



Identification of some transgenic maize genotypes collected from local markets at Baghdad

Dina K. Ibraheem¹ & Majid Sh. Hamadalla^{2,*}

Department of Genetic Engineering, Institution of Genetic Engineering and Bio-technique for Postgraduate Studies

*Corresponding author email: M.Sh.H.: majid.s@ige.uobaghdad.edu.iq; D.k.I.: dinaibraheem@gmail.com

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Abstract: Genetically modified organisms (GMO) are the organisms in which genes are altered by a gene modification technique but not by natural mating or DNA recombination. Twenty-two maize genotypes have been screened for detection the genetic modification, which collected from local markets in Baghdad. Three samples were taken for each genotype in the period from December 2023 to January 2024. DNA extraction was done using plantZol kit. The reference material, for positive control of Bt-11, CTAB DNA extraction method was used. Conventional PCR and Quantitative Real-time PCR were used for detection the transgenic maize genotypes. Three pairs of primers, namely, CaMV-35S, NOS and Bt-11 were used, in addition to genes zein and β -actin. A 77%, 68% and 100% 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 modified 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 1.09 and 1.05 respectively. The copy number average of 22 genotypes of maize samples for Bt-11 was 28.4. The current study showed spread of modified genes and contamination of local genotypes of maize with genetically modified genes. This matter requires a continuous survey of local genotypes of crops grown in the Iraqi environment, with the necessity of activating legislation that limits the spread of genetically modified materials.

Keywords: Bt-11, CamV35S, GMO, Maize, T-Nos.

Introduction

Genetically modified crops (GMCs) contain an exogenous gene(s) which is able to express a new protein that confers new traits, i.e. resistance to virus, insect, herbicide tolerance and or improved nutritional value. In 2011, 160 million hectares were cultivated with

genetically modified crops mainly soybean,maize, cotton and canola (ISAAA, 2011).

Genetic modifications in grains, such as Wheat, Rice and Maize have commenced in the late 1980s (Majeed, 2018; Al-Saadi *et al.*, 2024). A *Zea may* is one of the strategic crops, and it is one of the main genetically

modified crops grown globally (Hassan *et al*, 2019). It has been modified mainly to tolerate herbicides and environmental stress and insect resistance, in addition to produce maize with high lysine content. The first products of genetically modified maize (GMM) reached in the US and Canadian markets in 1997. In the European Union (EU), they approved the first lines of GMM in 1997. During 2013, total world maize cultivated area was 177 million hectares out of which 32% was GMM (Tuteja *et al.*, 2012). Maize is cultivated in most governorates of Iraq, but the average yield is still lower than the average of developed agricultural countries (Zaidan, *et al.*, 2019). Among the genes inserted in maize, tolerance/resistance against herbicides, including oxinyl, gluphosinate and glyphosate, and resistance against insects, including different *Bacillus thuringiensis* (Bt) forms (Zimmerman *et al*, 1998). Insect resistance through the production of the Bt protein represents about 16.2% of the area used in growing genetically modified crops (Ali *et al*, 2024). They are known as Bt crops, where the cry gene is transferred from the *B. thurngeinsis* bacterium, which is found in the soil, this bacterium is used as a bio-pesticide because it secretes a toxin specialized for the group of lepidopteran insects, the toxins of this bacterium are among the most important toxins produced commercially, due to the fact that they do not affect birds, mammals, amphibians, and reptiles (Yeung *et al*, 2019). To obtain tolerance to the herbicide Glufosinate ammonium, a most widely used herbicide; the pat gene is transferred from the *Bacillus thuringiensis* to the plant. The gene 4cp epsps, isolated from the *Agrobacterium* bacterium and responsible for producing the EPSPS protein, can also be transferred to the plant to create a resistance to the Glyphosate herbicide (Querci *et al*, 2006). There is another type of modification that carries both two characteristics: tolerance to the herbicide

and resistance to insects, the modification is called gene stacked, more than one gene may be placed and it is called Triple-gene stacked, as in maize resistant to the Root Worm insect and Maize beavers, this modification costs 5 times the cost of the modification containing the One gene (James, 2012). This study aims to identify some transgenic maize genotypes collected from local markets at Baghdad.

Materials & Methods

Sample collection and reference material

Twenty-two genotypes of maize were collected from local markets in Baghdad, Iraqi Ministry of Agriculture and agricultural companies in the period from December 2023 to January 2024 (table 1). Three samples were taken for each genotype. The study was conducted at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad-Iraq.

The reference material, for positive control of Bt11 is purchased from EC Joint Research Center (JRC), Institute for Reference Materials and Measurements (IRMM), Geel, Belgium. The certified reference materials (CRM) comprised a set of different mass fractions of dried powder from GM maize containing Bt11 gene (ERM-BF412bk) at 100% content GMO. For the CRM, 100 mg of material powder was taken, and DNA was extracted using the CTAB extraction method.

Germination

Maize seeds were cultured in sterile Petri dishes on filter papers moist with distilled water and grown in the growth chamber. Germination and growth conditions were 23°C, 55% humidity and continuous light: the obtained plants contain 5-7 young leaves were used for DNA extraction (Al-Salihy *et al*, 2020).

Table (1): Maize Genotypes collected from Iraqi markets

No.	Genotype	Origin
1	Al-Maha	Iraq
2	AL-Fajir 1	Iraq
3	Baghdad 3	Iraq
4	Sumer	Iraq
5	5018	Iraq
6	Sarah	Iraq
7	AGN	Gold Seed Company/Canada
8	Buhooth 106	Iraq
9	Gyson	Gold Seed Company/Canada
10	Julce	Gold Seed Company/Canada
11	EB-110F1	Gold Seed Company/Canada
12	BABEL/F1	Iraq
13	Cadez	Unknown
14	Furat	Iraq
15	TW-78	Egypt
16	TW-345	Egypt
17	IY-355	Egypt
18	IY-207	Egypt
19	Drachma	Syngenta/Italy
20	666	Unknown
21	777	Unknown
22	PL-700	Unknown

DNA extraction, concentration and purity

All the samples were grounded and homogenized with an electric mill (IKA m20 WERKE). A plantZol kit was utilized to extract DNA from one hundred milligrams of each sample that had been completely crushed and homogenized. After extracting the DNA, it was resuspended in 50–100 µl of DNA-grade water and stored at -20°C. For the purpose of obtaining a positive control, the cetyltrimethylammonium bromide (CTAB) technique was utilized to extract DNA from reference material (Bt11) (Yousif, 2023).

The concentration and purities of DNA were determined through the use of UV spectrophotometry at 230, 260, and 280 nm with a NanoDrop 2000c instrument.

According to Russell and Sambrook (2006), ratio between 1.8 and 2.0 for A260/A280 is considered to be an indication of pure DNA (Wuhaib, 2018). An agarose gel electrophoresis was performed on 1% agarose gels that included GelRed nucleic acid stain (Biotium, Hayward, California, United States) in 0.59 TBE buffer. The gels were viewed with a GelDoc EZ instrument (Bio-Rad, Hercules, California, United States). This provided an evaluation of the DNA integrity.

Method of Detection

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

Oligonucleotide primers

In this work four pairs of PCR primers was utilized, as detailed in Table (2). The sequencing primers are formulated in accordance with the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF, 2024). The primers were produced and lyophilized by Alpha DNA Ltd. in Canada. The primers were diluted to a final concentration of 10 µM using sterile double-distilled water and stored at -20°C until required.

PCR conditions

DNA amplification was followed by qualitative PCR protocols using different set of primers. Conventional PCR for the detection of *zein*, CaMV-35S, Bt-11, T-nos and *zein* genes were carried out using a Mastercycler gradient instrument (Eppendorf) in a final volume of 20 µL.

Agarose gel electrophoresis

PCR products were analyzed using agarose gel electrophoresis. The gel was prepared with 1% for DNA and 1.5% for genes agarose in Tris Borate EDTA (TBE) 1x (Sigma) with 10 µg/mL of DNA (Suryani et al., 2024). 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.

Quantitative detection by Real-time PCR

Quantitative PCR analysis was utilizing with the housekeeping B-actin gene as a control for experimental variability (Wong & Medrano, 2005). Quantification of copies was achieved through the standard curve method based on the Threshold cycle (Ct) for both the housekeeping gene and the genetically modified gene (Bt11) as outlined by Turkec *et al.* (2016).

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 (Yenilmez *et al.*, 2013) as follow:

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

MoM= Multiple of Median.

SC= standard curve (β-actin).

GM= genetic modified.

Ct= threshold.

Table (2): Primers utilized for detection and identification in PCR.

Gene	Primer	Amplicom length	Reference
T-nos (QL-ELE-00-007)	F 5'-GAATCCTGTTGCCGGTCTTG-3' R 5'-TTATCCTAGTTTGC GCGCTA-3'	180 bp	EURL GMFF, 2014
P35S(QL-ELE-00-04)	F 5'-CCACGTCTTCAAAGCAAGTGG-3' R5- 'TCCTCTCCAAATGAAATGAACTTCC-3'	123 bp	Lipp <i>et al</i> (1999)
Bt11	CTGGGAGGCCAAGGTATCTAAT GCTGCTGTAGCTGGCCTAATCT	189 bp	EURL GMFF, 2014
Zein	R-AGTGCGACCCATATTCCAG F-GACATTGTGGCATCATCATTT	277 bp	(Ismail, 2013)
Housekeepi ng gene β -actin	5'-TGG CAC CCG AGG AGC ACC CTG-3' 5'-GCG ACG TAC ATG GCA GGA ACA-3'	118bp	(Ismail, 2013)

Results and Discussion

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 1.8-2.1 and the DNA concentration range from 415 to 930 ng/ μ l.

Revelation of GM maize by Conventional PCR

PCR-based screening was conducted to detect four genes: i.e CaMV-35S promoter (a regular promoter found in GM constructs), BT-11 (a gene encoding

resistance proteins of an insecticidal and Glufosinate ammonium), the terminator gene. Plant- specific primers for the maize intrinsic *zein* (an endogenous maize gene) which is used to confirm the presence the samples of amplifiable maize DNA extracted from the samples. All samples did show presence of the *zein* gene as expected to show successful DNA extraction and amplification. Genetic control elements such as the CaMV-35S promoter and T-Nos are present in around 77% and 68% respectively of the maize genotype samples (Table 3 and figure 1). Interestingly, across the tested samples, the BT-11 gene showed a high prevalence and all samples showed positive amplification.

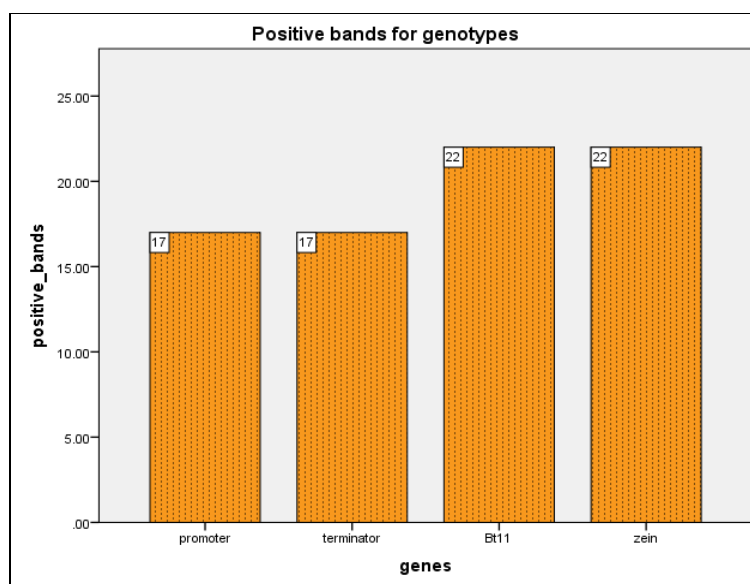


Figure (1): Number of positive bands for CaMV-35S, zein, BT-11 and NOS terminator genes.

Table (3): Detection of CamV35S, zein, BT-11 and NOS terminator genes in twenty-two genotypes of maize.

No.	Genotype	CaMV-35S promoter	zein	BT-11	NOS Terminator
1	Al-Maha	positive	positive	positive	positive
2	AL-Fajir 1	positive	positive	positive	negative
3	Baghdad 3	positive	positive	positive	positive
4	Sumer	positive	positive	positive	positive
5	5018	positive	positive	positive	negative
6	Sarah	positive	positive	positive	positive
7	AGN	positive	positive	positive	positive
8	Buhooth 106	negative	positive	positive	negative
9	Gyson	positive	positive	positive	positive
10	Julee	positive	positive	positive	positive
11	EB-110F1	positive	positive	positive	positive
12	BABEL/F1	positive	positive	positive	positive
13	Cadez	positive	positive	positive	negative
14	Furat	negative	positive	positive	negative
15	TW-78	positive	positive	positive	positive
16	TW-345	positive	positive	positive	positive
17	IY-355	negative	positive	positive	negative
18	IY-207	positive	positive	positive	positive
19	Drachma	negative	positive	positive	positive
20	666	positive	positive	positive	positive
21	777	negative	positive	positive	negative
22	PL-700	positive	positive	positive	positive

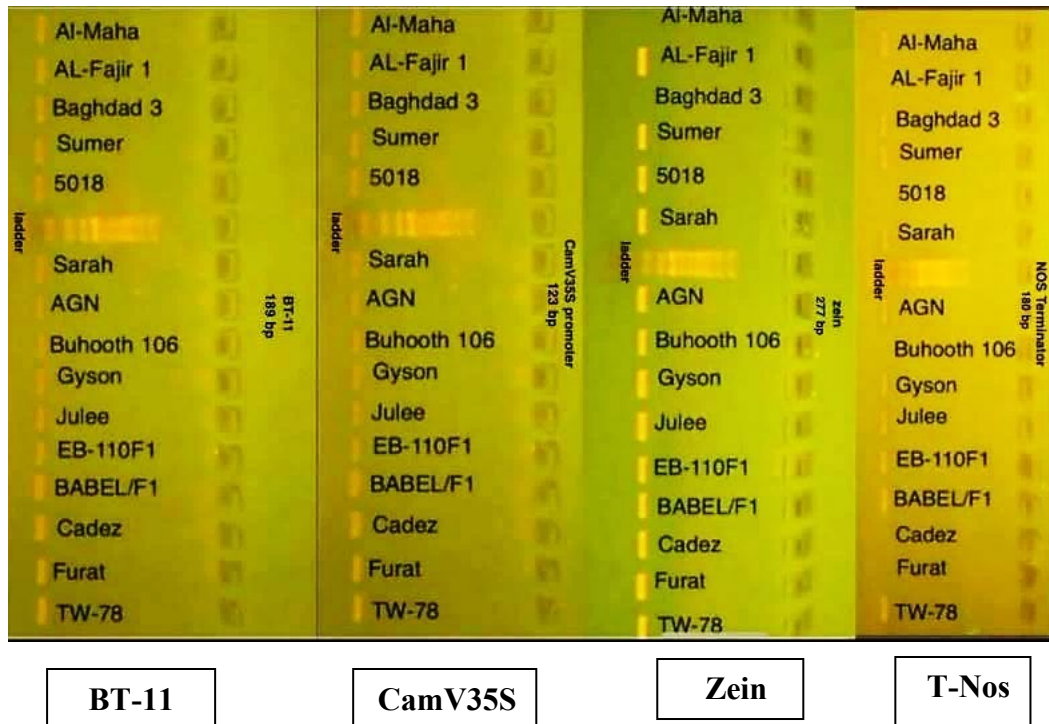


Figure (2): Electrophoresis of four genes for some genotypes of maize

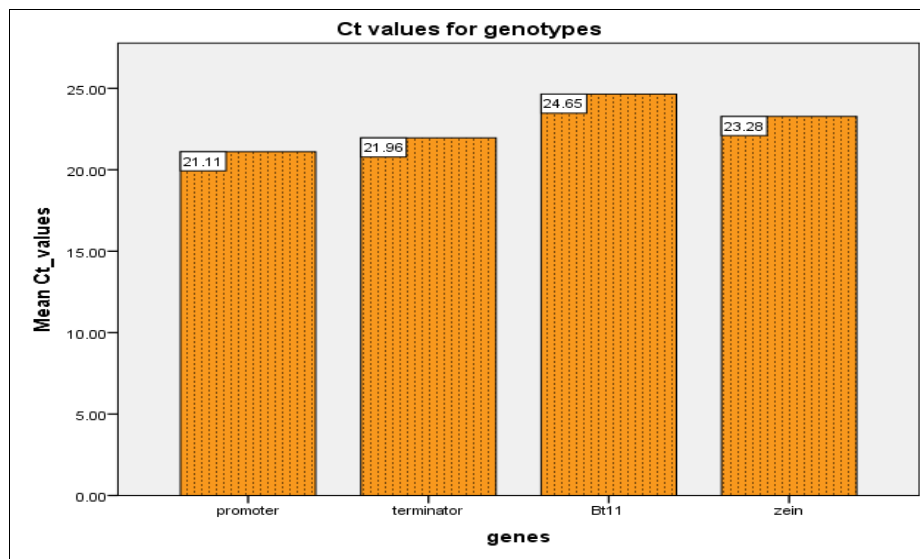


Figure (3): Mean Ct values for CaMV-35S , BT-11and NOS terminator genes for twenty-two genotypes of maize.

Revelation of GM maize by Real-time PCR

Through the current study, it has been found that Ct values ranged 17.35-23.13 for CaMV-35S with average 21.11 (table 4 and figure 3). Eight genotypes don't have the CaMV-35S, including Sumer, AGN, Buhooth 106, Julee, Furat, IY-355, Drachma and 777. Ct values

ranged 20.66-23.25 for Not-T with average 21.95. Amplification of the T-NOS was not detected in 7 genotypes including AL-Fajir 1, Buhooth 106, Julee, Cadez, Furat, IY-355 and 777. Amplification of the Bt11 gene was observed in all genotypes with average 24.64.

Table (4): Multiple of Median (MoM) for CaMV-35S promoter in twenty-two genotypes of maize.

No.	Plant	Ct (pro)	Ct (ter)	Ct Bt11	Ct T actin
1	Al-Maha	17.35	21.45	23.39	23.34
2	AL-Fajir 1	22.26	-	23.41	24.43
3	Baghdad 3	20.43	23.1	23.59	23.11
4	Sumer	-	21.38	24.79	23.29
5	5018	23.13	23.25	27.67	23.16
6	Sarah	21.06	22.47	25.78	23.59
7	AGN	-	23.01	27.76	23.83
8	Buhooth 106	-	-	28.1	23.4
9	Gyson	21.43	22.09	23.61	22.66
10	Julee	-	-	23.85	23.15
11	EB-110F1	22.32	22.41	22.97	22.89
12	BABEL/F1	21.68	21.71	24.09	23.5
13	Cadez	21.61	-	24.97	23.48
14	Furat	-	-	25.63	23.23
15	TW-78	21.07	21.26	23.64	22.59
16	TW-345	20.66	20.66	24.78	22.87
17	IY-355	-	-	24.73	23.78
18	IY-207	21.02	22.46	24.53	23.23
19	Drachma	-	21.81	24	23.31
20	666	21.33	20.75	24.3	23.2
21	777	-	-	23.45	23.24
22	PL-700	20.17	21.58	23.24	22.78
Avr		21.11±0.35	21.96±0.21	24.65±0.32	23.27±0.08

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

The Ct values were consistent 22.59 to 24.43 with an average 23.27 for the endogenous gene and 17.35 to 23.13 with an average while the lowest values of MoM were 1.039 for genotypes sarah and EB 110F1 respectively.

The Ct average for housekeeping gene for maize genotypes was 23.27 while the Ct

21.11 for the studied genotypes of CaMV-35S, suggesting a high representation of GMO maize in the Iraqi market (table 5). The highest of MoM values were 1.25 and 1.12 Al-maha and Baghdad 3 genotypes respectively, average for T-Nos terminator was 21.96 for the studied genotypes, therefore most MOM values were higher than 1. Nevertheless, some genotypes showed MoM values less than 1 (Baghdad, 5018, sarah and AGN) that

represent low gene expression. The results of the amplification of the specific primer of T-Nos terminator showed that the lowest records of MOM value was 0.95 for genotype

Sarah genotypes, Whereas the highest MOM value was 1.24 for PL-700 genotype (table 5)

Table (5): Multiple of Median (MoM) for CaMV-35S promoter and T-Nos in twenty-two genotypes of maize.

No.	genotype	CaMV-35S	T-Nos
1	Al-Maha	1.253120964	1.076625054
2	AL-Fajir 1	1.04175635	-
3	Baghdad 3	1.120533793	0.999566912
4	Sumer	-	1.013837638
5	5018	1.004304778	0.986044483
6	Sarah	1.093413689	0.958275382
7	AGN	-	0.998258598
8	Buhooth 106	-	-
9	Gyson	1.077486009	1.049892473
10	Julee	-	-
11	EB-110F1	1.039173483	1.063518596
12	BABEL/F1	1.066724064	1.084738617
13	Cadez	1.069737409	-
14	Furat	-	-
15	TW-78	1.092983211	1.054270463
16	TW-345	1.110632802	1.092665788
17	IY-355	-	-
18	IY-207	1.095135601	1.013181019
19	Drachma	-	1.061531842
20	666	1.081790788	1.078187472
21	777	-	-
22	PL-700	1.131726216	1.249913104
Avr		1.09±0.01	1.05±0.01

Estimation of Copy Number of Bt11 gene

Copy numbers were estimated in the range of 27-28 with average 28. The EB-110F1

genotype had greatest copy number, while lowest value was for Buhooth 106 genotype (table 6). About 12 genotypes had copy number above the average.

Table (6): Copy Number of Bt11 for twenty-two genotypes of maize.

No.	Plant	Copy Number
1	Al-Maha	28.8
2	AL-Fajir 1	28.8
3	Baghdad 3	28.7
4	Sumer	28.3
5	5018	27.3
6	Sarah	28.0
7	AGN	27.3
8	Buhooth 106	27.1
9	Gyson	28.7
10	Julee	28.6
11	EB-110F1	28.9
12	BABEL/F1	28.5
13	Cadez	28.2
14	Furat	28.0
15	TW-78	28.7
16	TW-345	28.3
17	IY-355	28.3
18	IY-207	28.4
19	Drachma	28.6
20	666	28.5
21	777	28.8
22	PL-700	28.8
Avr		28.4± 0.11

Discussion

This work aimed to identify genetically modified maize (GMM) in Iraqi seeds utilizing the 35 S promoter, T-nos terminator, and Bt11 for the comprehensive screening of transgenic materials. The maize crop Bt-11 is engineered for pest resistance and herbicide tolerance and has received approval from the EU for use as food or feed. The Cauliflower Mosaic Virus 35S promoter is found in approximately 95% of

commercially commercialized GMO plants in the EU (Rabiei *et al.*, 2013). The endogenous gene zein was utilized to verify the presence of amplifiable maize DNA isolated from maize genotypes. All genotypes have exhibited the zein gene. This gene comprises plant-specific primers for maize intrinsic, utilized by numerous researches to verify the presence of amplifiable maize DNA isolated from maize (Branquinho *et al.*, 2013; Rabiei *et al.* 2013; Avsar *et al.*, 2020).

Conventional PCR was used to detect GM genotypes. 22 (100%) of the positive references (Bt 11) have been detected, while CaMV-35S promoter and T-Nos are present in around 17 (77%) and 15 (68%) respectively of the corn genotype samples. Four genotypes (Buhooth 106, Furat, IY-355, and 777) were not owned both CMV 35S and Nos-T. Notably, the genotypes Buhooth 106 and Furat are local, while the two others are introduced. Aburummana *et al.* (2020) showed a positive response to the 35 S promoter by 100%, while Rabiei *et al.* (2013) found 20% positive results with CaMV35s primers in a study of the detection of genetically modified maize in processed foods sold commercially in Iran. However, varied results could be obtained using the 35S-primer for PCR analysis (Lipp *et al.*, 1999; Lin *et al.*, 2000).

Revelation of GM maize by Real-time PCR was also used. This technique depends on Ct values. The average of Ct values was 21.11 in fourteen genotypes for CaMV-35S. The Ct values of Nos-T was tiny, bigger than CaMV-35S (21.95) in 15 genotypes, while the Ct value of the Bt11 gene was observed in all genotypes with a higher mean (24.64). A potential false positive result may arise if test plant samples are contaminated or infected by the cruciferae crops, as the 35S-promoter is derived from the Cauliflower mosaic virus. The non-GM plant is devoid of the NOS-terminator. Nonetheless, a NOS-terminator may be present in the roots of non-GM plants, leading to a false positive PCR result. The NOS-terminator derives from *Agrobacterium tumefaciens*, a soil-dwelling bacteria that can taint plant roots (Lin *et al.*, 2000). Multiple of Median (MoM) equation is applied to find out the level of the gene expression of the copy of genetically modified genes depending on

Ct values. The lower Ct value of the interested gene compared to the housekeeping gene is an indicator of increased genetic transgenic content.

Multiple of Median (MoM) equation has used for both CaMV-35S NOS terminator. MOM values were adopted as 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 an abnormal limit (Heinemann, 2012). A similar MOM average has been obtained between promoter and terminator for both genotypes. Most genotypes have shown MOM values of more than 1. For promoter, the MOM average was 1.09. For Terminator, the MOM average was 1.05. However, some genotypes own MOM values less than one.

Copy numbers of the Bt11 gene were estimated using a dilution series of positive control (certified reference materials). The average copy number of genotypes was 28. The EB-110F1 genotype had the greatest copy number, while the lowest value was for the Buhooth 106 genotype (table 6). Aburummana *et al* (2020) reported that seven out of 11 (63.6%) of the tested maize seed samples were genetically modified by the Bt11. Evolutionary processes—such as gene duplication, polyploidization, and segmental duplications can also cause this variation in copy number as a source of genetic diversity or as a source of functional adaptations (Birchler & Yang, 2022). The presence of modified genes in some genotypes does not necessarily mean the existence of transgene(s) in this genotype, as genetic mixing between local varieties and introduced genotypes is a common problem that is difficult to control. The transfer of genes from genetically modified crops (GMC) to unrelated crops that are

incompatible (whether they belong to the same species or different species or genera) is called horizontal gene transfer (HGT). Genetic contamination can occur through the transfer of pollen grains or the mechanical mixing of seeds. Although the numbers and sizes of chromosomes differ between different species, cross-pollination contamination is likely to occur, especially in the absence of appropriate isolation distances (Brock *et al.*, 2020).

Conclusion

Twenty-two genotypes of maize samples were screened for the presence of GMOs. CaMV-35S, T-Nos and Bt-11 genes were detected in most the studied maize genotypes using conventional PCR and real time qPCR, reinforcing the need for a mandatory labeling system . Revision of the laws and establishment of consistent national screening protocols could increase both food industry compliance, and consumer confidence.

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Contributions of authors

D.K.I.: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

M.Sh.H.: Suggestion the proposal of the article, wrote and revised the manuscript, identification of the parasite.

ORCID

M.Sh.H.: <https://orcid.org/0000-0003-1531-411X>

Conflicts of interest

The authors declare that they have no conflict of interests.

References

- Aburumman A., Migdadi H., Akash M., Al-Abdallat A., Dewir YH., & Farooq M. (2020). Detection of Genetically Modified Maize in Jordan. *GM crops & food*, 11(3), 164-70. <https://doi.org/10.1080/21645698.2020.1747353>
- Al-Salihy, A.; Ismail, E., & Salih, M. (2020). Determination of ZmHKT1, 5 Gene Expression under Different Salt Stresses Using Plant Tissue Culture of Maize (*Zea mays* L.). *Iraqi Journal of Agricultural Science*, 51(4), 1226-1230. <https://doi.org/10.36103/ijas.v51i4.1102>
- Ali, A. M., Ali, A. A., & Abbas, B. A. (2024). Effect of Shelling Time and Sheller Feeding Rate of Locally Sheller in Some Mechanical and Physical Traits of The Process of Maize Shelling. *Kufa Journal for Agricultural Sciences*, 16(1), 65-72. <https://doi.org/10.36077/kjas/2024/v16i1.10766>
- Al-Saadi, W.K., Hamdalla, M.S., Aljuaifari, W., 2024. Morphological, Biochemical and Proline-Related Genes Analyses in Resistant and Susceptible Wheat Cultivars in Iraq. *Basrah Journal of Agricultural Sciences* 37, 119-133.
- Avsar, B., Sadeghil,S., Turkec, A., & Lucas, S.J.(2020). Identification and Quantitation of Genetically Modified (GM) Ingredients in Maize, Rice, Soybean and Wheat-Containing Retail Foods and Feeds in Turkey. *Food Science and Technology*, 57(2), 787–793. <https://doi.org/10.1007/s13197-019-04080-2>
- Birchler J.A., & Yang H.(2022). The Multiple Fates of Gene Duplications: Deletion, Hypofunctionalization, Subfunctionalization, Neofunctionalization, Dosage Balance Constraints, and Neutral Variation. *Plant Cell*, 34(7), 2466-74. <https://doi.org/10.1093/plcell/koac076>
- Branquinho, M.R., Gomes, D.M., Ferreira, R.T., Ferreira, R.L., & Leitte, P.C. (2013). Detection of Genetically Modified Maize Events in Brazilian Maize-Derived Food Products. *Food Science and Technology*, 33(3), 399-403. DOI: <http://doi.org/10.1590/S0101-20612013005000063>

- Brock, J. R., Scott, T., Lee, A. Y., Mosyakin, S. L., & Olsen, K. M. (2020). Interactions between Genetics and environment Shape Camelina Seed Oil Composition. *BMC Plant Biology*, 20, 1-15.
<https://doi.org/10.1186/s12870-020-02641-8>
- European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF). (2024).
<https://gmo-crl.jrc.ec.europa.eu/>
- Hassan, W., Hadi, B.H., & Hamdalla, M.Sh.(2019). Evaluation of Maize Hybrids, Their Inbred Lines and Estimation of Genetic Divergence Based on Cluster Analysis. *Indian Journal of Ecology*, 46 Special Issue (8,): 102-107.
https://www.researchgate.net/publication/339213000_Evaluation_of_Maize_Hybrids_their_Inbred_Lines_and_Estimation_of_Genetic_Divergence_Based_on_Cluster_Analysis
- Heinemann, J. A. (2012). Evaluation of Risks from Creation of Novel RNA Molecules in Genetically Engineered Wheat Plants and Recommendations for Risk Assessment. *UCResearch repository; Report 40*.
- ISAAA. (2011). *International Service for The Acquisition of Agri-biotech Application*. Available from: URL. <http://www.isaaa.org>
- Ismail, E. N. (2013). *Determination of Gene Expression of Salt Tolerant Gene TaGSK1 in Wheat Cultivars*. M.S.C. Thesis, institution of Genetic Engineering and Biotechnology, University of Baghdad, Iraq. PP: 128.
- [HTTPS://DOI.ORG/10.21608/EAJBSH.2014.16832](https://doi.org/10.21608/EAJBSH.2014.16832)
- James, C. (2012). *ISAAA briefs. Global Status of Commercialized Biotech/GM Crops*.
<https://www.isaaa.org/resources/publications/briefs/44/>
- Lin, H.Y., Chiueh, L.C., & Shih, D.C. (2000). Detection of Genetically Modified Soybeans and Maize by the Polymerase Chain Reaction Method. *Journal of Food and Drug Analysis*, 8(3), 8. <https://doi.org/10.38212/2224-6614.2836>
- Lipp, M., Brodmann, P., Pietsch, K., Pauwels, J., & Anklam, E. (1999). IUPAC Collaborative Trial Study of A Method to Detect Genetically Modified Soybeans and Maize in Dried Powder. *Journal of Analytical Chemistry and Microbiology International*, 82(4), 923-928.
<https://pubmed.ncbi.nlm.nih.gov/10490320/>
- Majeed, D. (2018). Detection of Salt Tolerance Gene in Three Selected Wheat Genotypes for Salinity Stress under Salinity Conditions. *Iraqi Journal of Agricultural Sciences*, 49 (3).
<https://doi.org/10.36103/ijas.v49i3.120>
- Querci, M., Kagkli, D. M., Gatto, F., Foti, N., Maretti, M., & Mazzara, M. (2006). The Analysis of Food Samples for The Presence of Genetically Modified Organisms. Technical Reports-User Manual. <https://data.europa.eu/doi/10.2760/5277>
- Rabiei, M., Mehdizadeh, M., Rastegar, H., Vahidi, H., & Alebouyeh, M. (2013). Detection of Genetically Modified Maize in Processed Foods Sold Commercially in Iran by Qualitative PCR. *Iranian journal of pharmaceutical research*, 12(1), 25. PMID: PMC3813200
<https://pubmed.ncbi.nlm.nih.gov/24250568/>
- Russell, D. W. S & Sambrook, J. (2006). *The Condensed Protocols From Molecular Cloning: A laboratory Manual*, Press, Cold Spring Harbor Laboratory Press. ISBN.0879697725, 9780879697723
https://books.google.iq/books/about/The_Condensed_Protocols_from_Molecular_C.html?id=iOloQgAACAAJ&redir_esc=y
- Suryani, R. ., Suliansyah, I. ., Warnita, W. ., Zainal, A. ., & Sukartini, S. (2024). The Molecular Characterization of Local Bengkulu Ambon Banana Through Chloroplast Simple Sequence Repeats (SSR) Markers. *Basrah Journal of Agricultural Sciences*, 37(1), 36-46.
<https://doi.org/10.37077/25200860.2024.37.1.03>
- Turkec, A., Lucas, S. J., & Karlik, E. (2016). Monitoring The Prevalence of Genetically Modified (GM) Soybean in Turkish Food and Feed Products. *Food Control*, 59, 766-772.
<https://doi.org/10.1016/j.foodcont.2015.06.052>
- Tuteja N, Verma S, Sahoo R, Raveendar S., & Reddy I.(2012). Recent Advances in The Development of Marker-free Transgenic Plants: Regulation and Biosafety Concern. *Journal of Biosciences*, 37:167-97.
<https://doi.org/10.1007/s12038-012-9187-5>
- Wong, M. L., & Medrano, J. F. (2005). Real-time PCR for mRNA Quantitation. *Biotechniques*,

- 39(1), 75-85.
https://doi.org/10.2144/05391RV01
- Wuhaib, K. (2018). Genotypic and Phenotypic Correlation in Maize and Path Coefficient I-Agronomic traits. *Iraqi Journal of Agricultural Sciences*, 49(2).
https://doi.org/10.36103/ijas.v49i2.462
- Yenilmez, E. D., Tuli, A., & Evrücke, İ. C. (2013). Noninvasive Prenatal Diagnosis Experience in The Çukurova Region of Southern Turkey: Detecting Paternal Mutations of Sickle Cell Anemia and β -thalassemia in Cell-free Fetal DNA Using High-resolution Melting Analysis. *Prenatal diagnosis*, 33(11), 1054-1062.
https://doi.org/10.1002/pd.4196
- Yeung, A. W. K., Tzvetkov, N. T., Gupta, V. K., Gupta, S. C., Orive, G., Bonn G. K., & et al. (2019). Current Research in Biotechnology: Exploring the Biotech Forefront. *Current Research in Biotechnology*, 1, 34-40.
https://doi.org/10.1016/j.crbiot.2019.08.003
- Yousif, E. (2023). Molecular Detection of New *Stretomyces* SPP. from Iraqi Oil Contaminated Soil. *Iraqi Journal of Agricultural Sciences*, 54(5), 1298-1304.
https://doi.org/10.36103/ijas.v54i5.1828
- Zaidan, S. A., Mohsin, K. H., & Muhsin, S. J. . (2019). Effect of Genotypes and Tillage Systems in some Growth Characteristic of Maize (*Zea mays* L.). *Basrah Journal of Agricultural Sciences*, 32(2), 7-15.
https://doi.org/10.37077/25200860.2019.182
- Zimmermann, A., Hemmer, W., Liniger, M., Lüthy, J., & Pauli, U. (1998). A Sensitive Detection Method for Genetically Modified MaisgardTMcorn Using A Nested PCR-System. *LWT-Food Science and Technology*, 31(7-8), 664-667.
https://doi.org/10.1006/fstl.1998.0422

تحديد بعض الطرز الوراثية للذرة المحورة وراثيا التي تم جمعها من الأسواق المحلية في بغداد

دينا كريم أبراهيم¹ و ماجد شايح حمدالله²

^{2,1} فرع الهندسة الوراثية، معهد الهندسة الوراثية والتقانات الاحيائية للدراسات العليا، جامعة بغداد، العراق

المستخلص: الكائنات المعدلة وراثيا هي تلك الكائنات التي يتم تغيير الجينات فيها بواسطة تقانة تعديل الجينات (gene modification) وليس عن طريق التهجين الطبيعي أو إعادة تركيب الحمض النووي (DNA recombination). تم فحص اثنين وعشرين تركيبا وراثيا من الذرة للكشف عن التعديل الوراثي فيها والتي تم جمعها من الأسواق المحلية في بغداد. تم أخذ ثلاث عينات لكل تركيب وراثي في الفترة من كانون الاول 2023 إلى كانون الثاني 2024. تم استخراج الحمض النووي باستخدام مجموعة الاستخلاص plantZol. تم أستخلاص الحمض النووي بطريقة CTAB للمادة المرجعية (reference material) لعينة المقارنة الإيجابية لجين Bt-11. تم استخدام تفاعل البوليميراز المتسلسل التقليدي (PCR) وتفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي (qPCR) للكشف عن تراكيب الذرة المعدلة وراثيا. تم استخدام ثلاثة أزواج من البادئات لجينات CaMV-35S و NOS و Bt-11، بالإضافة إلى بادئات الجينات zein و β -actin. وجد أن 77% و 68% و 100% من العينات تحمل جينات CaMV35s و Nos و Bt-11 حسب تقانة PCR التقليدية مما يشير إلى انتشار واسع للذرة المعدلة وراثيا في السوق العراقية. كانت قيم Ct للجينات المعدلة أعلى من نظيرتها من جين housekeeping (β -actin) مما يشير إلى احتمال ارتفاع تواجد الذرة المعدلة وراثيا في الاسواق العراقية. كان متوسط قيم MOM لجينات CaMV-35S و T-Nos 1.09 و 1.05 على التوالي. وكان متوسط عدد النسخ لـ 22 تركيبا وراثيا من عينات الذرة لجين Bt-11 28.4. أظهرت الدراسة الحالية انتشار الجينات المعدلة وراثيا وتلوث التراكيب المحلية للذرة بالجينات المعدلة وراثيا وهذا الأمر يتطلب إجراء مسح مستمر للجينات المحلية للمحاصيل المنزرعة في البيئة العراقية مع ضرورة تفعيل التشريعات التي تحد من انتشار المواد المعدلة وراثيا.

الكلمات المفتاحية: الذرة، الكائنات المعدلة وراثيا (GMO), GMO, Bt-11, CamV35S, T-N