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DETECTION OF GM SOYA AND CORN IN SOME OF THEIR FOOD PRODUCTS IN SULEIMANIA/KURDISTAN REGION OF IRAQ

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ABSTRACT

This study was aimed to investigate Genetically Modified (GM) Soybean and corn, in some foodstuffs, using molecular screening method based on Polymerase chain Reaction (PCR). To achieve that, a total of 60 food product samples containing corn and soy bean were collected from markets of Suleimania /Iraq. PCR was first applied to detect the presence of soybeans and corn using species specif ic primers. For the general screening of the GM foods, primers targeting the genetic elements that located in the majority of transgenic crops were used, which named NOS terminator and CaMV 35S promoter. Then primers targets specific events in these crops were used, which are GTS 40-3-2, or (RRS) and B t11 in soybeans and corn respectively. The results of this study showed the presence of genes representing soybeans and corn in all foods containing them. General screening of food samples also showed that the incidence of GMOs in the studied samples were 75 % and 82% for P35S and t NOS respectively. Regarding to the events specific, the Bt-11 gene detected in about 34% of food samples contain corn and GTS 40-3-2gene detected in 70 % of food samples contain soya.

Key words: Detection, food safety, genetically modified foods, Polymerase chain Reaction.

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INTRODUCTION

Genetically modified foods (GMF) are foods resulting from genetically modified organisms (GMOs) that have had certain alterations in their DNA using recombinant DNA technology. GMF are produce in the quest to solve food insecurity challenge, by producing GM plants with as example resistance to biotic stress (viruses, bacteria, and fungi, insect...etc.) and abiotic stress (cold, drought, saltiness) and improve yield in various crops as well as producing more nutritious food. Due to their benefits, the production of GM crops has increased recently on a worldwide level; 387 (Events from 27 yields were approved by 70 states up to 2018 and the area where GMF yields were planted are extended to 191.7 million hectares worldwide (Ricroch et al., 2022). Although there are still uncertainty and controversies regarding to the effects of these modified food, however some studies recorded the adverse effect of some of GMF on human health, that they cause an alteration of natural food nutrient, tendency to cause foot toxicity and allergic reactions (Das & Bhartia, 2020). Hence, the EU and many other countries has founded a rigorous monitoring system to detect and quantify GMOs in foods ingredients for helping users in creating informed consumption choices. These processes requires establishing reliable and sensitive methods, hence many techniques have been developed for this purpose (Bruetschy, 2019), these include primarily protein based (immunological) assays and DNA based assays (mainly polymerase chain reaction (PCR) technology (Randhawa et al., 2014). ELISA is the mainly Protein- based techniques that used for detection of the proteins that expressed from the transferring genes to the

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new host (Mahgoub et al., 2019 and Wu et al., 2019). However, these techniques are considered unsuitable for the detection of GMOs in processed food, due to that protein denature during Food processing reactions (Gomez Morte et al., 2019) , moreover, protein are affected by environmental factors .On the other hands , DNA based techniques are considered as a gold standard for detection of GMF, due to that DNA are more stable than Protein , hence they are particularly applied on processed food ,moreover ,DNA based techniques are more accurate, sensitive and specific (Zhang et al., 2020). DNA –based detecting procedures of GMOs categorized as: Screening, event-specific, gene-specific, and construct-specific, according to their level of specificity (Marmiroli et al., 2008). Screening detection is one of the greatest cost-effective techniques, which performances as an origin for more verifying GMOs identity. Several PCR based analytical methods have been developed to quality detection or quantity determination the presence of GMOs in both raw and processed food (Rosa et al., 2016 and Wang et al., 2019). Qualitative analysis may be screening methods or event-specific methods. Since the most of the crops altered with *Agrobacterium tumefaciens* nopaline synthase terminator (Tnos), and Cauliflower Mosaic Virus (CaMV) 35S promoter (p35S), hence to check the GM status, screening methods aiming these elements can be professionally employed and considered the first point of GMF detection System (Arshad et al., 2025). Whereas, the different insert DNA sequences crossing with the next DNA genome of the host was targeted by event specific, which usually followed the screening methods. Due Iraq imported different types of GM Food, especially that include soy and maize and due to increase concerns about them, it became necessary to establish legislation to control them which need to develop detection methods of GMF. This study was aim to detect GM maize and soybean in some of their products (chips, crackers, ,etc.) that collected from Suleimania market using PCR based screening and event-specific methods.

MATERIALS AND METHODS

Sampling : A total of 60 samples of generally consumed food products, containing corn and soybean (snacks, chips and, crackers, sweet corn frozen or fresh, popcorn, dried soy bean, soy sauce …etc.) witch collected from the Suleimania markets during the period of September 2021 to 2022 and transferred to the lab for analysis. Certified reference material (CRMs), were purchased from the Institute of Reference Materials and Measurements (JRC-IRMM, Geel, Belgium), standard of Bt-11 and RRS DNA were used as positive control samples. Rice DNA was used in species-specific PCR as negative control.

DNA Extraction and Quantification

Genomic DNA extraction was done from 100 mg of all selected food samples and reference material. Two protocol of DNA extraction methods from the food samples used in this study, one of them based on using the cetyltrimethyl ammonium bromide (CTAB) method with minor modification that applied by (Aksoy & Sonmezoglu, 2022) as follows : after homogenization of the food samples , 100 mg of each sample was transferred to a 1.5 mL micro centrifuge tube, and mix with 400 µL deionized water, 600 µL CTAB buffer, and 30 µL Proteinase K (20 mg/mL), shaken and incubation for 65 °C for 50 Min then centrifugation at 10000 g, transfer the supernatant to a micro centrifuge tube containing 500 µL chloroform and shaken for 90 s. Centrifuge for 10 min .This step was repeated again . The superior layer was transmitted to another tube after centrifugation, 350 µL NaCl (1.2 M) and a 0.6 volume of isopropanol were added then mixing and centrifugation for 10 min. Discard the supernatant and wash the pellet with 70% ethanol, after air-dried dissolve again in 50 µL sterile deionized water and stored at 4C for later analysis. Another method used for DNA extraction from the samples based on using two kits, Gene aid, Singon biotech kit (South Korea and Taiwan) which applied based on manufacture constriction. DNA concentrations were measured using a Nano drop and confirmed using gel electrophoresis.

Application of PCR for detecting GMF : In PCR application a thermal cycler (Labnet

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international) was used by mix of 25 µl that contain the follows: (2.5 µl of 10 x PCR buffer, 10 ng of genomic DNA; 0.1 to 0.5 µM of each primers; 200 µM of dNTPs mix; and 0.2 unit/reaction of taq DNA polymerase . The sequences of the primers (listed in Table 1) include Lectin gene , Starch synthase IIb (SSIIb) as species specific primers for corn and soy bean respectively , and the primers CaMV35s with T NOS for general screening for GMO genes , and the last two primers were Bt-11 and RR™ soybeans which used as event specific primers**.**The conditions of PCR were as follows:(preincubation at 93°C for 200s, 34 cycles consisting of dsDNA denaturation at 95°C for 50 sec) ,deferent annealing temperature used *(57.5°C, 58°C, 60°C and 62°C*) depending on the kinds of the primers for 45 sec, then elongation at a72°C for 50 sec. Last 3 min in 72°C added to these cycles.

Table 1. Represent the sequences of the primers used in this study

Primer name	Sequence (5′–3′)	target	Length (bp)	Ref
Lectin F	GCCCTCTACTCCACCCCCATCC	Soybean	118	(2,9)
Lectin R	GCCCATCTGCAAGCCTTTTTGTG			
SSIIb-F	GTACCGGAACTACAAGGAGA	StarchsynthaseIIb	100	17,18
SSIIb-R	GAGCACGTCCTCATACAGC			
NOS F	TCACCAGTCTCTCTCTACAAATCTATCTC	Tnos	260	(21,29)
NOS R	GCTGCTGTAGCTGGCCT			
CaMV F	GCTCCTACAAATGCCATC	35S	195	(2,9,21)
CaMV R	GATAGTGGGATTGTGCGT			
Bt -11 F	CTG GGA GGC CAA GGT ATC TAA T	intron ivs2-2	189	(2,9)
Bt -11 R	GCT GCT GTA GCT GGC CTA ATC T	Pat – B		
petu-F	TGA TGT GAT ATC TCC ACT GAC G	RR™ soybeans	172	26
-R	TGTATC CCT TGA GCC ATG TTG T			

RESULTS AND DISCUSSION

1. Evaluation of DNA yield

Accurate detection of GMF depends initially on the quality of the genomic DNA obtained from the food, then on the sensitivity and specificity of the PCR technique that used for their detection. Different DNA isolation methods (manually and kit) were applied for DNA extraction from the food products especially, the deep processed food which can be difficult to isolate due to the presence of impurities in these kinds of foods such as proteins, lipids and other organic compounds. Moreover the DNA in these processed products, is exposed to harsh condition during their processing include heat and chemical or physical treatment, which may result in the degradation of DNA molecules (Camma et al., 2012). Through the use the manual method, the quality of obtained DNA (in terms of purity and concentration) are low in many of these samples as presented in **(Figure 1).** However by using gene aid kit, which is specific for extraction of DNA from deep processed foods, an adequate amount of genomic DNA were obtained from all the sixty samples used in this study, which represent some of them in **(Figure 2)** that show the DNA bands visualized using gel electrophoresis with 1% agarose.

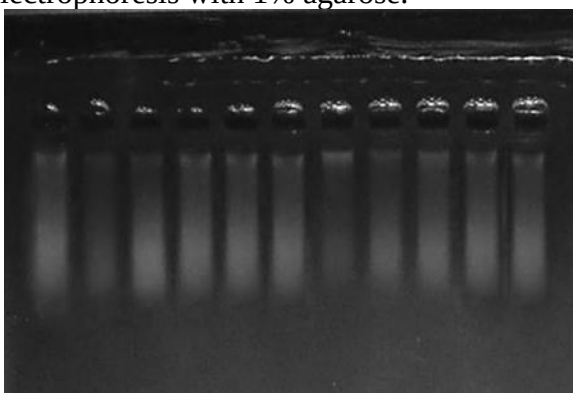

Figure1. Represent Electrophoresis Agarose Gel from some of the studied samples of DNA products using CTAB Methods

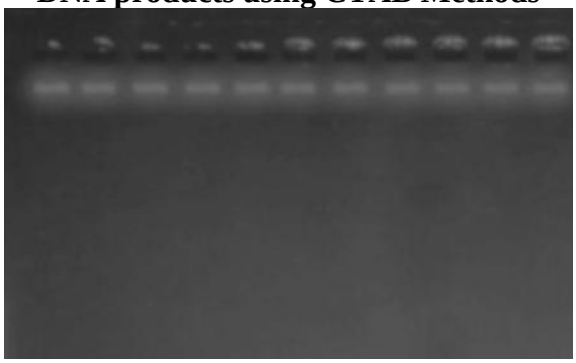

Figure2. Represent Electrophoresis Agarose Gel of DNA yields from maize and soybean crop using Kit

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The concentration of DNA extracted from food samples used in this study, which are determined by the absorbance in the ultraviolet (UV) region at 260-nm wavelength, was 20 to 100 ng/µl. DNA concentrations in food samples studied by Aksoy and Sonmezoglu (Aksoy& Sonmezoglu, 2022) ranged between 8.27 ng/µl in Cake to 593.58 ng/µl in soy flour. The quality of the extracted DNA based on the purity values of 260/280 ratio .The ratio of the most DNA samples extracted in this study ranged between 1.7- 1.9 which considered acceptable for further analysis by PCR, since this ratio indicates the minimal nucleic acid contamination with protein and some phenolic compounds (Hassan and Ali, 2012). However, It should take in consideration that A 260-nm wavelength are also sensitive to free nucleotides, single strand DNAs and single strand RNAs, beside some other organic contaminants, which can interfere with spectrophotometric analysis and may result in overestimation of the DNA yield. Since it should be confirmed by other techniques. Agarose gel electrophoresis is used to check and verify the DNA integrity that extracted from all samples used in this study. Smear of DNA obtained from samples exposed to deep processing methods, due to fragmentation of the DNA by processing into smaller fragments which may lead to limit the efficiency in detecting GMOs ((Aksoy & Sonmezoglu, 2022)), hence more numbers of each food samples were used for DNA extraction to obtain sharp bands of high molecular weight of DNA. A smear pattern of DNA degradation of some samples of DNA extracted from heat-treated foods were also recorded in other studies (Piskata et al., 2012 ; Piskata et al., 2019). It is also reported that baking affect negatively on the DNA quality (Arun, 2016). It was concluded that the yield and integrity of DNA obtained from deeply processed (heat-treated samples) food were influenced by different ingredients of sample matrix, the type of food and degree of processing, extraction procedure, and inhibitors . Food preservatives and additives also affect the extraction technique efficiency, Lo and Shaw (Lo and Shaw, 2018) found that addition of certain food supplements changes the DNA quality and creates an inhibitor for PCR amplification and increased the detection limit, these suggest using more efficient extraction technique for such samples. In this study Conventional CTAB was less efficient in extraction DNA from deeply processed food compared to the Gene aid kit, which also have other advantages because using kit is quick and don't use a lot of chemicals. However this can be expensive and sometimes less DNA get as wanted for farther analysis. So it's important to select a method that is fast, easy, and gives enough good-quality DNA for testing and analyzing by PCR.

2. Species specific PCR to proof the ingredient of the food samples: The first step in detecting GM soy and maize in the studied food samples in this study was proving the presence of soy and maize in these products, by applying PCR using species specific primers targeting lectin gene in soybean **(Figure 3)** and starch synthase IIb (Synthase IIb) (zSSIIb) gene in maize **(Figure 4)** ,which were selected as an endogenous reference (Kim& Kimm, 2009 ; Kim et al., 2014) to proof the ingredient of each sample. the second step of GMF detection system is general screening the existence of modified gene in the food samples which prof by amplification of definite sequences of GM plant, which was the 35S promoter and NOS terminator , and lastly, detection the type of GM crop line that inserted in these foods assessed by event specific PCR. The results of the current study show an amplified product of 118 bp size which was obtained using primers targeting the endogenous soy bean gene, (Lectin gene), in all the 30 tested samples (100%) as shown in **(Figure 1)**, this result is close to that of Mandaci et al. (2014). Amplified product of 100 bp size was obtained in 100% of the maize product indicating their contained of the endogenous genes (starch synthase gene) in maize (Kim, 2009 ; Kim, 2014). These results indicated that the extracted DNA were amplifiable and free from inhibitor compounds. However they not improve the modification of their DNA, due these amplicon produce in GMOs as well as in that are not modified.

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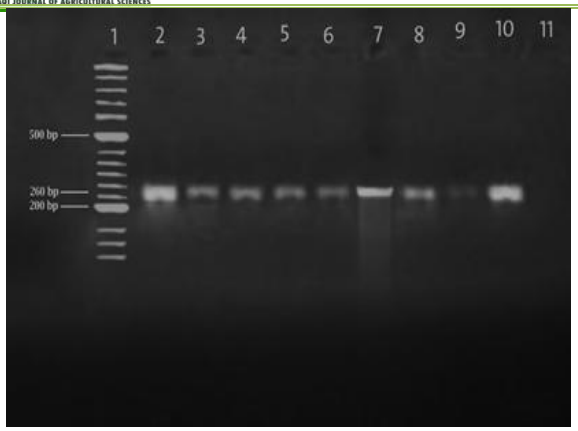

Figure 3. Electrophoresis Agarose gel of PCR yields from soy bean samples for investigation of (lectin gene) (118bp) line

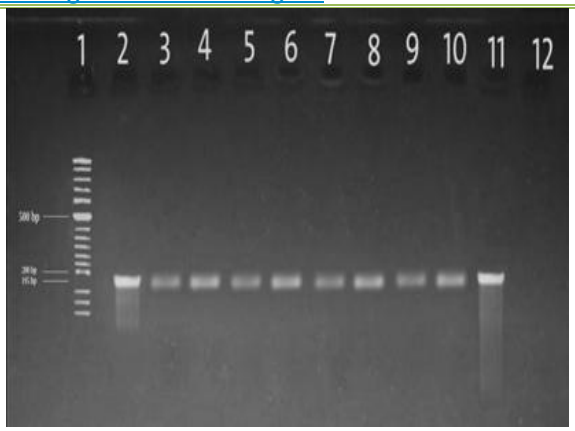

Figure 5. Electrophoresis Agarose Gel of PCR products from maize and soy bean analysis samples of p35 S (195bp) line.

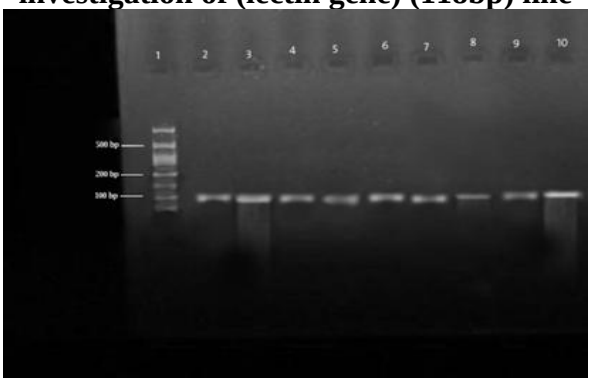

Figure 4. Electrophoresis Agarose Gel of PCR products from maize examples for analysis of zSSIIb gene (100bp).

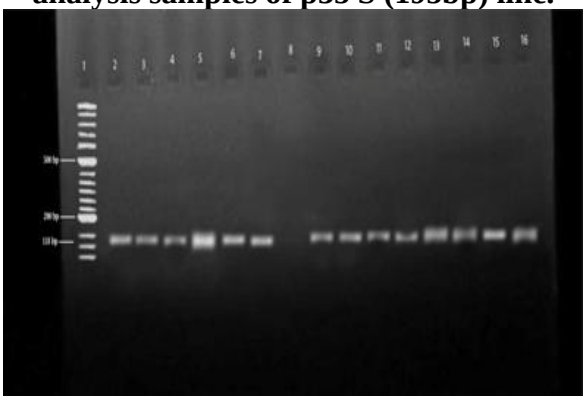

Figure 6. Electrophoresis Agarose Gel of PCR products from maize and soy bean examples for analysis of TNOS (260bp) line

3. Screening method of detecting GMF based on PCR application: General screening procedure is specific for detecting GMOs in food samples, by the detection of the common sequences introduced in most GM products include Cauliflower Mosaic Virus gene which termed 35S Promoter (CaMV P35S) and the Agrobacterium tumefaciens Terminator (tNOS).The results of the current study show Amplified product of 260 bp **(Figure 5)** and 195 bp **(Figure 6)** which obtained by applying PCR using primers targeting P35S and tNOS respectively. The incidences of GMOs in the studied samples were 55 % and 62% for P35S and tNOS respectively.

Holden, 2010 found CaMV 35 S in 95% of GM foods in Europe. Al-Hmoud et al. (2014) showed that the prevalence of GMOs was 72.5% of the examined products of maize in Jordan based on amplifying just the 35 S promoter by PCR. In screening 45 soybean samples from Poland, the incidence of GMOs was 97% (Sieradzki et.al., 2006). It was likewise proven that 91.2% of soy yields from the Hungarian market contained the (35S- promoter or NOS- terminator) elements in their genome (Ujhelyi et.al., 2008). Liu *et.al.*, 2020 emphasized the high- competence usage of p35 S and T-Nos for screening of transgenic seed in soybean and maize. For over a period of time, the Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens* and Cauliflower mosaic virus (CaMV) P35S promoter (P-35S) have been used as effective screening targets to detect GM crop.They are the two essential selection origins for qualitative examines by PCR. However, they are not cover the new produced GM event , hence, some studies suggest to add other structural genes such as *cry* genes derivative of *Bacillus thuringiensis* and phosphinothricin acetyltransferase gene derived from *Streptomyces*

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hygroscopicus (*bar*), to the screening step (Park et al., 2015). Screening methods that are used for control labeling regulations are very essential for routine analysis of GMOS and improvement of a practical finding method is necessary to check the labels on food if contain GMOs and to observer the status of spread of GMOs in food products.

4. Event specific detection of GMF using PCR: GM events derived from the transformation event that used in the course of GM crop production. For the selective finding of the transgenic corn and soy bean, an amplified product of 189 bp was obtained **(Figure 7)** in 34% of food processed samples that contain maize product by applying PCR using primers targeting the Bt-11 gene . These results agreed with the results of the study of Rabiei *et al.*(2013) Who found that 42 % of GM maize samples were positive for (Bt11 event) in Iran .However the percentage of Bt11 event in GM corn decrease in the study of Kim *et al.*(2014) to 22%. Branquinho *et al.*, 2013 also examined Bt11 line in incorrect labeled Brazilian maize samples and demonstrated that 90% of the samples were positive for Bt11.It is worth to mention that maize event Bt-11 is designed to be both herbicide tolerant and insect resistant, the EU approved it as food or feed. Regarding to soybean , an amplified product of 172 bp was obtained **(Figure 8)** in 70 % of food samples that contain soya ,by applying PCR using primers targeting the event specific gene for GTS 40-3-2 , which also known as roundup ready soybean (RRS)

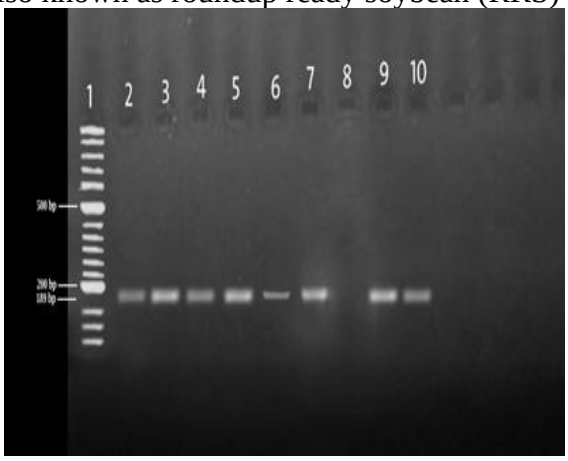

Figure 7. Electrophoresis Agarose Gel of PCR products from maize samples for analysis of Bt11 (189 bp).

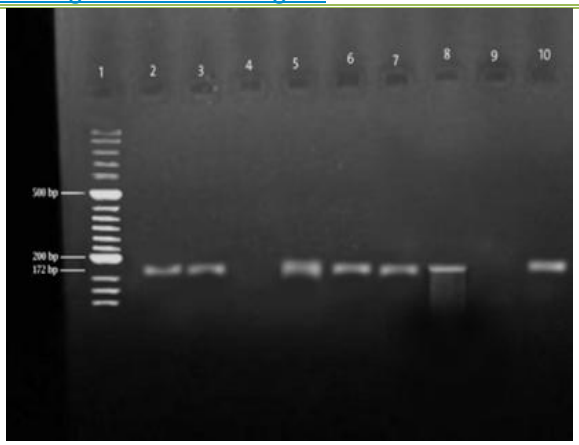

Figure 8. Electrophoresis Agarose Gel of PCR products from soybean samples for analysis of RRS (172 bp).

It was used by Nikolic et al., (2014) in his study of detecting of GM soybean in crude soybean oil .RRS was also selected by the study of Manzanares-Palenzuela et al.(2016)in detecting and quantification of RRS in some foods. RRS event was one of the thirty different types of GM soybeans events (such as A2704-12, A5547-127, and MON89788) that have been approved for use in food and animal feed in most of countries .The reason behind selecting RRS among those is that RRS is the main GM soybean events that have been cultivated for commercial release in worldwide shops since 2015 (Crop Life International). Moreover it is the first transgenic Soybean that developed by Monsanto and accepted for food production. It was confers tolerance to Roundup herbicide(Safaei et al., 2020). It's worth to mention that selecting amplified product size less than 200 bp in application of PCR is preferred, because it was considered a most important causes to successfully and efficiently identify GMOs in processed foods, cause DNA degradation and fragmentation during the processing reaction. Event-specific PCR is more precise strategy for GMOS finding than (gene- or construct-specific) PCR, since the crossing unit is targeted among the host DNA and the transgenic insert. Therefore for this kind of study, one primer was planned to aim the next region of the genome host and the other to precisely target the transgenic insert for each event.

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CONCLUSION

It could be concluded from this study, that it can be use qualitative PCR for detection of GMOs in processed food products by initially selecting a reliable method for extraction DNA from these kinds of food, since more of them exposed to harsh condition during processing technique, such as high temperature. Then application of PCR to detect the food ingredients using primer targeting endogenous genes in the food samples. A public approach for detecting transgenic products is general screening using primers targeting the genetic elements that located in the majority of transgenic crops proceeding the specific sequences identification (Event-specific method),which approved in this study. By the prevalence of transgenic sequences which was Bt11 in GM corn product and GTS 40-3-2, or (RRS) in GM soya product. Detecting more event-specific genes for maize and soya are recommended in the future studies.

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We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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الكشف عن التحور الجيني في بعض الاغذية التي تحتوي على فول الصويا و الذرة في الاسواق المحلية لمدينة السليمانية/ كردستان العراق

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المستخلص

تهدف هذه الدراسة الى الكشف عن فول الصويا والذرة المحورة وراثيا في بعض الاغذية التي تحتويها وذلك باستخدام طريقة الفحص الجزيئي المبني على استخدام التفاعل التضاغفي المتسلسل .(PCR) و لتحقيق ذلك الهدف تم جمع 60 عينة من المنتجات الغذائية التي تحتوي على الذرة وفول من أسواق السليمانية / العراق. اذ تم استخدام ال PCRاولا للكشف عن وجود فول الصويا والذرة باستخدام بادئات متخصصة للنوع و للفحص العام لتلك الأغذية فيما اذا كانت جيناتها محوره, تم استخدام بادئات ال Ca MV P35s و ال. TNOS بعدهاتم تطبيق ال PCR والذي يستهدف تغييرات محدده لبعض الجينات في تلك الاغذية. أظهرت نتائج هذه الدراسة وجود جينات تمثل فول الصويا والذرة في جميع الأطعمة التي تحتوي على فول الصويا والذرة على التوالي. كما أظهر الفحص العام (Screening) لعينات الأغذية أن نسبة ظهور الكائنات المحوره وراثيًا في العينات المدروسة كانت 75٪ و 82٪ للبادئين P35S و TNOS على التوالي. وفيما يتعلق بالجينات المحوره بطريقة خاصة (event-specific)، تم اكتشاف الجين Bt-11 في حوالي 34٪ من عينات الأغذية التي تحتوي على الذرة والجين GTS 40-3-2في 70٪ من عينات الأغذية التي تحتوي على فول الصويا. لذلك بانه يمكن استخدام ال PCR بنجاح للكشف عن الكائنات المحورة وراثيًا في المنتجات الغذائية.

الكلمات المفتاحية: سلامة الغذاء, الاغذية المحورة وراثيا,الكشف ,التفاعل التضاغفي المتسلسل.