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DETECTION OF GENETICALLY MODIFIED SOYBEAN SEED, SOYBEAN MEAL AND RICE IN KARBALA CITY OF IRAQ

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ABSTRACT: Rice and soybean are two high-demanded grains for human foods and animal feeds. The current study aimed as first time in one of Iraqi region to find genetically modified soybean seed, soybean meal and rice grain samples utilizing the *Cauliflower mosaic virus* (CaMV) 35S promoter and nopaline synthase (NOS) terminator catalyst like *Agrobacterium tumefaciens* NOS (ANOS) terminator, in PCR tests. A total of 55 samples of soybeans seed, soybean meal, and rice cereal were collected from the market in Karbala, Iraq. The samples were collected from markets in Karbala city during January-March 2021, and evaluated in the Food Laboratory, College of Al Safwa University of Karbala, Iraq. DNA was isolated from dry vegetable samples. Two genes, including CaMV-35S and NOS terminator, that are routinely used in genetic engineering were employed to evaluate genetically modified crops. The present study revealed CaMV-35S and NOS genes in soybean meals. In conclusion, the obtained results indicated that all rice samples tested with the same primers were genetically unaltered. Whereas, there is genetically alternations in soybean seeds and soybean meal.

Keywords: Genetically modified seeds, Feedstuff, Soybean meal, Rice, CaMV-35S, NOS terminator.

INTRODUCTION

DNA is the molecule that contains the genetic information of each living organism, and its discovery has revolutionized the field of life sciences (Chawla, 2011). So, due to the universality of genetic codes, scientists have used molecular biology techniques to connect DNA sequences from other animals and insert foreign DNA into plants (Holme et al., 2018). As a result of gene transfer, these genetically modified organisms commonly known as GMOs can synthesize extra proteins that allow them to adapt to their environment, one of the hoped-for benefits of gene transfer (Spök et al., 2007; Kircher et al., 2020).

Any new food items containing genetically modified soy or maize must be labeled under European legislation. Labeling is based on identifying foreign DNA sequences from GMOs (Böschen et al., 2006) using PCR (Broeders et al., 2012). DNA molecules are more thermostable than proteins; hence this approach can be beneficial for their sequence detection (Fraiture et al., 2015). Since any genetic information may be produced in an organism, genetic engineering has been used to address a wide range of issues, including crop protection in agriculture and high value added compound synthesis, such as vitamins or biopolymers (Broeders et al., 2012; Turnbull et al., 2022). A wide range of GMOs is also being investigated and produced, such as genetically modified maize engineered to generate a healthier cooking oil by decreasing the amount of its saturated fat (Uzogara, 2000). In this regard, antifreeze proteins generated from the winter flounder might be used to help strawberries flourish in cold climates and create nutritionally improved strawberries that include greater concentrations of ellagic acid, a natural anti-cancer agent (Mezzetti, 2013).

GMOs detection in different regions of Iraq has been started with using PCR method (Saadedin et al., 2019; Jasur et al., 2020). The study aimed to find genetically modified soybean products and rice yield in Karbala market samples utilizing the CaMV-35S promoter and NOS terminator catalyst in PCR tests.

MATERIALS AND METHODS

The current study included 55 food and feed samples collected randomly from a variety of sources, including the markets in Karbala, as well as other locations in this region, from January to March of 2021. The samples were sent to Kerbala

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University Food Laboratory, College of Alsafwa, Iraq, for analysis. The food samples were pulverized in a ceramic mortar, and liquid nitrogen was used. DNA was isolated from dried vegetable meals, including soybeans, soybean meal, and rice grains. Following that, a spectrophotometer was used to measure the concentration and purity of each DNA sample using a genomic DNA extraction kit (Intron Company, Korea). DNA collected from the samples was studied according to the manufacturer's guidelines. To evaluate the quality of the extracted DNA samples, spectrophotometer readings at two wavelengths (260 and 280 nm) were employed. Absorptance ratios between 1.7 and 2.0 indicate that the extracted DNA was of high purity.

Special primers were employed to identify GMOs in soybeans and their derivatives (soybean feed). The 195-bp primer CaMV35S and 118-bp terminator NOS were employed to identify GM genes in soybeans and their derivatives (Zaulet et al., 2009). Most PCR screening methods rely on identifying the CaMV-35S mosaic virus initiator or the NOS terminator in the examined product. In the current investigation, 123-bp primer CaMV35S and 118-bp terminator NOS were employed to detect genetically modified genes in rice grain genotypes (Safaei et al., 2019). These primers are shown in Table 1.

In addition to utilizing the components of the PCR kit and primer solutions, it was also important to chill the kit by storing it in an ice box, as daily work solutions for PCR interactions. In PCR reaction components, all target genes were examined with primer concentrations of 10 Pmol. The final findings were from 25-ul PCR runs. The gel was prepared in a electrophoresis procedure by dissolving 1 g agarose in 100 ml buffer TBE. Electrical migration commenced (IX) following Sambrook and Russell (2001). Once the agarose was completely dissolved, the mixture was cooled to 50°C and microwaved until the agarose was completely dissolved. Polymerization reaction mixture and DNA marker were each applied to each gel well in 5 microliter portions. Each gel well was filled with 0.5 microliters of ethidium bromide solution per milliliter of gel. At a voltage of 100 volts, the electrodes reached their target, and the models moved electrically for an hour.

Table 1 - The primers used in the present study.

Primer name	Origin sequence	Target sequence	Amplicon (bp)	Sequence
Detection Primer	*Cauliflower mosaic virus*	35S1 Forward	195 bp	F-5'-"GCTCCTACAAATGCCATCA-3"
		35S2 Reverse		R-5'-"GATAGTGGGATTGTGCGTCA-3'"
Detection Primer	*Agrobacterium tumefaciens*	HANos-118 Forward	118 bp	R-5'-GCATGACGTTATTTATGAGATGGG-3'
		HANos-118 Reverse		R-"GACACCGCGCGCGATAATTTATCC-3'"

RESULTS AND DISCUSSION

The GMO screening methods focused on uncovering the regulatory elements and genes responsible for GMOs' unique traits. CaMV-35S promoter (P35S), *Agrobacterium tumefaciens* NOS terminator (ANOS), and others were chosen (TNOS). In many CaMV-transgenic plants, the 35S promoter and/or NOS terminator are present (Querci et al., 2010).

Traditional PCR methods 1 and 2 were employed to detect the CaMV-35S encoder gene and no-terminator in soybean gain, soybean meal, and rice gain. Primers for detecting P-35S promoters for the existence of a package from the inflation process in the fourth, fifth, and sixth wells at 195 base pairs utilizing a soybean meal sample yielded experimental observations (Figures 1 and 2).

Rice is main daily meal in many countries, genetically modified rice strains can be hazardous for consumers (Fu et al., 2019; Hajimohammadi et al., 2022). Consumption of genetically modified foods and feeds are rising and it makes concerns for human and animal health (Nawaz et al., 2019; Turnbull et al., 2021). The discovery of transgenic wheat and soybean meal, which are widely used in livestock feeding, is of great importance in all parts of the world (Matovu, 2021; Sieradzki et al., 2021; Singh et al., 2021).

There are no restrictions on the kinds of GMOs that can be screened using this technology as long as the samples include one of the amplifying genes found by this technique (Nikolić et al., 2008). The advancement of PCR methods has made it possible to swiftly and accurately detect GMOs in food (Chen et al., 2021). There are two important GM features that may be detected using these methods; the CaMV 35S promoter and the Agrobacterium NOS terminator as general recombinant markers, as well as two of the most commonly occurring targets in GMOs so far (Barbau-Piednoir et al., 2012).

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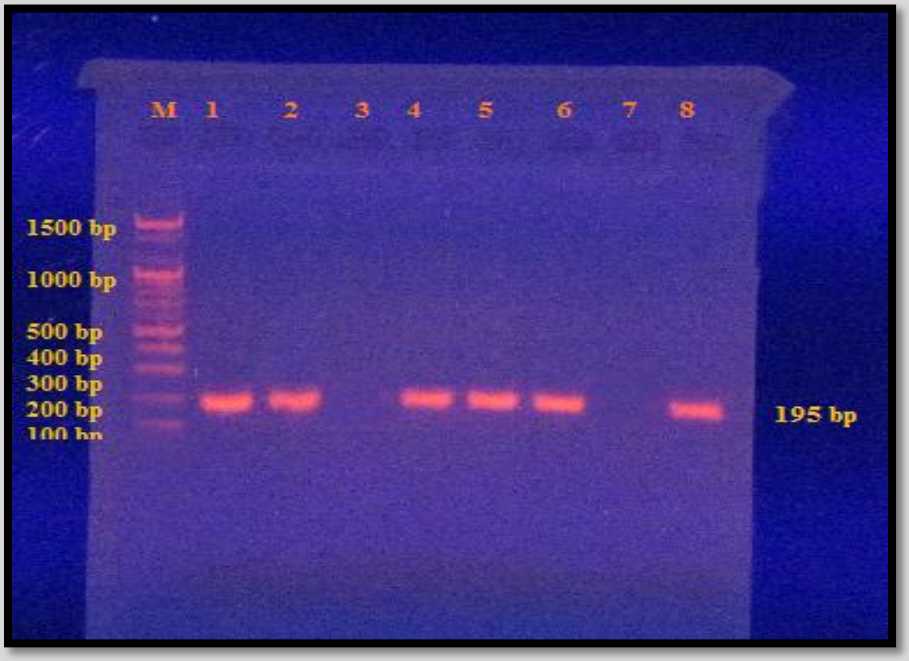

Figure 1 - Uniplex PCR amplification of GMO-specific areas employing primer pairs": P35S (*Cauliflower mosaic virus*), M as DNA ladder 100 base pair, 1,2,4,5,6 and 8 wells GMO positive, 3 and 7 wells without GMO.

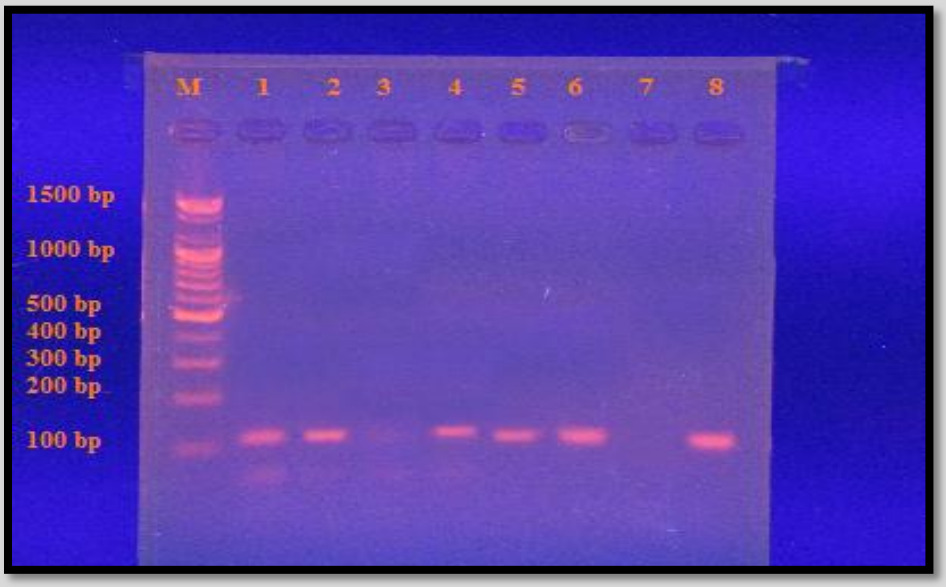

Figure 2 - Uniplex PCR amplification of GMO-specific areas employing primer pairs: HANOS (*Agrobacterium tumefaciens*), M as DNA ladder 100 base pair, 1,2,4,5,6 and 8 wells GMO positive, 3 and 7 wells without GMO.

CONCLUSION

PCR methods developed for the detection of genetically modified soybeans have also been effectively applied to a number of other crops and may be simply adopted to restrict the distribution and usage of genetically modified food. Two genes, including CaMV-35S and NOS terminator, were employed to evaluate genetically engineered crops throughout the current study period. These genes are routinely used in genetic engineering. The present study revealed CaMV-35S and NOS genes in soybean meals. The obtained results indicated that all rice samples tested with the same primers were genetically unaltered.

DECLARATIONS

Novelty statement

Genetically-modified seeds are identified in one of Iraqi region (Karbala city) as first time. Especially, these kinds of grains (rice and soybean) are high-demanded for human foods and animal feeds.

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Author's contribution

Kamal Mathlum Al-khafaji, Ashraf Ayyal Mutar Alrashedi, Wafaa Fawzi Al-Mosawy, and Hayder Ali Muhammed had similar roles and attempts in study, experiments, writing and proof-reading of article.

Conflict of interest

The authors have declared no conflict of interest.

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