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Original Article

Detection and occurrence of genetically modified rice and potato in the Saudi food market

Detecção e ocorrência de arroz e batata geneticamente modificados no mercado de alimentos saudita

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Abstract

The number of food products with genetically modified (GM) crops on the global market has increased due to advancements in genetic engineering technology. Legislation regulating the labeling and use of GM crops has increased considerably worldwide to provide consumers with health and safety assurance. It is still unclear whether genetically modified organisms (GMOs) are present in the food market of the Kingdom of Saudi Arabia due to a lack of scientific studies. This work was planned to detect GM rice and GM potatoes in the Saudi food market. One hundred non-labeled rice and rice product samples and 50 potato and potato samples were collected randomly from different market sites of Makkah, Riyadh and Jeddah during 2022-2023. The cetyl trimethyl ammonium bromide (CTAB) method was used to extract DNA. Viviants DNA extraction kit was used to extract DNA from rice starch and potato chips. To find GMOs in samples, CMOScreen 35S and NOS test kits were utilized. DNA-based qualitative and quantitative approaches were used to screen targets for PCR detection of GM rice sequences. The results indicated that 32 (32%) rice samples were positive for CaMV 35S promoter, while no positive result was detected for the NOS terminator. Besides, 30% of potato samples were positive for the CaMV 35S promoter, and the same samples were positive for the presence of the Cry V gene. It could be concluded that there were GM rice and potatoes in the Kingdom of Saudi Arabia's food markets. Establishing strong regulations and certified laboratories to monitor genetically modified foods (GMF) or crops in the Saudi market is recommended.

Keywords: genetically modified organisms, GMOs, detection method, DNA, extraction, polymerase chain reaction (PCR), transgenic plants, food safety.

Resumo

O número de produtos alimentícios com culturas geneticamente modificadas (GM) no mercado global cresceu devido aos avanços na tecnologia de engenharia genética. A legislação que regulamenta a rotulagem e o uso de culturas GM aumentou consideravelmente em todo o mundo para fornecer aos consumidores garantia de saúde e segurança. Ainda não está claro se organismos geneticamente modificados (OGM) estão presentes no mercado de alimentos do Reino da Arábia Saudita em razão da falta de estudos científicos. Este trabalho foi planejado para detectar arroz e batatas GM no mercado de alimentos saudita. Cem amostras de arroz e produtos de arroz não rotulados e 50 amostras de batata e produtos à base de batata foram coletadas aleatoriamente de diferentes mercados de Meca, Riad e Jeddah durante 2022-2023. O método de brometo de cetiltrimetilamônio (CTAB) foi usado para extrair DNA. O kit de extração de DNA Viviants foi usado para extrair DNA de amido de arroz e batatas fritas. Para encontrar OGMs em amostras, foram utilizados os kits de teste CMOScreen 35S e NOS. Abordagens qualitativas e quantitativas baseadas em DNA foram usadas para rastrear alvos para detecção de PCR de sequências de arroz GM. Os resultados indicaram que 32 (32%) amostras de arroz foram positivas para o promotor CaMV 35S, enquanto nenhum resultado positivo foi detectado para o terminador NOS. Além disso, 30% das amostras de batata foram positivas para o promotor CaMV 35S, e as mesmas amostras foram positivas para a presença do gene Cry V. Pode-se concluir que havia arroz e batatas GM nos mercados de alimentos do Reino da Arábia Saudita. É recomendável estabelecer regulamentações rigorosas e laboratórios certificados para monitorar alimentos geneticamente modificados (GMF) ou safras no mercado saudita.

Palavras-chave: organismos geneticamente modificados, OGMs, método de detecção, DNA, extração, reação em cadeia da polimerase (PCR), plantas transgênicas, segurança alimentar.

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1. Introduction

One of the main crops grown worldwide is rice, which provides body calories to about 50% of the world's population (Chen et al., 2011; Rahnama et al., 2023; Shin et al., 2023; Huang et al., 2024). Genetic engineering can increase and stabilize productivity, improve nutrition, fight disease and insects, tolerate herbicides, and endure abiotic challenges compared to traditional methods. Plant transformation techniques were used to create genetically modified (GM) rice for the first time in 1988 (Fraiture et al., 2016; Bajaj and Mohanty, 2005; Rahnama et al., 2023). Quality improvement and decreased field-applied pesticides and herbicides were the goals of GM rice cultivation, which could not be accomplished using conventional breeding techniques (Helliwell and Yang, 2013; Tang et al., 2009; Shin et al., 2022; Singh et al., 2023). Meanwhile, 185.1 million hectares of GM crops were grown worldwide in 2016. Even with most engineering plants (soybean, maize, cotton, and canola), transgenic rice is still very rare and primarily developed in Asia, where commercialization of these GM variants has not been permitted. Despite prohibitions, unauthorized genetically modified (GM) rice has been reported (Price and Cotter, 2014). China is the world's biggest producer of rice, and rice cultivation accounts for 20% of the planted area (Chen et al., 2011).

Transgenic rice, genetically altered in Iran thirteen years ago by adding the Cry1Ab gene from the *Bacillus thuringiensis* bacteria, is not allowed commercially. This gene makes the plant more insect-resistant, increasing yield (De Steur et al., 2014). According to Statistics Korea, 3.74 million tons of rice were produced in Korea in 2019. The first GM rice was created in 1988 using techniques such as protoplast transformation mediated by polyethylene glycol or electroporation (Fraiture et al., 2016). Since 1988, rice has been genetically modified to acquire several characteristics, including resistance to abiotic stressors, higher nutritional value, and resistance to pests and illnesses (Bajaj and Mohanty, 2005). In 2020, according to the GM Approval Database (ISAAA, 2024) of the International Service for the Acquisition of Agri-biotech Applications (ISAAA), seven GM rice events have been developed, namely GM shanyou63, GR2E, Huahui-1/ TT51-1, LLRice06, LLRice601, LLRice62, and Tarommolaii +cry1Ab. China is home to well-known transgenic rice lines Bt63 (TT51-1), Kemingdao1 (KMD1), Kefeng 6, and Kefeng 8. China obtained the safety clearance for the hybrid Cry1Ab/Ac gene, an insect-resistant characteristic, in 2009. This gene is included in the Bt63. With a synthetic cry1Ab gene, the KMD1 rice line is resistant to lepidopteran pest species. Two insect-resistant genes, cry1Ac and cowpea trypsin inhibitor (CpTI) gene, are present in Kefeng6 and Kefeng8 (Lu et al., 2016; Wang et al., 2012). According to Oberdoerfer et al. (2005), the LLRice62 carries the glufosinate herbicide-tolerant bar gene and phosphinothricin-Nacetyltransferase (PAT) proteins. China was found to be cultivating unapproved GM rice illegally in 2005 (Zi, 2005). Bayer's LLRice601 and LLRice62 were discovered in 2006 (Greenpeace International, 2006). The Rapid Alert System for Food and Feed (RASFF) reported that Bt63 was also found in imported food products and detected in Europe (Lu et al., 2016; Price and Cotter, 2014).

For these reasons, identifying and analyzing unapproved GM crops or goods requires precise and trustworthy detection techniques. Bt63 was also discovered in imported food products and detected in Europe, according to the Rapid Alert System for Food and Feed (RASFF) (Lu et al., 2016; Price and Cotter, 2014). For these reasons, accurate and reliable detection procedures are necessary to identify and analyze unapproved GM commodities or crops. Most studies investigating GM rice screening assays sought to identify GMOs using easy-to-use and reasonably priced techniques (Safaei et al., 2019; Zhang et al., 2015). The detection techniques for more GM rice varieties, like M12 and G6H1, have recently been developed (Deng et al., 2020; Xu et al., 2019). PCR and real-time PCR are the traditional and most commonly used techniques for detecting GMOs. Several PCR-based methods have been developed to identify and measure GMOs in food and feed (Deisingh and Badrie, 2005). Corn events like Bt176 (Kuribara et al., 2002), Bt11 (Kuribara et al., 2002), NK603 (Huang and Pan, 2004), CBH-351 (Windels et al., 2003), T25 (Collonnier et al., 2005), GA21 (Kuribara et al., 2002), and GM potato spunta (Elsanhoty et al., 2005, 2006; Ramadan and Elsanhoty, 2012) were all characterized using real-time PCR. Furthermore, Elsanhoty et al. (2011) compared six distinct techniques for obtaining DNA from unprocessed maize and its derived products. When Cardarelli et al. (2005) examined food products in Brazil for the presence of the Cauliflower Mosaic Virus promoter (CaMV 35S and (nos) 3-terminator, and Agrobacterium tumefaciens nopaline synthase terminator (NOS), they detected positive samples for GMOs. Elsanhoty et al. (2002) examined samples of maize and soybeans from the Egyptian food market. The findings unequivocally showed that GM maize and soybeans are common in the Egyptian market. Rott et al. (2004) discovered that soy samples from Canadian stores tested positive for GMOs. To identify GM soy (Roundup-Ready® (RR) soy) and maize (Bt176 Maximizer maize; Bt11 maize, MON 810 Yield Gard corn, and T25 LibertyR Link maize) in Brazilian processed foods. Greiner et al. (2005) used qualitative and quantitative PCR-based approaches. In 18 out of 80 samples, Abdullah et al. (2006) discovered the NOS terminator and the 35S promoter. Also, GMOs were screened in food products (soya bean products, maize flour, and rice)from the Czech markets (Kyrova et al., 2018).

In a recent study, Suad and Alreshidi (2024) screened the GMOs in food and feed products in Kuwait's market using DNA-based methods and GMO sequences in products derived from corn, soybean, and rice. In contemporary plant breeding, genetic engineering, or recombinant DNA technology, has created plants with enhanced nutritional and agronomic qualities. Huang et al. (2024) used RapiSense to detect specific DNA and fragmented RNA in GM variants of sweet potato and rice, showcasing its potential for rapid, on-site GM plant screening. Besides, Rahnama et al. (2023) compared the morphology and composition of a GM potato expressing mannitol-1-phosphate dehydrogenase (mtlD) with its non-GM counterpart. The analysis of the mtlD-GM potato plant revealed substantial equivalence

with its non-GM counterpart. Moreover, Singh et al. (2023) developed visual and real-time LAMP assays targeting the T-pinII terminator sequence for screening GM crops/events, including potatoes.

It became essential to detect GMO crops to give consumers the freedom to select products and adhere to labeling laws. Saudi Arabia mostly depends on imported (60-75%) food and crops, but genetic alteration is not considered; instead, the control of these foods and crops is based solely on their nutritional value, allowable mycotoxin levels and heavy metals pollution. Therefore, this work was designed to investigate and identify GM varieties in rice and potato samples collected from Saudi traditional markets, supermarkets, and grocery shops.

2. Materials and Methods

2.1. Certified reference materials

The EU's Institute of Reference Materials and Measurements (IRMM, Geel, Belgium) provided certified reference materials (CRMs). The references were two commercially available GM varieties (Roundup Ready soy 5% and Bt 11 maize 5%) with GM target sequences, and they were utilized as the study's positive controls.

2.2. Rice and potato sample collection

Tables 1 and 2 present rice samples and potato samples under investigation. One hundred (100) rice and rice product samples and 50 samples of potato products were collected from traditional markets, supermarkets, and grocery shops in Saudi Arabia from 2022-2023. All the samples were homogenized and ground using an electric homogenizer and stored at -20°C before DNA extraction.

2.3. Extraction of genomic DNA

Using the cetyl trimethyl ammonium bromide (CTAB) method, DNA was extracted using the official German maize and soybeans methods (Germany, 1998, 2002). Two separate techniques were used to obtain DNA. A 200 µL of autoclaved bidistilled water was used as a blank sample to control the reagents. After air drying, the DNA pellet was reconstituted in 100 µL of sterile water, bidistilled and deionized (Roche, Germany). The extracted DNA was kept at -20 °C until needed for the following procedures.

2.4. Assessment of the purity and quality of extracted DNA

Using an Ultra-Spec 2000 spectrophotometer (Pharmacia Biotech, USA) in comparison to a DNA standard with established quantities (Calf Thymus, final concentration of 25 ng/µL), the concentration and purity

Table 1. Rice products screened by 35S promoter and NOS terminator and analyzed for the presence of GM rice.

Tested raw material and processed products	Number of samples	Number of samples positive for 35S	Number of samples positive for NOS terminator
Egyptian rice	18	2	-
Indiana rice	18	6	-
Thailand rice	16	6	-
Rice starch	12	0	-
American rice	18	6	-
Rice imported from China	16	12	-
Total number of samples	100		-
Total number of positive samples		32	-

Table 2. Potato and potato products samples screened by 35S promoter and NOS terminator and analyzed for the presence of GM potato.

Tested raw materials and processed products	Number of samples	Number of samples positive for 35S	Number of samples positive for NOS terminator
Fresh potato from Makkah	9	3	5
Fresh potato from Jeddah	9	3	5
Fresh potato from Riyadh	9	3	5
Potato chips from Makkah	9	2	-
Potato chips from Jeddah	8	2	-
Potato chips from Riyadh	6	2	-
Total number of samples	50		15
Total number of positive samples		15	15

of extracted DNA were evaluated at 260 and 280 nm. Concentrations (ng/µL) and A260/A280 readings were noted for every sample. The extracted DNA concentration was determined and corrected to 20-25 ng/µL before PCR analysis using bidistilled and deionized water.

2.5. Oligonucleotide primers

Four primer pairs have been used to detect GM rice. Moreover, the sucrose phosphate synthase (SPS), soy lectin and maize invertase genes were utilized as rice-specific, soy-specific, and maize-specific endogenous reference genes, respectively. Table 3 indicates the oligonucleotide primer pair sequences and their target elements. Consequently, the quality and existence of extracted DNA from rice samples, soy CRM, and maize CRM were confirmed using the tree primer pairs, SPS-F/R, Lectin-F/R, and Invertase-F/R. Accurate detection of GM rice samples necessitates the CRMs' DNA quality determination. Given that these materials are provided as a control group during the investigation. Following the guidelines provided by the International Standard Organization (ISO, 2005), the primer pairs P-35S and T-nos were created (ISO, 2005). Based on previously released research, the SPS, lectin, and invertase gene sequences were acquired for primer design (Lipp et al., 2001; Cardarelli et al., 2005). Bio Synthesis (Inc., USA) produced all of the primers, which were all received lyophilized. All primers were dissolved before usage to yield a final 20 pmol/µL concentration.PCR was performed using a master mix and a thermocycler (Biometra, T1, Göttingen, Germany). A 2.5 µL of Reddy Mix buffer (10× concentrate, Thermo Scientific), 2 µL of MgCl2 solution (25 mM), 1 µL of deoxynucleotide (dNTP) solution (0.2 mM each of dATP, dCTP, dGTP, and dTTP), 0.5 µM of each primer, 0.625 Unit ThermoprimeTaq polymerase (Thermo Scientific), and 2 µL of template-extracted DNA were included in each PCR reaction mix's 25 µL total volume.

2.6. PCR conditions

Amplification reactions contained 2 µL of genomic DNA and an appropriate PCR reaction mixture. PCR reaction mixture including 12 µLready-to-use PCR master mix 2× (the composition: Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 3 mMMgCl$_2$, 0.2% Tween 20, 0.4 mM dNTPs, 0.2 units/µL AmpliqonTaq DNA polymerase, Inert red dye and stabilizer), 1 µL of each primer, and 9 µL sterile free ions distill water. The concentration of primers for all target genes was 0.1 µL. Finally, PCR assays were performed in a volume of 25 µL. The reaction conditions of PCR are as follows in Table 3. All amplicons were stored at 4°C until gel electrophoresis. PCR profile was indicated in Table 4.

2.7. Agarose gel electrophoresis

Tris-base/borate (TBE) buffer solution (pH 8.0) containing 45 mmol/L Tris-base/boric acid and 1 mmol/L EDTA adjusted with hydrochloric acid was used for agarose gel preparations and electrophoresis. DNA of known size (50 and 100 bp DNA marker, Roche Germany) and various amplicons were separated on 2% (w/v) agarose gel (LE, Roche) and stained with 0.01% ethidium bromide solution (0.5 mg/L) to ascertain the size of the DNA fragments. Before gel electrophoresis, ten microliters of each amplicon and DNA marker were stained with a 2 µLxylenecyanol dye solution (1 mg xylenecyanol, 400 mg sucrose, and 1 mL water). The electrophoresis was then run for 45 min. Ethidium bromide staining was used to

Table 3. Target elements and oligonucleotide primer pair sequences.

Primer	Sequence (5′-3′)	Fragment length	Target element	Reference
SPS-F	TTG CGC CTG AAC GGA TAT	277	SPS	(Cardarelli et al., 2005)
SPS-R	GGA GAA GCA CTG GAC GAG G			
P35S-cf3	CCA CGT CTT CAA AGC AAG TGG	123	P-35S	(Cardarelli et al., 2005; Lipp et al., 2001)
P35S-cr4	CCA CGT CTT CAA AGC AAG TGG			
HA-nos-118f	GCA TGA CGT TAT TTA TGA GAT GGG	118	T-NOS	(Cardarelli et al., 2005; Lipp et al., 2001)
HA-nos-118r	GAC ACC GCG CGC GAT AAT TTA TCC			
GM03	GCC CTC TAC TCC ACC CCC ATC C	118	Lectin	(ISO, 2005)
GM04	GCC CAT CTG CAA GCC TTT TTG TG			
IVR1-F	CCG CTG TAT CAC AAG GGC TGG TAC C	226	Invertase	(ISO, 2005)
IVR1-R	GGA GCC CGT GTA GAG CAT GAC GAT C			
CaMV35SF/	TCC ACT GAC GTA AGG GAT GAC	105	CaMV 35S promoter	(Franck et al., 1980)
CaMV35S	CTG GTG ATT TCA GCG TGTCC			
Spu-35S1_F/	CTTCGCAAGACCCTTCCTC	122	CaMV promoter and Cry V gene from Bacillus Thuringiensis in genetically modified potato *Spunta*	(El Sanhoty, 2004)
Spu-cryVm_R	GCTGGAGAACGATTGGTGC			

Table 4. Profiles of time/temperature for qualitative PCR with DNA extracted from certified reference materials of maize, soybean and rice samples using primer pairs.

Primer pair	Initial denaturation	Denaturation	Annealing	Extension	Number of cycles	Final elongation
GM03/GM04	10 min at 95°C	30 s at 95°C	30 s at 60 °C	1 min at 72°C	35	3 min at 72°C
P35s-f2/petu-r1	10 min at 95°C	30 s at 95°C	30 s at 62 °C	25 s at 72°C	35-40	10 min at 72°C
IVR1-F/IVR1-R	12 min at 95°C	30 s at 95°C	30 s at 64 °C	30 s at 72°C	42	10 min at 72°C
Cry03/Cry04	12 min at 95°C	30 s at 95°C	30 s at 63 °C	30 s at 72°C	38	10 min at 72°C
SPS-F/SPS-R	5 min at 94°C	30 s at 94°C	45s at58 °C	75 s at 72°C	35	8 min at 72°C
HA-nos-118f HA-nos-118r	5 min at 94°C	1 min s at 94°C	40 s for,60°C	1 min for 94°C	35	8 min at 72°C.
CaMV35S-F/ CaMV35S-R	2 min at 95°C	30s at 95°C	30s for 60°C	30 S at 72 C°	35	5 min at 72°C.
Spu-35S1_F/ Spu-cryVm_R	2 min at 95°C°	30 S at 95°C	30 S at 60°C	40 S at 72 °C	35	5 min at 72°C

make the amplicons visible, and Dolphine-View WealTech and UV transillumination were used to record the results.

3. Results and Discussion

3.1. DNA extraction

DNA was extracted based on the intricacy of the technology used and the makeup of the rice product samples. The food utilized in this investigation was categorized and explained in Table 1. Fatty acids, polysaccharides, polyphenols, and other substances that could obstruct or even destroy DNA isolation are typically present in the samples and can impede PCR (Holden et al., 2003; Porebski et al., 1997). To identify the band corresponding to the genomic DNA, DNA was quantified on agarose for all samples and various DNA extractions (Figure 1 and Figure 2). According to the results, there were variations in the DNA extracted using different extraction techniques from rice and rice products that had been heated or mechanically handled. A spectrophotometer or agarose gel could not be used for genomic quantification except for the samples that yielded the maximum amount of DNA when the Vivantis kit was used. The degree of DNA damage (such as depurination), the existence of PCR inhibitors in food matrices, and the average length of the extracted fragments all impact the overall quality of the DNA recovered from food products. These variables rely on the samples themselves, the procedures followed in the food's manufacture, and the physicochemical characteristics of the extraction process (Peano et al., 2004; Elsanhoty et al., 2011; Elsanhoty et al., 2013; Ramdan et al., 2016). High molecular weight DNA fragmentation occurs when exposed to heat. However, random breaks in DNA strands caused by physical and chemical treatments result in smaller average DNA fragments (Hupfer et al., 1998; Toyota et al., 2006; Elsanhoty, 2009). Since many foods are acidic, heat treatments involving acid-catalyzed processes are accelerated (Anklam et al., 2002; Yamaguchi et al., 2003). The yield of the extracted DNA should be the sole factor influenced by the matrix, provided that the extracted DNA is of a quality suitable for PCR. The amount of DNA utilized in PCR can vary from 20 pg to 200 ng, based on various studies. In cases where there are insufficient copies of the gene available for PCR amplification, the amount of template DNA utilized in the PCR may be increased. However, too much template DNA is not a good idea because it lowers PCR efficiency (Miraglia et al., 2004). Good results were obtained when the Vivantis DNA extraction kit was applied to processed foods. DNA extracted with a Vivantis kit gave higher concentrations and purities. Similar results were obtained by Smith et al. (2005), Yohimitsu and Hori (2003), Sisea and Pamfil (2007) and Milia et al. (2008).

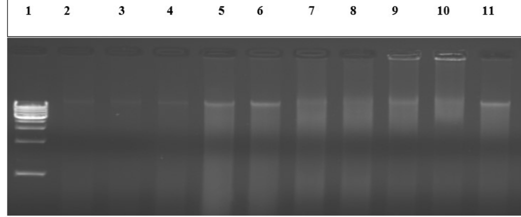

Figure 1. Example of DNA electrophoresis on 0.5 agarose gel of DNA extracted from rice and rice products. Lanes 1: M 1 Kbp, lanes 2, 3 and 4: DNA from some rice starch, lanes 5 and 6: DNA from some rice samples from Egyptm, lanes 7 and 8: DNA from some rice samples from China, lanes 9 and 10 DNA from rice samples from Thailand, lanes 11 DNA from rice samples from India.

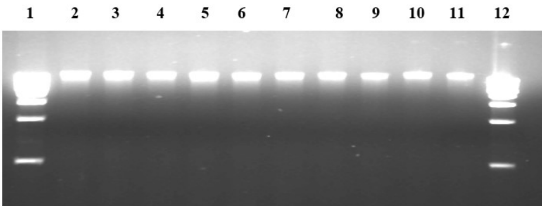

Figure 2. Agrose gel electrophoresis of total DNA was extracted from different rice samples using the Vivantis kit. Lane 1 and 12: DNA ladder 1 Kbp. Lanes 2 and 3: DNA extracted from rice starch; Lanes 4 and 5: DNA extracted from rise from Thailand; Lanes 6 and 7: DNA extracted from rise granules from USA; Lanes 8 and 9: DNA from rise granules from Egypt; Lane 10 and 11: DNA from rise granules from India.

3.2. Detection of GMO-specific genetic elements (35S promoter or NOS terminator)

All extracted DNA was screened using a GMOScreen 35S/NOS test kit to detect GMO varieties. The amplicon was specific for GMO-specific genetic elements (35S promoter or NOS terminator). Soybean samples gave positive results from GMScreen 35S/NOS, and the amplicon rose at the expected size of 123 bp (Figure 3). Thirty-two rice samples showed results for the presence of 35S promoter or NOS terminator. The results indicated that a GM construct (35S promoter or NOS terminator) was found in the rise samples under investigation; therefore, the samples that give positive results will be confirmed by specific primer SPS. The results obtained agreed with the results by Elsanhoty (2009). On the other hand, the results disagreed with the results obtained by Elsanhoty et al. (2013), who indicated that the rice samples gave negative results in the presence of a 35S promoter or NOS terminator.

3.3. Detection of genetically modified SPS gene in rice products

The CaMV 35S promoter and NOS terminator were examined in each of the 32 rice samples that tested positive for the SPS gene to determine whether any GMO targets were present. The DNA from GM soy was isolated and used to control these samples. Using the primer pair P35S-cf3/P35S-cf4, the CaMV 35S promoter sequences were only detected in 32 in 123 bp DNA rice samples (Figure 3). However, none of the examined samples showed evidence of a NOS terminator using the 118 bp primer pair HA-nos-118f/HA-nos-118r. Similarly, other researchers (Fernandes et al., 2014; Rabiei et al., 2013) announced that the PCR approach and these primer pairs could detect GM organisms. Figure 3 displays the gel electrophoresis results for the positive samples. The nucleotide sequences of the PCR products, including DNA isolated from positive samples, were ascertained to guarantee the desired outcomes. Following analysis of the sequencing data using the NCBI's BLAST search, it was discovered that 32 of the 100 samples had the 35S promoter. In a different study, Arun et al. (2013) used a PCR approach to screen processed food containing maize and soy for the presence of the CaMV 35S promoter and nos terminator. The results showed that 25 out of 100 (25%) samples were GM-positive. 12.5% of the food products examined had positive results for CaMV 35S, whereas NOS primer yielded negative results, according to Oraby et al. (2005). In a similar vein, Erkan and Dastan (2017) discovered that GM targets were present in 11 samples of rice and rice flour products. However, more event-specific methodology is needed to identify the transgenic rice event. However, because of safety concerns, rice events are prohibited in Iran and most other nations; as a result, consuming them may have unfavorable effects on human health. In research on risk assessment, Xue et al. (2012) noted that biotech rice may raise worries about hazards to human health. According to a Chinese study, one of the two rice samples tested positive for the CaMV 35S promoter (Made et al., 2007). Two hundred samples, including rice, soy, and maize, were examined in a different investigation to check for genetic modification. Two primer pairs, p35S and NOS, were employed for the detection technique. These results showed that, for these two primers, 26 and 44% of the samples, including soybean and maize, respectively, were positive; in contrast, every sample containing rice tested negative (Elsanhoty et al., 2013). According to the findings, PCR is a viable and preferable approach for successfully screening GMO targets in food products (Kim et al., 2017; Miraglia, 2004; Arun et al., 2013; Safaei et al., 2019; Shin et al., 2022).

Meanwhile, 32 of the 100 rice samples in our investigation had positive results for the primer pair P35S-cf3/P35S-cr4, suggesting that the rice may have undergone genetic modification. However, none of them had been given a label. The Food and Drug Administration's assessment indicates that the product's illegal entry into Saudi Arabia or its possible CaMV virus infection could be the reason for the positive results. Al Mazrooei and Alreshidi (2024) recently used PCR screening of GMOs in food and feed products sold in Kuwait's market and obtained similar results. The results showed that, among the 21 products derived from corn, soybean, and rice, 6 out of 21, 1 out of 5, and 2 out of 5 contained GMO sequences. According to the study, GMOs have been found in animal feed products made from corn. Currently, Kuwait lacks a regular, approved system to control the import of genetically modified crops. This study emphasizes Kuwait's need to create stable regulations and labeling systems for imported biotech crops, their derivatives, and legislation regarding GMOs.

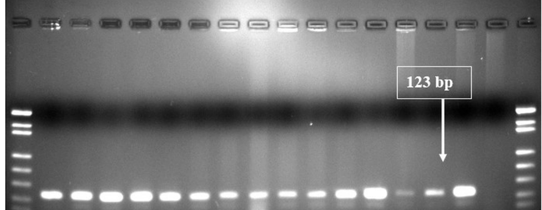

Figure 3. Example of analysis of rice samples for the presence of 35S promoter-specific DNA. Analysis was performed as described in materials and methods, except that the primer pair SPS-F/SPS-R was used for PCR analysis. Lanes 1 and 18: DNA ladder 50 pb, Lanes 2, 3, 4 and 5: PCR products of DNA from rice from USA, Lanes 6, 7, 8, 9: PCR products DNA from Thailand, Lanes 10, 11, 12 and 13 PCR products of DNA of rice from India, Lanes 14, 15, 17: PCR products of DNA of rice from China, Land 17 PCR control.

3.4. Detection of CaMV 35S promoter sequence in potato products

Figure 4 shows the results of potato samples for detecting the CaMV 35S promoter sequence derived from the cauliflower mosaic virus. The samples under investigation were positive for CaMV 35S promoter sequence in potatoes according to the procedure used in the detection. Data indicated that 30% of the samples exhibited positive CaMV 35S promoter sequence, and PCR products were observed at 105 bp. Meriç et al. (2014) obtained similar results in maize and soybean, demonstrating that

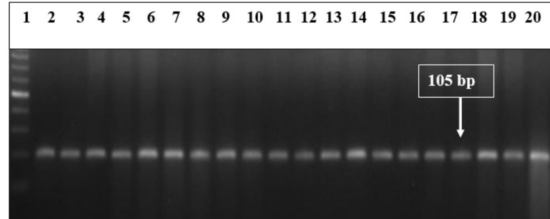

Figure 4. Detection of 105 bp on the CaMV35S gene using the primer pair CaMV35SF/and CaMV35S-R. From the left: lane 1, 50 bp, lanes 2-20: positive samples from fresh potato for the presence of CaMV35S.

Figure 5. Example of detecting the NOS terminator in potato samples collected from the Saudi Arabia food market. Analysis was performed and is documented as described in the legend in Figure 1, except that the GMOScreen 35S/NOS test kit for the qualitative detection of GMO varieties in food, Agarose gels of total DNA extracted from potato samples using the CTAB method and Vivants DNA extraction Kit. Lines 1 to 15: example of PCR products of DNA from potato samples Lane 16 PCR control with DNA positive provided with the kit. M: DNA ladder 100 bp.

all samples were transgenic for CaMV 35S promoter due to the PCR-based method. Khider et al. (2018) found that 100% of the potato samples exhibited positive CaMV 35S promoter sequence, and PCR products were observed at 105 bp. Safaei et al. (2019), Elsanhoty et al. (2013), and Shin et al. (2022) found soybean samples and maize samples were positive for the presence of CaMV 35S in the samples collected from Iran and the Kingdom of Saudi Arabia. From these results, it could be concluded that there may have been horizontal gene transfers between the GM plants and non-GMplants and/or the infection of plants by the virus in the field. This could explain why all samples under investigation were positive for the presence of CaMV 35S promoter.

3.5. Detection of NOS terminator in positive potato samples for the presence of CaMV35S using GMOScreen Kit

The findings of utilizing the GMOScreen Kit to detect NOS terminator in the same samples that showed positive results for the presence of CaMV35S were displayed in Figure 5. The GMOScreen Kit indicated that the positive potato sample included both CaMV35S and NOS terminator, based on data indicating that the DNA samples of the same samples had positive results for the presence of NOS terminator. The outcomes coincided with those reported by Elsanhoty et al. (2013), who discovered CaMV35S and NOS terminator in soybean samples from the Kingdom of Saudi Arabia. Unlabeled material produced from GMOs was found. The distribution of GMOs in commercial food has been studied previously, and these findings matched those findings (Elsanhoty et al., 2002; Cardarelli et al., 2005; Greiner et al., 2005; Margarit et al., 2006; Brod and Arisi, 2008; Park et al., 2010). Greiner and Konietzny (2008) examined the presence of MON 810, Bt11, Bt176, and T25 events in 100 Brazilian meals made with maize. Of the 18 samples tested, 4 contained GM maize flour, and 3 had GM maize polenta. A total of 11 samples tested positive for GM maize. Most items containing genetically modified maize were not Brazilian in origin. When 32 commercially available foods were evaluated for the presence of genetically modified maize, eight of the 32 samples had positive results (Margarit et al., 2006). According to Park et al. (2010), most maize in five Korean provinces' storage products was GM, with almost 50% of the grains germinating.

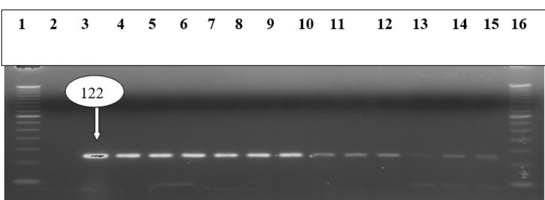

Figure 6. An example of detecting genetically modified potato spunta in potato samples collected from the Saudi Arabia food market is Analysis was performed and is documented as described in the legend to figure 1 except that the Spu-35S1-F/ Spu-cryVm-R primer was used Lines 1 and 16DNA ladder 50 bp, Lan2: positive control genetically modified potato spunta obtained from Agriculture genetic Engineering Research Institute, Egypt, Lanes 4 to 15: examples of PCR products of DNA of fresh potato samples.

3.6. Detection of Cry V and CaMV35S in GM potato in potato samples

The DNA of the 50 samples was examined for the presence of GM potato *Spunts*using primers Spu-35S1- F/ Spu-cryVm-R developed by Elsanhoty (2004). According to data in Figure 6, 15 samples (30%) out of 50 potatoes tested positive for the genetic elements present in the vector (pSPUD5), which included a gene cassette containing the following: the CaMV35S promoter, the Cry5-Bt gene, and the NOS terminator (Mohammed et al., 2000). The findings were somewhat consistent with those of Elsanhoty et al. (2013), who discovered GM plants in food samples taken from Saudi Arabia, and Song et al. (2017), who provided a helpful technique for recognizing GM potatoes and utilized multiplex polymerase chain reaction to locate unapproved GM potatoes in Korea. Furthermore, the data obtained agreed with the findings published by Greiner and Konietzny (2008), who examined 100 Brazilian items, including maize, to determine whether MON 810, Bt 11, Bt 176, and T25 events were present. They found 11 positive samples for GM maize, 4 of 18 maize flour, and 3 of 18 polenta samples. Brazil was not the source of the approved bulk of GM maize products. Eight of the 32 food product samples regularly tested for GM

maize in Argentinean markets yielded positive results (Margarit et al., 2006). Roughly half of the GM maize grains germinated in storage products from five Korean provinces, according to Park et al. (2010). Previous studies (Elsanhoty et al., 2002; Cardarelli et al., 2005; Greiner et al., 2005; Margarit et al., 2006; Park et al., 2010) on the dispersion of GMOs in commercial food yielded similar results.

In our investigation, 32% of the rice and 30% of the potato samples tested positive for CaMV promoter and genetically modified potato Spunta, indicating that the latter may be genetically modified even though none have been labeled. However, according to the Food and Drug Administration's assessment, the positive results could be the product being illegally imported into Saudi Arabia or infected with the CaMV virus.

4. Conclusion

It could be concluded that the DNA isolation methods were suitable for most food products. DNA was successfully isolated in all samples using the CTAB or Vivantis kits. The results demonstrated GM rice's presence and sin in the Saudi food market. The existence of unauthorized GMOs for food use in Saudi Arabia. The product labels did not indicate the presence of GMO ingredients to allow the consumers to select food products. Controlling all imported raw materials and food products would be advisable, depending on the results. Establishing strong regulations and certified laboratories to monitor GM foods or crops is recommended. The results showed the importance of a monitoring system to ensure that GM ingredients are adequately controlled in food products and, therefore, on their labeling. To comply with EU and worldwide rules requiring the labeling of GMF when their presence in the food exceeds 0.9%, the amount of genetically modified goods in food commodities should be monitored and managed. To successfully introduce a significant quantity of genetically modified food into global markets, it will be imperative to maintain constant surveillance for the presence of genetically modified elements in food products.

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