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Review

Advances in Electrochemical Techniques for the Detection and Analysis of Genetically Modified Organisms: An Analysis Based on Bibliometrics

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Abstract: Since the first successful transgenic plants obtained in 1983, dozens of plants have been tested. On the one hand, genetically modified plants solve the problems of agricultural production. However, due to exogenous genes of transgenic plants, such as its seeds or pollen drift, diffusion between populations will likely lead to superweeds or affect the original traits. The detection technology of transgenic plants and their products have received considerable attention. Electrochemical sensing technology is a fast, low-cost, and portable analysis technology. This review interprets the application of electrochemical technology in the analysis and detection of transgenic products through bibliometrics. A total of 83 research articles were analyzed, spanning 2001 to 2021. We described the different stages in the development history of the subject and the contributions of countries and institutions to the topic. Although there were more annual publications in some years, there was no explosive growth in any period. The lack of breakthroughs in this technology is a significant factor in the lack of experts from other fields cross-examining the subject. Through keyword co-occurrence analysis, different research directions on this topic were discussed. The use of nanomaterials with excellent electrical conductivity allows for more sensitive detection of GM crops by electrochemical sensors. Furthermore, co-citation analysis was used to interpret the most popular reports on the topic. In the end, we predict the future development of this topic according to the analysis results.

Keywords: GMO; sensors; transgenic food; electrochemical sensor; bibliometrics; genetically modified food; analytical chemistry; food analysis; biosensor

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1. Introduction

The first successfully obtained genetically modified (GM) plants and the first commercialized GM crops can be traced back in 1983 and 1996, respectively [1]. Nowadays, the research scope of GM technology is constantly expanding, and the planting area of GM crops continues to increase worldwide. GM crops are plants in which genes with target traits are modified by genetic engineering techniques and then introduced into the genome of the recipient plant [2]. These exogenously transferred target genes are not only stably inherited in the offspring but also enable transgenic plants to produce new agronomic traits, such as insect resistance, herbicide tolerance, stress resistance, disease resistance, improved crop nutrition, and quality [3], etc. Since the commercialization of transgenic crops in 1996, the area under cultivation has increased from the initial 1.7 million hm^2 to

191.7 million hm^2 in 2018 [4]. There are currently 70 national/regional GM safety regulators worldwide that allow GM crops to be used for food, feed, and commercial cultivation. The continued increase in the global cultivation and import of GM crops is evidence of the range of agricultural, economic, and environmental benefits that GM crops provide [5]. While GM crops bring enormous economic and ecological benefits, their potential risks have always been controversial [6]. Therefore, establishing a rapid and accurate GM crop detection system has become a topic in this field. According to the target substances in GM crops, there are three main methods for the ingredient detection of GM crops: (1) gene nucleic acid detection. The main methods are qualitative polymerase chain reaction (PCR) and real-time fluorescence quantitative PCR; (2) detection of proteins. The main methods are western blotting, enzyme-linked immunosorbent assay, and immunochromatography. (3) metabolite detection. The main methods are high-performance liquid chromatography and two-dimensional electrophoresis. The results of conventional GM crop detection methods are more reliable, but they cannot meet the actual need for speed and low cost; so many rapid detection technologies are generated [7]. Among them, electrochemical-based analysis technology has significant application potential in rapid detection. Currently, electrochemical sensors are favored by researchers for their rapidity, sensitivity, and simplicity of operation, and research on electrochemical sensors is increasing year by year.

The detection and analysis of GM organisms by electrochemical sensors mainly converts DNA hybridization signals into detectable electrochemical signals. Typically, probe single-strand DNA is firstly fixed to the surface of signal transducers (glassy carbon electrode, gold electrode, indium tin oxide, etc.). When the target gene in the test solution is hybridized with the probe on the electrode surface by complementary base pairing to form a double-stranded structure, the single-double-stranded DNA information is converted into detectable electrochemical signals (such as electrochemical impedance, cyclic voltammetry, photocurrent, etc.) through the signal converter, and then the detection of specific genes is achieved. Since the immobilization of the probe and the hybridization reaction with the target are carried out on the surface of the electrode, the material and surface properties of the electrode have a great influence on the electrochemical detection results [8–10]. To further improve the sensitivity of electrochemical sensors, the electrode surface is often modified to enhance the electrical signal. Chemical modification of electrodes refers to the attachment of chemical groups or substances with special functions to the electrode surface by covalent bonding, adsorption, or polymer coating in order to achieve a desired effect [11–15]. Considerable attention has been focused on modification materials and methods to achieve the artificial control of the electrode surface through the use of modification materials, which can effectively increase the specific surface area of electrodes, increase the fixed amount of probes, and improve the efficiency of electron transmission, and thus optimize the detection results. In recent years, this detection method has attracted wide attention and developed rapidly in various fields of detection. However, electrochemical sensors have not been applied commercially in detecting GM organisms.

This review summarizes the development of electrochemical technology in detecting and analyzing GM organisms. Although reviews on this topic are often published [16–19], this review attempts to summarize and explain the following issues using bibliometrics:

(1) Has electrochemical analysis always attracted the interest of scientists in the detection of GM crops?

(2) Is there extensive international collaboration on this topic?

(3) Are the labeled or label-free electrochemical sensors more suitable for practical applications?

(4) Have advances in materials science improved the accuracy of electrochemical sensors in identifying GM crops?

(5) Investigating the main reasons why electrochemical technology has not been commercialized to detect GM crops.

Two bibliometric software have been used in this work. The first is CiteSpace (Philadelphia, PA, USA), developed by Dr. Chaomei Chen, a professor at the Drexel University

School of Information Science and Technology [20–23]. It has become one of the commonly used software in bibliometrics analysis. CiteSpace 5.8R3 was used to calculate and analyze all documents. COOC (Beijing, China) is another emerging bibliometrics software [24]. This software will supplement CiteSpace analysis by providing additional measurement results. COOC11.8 was used to calculate and analyze all documents. We used the core collection on Web of Science as a database to assure the integrity and academic quality of the studied material. "GMO electrochemical", "GM electrochemical", and "genetically modified electrochemical" were used as a "topic." The retrieval period was indefinite, and the date of retrieval was 30 December 2021. A total of 505 research articles were retrieved, spanning the years 1993 to 2021. After careful screening, only 83 papers were actually on the subject.

2. Developments in the Research Field

2.1. Literature Development Trends

The number of papers published on a topic each year is an important indicator of whether the topic continues to attract academic attention. Figure 1 shows annual and accumulated publications from 1993 to 2021 after searching on the Web of Science about electrochemical techniques for the detection and analysis of GM organisms. Although the earliest publications on the subject date back to 1993, according to a literature search, the first commercial GM crops were not introduced until 1996. Therefore, papers retrieved before 1996 were included because of deviations. We carefully examined the papers and found other academic abbreviations that might be mistaken for "GM" or "GMO," such as the generalized model, glutamate oxidase, and gastrocnemius. These works were mistakenly retrieved in the results when they involved electrochemistry. The first actual paper on electrochemistry as an analytical technique for detecting GM organisms was published in 2001 [25]. This work describes three different biosensors for detecting GM organisms, one of which is electrochemical technology. The principle of detection is based on the affinity interaction between nucleic acids. Probes immobilized to the electrode surface can be specific to inserted sequences in GM organisms. The oxidation peak of daunomycin was used as a marker in this electrochemical sensor. If hybridization occurs, the daunomycin can be inserted into the double helix, and its oxidation peak can be observed in electrochemical voltammetry. We went through the papers one by one and found that only 83 papers really belonged to the topic. The introduction of topic-irrelevant results in bibliometrics analysis is likely to lead to decreased accuracy in the analysis results, especially when some abbreviations represent entirely different meanings in different fields. However, abbreviations on some topics have become commonplace in academic papers. They are used directly in headings and summaries without full name interpretation. In this particular case, the abbreviations GM and GMO are used directly in some papers [26,27].

Figure 1 shows the annual publications and accumulated publications in this topic. Although there were more annual publications in some years, there was no explosive growth in all periods. There are generally two reasons behind this phenomenon. The first is that the topic has only attracted experts in a specific field. In this particular case, it is possible that experts in the field of electrochemical analysis led most of the investigations. Another reason is that the topic has not broken new ground, so it has not attracted much attention. Of course, there is a connection between the two reasons. The lack of breakthroughs in this technology is a significant factor in the absence of experts from other fields to cross-examine the subject. In addition, there are still papers published every year, indicating that the investigation on this topic has not stopped. Electrochemical techniques remain an option for the detection and analysis of GM crops.

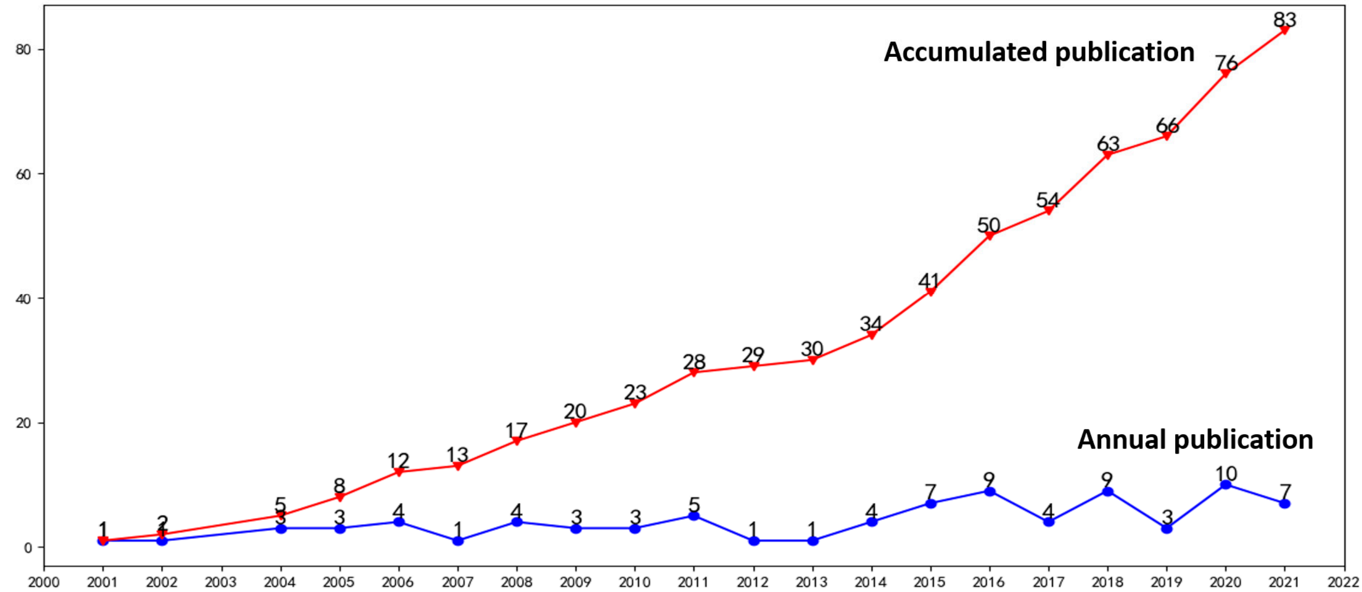

Figure 1. Annual and accumulated publications from 1993 to 2021 found in the Web of Science in a search about electrochemical techniques for the detection and analysis of GM crops.

2.2. Journals, Cited Journals, and Research Subjects

Figure 2 shows the five journals with the highest number of publications on electrochemical techniques for the detection and analysis of GM crops. The five most frequently published journals on the topic are in analytical chemistry, with the two most frequently published journals focusing on sensors. *Biosensors & Bioelectronics* and *Sensor and Actuators B: Chemical* have published 13 and 9 papers. Although this topic emphasizes electrochemical techniques, its application areas are in biology and agriculture. However, most of the work on this topic has been published in journals not directly related to agriculture/biology, meaning that this testing technique is currently being explored and established in the methodology rather than the actual testing of GM crops. This is often the case when a new analytical technique has not yet been widely used in a particular application.

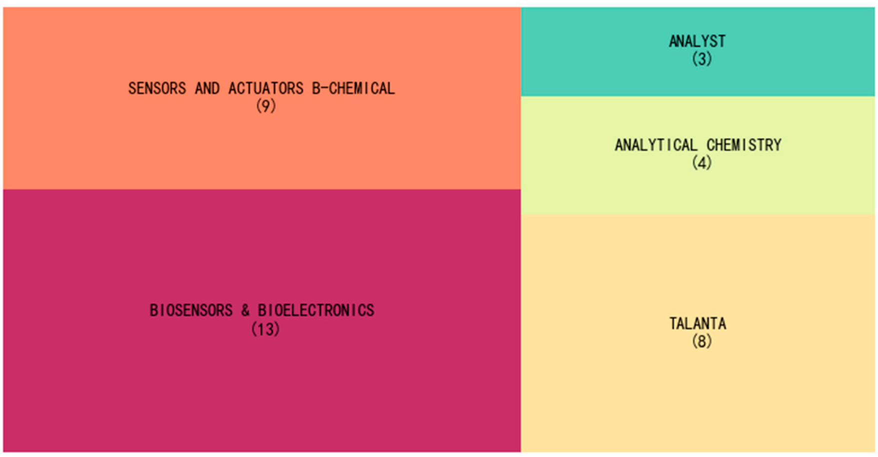

Figure 2. Top 5 journals that published articles on the electrochemical techniques for the detection and analysis of GM crops.

We further analyzed cited journals to understand the areas and journals associated with this topic. Table 1 shows the top 15 cited journals. The journals with the highest frequency in the table generally correspond to those in Figure 2. The results indicated that the most frequently published and cited journal on the topic was related to analytical chemistry. It also means that the journals that publish articles on the topic have a significant impact. On the other hand, Table 2 provides some additional information. In addition to the journals related to analytical chemistry, several journals are related to food and agriculture, such as the *Journal of Agricultural and Food Chemistry*, *Food Control* and *Food Chemistry*. This is because GM crop detection is a widely known topic in food and agriculture. Even though GM crop detection based on electrochemical technology is still in the stage of methodological innovation, it is still useful to conduct a horizontal comparison with other analysis technologies. More information will be presented in the subsequent keyword analysis.

Table 1. Top 15 cited journals on the electrochemical techniques for the detection and analysis of GM crops.

No.	Citation	Cited Journal
1	70	*Biosensors & Bioelectronics*
2	66	*Analytical Chemistry*
3	57	*Analytica Chimica Acta*
4	52	*Talanta*
5	37	*Electroanalysis*
6	36	*Sensor and Actuators B: Chemical*
7	36	*Analytical and Bioanalytical Chemistry*
8	34	*Journal of the American Chemical Society*
9	27	*Journal of Agricultural and Food Chemistry*
10	25	*Analyst*
11	25	*Analytical Biochemistry*
12	21	*Electrochimica Acta*
13	20	*Food Control*
14	16	*Food Chemistry*
15	16	*Microchimica Acta*

Table 2. List of journals that have appeared in the co-occurrence network in the last three years.

Year	Journal Name
2021	*ACS Applied Nano Materials; Environment International; Journal of The Electrochemical Society; Pest Management Science; Science of the Total Environment*
2020	*Critical Reviews in Food Science and Nutrition; Gene; Journal of Colloid and Interface Science; Nanoscale*
2019	*Applied Materials Today; Biochemistry; Chemistry & Biodiversity*

Figure 3 shows the co-occurrence network of cited journals associated with electrochemical techniques for the detection and analysis of GM crops (the lighter nodes and lines represent the more recent behavior). The vast majority of journals are clustered together, forming a dense network. The journals in this dense network are very similar to Table 1, confirming their extensive influence on the topic. The highest-ranked journals in Figure 2 and Table 1 do not show the strongest impact in this dense network. In contrast, *Electroanalysis* and *Analytical and Bioanalytical Chemistry* have very thick envelope edges, which means they link to a broader range of investigations. In addition to this dense network, there is a smaller, loosely connected network in the upper part of Figure 3. This small network mainly contains *Sensors-Basel*, *Food Control,* and *Food Chemistry*, and a series of other journals. Among them, Sensors-Basel is not cited very frequently, but it links to several journals, which means that it contributes crucial value to the diversified development of this topic.

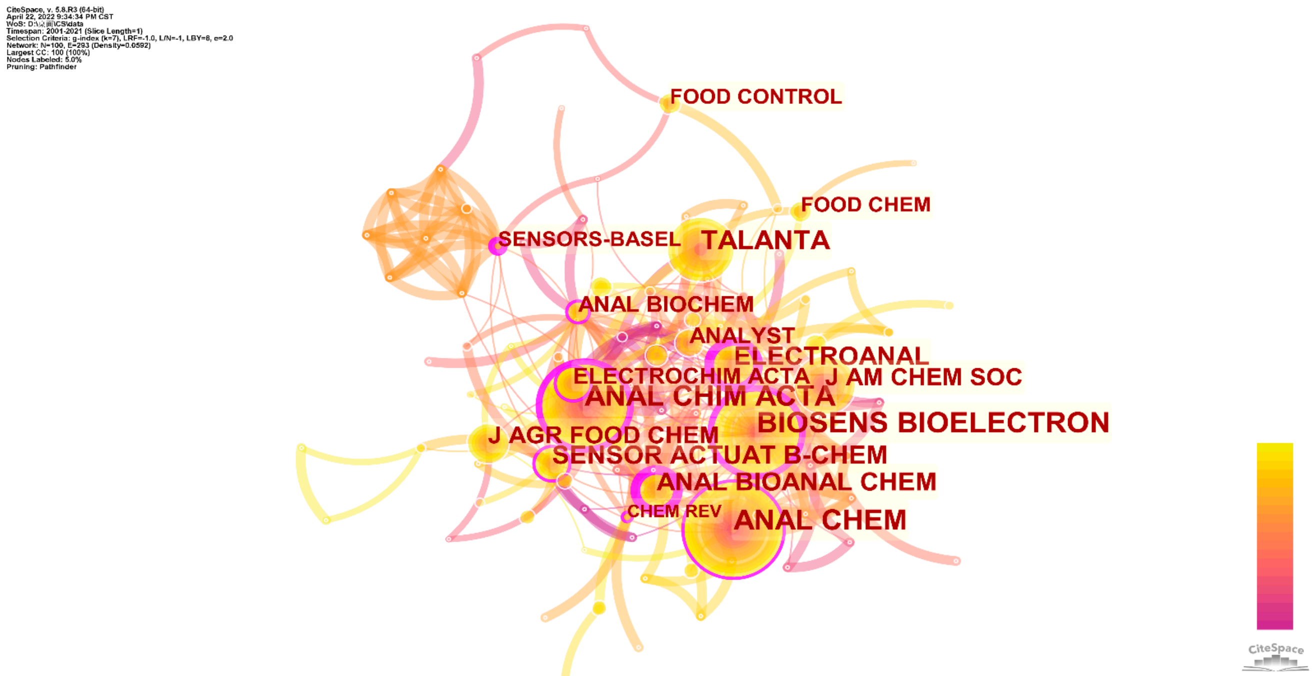

Figure 3. Co-occurrence network of cited journals for electrochemical techniques for detection and analysis of GM crops.

In the co-occurrence network of journals, we can also know which journals started to publish papers on this topic in recent years, showing the most cutting-edge progress. Table 2 shows journals that have begun publishing papers related to electrochemical techniques for detecting and analyzing GM crops for the first time in the last three years. Two electrochemical-related journals and one food-related review journal are directly related to this topic. However, most of the journals in this table are related to materials science, and there are four in total. At the same time, there are three journals related to environmental science. Therefore, the investigation of electrochemical techniques for detection and analysis of GM crops is no longer limited to analytical chemistry and agriculture but has begun to interact across disciplines with other fields.

In order to understand the evolution of a topic from one field to another, category analysis of papers can give clues. Figure 4 shows the time-zone view (a method to reflect the relationship between documents in different years by time slice) of categories for electrochemical techniques for the detection and analysis of GM crops. The topic was first published in the journal category of chemistry in 2001. Three years later, the journal categories covered by this topic include electrochemistry, analytical chemistry, science and technology, food science and technology, biotechnology and applied microbiology, and biophysics. Biotechnology and applied microbiology and biophysics are categories not covered in the discussion above. These two categories contain two papers published in 2004. Carpini et al. [28] prepared an electrochemical gene-sensor for detecting specific DNA sequences using a disposable screen-printed gold electrode. The surface of the sensor was modified with a specific DNA probe whose sequence is located within the 35S promoter sequence and thus can be used for GM crops detection. After modifying the probe, a streptavidin-alkaline phosphatase conjugate and biotinylated target sequences were used to amplify electrochemical signals further and achieve highly sensitive detection purposes. Marques et al. [29] studied the detection of methamidophos insecticide by using genetically modified acetylcholinesterase as a biological receptor. The results showed that genetically modified acetylcholinesterase was more sensitive to methamidophos insecticide. In 2007, papers on the topic entered the agriculture category for the first time. Petrlova et al. [30] modified a carbon paste electrode with avidin. The modified electrode can detect avidin in GM maize extract with high sensitivity. Because GM maize produces extra avidin as a pesticide, the concentration of avidin in its extract will be higher than that in non-GM maize extract. This sensor can detect avidin at 3 pM-170 nM, which is suitable for screening whether maize seeds are genetically modified or not. The construction of electrochemical sensors is an instrument science, so innovations in instrument technology and methodology are indispensable for the assembly of purpose-specific sensors. This topic was first published in the category of instruments and instrumentation in 2008. This work reports an electrochemical DNA biosensor for detecting NOS gene sequences in GM organisms [31]. The electrochemical behavior of methylene blue on single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) was investigated. Compared with the ssDNA-modified electrode, the redox peak current of MB on the dsDNA-modified electrode was increasingly used for the recognition of DNA hybridization. The sensor successfully detected polymerase chain reaction products produced when the GM organism was inserted with NOS terminators in the actual sample test. Because electrochemical sensors involve the transfer of electrons at electrode interfaces, physics was introduced to this topic in 2011. Shkil et al. [32] investigated the electron transfer of genetically modified Hansenula polymorpha yeast cells at the electrode interface. In order to further improve the performance of electrochemical sensors, nanomaterials have been used in the preparation of electrodes. Compared with the traditional electrode, the electrode modified by nanomaterials often presents a wider linear detection range and a lower detection limit [13,33–37]. Therefore, this topic began to expand to materials science and nanoscience and nanotechnology in 2015. Even in 2021, the topic is still breaking new ground. Specifically, nutrition and dietetics and materials science, coatings, and films have entered this topic for the first time.

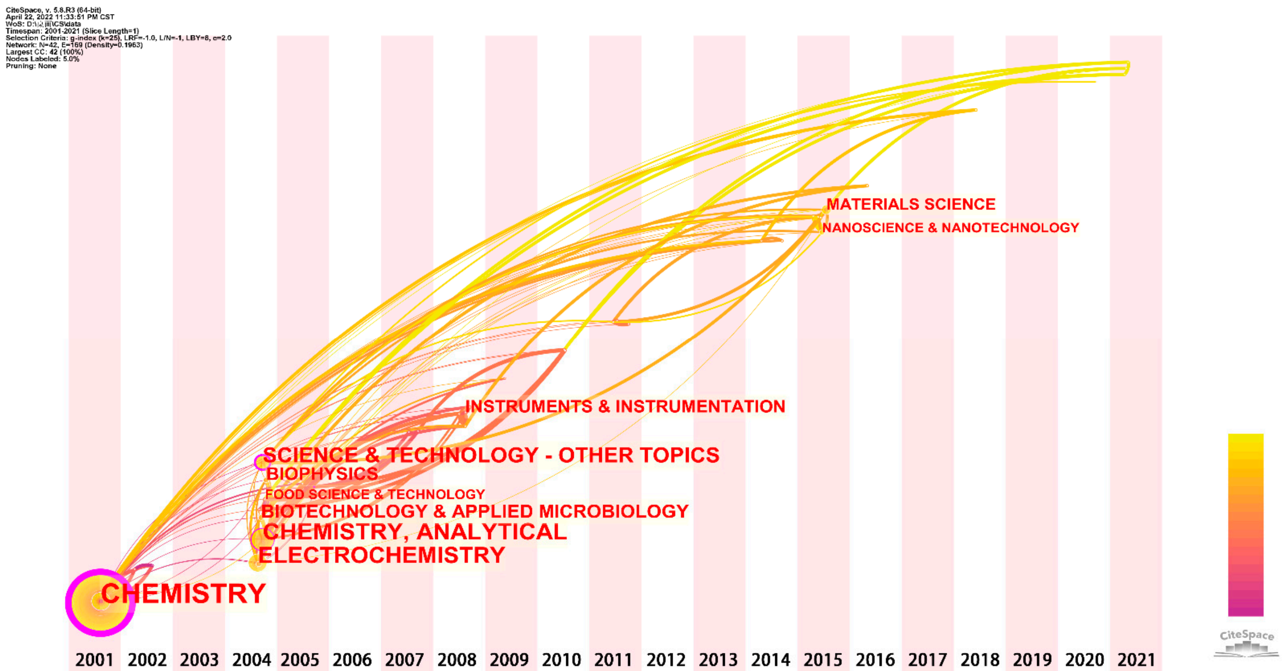

Figure 4. Time-zone view of research categories for electrochemical techniques for the detection and analysis of GM crops.

2.3. Geographic Distribution

Figure 5 shows the seven countries with the most publications. China has the most significant number of publications, contributing more than 30%. Spain, Italy, and the USA each contributed to more than 10% of publications. This trend is not consistent with our other bibliometrics-based investigations of electrochemical sensors. Electrochemical sensor research has traditionally attracted scientists from the Middle East and South Asia. However, an unusually large number of European scientists have been involved in investigating the topic. Portugal and Germany are also very active in this topic, as shown in Figure 5.

This particular phenomenon should be related to the negative attitude towards GM crops in Europe. For example, only 0.05% of arable land in Europe was used for GM crops in 2009, compared to 1% of the global average of land used for GM crop cultivation [38]. European countries, especially the European Union, are committed to the principle of precaution in GM technology and products. A range of potential risks associated with GM technology should be fully considered before its developing and commercialization. These risks need to be kept within strict regulatory limits. Applications for GM food, feed, and imports from the 27 EU member states need to be approved by the EU Food Safety Authority (ESFA). The applications are also subject to joint approval by the EU regulatory authorities and the Member States. Composite traits of GM products are considered new GM products in the EU and must go through the same authorization process as individual GM products [39–41]. The whole approval cycle takes a long time. For example, the varieties approved in 2018 took about 6 years on average from application to market access permission, much longer than in the US, Brazil, or South Korea (generally about 2~3 years). In such a political and cultural context, detection techniques for GM products have attracted the attention of scientists in these regions.

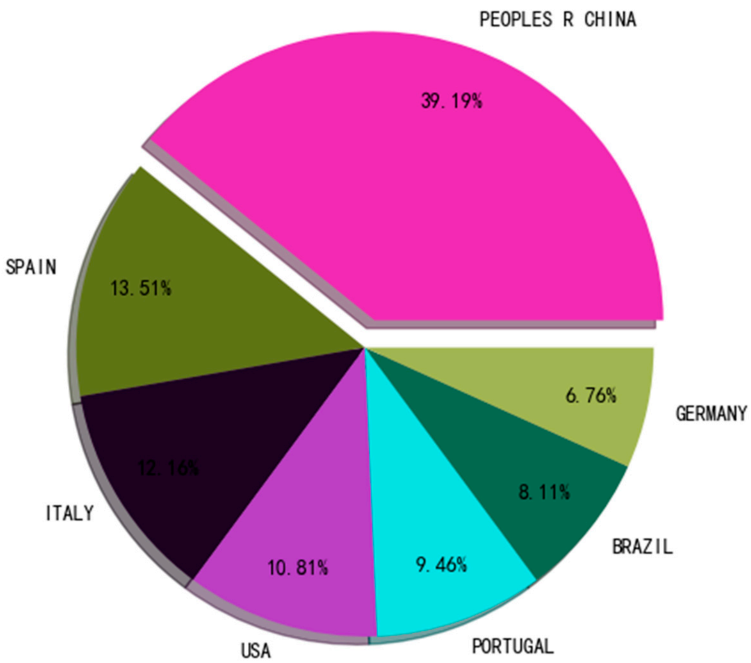

Figure 5. Pie chart of papers contributed by different countries.

Figure 6 shows the time-zone view of the geographic distribution for electrochemical techniques for the detection and analysis of GM crops. Links between different countries are established based on papers published in those countries being directly cited. Although China and Spain contribute the most papers on this topic in Figure 5, neither was involved in this topic at the earliest stage. Italian scientists started the investigation of this topic in 2001. Their work allowed scientists in Turkey to start an investigation on this topic in 2004. At the same time, scientists from Libya and China were influenced to start investigations in 2006. In 2002, scientists from Romania and France also started investigations on this topic, and their work caused scientists from Brazil to join the topic. Figure 6 shows no lines connecting these studies to the following countries that emerged, indicating no straightforward relationship between these studies and many investigations on this topic that began after 2006. In 2007, the Czech Republic and the USA began to cooperate on this topic. Their work directly affects some of the most important countries on the topic. In 2008, Chinese scientists began investigating the topic again and were on their way to becoming the country with the most contributions. Poland also launched a survey on the topic in the same year. From 2009 to 2012, a series of countries participated in the topic, including Japan, Germany, South Korea, Italy, France, Vietnam, and Thailand. The work in these countries is more or less related to papers published between 2007 and 2008. A series of other countries have been involved in the topic since 2015, with some emerging as major players in the topic, such as Spain, Brazil, and Portugal. At this stage, several countries began to investigate the topic independently. There is no connection between them in Figure 6 and other countries, such as Iran, Denmark, and Mexico. The latest country to participate in this topic is Singapore, which joined in 2020. As can be seen from the above analysis, the topic has attracted academic interest from different regions, especially from scientists in Asia and Europe. This may be because GM crops are more controversial in some countries in these regions, so the need for testing technology is more urgent.

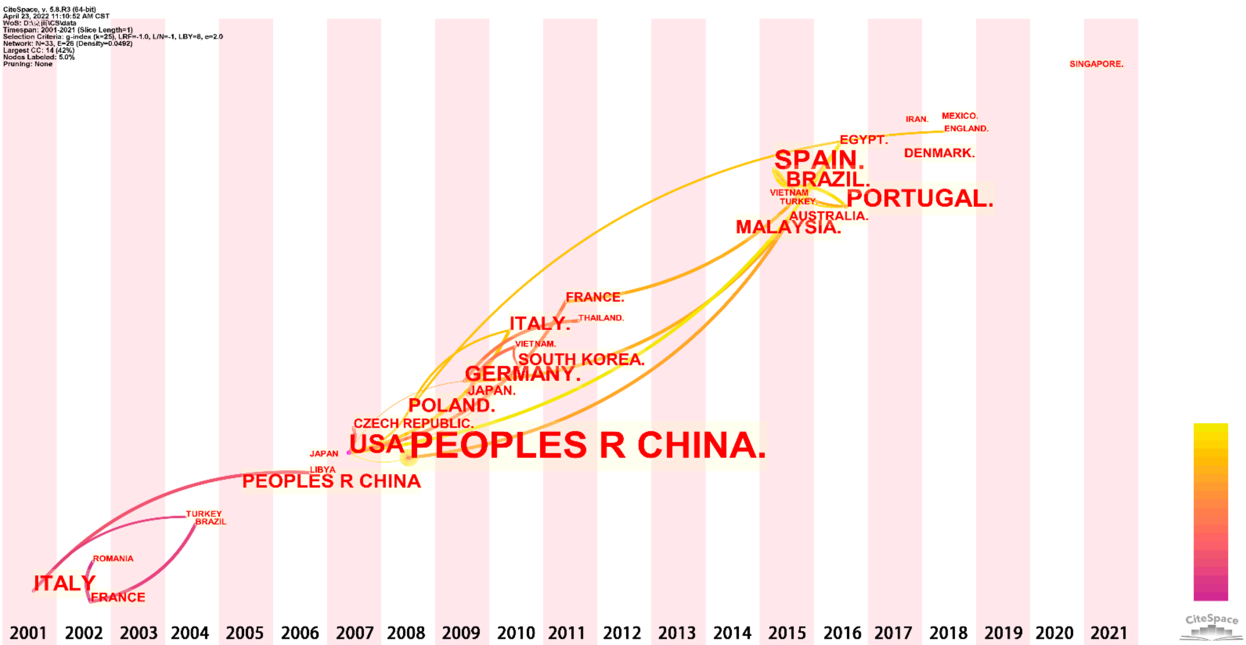

Figure 6. Time-zone view of geographic distribution for electrochemical techniques for detection and analysis of GM crops.

Figure 7 shows the collaborative network on this topic across different institutions. It can be seen from the figure that there is a relatively large cooperation network on this topic, mainly led by Universidad de Oviedo, Universidade do Porto, and The Instituto Politécnico do Porto. They and the other nodes in the network are from Spanish research institutions, which represents a very broad pattern of regional cooperation on the topic within Spain. However, the network did not lead to international cooperation. On the contrary, China, the country with the most published papers on this topic, does not even present pervasive collaboration. Chinese institutions have developed only three very small collaborative networks. The first network consists of the Shanghai Academy of Agricultural Sciences, Shanghai Jiao Tong University, Lanzhou University of Technology, and the Ministry of Agriculture and Rural Affairs. The second network, led by the Chinese Academy of Sciences and Southeast University, included Fuzhou University, Ocean University of China, and Shanghai Novamab Biopharmaceuticals Co., Ltd. (Shanghai, China) The third network, led by the Chinese Academy of Agricultural Sciences, includes Huazhong Agricultural University, Shanghai University of Engineering Science, Changchun University of Science and Technology, and China Agricultural University. Similar situations have occurred in other countries. For example, the investigation of this topic in Malaysia is mainly led by Universiti Kebangsaan Malaysia, including Universiti Malaysia Pahang, Malaysian Agricultural Research and Development Institute, and Universiti Sains Malaysia. Similarly, the investigation of this topic in Czech Republic is mainly led by the Czech Academy of

Sciences, including Mendel University in Brno, Brno University of Technology, and the Institute of Chemical Technology in Prague. Except for one example, Japan, Vietnam, and South Korea have a cooperative relationship on this topic. The lack of international cooperation on this topic may be due to different attitudes and regulations on GM crops in different countries. However, an electrochemical sensing approach analysis could not give such a definitive conclusion. Therefore, the bibliometric analysis of GM crop detection based on other analysis techniques is worth investigating in the future.

Figure 7. Institution cooperation network of published papers for electrochemical techniques for the detection and analysis of GM crops.

3. Keyword Analysis and Evolution of the Field

A keyword analysis is critical to understanding the different research focuses on the topic. Table 3 lists the top 15 keywords in this topic. Since this topic uses electrochemical strategies for GM crop detection, the two most frequently occurring keywords listed are biosensor and sensor. The keywords electrochemical detection and assay, ranked 12th and 14th, also show how to reach the goal of this topic. Similarly, the purpose of the topic is also an important keyword. Specifically, GMO and modified organism ranked 6th and 10th in the keyword list. The electrode is the element that receives the signal in the electrochemical sensor, so it also has a very high frequency of occurrence. A merely conductive electrode cannot by itself have the ability to identify GM crops precisely, so a specific identification element immobilized on the electrode surface is the key to achieving detection purposes. Therefore, the immobilization of probe DNA on the electrode surface and subsequent DNA hybridization during the assay is the most common method to identify specific sequences in GM crop genes. This methodology can be applied in electrochemical analysis and is also widely used in other analytical techniques [42–46]. Therefore, hybridization, DNA, nucleic acid, and immobilization appear in high frequency in the keyword list. Electrochemical sensors are a technique for qualitative and quantitative analysis of analytes on the surface of electrodes through changes in electrical signals [47]. The sensitivity of the sensor is directly related to the concentration range and detection limit of the method. Since the recognized abovementioned elements immobilized on the electrode surface are macromolecules, which do not possess excellent electrical properties, the performance of commonly used electrodes will be significantly degraded. Modifying nanomaterials on the electrode surface, especially those with high electrical properties, can effectively amplify the electrical signal, improve the detection range, and reduce the detection limit. Nanoparticle, amplification, and gold nanoparticles in Table 3 are then related to electrode surface modification. Finally, the PCR is also in Table 3. PCR is a technique that has been widely used for the identification of GM crops [48–51]. Its high-frequency presence represents being used as a validation technique to measure the electrochemical strategies proposed in different works.

Table 3. List of top 15 keywords for electrochemical techniques for the detection and analysis of GM crops.

No.	Freq	Centrality	Keywords
1	16	0.28	Biosensor
2	14	0.16	Sensor
3	13	0.22	Electrode
4	11	0.15	Hybridization
5	11	0.12	DNA
6	10	0.08	GMO
7	10	0.04	PCR
8	9	0.10	Nanoparticle
9	9	0.12	Modified organism
10	9	0.08	Immobilization
11	8	0.07	Amplification
12	7	0.15	Electrochemical detection
13	7	0.09	Nucleic acid
14	7	0.08	Assay
15	6	0.04	Gold nanoparticle

Burst detection is a more advanced method than citation counts for identifying keywords receiving significant attention from the research community at various stages of development. Table 4 shows the 10 keywords with the most powerful citation bursts during the research history of the electrochemical techniques for the detection and analysis of GM crops. Although research on this topic started in 2001, the first burst keyword, nucleic acid, appeared in 2006. This means that this topic did not receive much attention in the early stage. Combined with the results in Figure 6, the emergence of the burst keyword in 2006 is likely responsible for triggering the series of research that began in 2007. The paper containing this keyword proposes a voltammetric method for determining trace amounts of double-stranded DNA in fish sperm [52]. The mechanism of this method is based on the interaction of fish sperm double-stranded DNA with polysulfone. A significant decrease in the reduction peak current occurs after adding dsDNA to the solution containing polysulfone. This is because dsDNA interacts with polysulfone to form a new supramolecular complex, decreasing the diffusion coefficient. This assay has also been successfully used to determine GM samples and NOS DNA polymerase chain reaction products with satisfactory results. The second burst keyword is film which appeared in 2009. The performance enhancement of conventional electrodes is often modified with a thin film of nanomaterials on their surface. A thin film with good electrical conductivity and biocompatibility is the best choice for this topic. Yang et al. [53] prepared polyaniline nanofibers/multi-walled carbon nanotubes/chitosan composite film for carbon past electrode surface modification. The probe DNA can be efficiently immobilized on this film and provide a sensitive EIS signal during hybridization. Electrochemical detection, assay, PCR, genosensor, and sequence became burst keywords over the next few years, but they did not last very long. In 2018, amplification and reduced graphene oxide became the new burst keywords until 2021. This representsthe fact that the hottest direction of this topic at the moment is how to amplify the signal. Reduced graphene oxide is a very useful material for this purpose. It is a material resulting from graphene oxide after removing most of the oxygen-containing functional groups on the surface by specific methods. In the graphene oxide phase, it has excellent dispersion and is suitable for forming composites with other materials in different solvents. However, the poor electrical conductivity of graphene oxide does not improve the detection sensitivity of electrochemical sensors. Therefore, the reduction of graphene oxide can be used to restore its electrical conductivity. For nearly a decade, reduced graphene oxide has been a frequently chosen material for electrochemical sensor assembly [54–57]. Label-free also became a burst keyword in 2018. Although DNA hybridization with altered electrode surface properties is the most direct way to identify GM crops, changes in electrode properties are not always as sensitive. Therefore, most work based on this methodology requires the addition of redox-labeled molecules to the detection process to achieve highly sensitive identification. However, this labeling can be avoided if the electrodes are sufficiently sensitive. With the help of an excellent electrode modifier, label-free sensors are starting to become a popular direction in this topic [58,59].

Table 4. The 10 keywords with the strongest citation bursts during the research history of the electrochemical techniques for the detection and analysis of GM crops.

Keywords	Strength	Begin	End	2001–2021
Nucleic acid	1.69	2006	2015	
Film	1.56	2009	2014	
Electrochemical detection	2.17	2014	2018	
Assay	2.00	2015	2017	
PCR	1.94	2015	2016	
Genosensor	1.58	2016	2017	
Sequence	1.59	2017	2018	
Amplification	2.25	2018	2021	
Reduced graphene oxide	1.81	2018	2021	
Label-free	1.81	2018	2021	

Cluster analysis of keywords can exhibit the research focus formed by different keywords in this topic. Figure 8 shows the clustering results of keywords, with 11 clusters formed based on similarity of content. It can be observed in the figure that many clusters have overlapping areas between them, representing that their contents have more similarities with each other. On the contrary, two clusters are entirely outside of other clusters, representing that their contents do not have a very strong connection with other clusters. Table 5 shows a detailed description of the clusters and their ID, size (number of papers), silhouette (homogeneity of a cluster, the higher the silhouette score, the more consistent of the cluster members are, provided the clusters in comparison have similar sizes), and respective keywords. The following is a short explanation of each cluster:

#0 (Electrochemical DNA biosensor) This cluster contains a series of DNA electrochemical biosensors for detecting GM crops, especially GM soybeans. Jamaluddin et al. [60] used anthraquinone-2-sulphonic acid as a marker. Deng et al. [61] used a label-free sensing technology to directly reflect the differences in DNA hybridization with changes in electrode performance. Ge et al. [62] recently reported a CRISPR/Cas12a-mediated dual-mode for electrochemical detection of GM soybean. In addition to soybeans, GM rice [63] and *E. coli* [64] tests are also included in this cluster.

#1 (Genosensor) The genosensor is emphasized in the title of the papers in this cluster. Genosensor is a terminology used in sensor analysis to indicate either that the analyte has a specific gene sequence or that a specific gene sequence has been used to construct the sensor. Three papers in this cluster used peptide nucleic acid to modify the sensor [65–67]. The remaining three papers used gold nanoparticles as electrode modifiers to amplify signals [58,68,69].

#2 (PCR product) This cluster emphasizes electrochemical sensors' rapid screening and multiplex identification of GM crops. Liao et al. [70] proposed a biomolecular analysis system with a unique biochemical activity that allows the interpretation of promoter, coding, and species genes through the signals of sensors. Moura-Melo et al. [71] identified the products of helicase-dependent isothermal amplification (HDA) by using an electrochemical platform and could identify the Cauliflower Mosaic Virus 35S Promoter (CaMV35S). In addition to qualitative analysis, quantitative analysis of the content of GM crops is also very necessary because of current EU regulations on the mandatory labeling of GMOs with a minimum content of 0.9%. Manzanares-Palenzuela et al. [72] developed a simple and sensitive composite electrochemical sensor for the quantitative analysis of Roundup-Ready Soybean. Fluorescein isothiocyanate or digoxin was used as a signal probe in this sensor.

#3 (Specific gene) This cluster is completely encapsulated in Cluster #0. This cluster emphasizes the detection of specific gene sequences in GM crops. For example, Xu et al. [73] used methylene blue as a marker to detect CaMV35S. The sensor prepared by Mix et al. [74] is particularly targeted at cryIa/B and the MON810 specific fragment.

#4 (Electrochemical genosensor) This cluster is completely encapsulated in Cluster #0 as well. The cluster also shares many papers with Cluster #0. In addition to DNA hybridization as a sensing strategy, this cluster also has papers on detecting molecules specifically produced by GM crops. GM maize produces more vaidin than non-GM maize, so that this molecule can be used as an indicator [30].

#5 (Polymer) The three electrochemical sensors in this cluster have polymers for electrode modification. Polyaniline nanofibers were used in the DNA sensor proposed by Yang et al. [53] Silva et al. [75] used poly(allylamine hydrochloride) in the sensor. Elmoghazy et al. [76] selected poly(vinyl alcohol) nanocomposites to construct sensors.

#6 (Laboratory analyses) This cluster contains only two papers. The authors of the first paper designed a biosensor platform based on photosynthetic biology. GM algae were used to enhance the sensing capabilities of the platform, which could be used to identify different herbicide subclasses. The authors of the second paper proposed a labeled electrochemical sensor to detect GM soybean [61]. This paper is also classified in Cluster #0.

#7 (GM crops) All of the papers in this cluster have realized the detection of actual GM crops. GM soybean, rice, and *Arabidopsis thaliana* were used as actual samples in the sensor proposed by Huang et al. [77]. Similarly, GM soybean and maize were used as real samples in reports published by Zeng et al. [78] Wang et al. [79] proposed an on-point detection sensor for GM rice identification.

#8 (Immunosensor) All three papers in the cluster used immunosensing strategies rather than DNA hybridization.

#9 (Yeast cells) The only paper in the cluster presents an electrochemical scanning microscope study of enzyme activity in yeast cells [80].

Table 5. Knowledge clusters in the field of the detection and analysis of GM crops on keyword co-occurrences for each cluster.

Cluster ID	Size	Silhouette	Keywords	References
0	35	0.847	BT63 detection; electrochemical DNA biosensor; transgenic event; Au-reduced graphene oxide nanocomposite; reduced graphene oxide	[29,60–64,81,82]
1	31	0.893	Electrochemical genosensor; nucleic acid-mediated PCR; asymmetric PCR technique; electrostatic interaction; metal cation; genosensor	[58,65–69]
2	30	0.874	Electrochemical sensor; logic-based biomolecular analysis; biotech crop; multiplex screening; PCR product	[70–72,83–86]
3	28	0.855	Electrochemical detection; using methylene blue; specific gene; ethylenediamine-modified glassy carbon electrode; PCR product	[31,73,74,87–89]
4	24	0.870	Genetic element present; disposable genosensor; new tool; voltammetric technique; DNA hybridization biosensor	[30,62,68,71,90–93]
5	22	0.905	Polymer; different configuration; screen-printed graphite electrode; gold nanoparticle; using polyaniline nanofiber	[53,75,76,94]

Table 5. *Cont.*

Cluster ID	Size	Silhouette	Keywords	References
6	22	0.977	Laboratory analyses; pesticide residue; biosensor; new platform; DNA-based biosensor	[61,95]
7	20	0.903	GM rice; CPA acceleration; on-point detection; FMO product; GM crops	[77–79,96]
8	13	0.991	Immunosensor; Cry1ab protein; of-care testing; MXene catalyzed Faraday cage	[97–99]
9	10	1.000	Yeast cells; gene expression; array	[80]

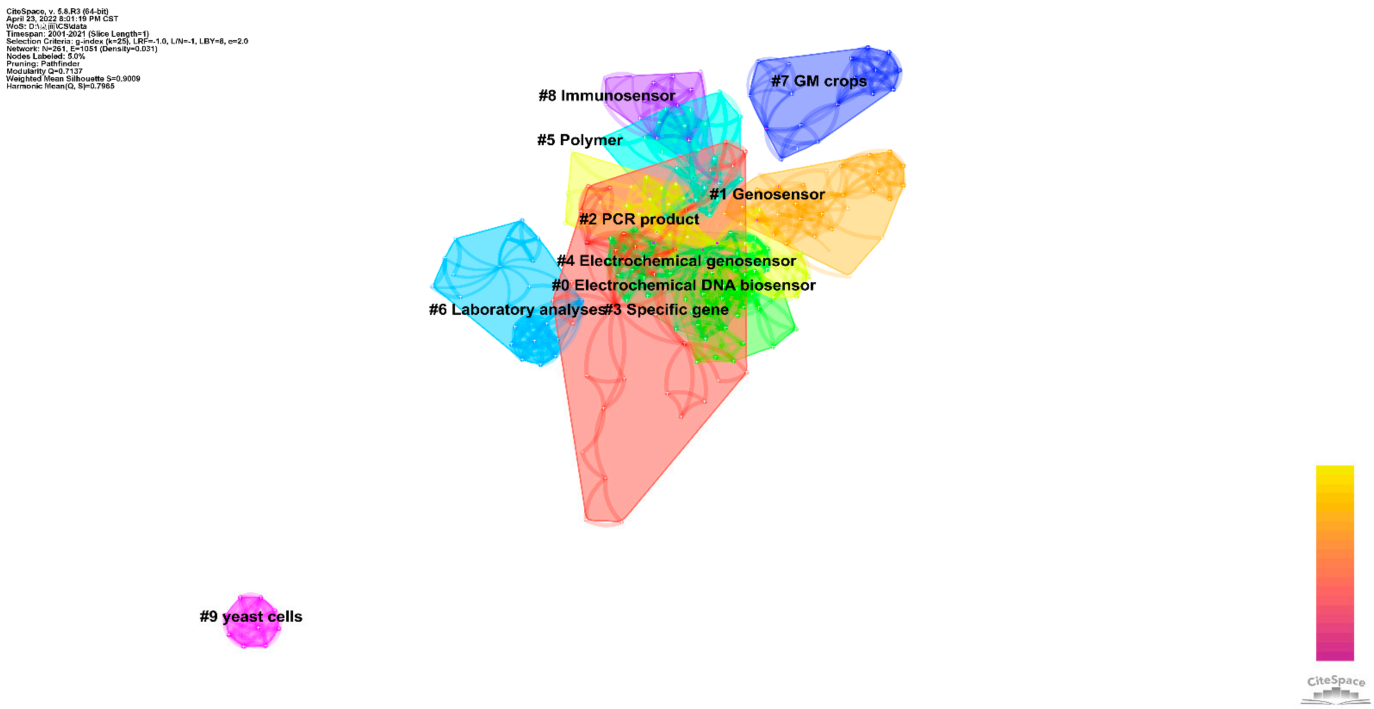

Figure 8. Grouping of keywords for electrochemical techniques for the detection and analysis of GM crops.

Figure 9 shows the frequency of occurrence between keywords. The most co-occurrence is between sensor and hybridization, indicating that the most common methodology used by electrochemical sensors for GM crop detection is based on signal changes generated during DNA hybridization. Gold nanoparticles and sensors also have a strong co-occurrence, indicating that gold nanoparticles are the most commonly used materials for electrode surface modification. Gold nanoparticles are often used in electrochemical sensors to amplify signals due to their excellent electrical conductivity [100]. On the other hand, gold nanoparticles are suitable for specific binding with some functional groups, which can be used for further modification [101]. Free gold nanoparticles tend to agglomerate, so they are usually first anchored to a substrate. Graphene and gold nanoparticles also co-occur in Figure 9, which indicates that graphene can be used as a substrate for the immobilization of gold nanoparticles [102].

Based on the above analysis of keywords, the investigation directions of the electrochemical techniques for the detection and analysis of GM crops can be summarized as follows:

(1) DNA hybridization is the most common strategy used for the electrochemical sensor detection of GM crops. Among them, labeled sensors have been widely reported, and some new work has begun to focus on the construction of label-free sensors.

(2) Electrochemical sensors based on other methodologies, such as electrochemical immunoassay, can also be used to detect GM crops.

(3) The application of new high-performance nanomaterials to amplify the detection signal further improves detection sensitivity and accuracy.

(4) In addition to DNA sequence-based detection, the direct detection of GM crops' specific substances is also a new identification method.

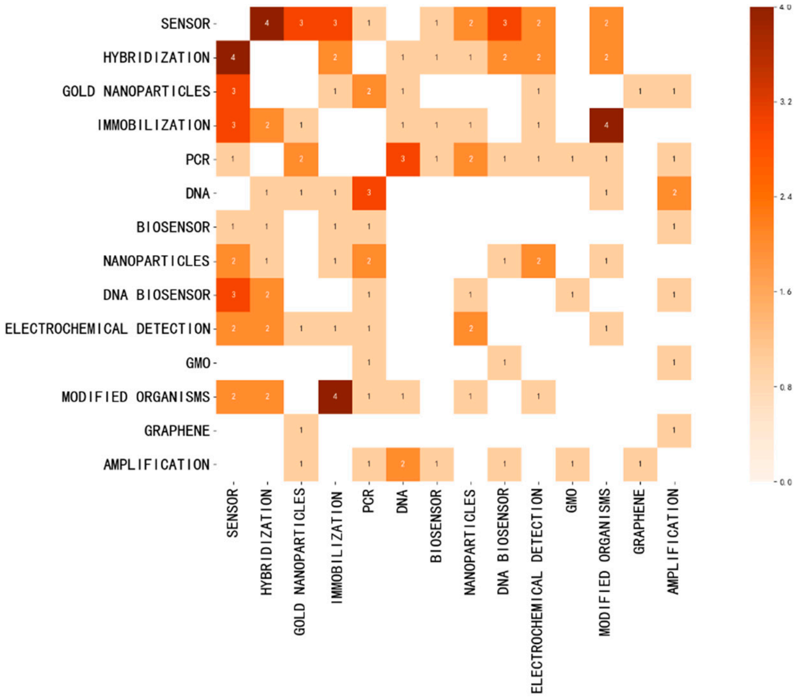

Figure 9. Keywords confusion matrix for electrochemical techniques for the detection and analysis of GM crops.

4. Co-Citation Analysis

Figure 10 shows a co-citation analysis for electrochemical techniques for the detection and analysis of GM crops. As can be seen, the co-citation network is mainly divided into three sub-networks. These three sub-networks can be divided and correspond to different time phases. Among them, the sub-network in the middle of the whole network has less content, and the other two sub-networks contain more references.

The rightmost sub-network corresponds to earlier references. Among them, the review on electrochemical DNA sensors published in *Nature Biotechnology* by Drummond et al. [103] has a very strong impact. Another review on electrochemical DNA hybridization biosensors plays a similar role [19]. These two papers are the most critical nodes connecting this sub-network with the central sub-network. The sub-network on the right can be further divided into two parts connected by a review. Specifically, this review summarizes the methodology of electrochemical nucleic acid biosensors [104]. The upper part of the sub-network references focuses on the interactions between DNA and other molecules that can affect double-stranded DNA hybridization [105–108]. These interactions were later used as signal variations for sensing purposes. The second half of the sub-network is the electrochemical detection of nucleic acids and GM nucleic acids [28,109–112]. The middle sub-network is based on the results of the electrochemical detection of nucleic acids and further enables the detection of PCR products [113–116]. Campuzano et al. [117] prepared an electrochemical sensor for DNA recognition that allows the detection of untreated clinical samples. This work links the middle sub-network to the densest sub-network on the left. There are many influential references in the densest middle region of the sub-network on the left. For example, Barroso et al. [118] used a 3D-nanostructured Au electrode to prepare DNA sensors to detect MON810GM maize. Arugula et al. [119] advocate that biosensors are the most crucial technology for detecting GM crops in the 21st century. The sensor prepared by Manzanares-Palenzuela et al. [72] allows the detection of GM soybeans at the femtomolar level. They also advocate electrochemical genosensor as an emerging technology for GM crop detection [16]. Freitas et al. [120] used monodisperse Fe_3O_4@Au superparamagnetic nanoparticles to detect GM organisms. Some other strategies for detection were also considered, such as enzymatic [121] and molecular imprinting assays [122]. Kamle and Ali [123] summarized the different detection strategies and corresponding biosafety issues. In addition, methods that do not utilize the label [124], immobilizing the label on the electrode [125], triple magnification model technology [126], and the use of magnetic materials [127] have been tried.

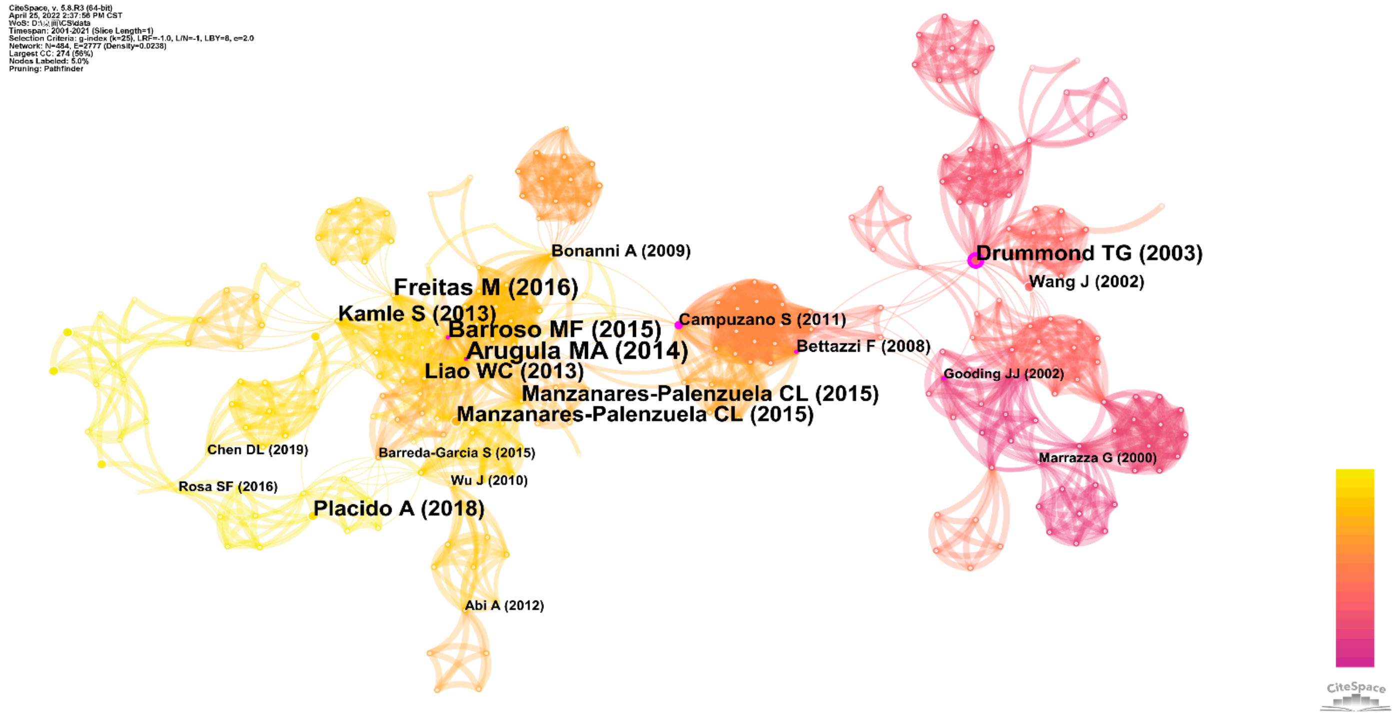

Figure 10. Co-citation analysis for electrochemical techniques for the detection and analysis of GM crops [19,70,72,98,103,104,109,117–119,123–127].

5. Conclusions and Perspectives

GM crops have always been controversial, and people in different regions present different attitudes toward GM. People in the Americas have a more tolerant attitude toward GM crops, but countries in Europe have set many strict rules for GM crops. The attitude of Asian countries is somewhere between that of the Americas and Europe. Although it is a controversial topic, it is undeniable that GM crops are gradually increasing in acreage worldwide. Therefore, technologies targeting GM crops have also been of interest to scientists in academic circles. The detection of GM crops has applications not only in food safety but also has the potential to play an essential role in commercial activities. Conventional GM crop detection techniques still require large laboratory-based instruments and tedious operational steps, so there is a need to develop technologies that can be used in the field. Electrochemical sensors are an analytical technique often used for field testing due to their miniaturization, ease of operation, and speed. This review summarizes the progress of electrochemical sensors in GM crop detection and analysis. Based on the above bibliometric analysis, the following conclusions can be drawn:

(1) Although electrochemical sensors possess some advantages that other analytical methods do not, they have not attracted much attention in detecting GM crops. This may be because GM crop detection in electrochemical sensors requires DNA hybridization on

the electrode surface. This methodology requires the customization of expensive DNA sequences and the incorporation of markers. The production cost, preservation conditions, and reproducibility of the sensors have all become challenges.

(2) The content of this topic has been showing a relatively slow trend in the number of papers published because of the lack of breakthroughs. These papers were mainly published in academic journals related to analytical chemistry, with only a small amount of content published in agriculture- and food-related journals, representing a topic that is still in a phase of methodological construction and innovation. Although many published papers used GM crops as actual samples, extensive practical testing experiments have not been conducted.

(3) The use of nanomaterials with excellent electrical conductivity allows for the more sensitive detection of GM crops by electrochemical sensors. Gold nanoparticles and reduced graphene oxide are the most selected nanomaterials.

Meanwhile, based on the review of this topic, we believe that the following issues need to be investigated regarding the electrochemical techniques for the detection and analysis of GM crops:

(1) DNA hybridization is a methodological borrowing from other analytical techniques, except that electrochemical sensors use changes in electrical signals in the process as an indicator for determining GM crops. In contrast, the main advantage of electrochemical sensors is the detection of substances with electrochemical redox activity. Therefore, it is necessary to develop new analytical methodologies for detecting GM crops.

(2) The modification of nanomaterials on the surface of the electrode is proven to be used to improve the sensitivity of electrical signals. However, whether nanomaterials can be poisoned during contact with the substance being tested, especially when the actual sample is tested, remains a factor worth considering. If the nanomaterial properties can be influenced by substances other than the analyte, then the signal generated by the sensor may be distorted.

(3) The electrochemical sensors that can be used for GM crop detection at this stage still need to be used in the laboratory, which means that it has not been able to replace the traditional detection methods. It is also a challenge to design the electrochemical sensors for GM crop detection in an intelligent and miniaturized way. In this process, the time and steps of detection need to be optimized, and the robustness of the sensor needs to be further enhanced.

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