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Investigation of Genetically Modified Foods in Iraqi Markets

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Abstract

Background. Plant genetic transformation is a pathway used to improve plant yield, quality, and tolerance to abiotic/biotic stress. **Aim**. of the current study was detection of genetically modified food products in maize products in Iraqi markets. Methods. Fifty food products of corn such as corn chips, starch, flakes, etc. were collected from local markets in Baghdad, Iraqi Ministry of Agriculture and agricultural companies. Three samples were taken for each item in the period from December 2023 to January 2024. DNA extraction was done using gene aid kit and CTAB DNA extraction method for positive control. Conventional PCR and Quantitative Real-time PCR for detection GM food products were used. **Results**. A 78%, 96% and 80% of the samples were found to carry the CaMV35s, Nos, Bt-11 genes according to conventional PCR suggesting widespread presence of GM maize in the Iraqi market. The Ct values of the transformed genes were higher than its corresponding of housekeeping gene suggesting a possible high representation of GMO maize in the Iraqi market. The MOM average of CaMV-35S and T-nos was 1.02 and 1.01 respectively. The copy number average of 36 food products of maize samples for Bt-11 was 25. **Conclusion**. The results presented in the present study demonstrate a clear risk of GMO maize availability in the Iraqi market.

Keywords: GMO, maize food products, MOM, Gene copy number

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Introduction

Genetically modified Organ (GMO) referred to possess genetic material that has been altered in a manner distinct from natural recombination or mating processes(1). These organisms have undergone genomic engineering to enhance the expression of specific biological products or desired physiological traits(2). Organisms with an altered genome are designated as GMOs. Genomic alterations are executed through the introduction of foreign genes that can express novel proteins, hence augmenting desirable traits such as tolerance (3). Genetic methodologies swiftly enhance the characteristics of commonly utilized flora and fauna. These strategies transcend natural limitations related to the exchange or reintegration of genetic material among distinct species by generating transgenes(4).

Genediting strategies have been employed for several agricultural products to enhance desired traits related to disease and pest resistance, nutritional value, feed quality, herbicide tolerance, and additional factors (5). Maize is one of the most thoroughly researched plant species. Maize, owing to its extensive genetic and genomic resources, has been employed in biological studies concerning plant domestication and evolution, epigenetics, heterosis, disease and insect resistance inheritance, doubling haploids, genome editing, and breeding

techniques (6, 7). This is accomplished due to the abundance of genetic and genomic resources that maize possesses. In the year 2019, genetically modified maize varieties were responsible for more than thirty percent of the total area of maize cultivation worldwide (8). The list of so-called biotech features that are currently accessible is no longer limited to herbicide and insect resistance, but it includes traits such as abiotic stress tolerance, high yield, and increased nutritional quality. Maize has been genetically modified to confer tolerance and resistance to herbicides such as vinyl, glufosinate, and glyphosate, as well as to many insect pests through the incorporation of Bacillus thuringiensis (Bt) Cry proteins(9). The emergence of GM crops has been contentious, mostly due to ethical dilemmas and sustainability challenges related to their adverse effects. These challenges encompass various aspects, including the adverse impacts of GM crops on the environment and human health, the ethical implications of generating new life forms inside society, and the intellectual property rights associated with GM crops that provide economic advantages to particular individuals(10). Presently, several systems for detecting genetically modified organisms (GMOs) rely on DNA and protein detection to enforce these restrictions. DNA analysis for the verification of food authenticity is rapidly increasing in popularity(7), and numerous PCR primers are employed for GMO evaluation. In this study, it has been examined the detection of recombinant DNA of genetically modified maize in several processed foodstuffs gathered from Iraqi markets.

Materials and Methods

Sample Collection

Various food products of corn (50 items) such as corn chips, starch, flakes, etc. were collected from local markets in Baghdad, Iraqi Ministry of Agriculture and agricultural companies in the period from December 2023 to January 2024. Three samples were taken for each item. The study was conducted in the Biological Laboratory of Education the institution of genetic engineering and biotechnique for postgraduate studies, Baghdad University.

DNA extraction

The samples were all ground and homogenized using an electric mill (IKA m20 WERKE) to achieve the desired results. A generated kit was utilized in order to extract DNA from one hundred milligrams of food that had been crushed and homogenized. After extracting the DNA, it was suspended in a volume of 50–100 µL of DNA-grade water and stored at a temperature of -20°C.

Reference material

Suitable reference materials for positive and negative controls establish the foundation for the validation of analytical techniques (11)**.** The reference material for the positive control of Bt11 is acquired from the EC Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), located in Geel, Belgium. The certified reference materials (CRM) consisted of various mass fractions of dried powder from GM maize harboring the Bt11 gene (ERM-BF412bk) with 100% GMO content. For the CRM, 100 mg of powdered material was utilized, and

DNA was extracted with the CTAB extraction technique.

Evaluation of the concentration and the purity of the extracts

DNA purity was estimated by UV spectrophotometry at 260 and 280 nm using a NanoDrop 2000c instrument. Ratio between 1.8 and 2.0 for A260/A280 are accepted as indication to pure DNA

Method of Detection

It was used conventional PCR and Quantitative Real-time PCR for detection GM food products.

Conventional PCR

To start the PCR, the reaction was repeated several times, thus the following optimum programs were adopted which produced clear and sharp bands in agarose gel . This protocol employs 2xEasyTaq® PCR SuperMix . All PCR reactions were carried out in a 25 µL final volume and according to the manufacturer's instructions (12-14)

Oligonucleotide primers

Four pairs of PCR primers listed in Table 1 were used in this study. The sequence primers are designed according to European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) (2024). The primers were synthesized and lyophilized by Alpha DNA Ltd. (Canada).The used primers were diluted to a final concentration of 10 µM with sterile double-distilled water and stored at - 20°C until use.

Table 1. Primers utilized for detection and identification in PCR

Gene	Primer	Amplicon length	PCR Assay	Chemistry method	Ref.
T-nos (QL-ELE-00-007)	F 5'-GAATCCTGTTGCCGGTCTTG-3' R 5'-TTATCCTAGTTTGCGCGCTA-3'	180 bp	single	Agarose gele Electro	
P35S(QL-ELE-00-04)	F 5'-CCACGTCTTCAAAGCAAGTGG-3' R 5'-TCCTCTCCAAATGAAATGAACTTCC-3'	123 bp	single	Agarose gel Electrophorsis	(15)
Bt11	CTGGGAGGCCAAGGTATCTAAT GCTGCTGTAGCTGGCCTAATCT	189 bp	single	Agarose gel Electrophorsis	
Zein	R-AGTGCGACCCATATTCCAG F-GACATTGTGGCATCATCATTT	277 bp		qPCR	

PCR conditions

Following the completion of the DNA extraction process, qualitative PCR methods were designed employing a variety of primer sets. In order to establish the presence of zein, CaMVp35s, Bt-11, T-nos, and zein genes, conventional polymerase chain reaction (PCR) was performed. The instrument used was a Mastercycler gradient apparatus from Eppendorf, and the final volume was 20 µL.

Agarose gel electrophoresis

PCR products were analyzed using agarose gel electrophoresis. The gel was prepared with 1% agarose in Tris Borate EDTA (TBE) 1x (Sigma) with 10 µg/mL of DNA. Electrophoresis was performed at 200 mA and voltage at 70 V for 45 m in TBE 0.5 x buffer using 1 Kb DNA ladder. To visualize the stained amplicons, Transilluminator Villber Lourmat® imaging system was employed(16).

Quantitative detection by Real-time PCR

The samples were tested using relative quantitative PCR depending on the housekeeping B-actin gene which is used as a control for experimental variability (17), and quantification of copy were accomplished by using the standard curve method depending on the Threshold cycle (*Ct*) for housekeeping gene and genetically modified gene (Bt11) according to (18). The primer sequences of β-actin used are shown in Table (2)

Table 2. The primer sequences of β-actin

Primer Name	Target	5 →3 Sequence	Product (bp)
Housekeeping gene β-actin	B-actin	5'-TGG CAC CCG AGG AGC ACC CTG-3' 5'-GCG ACG TAC ATG GCA GGA ACA-3'	118bp

Real-time qPCR

The program was applied by using the thermal cycler with the desired thermal cycling conditions as per the manufacturer's instructions and data were collected during the annealing step of each cycle.

MOM Formula

Multiple of Median (MoM) was calculated for T-nos and P35S to estimate the copy number (26) as follow:

$$\mathbf{MoM}=\frac{\mathbf{Ct(sc)+[\ Ct(sc)-\ Ct\ of\ GM\ gene]}}{\mathbf{Ct(sc)}}$$

MoM= Multiple of Median.

Ct= threshold

SC= standard curve (β-actin).GM= genetic modified

Data analysis

Data analysis of genetically modified organisms (GMOs) in various samples was carried out with each accession, and subsequent rating of reproducible primer products was carried out (Table 3). check manually for the presence or absence of the band. It was decided to create a binary qualitative data matrix.

Results

Genomic DNA Extraction.

Good quality of genomic DNA is essential for revealing of targeted genetic markers. Geneaid kit for food maize products commercially kit was used for the extraction of DNA. DNA extraction experiments showed that the purity ratio was 2.1 and the DNA concentration range from 415 to 1130 ng/µl.

Revelation of GM maize by Conventional PCR

Results showed high frequencies of zein genes as expected showing success in both extracting and amplifying maize DNA from the samples studied. As expected,. Agarose gel electrophoresis of the PCR amplified products resolved bands of 277 ,123, 180 and 189 bp for the introduced gene of zein, CaMV35s promoter, T-nos and Bt11 (figure 1). A 78%, 96% and 80% of the samples were

found to carry the CaMV35s, Nos, Bt-11 genes suggesting widespread presence of GM maize in the Iraqi market. Cauliflower Mosaic virus 35s promoter is presented in around 95% of currently commercialized GMO plants in EU(19). These findings are consistent with previous reports of the global prevalence of GM maize, in particular, that expressing the *Bt-11* gene (20, 21)

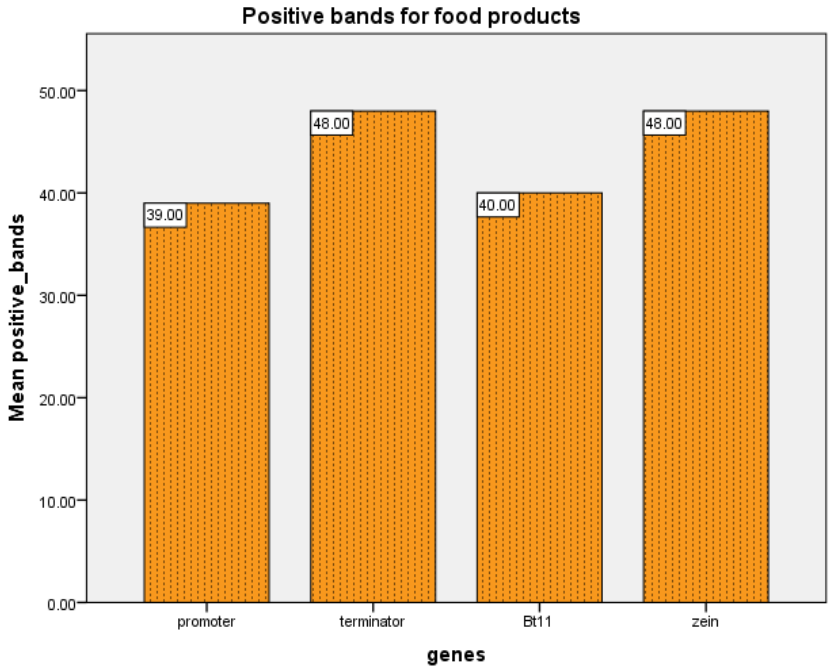

Figure 1. Mean positive band for food products

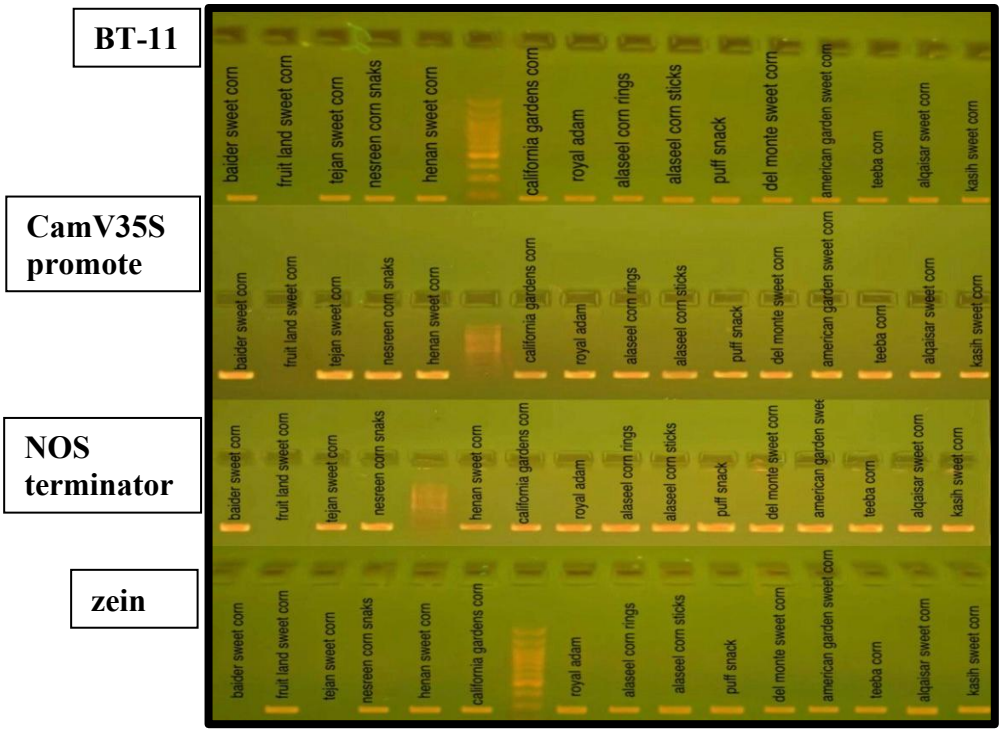

Figure 2. PCR product bands for different types of food products for *zein, CaMV35s promoter, T-nos* and *Bt11* genes.

Revelation of GM maize by Real-time PCR for food products

This study utilized real time PCR to screen 50 processed maize products from Iraqi markets for the presence of common genetic modifications, targeting the CaMV-35S promoter, NOS terminator, and Bt11 gene, with T. actin serving as an internal control. Notably, amplification of the CaMV-35S promoter was not observed in several products, including baider sweet corn, salsa sweet corn , doritis chips, super ring corn , Iraqi cinema popcorn , super mix corn flour , family corn flour and alshami max popcorn. Amplification of the NOS terminator was not detected in only three products; fruit land sweet corn, teeba corn and doritos chips. Amplification of the Bt11 gene was not observed in some products; royal adam, teeba corn, alqaisar sweet corn, kasih sweet corn, alaseel peanuts chips, super mix corn flour, family corn flour, family corn flour, dahab corn flour, albaiker corn flour, domo corn flour, ansak chips and fatfeet popcorn. The Ct values ranged 19.27-26.14 for CaMV-35S with average 21.34 in 40 products. The Ct values ranged 20.44 - 24.6 for Not-T with average 22.29 in 47 products. Amplification of the Bt11 gene was observed in 36 products with average 33.96 and range of 28.16-41.77.

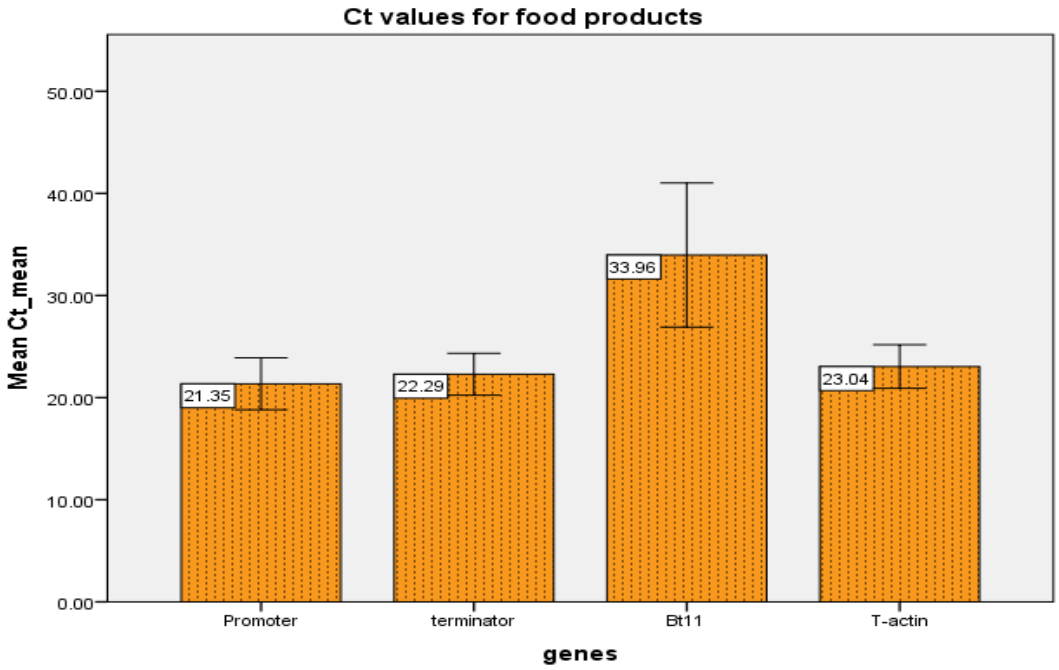

Figure 3. Mean Ct values of four genes for food product

Estimation Of Multiple of Median (MoM)

Estimation of Multiple of Median (MoM) for CaMV-35S promoter

The Ct values were consistent 23.1 to 24.43 with an average 23.3 for the endogenous maize gene and 17.35 to 23.13 with an average 21.10 for the 35S promoter, suggesting a possible high representation of GMO maize in the Iraqi market. The 1 MOM value represents the normal limit of copy and any increase in the copy of 1 is considered abnormal limit (23). Most of the Ct values for the housekeeping gene were higher than those

for the promoter in the food products, which was reflected in the high MOM values for most food products, except for five products which gave MOM values less than 1. The MOM average of **CaMV-35S was 1.02**. The highest MoM values are 1.17 for genotype (tortilla chips).

Estimation of Multiple of Median (MoM) for T-Nos

The Ct average for housekeeping gene for maize genotypes was 23.03 while the Ct average for T-Nos terminator was 22.28, reflecting on the MOM average which was 1.01. Teen food products own low MOM values (american garden, super ring corn, dana popcorn, mazaj popcorn, zer corn, super mix corn flour, family corn flour, american gourmet corn flour, dahab corn flour, and sport chips). The highest MOM value was 1.11 for alaseel corn sticks, tortilla chips and layonk chips .

Estimation of Copy Number of *Bt11* gene

Copy number variation is defined as the presence of variable numbers of copies of a particular DNA segment relative to a reference genome. Also, The gene copy number (also "copy number variants"or CNVs) is the number ofcopies of a particular gene in the genotype of an individual. The 100% certified reference materials (CRM) sample comprised a dried maize powder was used as a positive control.

A 78% (39 out of 50), 96% (48 out of 50) and 80% (40 out of 50) of the samples were found to carry the CaMV 35s, Nos, Bt-11 genes suggesting wide appearance of GM maize in the Iraqi market. Interestingly, the only food product that did not contain all three genes was fruit land sweet corn, while just three genotypes were not own both the promoter and Bt11 which are super mix corn flour, family corn flour and domo corn flour. Food products of maize are screened for GM by realtime PCR. The Ct values of CaMV-35S was higher than its corresponding, Nos-T (21.34 in 40 products and 22.29 in 47 products)(25). However, some samples lacked the Termintor and/ or promoter gene due to different GM constructs or possible degradation of the target DNA during processing (26)**.** The Bt11 gene was amplified in 36 products, with an average of 33.96.

NOS-terminator; The MOM values of **CaMV-35S** for most food products were higher than 1 with 1.02 average, except for ten products which gave MOM values less than 1. The highest MoM values are 1.17 for tortilla chips. The MOM average of T-nos was 1.01. Teen food products have low MOM values, while the highest MOM value was 1.14 for albaki chips.

The copy number average of 36 food products of maize samples for Bt-11 was 25. Results indicated variable transgene copy number of a 22-27 range of products analyzed. Several samples appeared negative free of GMO, i.e., had not Ct values, and this is may due to own a copy number below the range of detection of the quantification method, or had problems with the quantification.

Discussion

The results of this widespread detection of GM elements in processed maize products agree with previous findings in other countries in which many processed foods, including those with maize products, were found to contain GM ingredients (22).

The consistent detection of the 35S promoter in the present study strongly indicate that GMO maize is sold in Iraqi markets. These findings are consistent

with previous study of rising (global) all across the world prevalence of GMO crops, notably maize, in a variety of food products and markets (24)

A potential false positive result may arise if test plant samples are contaminated or infected by the Cruciferae family, as the 35S promoter is derived from the Cauliflower mosaic virus (27). The non-GM plant lacks a however, a NOS-terminator may be present in the roots of non-GM plants, potentially leading to a false positive in PCR testing. The NOS-terminator derives from Agrobacterium tumefaciens, a soil-dwelling bacteria that can taint plant roots.

MOM values adopted as a criteria for contamination by transgenes. The 1 MOM value represents the normal limit of copy and any increase in the copy of 1 is considered abnormal limit (28)

Some problems could arise in developing a PCR method for detection of GMO in foods because of: (1) Processed food: In highly processed or fermented foods, genetic alterations or protein denaturation may complicate identification. (2) Mixture of genetically modified products: various strains of genetically modified crops are combined either artificially or organically. Insufficient information concerning the gene sequence of the inserted gene may hinder the construction of an appropriate primer. Trade with nations without regulations on GMF may pose challenges in detecting GMF (27). Overall, the results presented in the study demonstrate a clear risk of GMO maize availability in the Iraqi market, particularly when closely matched 35S promoter and T-Nos are consistently detected in most tested samples. Therefore, consumers in Iraq to become aware and make informed choices about GM foods.

Conclusion

In the present study, genetically modified DNA was detected in the processed food products which were gathered from Iraqi market and institutions. The prevalence of GM crops and ingredients in food supply chains is attributable to the fact that GM crops and ingredients appear in Iraqi market.

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