO) system, aiming ninety-one targets for wider range detection coverage of GM events, was also reported by Shao and his coworkers.

DNA Biosensors

A biosensor is a device that combines a biological component with a physicochemical detector component and is used to detect analytes (samples). In the past 20 years, biosensors have gained tremendous popularity. Modern life greatly benefits from new biosensor research and developments. In recent years, the use of biosensors for ongoing monitoring of biological and synthetic processes in both industrial and clinical chemistry has increased. Biosensors can be used in small hospitals and laboratories in far-off places where there aren't any sophisticated instrument facilities because they are quick, easy, and affordable. It is growing in popularity in the fields of food analysis, environmental analysis, and human health monitoring and diagnostics. A biosensor is a small device that uses biological recognition properties to perform targeted bioanalysis [24]. In order to transform biological signals into electrical signals or other signals proportional to the concentration of analytes, these devices rely on the close coupling of a biological recognition element with a physical transducer [25]. Biosensors don't require sample preparation, so they hold great promise for a variety of on-site analytical applications that require quick, low-cost measurements [26].

A receptor, a transducer, and a processor are components of a basic biosensor assembly. The sensing components, which can be whole cells, antibodies, enzymes, or nucleic acids, form a recognition layer that is integrated with the transducer through immobilization by adsorption, cross-linking, or covalent binding. Due to their extensive range of physical, chemical, and biological activities, nucleic acids have been heavily utilized in recent years in a wide range of biosensors and bioanalytical assays. The sensing components in nucleic acid-based biosensors are oligonucleotides with a known base sequence or a DNA or RNA fragment. Nucleic acid biosensors are either based on the highly specific hybridization of complementary strands of DNA or RNA molecules or act as highly specific receptors of biochemical or chemical species. Due to their great potential to obtain sequence-specific information in a quicker, easier, and less expensive manner than conventional ones, nucleic acid biosensors are of significant interest. Nucleic acid recognition layers, as opposed to enzymes or antibodies, are simple to create and can be renewed for a variety of applications. When used with polymerase chain reaction (PCR) techniques, nucleic acid biosensors can be more precise and sensitive.

DNA Hybridization Biosensors: In hybridization biosensors, the bio-recognition process is based on complementary DNA base pairing. Single-stranded DNA segments with a short length of 20–40 base pairs and high target selectivity are immobilized on the electrode surface. It is necessary to immobilize the DNA fragments while maintaining their stability, reactivity, accessibility to the target analyte, and ideal orientation. In a process known as hybridization, target DNA binds to the complementary sequence of the captured or probe DNA to produce an electrical signal. An electrochemical indicator that preferentially binds to DNA duplexes and produces an electrochemical signal is ferrocenyl naphthalene diimide (FND). Alkaline phosphatase and horseradish peroxidase are the other enzyme labels that are used to gauge hybridization.

Electrochemical DNA Biosensors: The detection techniques play very important role in design of biosensors and are selected according to their specific application. Among the various devices designed so far, electrochemical DNA Biosensors have attracted more attention due to their high sensitivity and rapid response. Electrochemical devices are very useful for sequence-specific bio-sensing of DNA. The reduction of devices and advanced technology make them excellent tool for DNA diagnostics. Electrochemical detection of DNA hybridization usually involves monitoring a current at fixed potential. Electrical modes were developed for detection of both label-free and labeled objects [27]. The immobilization of the nucleic acid probe onto the transducer surface plays an important role in the overall performance of DNA biosensors and gene chips [28].

Label Based or Indirect Detection: For the purpose of detecting hybridization in label-based electrochemical biosensors, specific organic dyes, metal complexes, or enzymes are employed. The use of enzyme-labeled probes holds out a lot of promise for electrochemical DNA hybridization detection. When a substrate is added to the enzyme-modified electrode surface, the product's electrochemical activity makes it easier to detect hybridization [29].

Applications of DNA Biosensors for Detection of GMOs: The identification of a particular DNA sequence is significant in a variety of fields, including environmental, clinical, and food analysis. The detection of specific DNA sequences can be used to identify genetically modified organisms in the environment and food (GMO). The primary interest is in DNA biosensors and gene chips because of their enormous potential for obtaining sequence-specific information more quickly, easily, and affordably than with conventional hybridization.

Chromatography

The detection of differences in the chemical profile can be done using traditional chemical methods based on chromatography in cases where the composition of GMO ingredients, fatty acids, or triglycerides has been altered. This was shown with oils made from GM canola when high performance liquid chromatography (HPLC) and atmospheric pressure chemical ionization mass spectrometry (APCI-MS) were used to analyze the triglyceride patterns. The diacylglycerol fragments and the protonated triglyceride molecular ions served as the foundation for the spectral identification. A flame ionization detector was used for quantification (FID). When comparing the triglyceride patterns, it was found that the GM canola varieties' oils contained more triacylglycerols, indicating greater oxidative stability for canola oil with a high stearic acid content.

This outcome is in line with earlier studies on the oxidative stability of new soybean varieties and high-oleic-acid canola oils obtained using HPLC-FID. To support the HPLC results, the fatty acid compositions have also been measured using gas chromatography (GC) in conjunction with FID. It must be emphasized, though, that this methodology is only appropriate when the material characteristics of GM plants or their derivatives significantly change. Furthermore, rather than being a quantitative detection method, it is a qualitative detection method. Considering the natural variation in ingredient patterns as well, low additions of GM canola oil with an altered triglyceride composition to conventional canola oil are likely to go undetected.

DNA Sequencing Methods

Next-generation sequencing technology has established itself as a viable alternative in the field of GMO detection as it offers the opportunity to directly identify the modified gene in a given sample through the characterization of its sequences. For the purpose of identifying the unidentified GM events, new PCR markers could also be created from the sequences found. A bioinformatics analyst is needed to manipulate and analyze the data obtained by this technology, which is relatively expensive and necessitates the use of sophisticated equipment. A novel method known as "next generation sequencing" has recently been put forth in an effort to address the difficulties associated with the identification of GMO transgenic events. It is a promising technology that enables millions of sequencing reads for massively parallel DNA segment sequencing [17,20]. Even in the absence of sequence data for such events, NGS is a useful tool for detecting transgenic events. The method has typically been used for mutant-site detection [30]. Fullwood, et al. benefit from excellent quality, accuracy, and satisfactory information at the genome-wide level [31-33]. To date, a number of research trials have been conducted to evaluate the use of NGS in determining GM content. NGS is effectively used to characterize site addition, flanking regions, accidental addition, as well as to calculate the number of copies of a transgene. Two main approaches (targeted sequencing

strategy) or whole genome sequencing (WGS) strategy have been identified for sequencing samples that have previously been enriched with desired sequence regions.

Conclusion

Various techniques can be used to find the GM material. One of these techniques is qualitatively and quantitatively based on DNA. The most popular PCR methods for detecting DNA include PCR, Qc PCR, Real-time PCR, and multiplex PCR. The most effective method for quantitative detection is real-time PCR. When using probe hybridization to detect DNA, southern blotting is occasionally used. The second category of detection techniques uses proteins to determine the degree of expression and includes western blotting and ELISA for color detection. In both cases, quantification is preferable to qualification in order to determine a specific threshold level. There are also additional methods for quickly and simultaneously detecting multiple samples of DNA and protein. These techniques can help us save both time and energy. These are chromatography, biosensors, and DNA microarrays. The most effective method for simultaneously quantifying multiple samples using many primers is the DNA microarray, but this method has the disadvantage that primers are expensive. To regulate the presence of GMOs in crops, foods, and ingredients necessitated by the development of reliable and sensitive methods for GMO detection.

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Conflict of Interests

The author has not declared any conflict of interests.

References

1. Jensen AH (2001) GMO detection methods and validation. Oslo, Norway.
2. USDA (2000) Biotechnology. United States Department of Agriculture.
3. Peano C, Bordoni R, Gulli M, Mezzelani A, Samsonm MC, et al. (2005) Multiplex polymerase chain reaction and ligation detection reaction/universal array technology for the traceability of genetically modified organisms in foods. Anal Biochem 346(1): 90-100.
4. Giovannini T, Concilio L (2002) PCR detection of Genetically Modified Organisms. Wiley-VchVerlag GmbH & Co. KGaA 54: 321-327.
5. Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. Evolution 55(7): 1325-1335.
6. Marmiroli N, Maestri E, Gullì M, Malcevschi A, Peano C, et al. (2008) Methods for detection of GMOs in food and feed. Anal Bioanal Chem 392(3): 369-384.
7. Rizzi A, Sorlini C, Daffonchio D (2004) Practically detection of genetically modified organisms in food. AgBiotech 6: 1-9
8. Cazzola ML, Petruccelli S (2006) Semi-quantitative analysis of genetically modified maize and soybean in food. Electronic Journal of Biotechnology 9(3): 1-6.
9. Matsuoka T, Kuribara H, Akiyama H, Miura H, Goda Y, et al. (2001) A multiplex PCR method of detecting recombinant DNAs from five lines of genetically modified maize. Shokuhin Eiseigaku Zasshi 42(1): 24-32.
10. Marmiroli N, Maestri E, Gullì M, Malcevschi A, Peano C, et al. (2008) Methods for detection of GMOs in food and feed. Anal Bioanal Chem 392(3): 369-384.
11. Adugna A, Mesfin T (2008) Detection and quantification of genetically engineered crops. Journal of SAT Agricultural Research 6: 1-10
12. Tripathi L (2005) Techniques for detecting genetically modified crops and products. African Journal of Biotechnology 4(13): 1472-1479.
13. Lipton CR, Dautlick JX, Grothaus GD, Hunst PL, Magin KM, et al. (2000) Guidelines for the validation and use of immunoassays for determining of introduced proteins in biotechnology enhanced crops and derived food ingredients. Food Agric Immunol 12: 153-164.
14. Brett GM, Chambers SJ, Huang L, Morgan MRA (1999) Design and development of immunoassays for detection of proteins. Food Control 10(6): 401-406.
15. Markoulatos P, Siafakas N, Papathoma A, Nerantzis E, Betzios B, et al. (2004) Qualitative and quantitative detection of protein and genetic traits in Genetically Modified Food. Food Rev Internat 20(3): 275-296.
16. Turkec A, Lucas SJ, Karacanli B, Baykut A, Yuksel H (2016) Assessment of a direct hybridization microarray strategy for comprehensive monitoring of genetically modified organisms (GMOs). Food Chem 194: 399-409.
17. Willems S, Fraiture MA, Maecker SCJD. Roosens NH (2015) Next generation sequencing to identify GMO in

food and feed products. Lab info 13: 1-3.

18. Pla M, Nadal A, Baeten V, Bahrdt C, Berben G, et al. (2012) New multiplexing tools for reliable GMO detection. John Wiley & Sons, pp: 333-366.

19. Leimanis S, Hamels S, Nazé F, Mbella GM, Sneyers M, et al. (2008) Validation of the performance of a GMO multiplex screening assay based on microarray detection. Eur Food Res Technol 227: 1621-1632.

20. Fraiture MA, Herman P, Taverniers I, Loose MD, Deforce D, et al. (2015) Current and new approaches in GMO detection: challenges and solutions. Biomed Res Int 2015: 392872.

21. Prins TW, Dijk JPV, Beenen HG, Hoef AAV, Voorhuijzen MM, et al. (2008) Optimised padlock probe ligation and microarray detection of multiple (non-authorised) GMOs in a single reaction. BMC Genomics 9: 584.

22. Dobnik D, Morisset D, Gruden K (2010) NAIMA as a solution for future GMO diagnostics challenges. Anal Bioanal Chem 396(6): 2229-2233.

23. Milavec M, Dobnik D, Yang L, Zhang D, Gruden K, et al. (2014)GMO quantification: valuable experience and insights for the future. Anal Bioanal Chem 406(26): 6485-6497.

24. Drummond TG, Hill MG, Barton JK (2003) Electrochemical DNA sensors. Nat Biotechnol 21(10): 1192-1199.

25. Berdat D, Marin A, Herrera F, Gijs MAM (2006) DNA biosensors using fluorescence microscopy and impedance spectroscopy. Sensors and Actuators B: Chemical 118(1-2): 53-59.

26. Sharma SK, Sehgal N, Kumar A (2003) Biomolecules for development of biosensors and their application. Curr Appl Phys 3(2-3): 307-316.

27. Erdem A, Kerman K, Meric B, Ozsoz M (2001) Methylene blue as a novel electrochemical hybridization indicator. Electroanalysis 13(3): 219-223.

28. Wang J, Xu D, Kawde AN, Polsky R (2001) Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. Anal Chem 73(22): 5576-5581.

29. Lee B (2003) Review of the present status of optical fiber sensors. Optical Fiber Technology 9(2): 57-79.

30. Polko JK, Temanni MR, Zanten MV, Workum WV, Iburg S, et al. (2012) Illumina sequencing technology as a method of identifying T-DNA insertion loci in activation-tagged Arabidopsis thaliana plants. Mol Plant 5(4): 948-950.

31. Campbell PJ, Stephens PJ, Pleasance ED, O'Meara S, Li H, et al. (2008) Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. Nat Genet 40(6): 722-729.

32. Anjum V, Pundir CS (2007) Biosensors: future analytical tools. Journal of Sensors and Transducers 76(2): 937-944.

33. Erdem A, Kesman K, Mesie B, Akarea US, Osoz M (2000) Novel hybridization indicator methylene blue for the electrochemical detection of short DNA sequence related to Hepatitis B Virus. Anal Chim Acta 422(2): 139-149.