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Detection of genetically modified organisms by genetic markers in the local market of Al-Muthanna Province-Iraq

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Abstract

Genetically altered plants also called genetically modified organisms or GMOs, are contentious subjects concerning food regulation. There are several challenges concerning the potential dangers of GMOs' toxic, allergenic and dietary interactions. In this context, sixty-seven food specimens were evaluated soybeans *(Glycine max* L. Merr), barley (*Hordeum vulgare* L.), rice cereals *(Oryza sativa* L.), tomato (*Lycopersicum esculentum* L.), Corn chips, maize (*Zea mays* L.), and potato *(Solanum tuberosum* L.) for GMO content; these samples were obtained from Iraq, and DNA extraction was performed using a test kit. DNA amplification was conducted using polymerase chain reaction (PCR); subsequently, agarose gel and four primers were used to perform electrophoresis to establish the presence of augmented GMO sequences. Experimental outcomes of 67 specimens indicated that 15 specimens tested positive; they comprised three samples each of corn chips, rice, and maize, four soybean samples, and one sample for tomato and corn seed using four primers. These results showed that GMO screening is a suitable technique for detecting transgenic plants, testing and monitoring food specimens and controlling GM levels in foods.

1. Introduction

Genetically altered or genetically modified (GM) plants, called GM organisms (GMOs), are those whose genetic material is modified such that it is different from that produced by natural recombination or mating processes (Holst-Jensen *et al*., 2012), an organism whose genome has been engineered by the experiments in order the generation of favor the expression or desired biological products of desired physiological characters (Diaz and Fridovich-Keil, 2022). Living organisms having a modified genome are referred to as GMOs. Genome changes are implemented using external genes capable of expressing novel proteins that enhance desirable properties like tolerance (Mannelli *et al*., 2002).

Genetic techniques rapidly improve the attributes of widely used plants or animals. Such techniques surpass natural barriers concerning genetic material exchange or reintegration between different species by producing transgenes. Nevertheless, society may not perceive this short way appropriately because many are still not confident and conclusive about the safety aspects of genetic technology. Like other technologies, genetics might raise concerns (Holst-Jensen, 2009). Gene-altering mechanisms have been used for several agricultural items to add desirable characteristics concerning disease and pest resistance, food nutrition, feed products, herbicide resistance, and others (Safaei *et al*., 2020).

Developing and developed nations increasingly rely on commercial GMO use (Wana *et al*., 2021). Specifically, GMO production and commercial utilisation are contingent on most nations' stringent legal requirements. The present legal system primarily addresses hazards concerning the environmental release of GMOs and consumption effects on human and livestock health. GMO use might be restricted, e.g., GMOs might be permitted for feed products but limited for food (Holst-Jensen *et al*., 2012).

Challenges concerning GMO product sales in international markets are rising because of regulatory diversity and compliance required to transport products across the globe. The asynchronous authorization is a significant challenge causing low-level presence (LLP)

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of unofficial GM content in imported feed or food (Wana *et al*., 2021).

Using DNA indicators facilitates identifying genetic differences and associations between plant genotypes (Al -Janabi and Alhasnawi, 2021). Many amplification substances can be synthesised using PCR techniques using one primer for such regions. They can be used as a foremost multi-locus marker approach for assessing genetic alterations in organisms (Mandal *et al*., 2019; Alhasnawi, 2019). PCR-specific DNA identification techniques are prevalent; however, challenges arise because assessment reliability depends on precise information concerning the PCR primers used for experimentation (Safaei *et al*., 2020).

Presently, several genetically modified organism (GMO) identification techniques rely on DNA and protein detection to enforce such legislation. DNA assessment for authentic food is gaining traction rapidly (Rastegar *et al*., 2021). Despite using many PCR primers for GMO assessment, several such pairs might have constrained application (e.g., screening-specific primers only) (Holst-Jensen *et al*., 2003).

This study was performed to evaluate and determine whether PCR-based techniques are appropriate to decide on genetic markers indicating the presence of genetically modified organisms concerning food products sold in the local markets in the Al-Muthanna Province in Iraq.

2. Materials and methods

2.1 Sample gathering

Sixty-seven food specimens (soybeans, corn products, barley, rice cereals, tomato, maize, and potato) were gathered randomly from Al-Muthanna Governorate markets in Iraq. The study duration was January-April 2021, and all specimens were gathered during this period. The research was performed at the Biological Laboratory of the College of Basic Education, Al-Muthanna University.

2.2 DNA extraction

The extracted DNA was obtained using the Easy Pure® Plant Genomic DNA Kit, with certain modifications made to the manufacturer's protocols. The RB1 resuspension buffer1 was pre-heated in a 65°C water bath before adding powdered leaf tissue, and 130 mg of the fresh leaf was used. In addition, the tissue lysis incubation time was doubled from 15 to 20 mins, with three interval vertexing performed during the incubation period. The elution buffer was reduced from 200 to 70 µL in the last stage.

2.3 PCR reaction

2.3.1 PCR primer preparation

The primer formulation was based on the production instructions that required adding lyophilised primers to TE (Tris-EDTA) buffer specimens to allow a 100 pmole/ mL concentration mixture. The solution was subjected to spinning and allowed to cool at 4°C for one night. The working mix was prepared by diluting the stock using the TE buffer to obtain every primer's active mixture (10 pmole/ mL).

2.3.2 PCR master mix reaction synthesis

The PCR master mix reaction was synthesized using a quick-load PCR kit. Manufacturer recommendations were followed to prepare the sample; Table 1 lists the process – (1) Protocol for OneTaq® Quick-Load 2× Master Mix using Standard Buffer (M0486). Subsequently, the PCR master mix constituents were put in standard PCR tubes comprising lyophilised multiplex PCR and other substances needed for the analysis (*Taq* DNA Polymerase, 6 mM $MgCl_2$, dNTPs; pH 8.7). An Existing vortex centrifuge was used to spin the tube for 3 mins, and the mixture was then processed using the Multigene PCR thermocycler.

Table 1. Master mix reaction.

Component	25 µl reaction
10 µM Forward Primer	0.5 µL
10 µM Reverse Primer	0.5 µL
Template DNA	1.5 µL
OneTaq Quick-Load 2X Master Mix with Standard Buffer	12.5 µL
Nuclease-free water	10 µL
Total	25 µL

2.3.3 PCR amplification and DNA assessment

The typical PCR thermocycler approach was used for every gene under conditions specified in Table 2. Table 3 lists the research target and primer choice.

Table 2. PCR thermocycler approach.

Steps	Temperatures	Time
Initial Denaturation	94°C	30 s
30 Cycles	94°C Variable (as mentioned below every photo) 68°C	15-30 s 15-60 s 1 min per kb
Final Extension	68°C	5 mins
Hold	4-10°C	

2.4 Gel electrophoresis

2.4.1 Agarose gel (1%) preparation.

A solution was prepared in a flask using 1 g agarose mixed with 100 mL 1× TBE buffer. The solution was processed in a microwave oven till boiling commenced. The flask was removed from the microwave, and the

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Table 3. Primers and gene sequence.

Gene and gene sequences	Primer Sequences	PCR Product	References
35S -1	F 5'GCTCCTACAAATGCCATCA'3 R 5'GATAGTGGGATTGTGCGTCA'3	195 bp	Lipp *et al*. (1999); ISO (2005); Zaulet *et al*. (2009)
LE-103	F 5'GCCCTCTACTCCACCCCCATCC '3 R 5'GCCCATCTGCAAGCCTTTTTGTG '3	180 bp	Beuth (1998)
IVR1-1	F 5CCGCTGTATCACAAGGGCTGGTACC '3 R 5'GGAGCCCGTGTAGAGCATGACGATC '3	226 bp	Ehlers *et al*. (1999); ISO (2005)
HA-NOS118	F 5' GCATGACGTTATTTATGAGATGGG '3 R 5' GACACCGCGCGCGATAATTTATCC '3	118 bp	Cardarelli *et al*. (2005); Zaulet *et al*. (2009)

solution was stirred and again processed until boiling was observed. This process was repeated till flicks were eliminated. Subsequently, the agarose cooled between 50°C and 65°C. Ethidium bromide was then mixed with the prepared agarose gel (5 µL solution comprising 10 mg/mL stock for every 100 mL agarose gel).

2.4.2 Gel electrophoresis test

Multi-gene PCR substances were assessed using the electrophoresis technique using agarose gel using five steps. 1× TBE was mixed in a water bath for 15 mins at 100°C and then cooled to 50°C to synthesize the 1% Agarose gel sample, subsequently, the solution was mixed with 2 µL ethidium bromide stain then the gel solution was set in a tray, and the comb was attached in the required position. Fifteen mins cooling at room temperature allowed the solution to solidify; the comb was slowly removed, and 10 µL PCR was put in every comb along with 5 µL of (1500 bp Ladder) in a single well, the tray was mounted in the electrophoresis setup, and 1× TBE buffer was added. The solution was processed using 100 volts for 30 mins and 50 volts for 45 mins and finally, an ultraviolet transillumination instrument was employed to visualize PCR samples.

2.4 Data analysis

Data analysis of GMOs in different samples was performed with each accession and scoring of reproducible primer products (Table 3), was done manually for band absence or presence. A binary qualitative data matrix was done.

3. Results

Sixty-seven food specimens (soybeans *Glycine max* L. Merrill, corn products, barley *Hordeum vulgare* L., rice cereals *Oryza sativa* L., tomato *Lycopersicum esculentum* L., maize *Zea mays* L. and potato *Solanum tuberosum* L.) were used for DNA isolation. Bright bands validated DNA presence inside the specimens. Figure 1 depicts gel-specific observations.

Precise annealing temperatures for a primer and selective PCR amplification are determined by nucleotide sequence and length, primer concentration, and base constitution. This work used GMO screening techniques for sixty-seven samples based on GMO primers. The outcomes indicate 52 negative and 15 positive GMO samples; the latter comprised three samples each of corn chips, rice, and maize, four soybean samples, and one sample of tomato and corn seed. GMO sample details are listed in Table 5. PCR assessment using four primers (35S -1, LE-103, IVR1-1, and HA-NOS118) facilitated event-based GMO sequence identification for the selected food samples are shown in Table 4.

The 35S-1 primer was selected to evaluate DNA-based GMOs in food specimens (Lipp *et al*., 1999; ISO 2005; Zaulet *et al*., 2009). Specimen-based genetic data assessment indicated 195 bp for (R12, R17, T1, M3, M9,

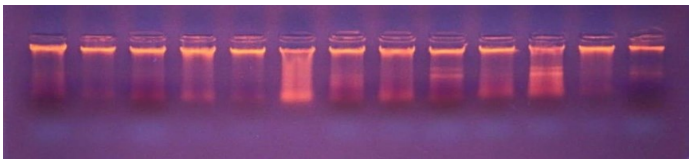

Figure 1. Ethidium-Bromide-stained gel electrophoresis appearance that displays DNA from various plant sources that were extracted.

Table 4. Annealing temperature and positive samples.

Gene and gene sequences	PCR product	TM (ºC)	Positive samples
35S -1	195 bp	59	6
LE-103	180 bp	57	0
IVR1-1	2261 bp	57	4
HA-NOS118	118 bp	55	5

Table 5. GMO positive and non-positive samples.

Code	Name	No. of samples	No. samples positive
R (R1-R22)	Rice Cereals	26	3
Ch (C1-C14)	Corn chips	10	3
M (M1-M13)	Maize	13	3
B (B1-B4)	Barley	4	0
S (S1-S7)	Soybean	7	4
CS (CS1-CS8)	Corn seeds	8	1
T (T1-T4)	Tomato	4	1
P (P1-P3)	Potato	3	0
Total samples		67	15

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and S4) for the positive outcomes (Figure 2).

The LE-103 primer in Figure 3 depicts genetic data assessment used for samples not indicative of 180 bp for (R, Ch, M, B, S, CS, T, and P), indicating negative outcomes. The IVR1-1 primer was selected to evaluate DNA-based GMOs in food specimens (Ehlers *et al*., 1999; ISO, 2005). Genetic data assessment used for samples indicating 226 bp for Ch10, Ch6, S1, M12, and CS4 indicated positive outcomes, as depicted in Figure 4.

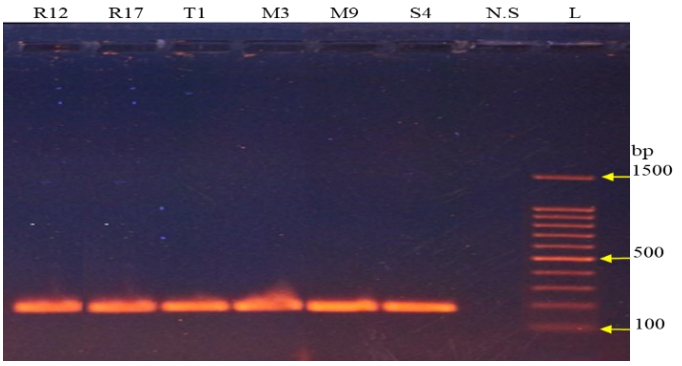

Figure 2. Agarose gel electrophoresis for PCR amplification of 35S-1 primer which shows 195 bp primer, annealing temperature at 59℃ to the identification of GMOs. Lane L: 1500-100 bp DNA ladder, Lane N.S represents negative control sample, Lanes R12, R17, T1, M3, M9 and S4 represents positive results.

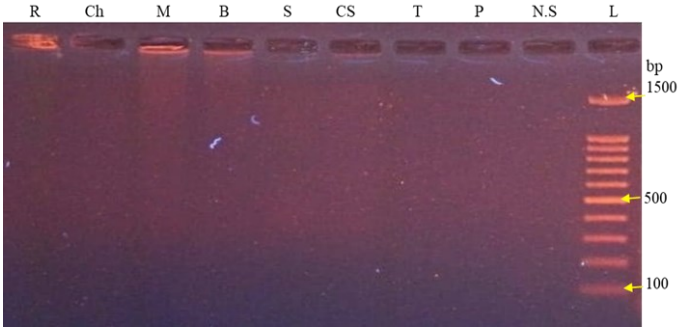

Figure 3. Agarose gel electrophoresis for PCR amplification of LE-103 primer which show 180 bp, annealing temperature at 57℃ to the identification of GMOs. Lane L: 1500-100 bp DNA ladder, Lane N.S represents negative control sample, Lanes R, Ch, M, B, S, CS, T and P represents positive results.

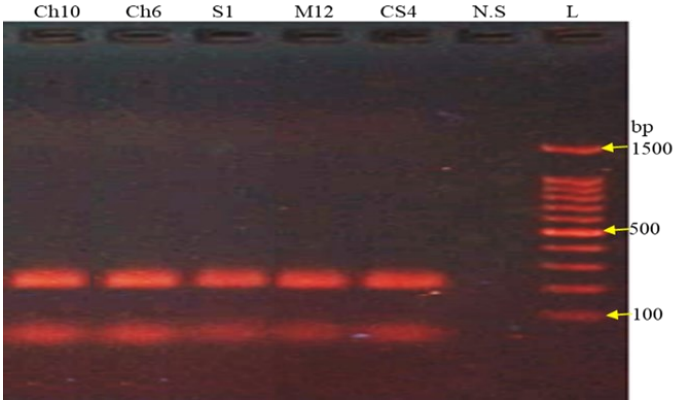

Figure 4. Agarose gel electrophoresis for PCR amplification of IVR1 primer which shows 226 bp, annealing temperature at 57℃ to the identification of GMOs. Lane L: 1500-100 bp DNA ladder, Lane N.S represents negative control sample, Lanes Ch10, Ch6, S1, M12 and CS4 represents positive results.

The HA-NOS118F primer was selected to evaluate DNA-based GMOs in food specimens (Cardarelli *et al*., 2005; Zaulet *et al*., 2009). Figure 5 depicts that genetic data assessment for samples indicating 118 bp for R8, Ch3, S6, and S2 indicated positive outcomes.

This work assessed the global production of soybeans, rice cereals, potatoes, barley, maize, corn produce, and tomatoes to identify GMO presence in the identified food-producing nations. Such PCR techniques can be employed only for GMO quantification using genome equivalence (relative constitution in DNA).

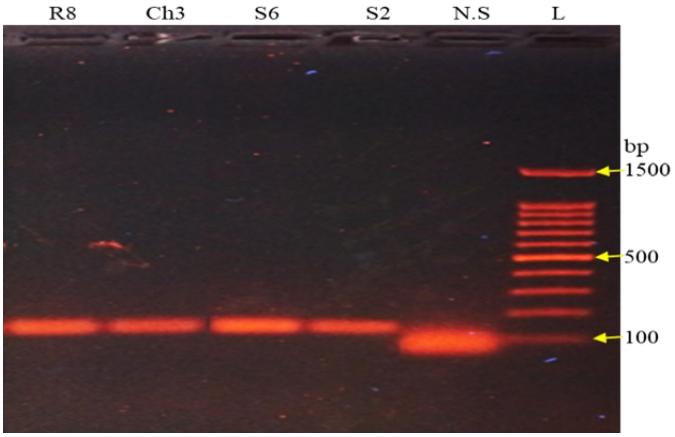

Figure 5. Agarose gel electrophoresis for PCR amplification of HA-NOS118F primer which shows 118bp primer, annealing temperature at 55℃ to the identification of GMOs. Lane L: 1500-100 bp DNA ladder, Lane N.S represents negative control sample, Lanes R8, Ch3, S6 and S2 represents positive results.

4. Discussion

Present genetic techniques include direct genome sampling using DNA, offering better perspectives on the similarities and distinct characteristics across genomes. Genetic correlation assessment precision is determined using molecular indicators comprising the location and number of such markers (Alhasnawi *et al*., 2019). The 35S-1 primer was selected to evaluate DNA-based GMO in food specimens (Lipp *et al*., 1999; ISO, 2005; Zaulet *et al*., 2009). The LE-103 primer was selected to evaluate DNA-based GMO in food specimens (Beuth, 1998). The IVR1-1 primer was selected to evaluate DNA-based GMO in food specimens (Ehlers *et al*., 1999; ISO, 2005).

The HA-NOS118F primer was selected to evaluate DNA-based GMO in food specimens (Cardarelli *et al*., 2005; Zaulet *et al*., 2009). Considering the DNA molecules might vary across one GM specimen compared to a non-GM, selecting the sequence motif is the critical aspect regulating PCR specificity. The target sequence is typically a subset of the changed gene sequence (Holst-Jensen *et al*., 2003). Molecular

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characterization of GMO yields basic information on DNA integration, including entire inserted sequences, integration sites and structures, copy number, and flanking sequences, providing key data for biosafety assessment (Zhang *et al.*, 2022). Ligation-mediated PCR anchored PCR, and inverse PCR are genome-traversing techniques employed to pinpoint sequence junctions and formulate event-based assessments for numerous transgenic cultivars (Akritidis *et al*., 2008). Hence, GMO identification using transcription is not typically usable and usually needs a comprehensive evaluation to understand false negatives and positives than the chosen DNA sequence assessments (Holst-Jensen *et al*., 2012). Also, DNA band intensity was proportional to the number of transgenic substances in the specimen (Datukishvili *et al*., 2015). GMO is an organism in which transgenes of one or more genes have been introduced into its genetic information from another organism by DNA methods (Gbashi *et al*., 2021).

Analytical techniques are essential for the detection of GMO in raw materials (Joelma *et al*., 2022). Most GMOs created to date were altered by adding additional DNA if it is produced from the receiving species or another species that can naturally transfer genes – such GMOs are classified cisgenic when the sequence of the added elements is not altered; in case the sequence is altered, it is categorised intragenic (Holst-Jensen *et al*., 2012). Nam and Lee (2022) reported differences in opinion about GMO are, according to reports, founded on disparities in interests between consumers, suppliers, and producers, as well as in scientific knowledge levels between consumers and experts. Particularly, consumers' ignorance of genetically modified organisms (GMOs) is associated with their fear of them, which eventually makes them less likely to prefer GMOs.

5. Conclusion

The rising commercial adoption of GMO crops might increase the influx of GMO products imported to Iraq. There are concerns regarding the legal status of such imported GM crops. The outcomes of this study indicate that typical PCR techniques are appropriate and reliable for assessing GMO aspects. Moreover, the study concluded that primer formation and the DNA extraction technique are suitable for sample evaluation. Consumers can choose GMO or non-GMO foods; they expect healthy and safe food. Even authorized GM food products do not indicate the potential adverse impact. Additional research concerning monitoring should emphasize detecting and understanding unapproved GMOs like soybeans, rice cereals, potatoes, tomatoes, corn, barley, and maize.

Conflict of interest

The authors whose names are in this study certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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