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Detection of genetically modified food products in indian market using PCR based GMO detection kit: a pilot study

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ABSTRACT

The Food Safety and Standards Authority of India (FSSAI) have not approved any genetically modified (GM) food products to be manufactured, distributed, sold/or imported in the country. Many countries across the globe are legally approved to cultivate GM crops like soybean, maize, canola, cotton seeds, etc. Many people living in urban India nowadays prefer to purchase imported food products. As a result, an increasing number of food items (without GM labels) are being imported in India. Nevertheless, these products are also easily available for buyers online. Thus, it is important to understand whether these imported food items available in the Indian market are GMO-free. The objective of this study is to check the availability of GM food products in raw and processed forms in the Indian local market through the use of conventional Polymerase Chain Reaction (PCR). The study is designed to screen for the presence of regulatory genes (35S promoter and NOS terminator) which are the most common sequences found in transgenic food products. Using the cetyl trimethyl ammonium bromide (CTAB) method, DNA was extracted from 12 food samples commercially available in the Indian market (locally and online) followed by PCR to detect the presence of GM DNA using HIMEDIA'S MBPCR055 GMO detection kit. Overall, 16.66% of the total samples were tested positive for GM DNA. Of the imported food items, 33.33% were tested positive. Products that were manufactured in the US and Netherlands were tested positive for GMOs. Their main ingredients were also soy and corn. Samples manufactured in India were GMO negative.

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1. Introduction

Usually people are very particular in choosing the type of food they consume in terms of safety, its effect on their health and well-being. In recent years, many genetically modified organism-based foods have been developed (1). Some of them have found its entry in the local markets. Nowadays people in the cosmopolitan cities prefer buying things from online markets where such GM Food products are readily available.

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These GM foods are produced through genetic engineering techniques. Using this technology, selected genes can be transferred between organisms of related as well as non-related species like from bacteria to plants (2). Many countries like the USA, Brazil, Argentina, Mexico, Australia, Canada widely use such GM foods (3). India is a leading producer of genetically modified cotton ie, BT cotton (4). GM crops are manufactured and marketed for several reasons like protection of crops from pests that cause disease in plants, tolerance to pesticides and herbicides, etc (2). Mostly such GM foods are not subjected to long term safety studies so many researchers are concerned that

such GM foods may cause antibiotic resistance, allergies, unnatural nutritional changes and toxicity (5). The Food Safety and Standards Authority of India (FSSAI) does not allow manufacture, distribution, sale or import of GM foods in the country. Center for Science and Environment (CSE) studies have shown that the availability of GM foods in the Indian market are mainly packaged foods imported from other countries (6). This study recommended action from FSSAI in interest of public health and make labelling of such GM foods mandatory which will give the consumers right to choose. The study was conducted to understand the current scenario of GM foods in India. The major GM foods are mostly made of genetically modified corn, soya bean, cotton seed oil, canola, sugar beet, alfalfa, squash and potato among others (7). The focus of this study was on GM foods made of corn and soybean. Both local supermarkets and online markets were explored in this study.

2. Materials and Methods

2.1. Sample collection

12 corn and soy-based samples (solid and liquid) were collected from the Indian local supermarkets and online markets. Processed and unprocessed samples both were used for the experiment. The samples used in this study included corn kernels, cornflakes, fruit loops, popcorn, tofu, lactose free soy instant formula milk, soy milk, soybean seeds, protein powder, soya sauce, corn snack, corn flour. Out of these 12 items, 6 were of Indian origin whereas the other 6 were manufactured in different countries like US, Netherlands and UAE.

2.2. Cetyl trimethylammonium bromide (CTAB) method for extraction of DNA: The CTAB method was used to extract DNA from the samples as described by Stefanova et. al, 2013 (8) with some modifications. The solid and liquid samples were standardized using different volumes of CTAB buffer. It was determined that 4g of solid sample in 20 mL of CTAB buffer and 5mL of liquid sample in 5 mL of buffer yielded appreciable amounts of DNA when visualized under agarose gel electrophoresis. The solid samples were homogenized with a CTAB buffer containing CTAB, 5 M NaCl, 0.5 M EDTA, 1 M Tris-HCl and β-mercaptoethanol. These samples were then filtered using Whatman filter paper No. 1 and transferred into centrifuge tubes. At the same time, the liquid sample was directly mixed with the CTAB buffer and transferred into centrifuge tubes. The tubes were vortexed thoroughly and kept in a water bath at 60°C for 30 min. After incubation, the tubes were centrifuged at 6000 rpm for 5 min. The supernatant was transferred into a new tube and equal volume of chloroform:isoamyl alcohol (24:1) was added. It was vortexed for 5 min and centrifuged at 10000 rpm for 1 min. The aqueous upper phase was transferred into a new tube. This step was repeated until a clear upper phase was obtained. To the clear upper phase, 0.7 volume of chilled isopropanol was added for DNA precipitation and incubated at -20°C for 15 min. The sample was centrifuged at 8000 rpm for 10 min. The pellet was collected and washed with 200 µL of ice cold 70% ethanol. The DNA was dissolved in a 20 µL TE buffer.

2.3. DNA concentration and purity

The concentration of DNA was determined by measuring the absorbance at 260 nm. The purity of DNA extracts was calculated by taking the ratio of the absorbance at 260 nm and 280 nm. A UV-VIS spectrophotometer (Shimadzu Corporation, Japan) was used for spectrophotometry analysis. The integrity of the extracted DNA was evaluated by electrophoresis on 1% agarose gel containing ethidium bromide and visualized under UV light using Gel doc.

2.4. PCR Amplification and DNA analysis

The samples were amplified to detect GM DNA using Himedia's MBPCR055 GMO detection kit. The kit is designed for specific detection of NOS terminator and 35S promoter. Under sterile conditions the PCR reaction mixture was prepared for each DNA sample. Sterile micropipette tips and Eppendorf tubes are used for addition of the PCR reaction mixture. Positive and negative control was also prepared. The components added to each sample tube and positive-negative control along with their volumes is given in table 1 and 2, respectively. After the addition of the

Table 1. PCR reaction for each DNA sample

gene	35S promoter	NOS terminator + rbcl (internal control)
TUBE	#1	#2
2X PCR Taq mixture (MBT061)	12.5 µL	12.5 µL
Primer mix	1 µL 35S primer	1 µL NOS primer + 1 µL rbcl primer
Template DNA	0.5 µL	0.5 µL
Molecular biology grade water for PCR (ML065)	25 µL	25 µL

Table 2. Description of the Controls

Positive control	0.5 µL positive control (Bt.cotton) + 0.5 µL rbcl gene
Negative control	0.5 µL negative control (non Bt.cotton) + 0.5 µL rbcl gene (Internal control of ribulose bisphosphate carboxylase/oxygenase gene for plants)

Table 3. PCR program. Number of cycles: 30

1.Initiation	94°C for 5 min
2. Cyclic parameters:	
a) Denaturation	94°C for 30 s
b) Annealing	60°C for 30 s
c) Extension	72°C for 30 s
3. Final extension	72°C for 5 min

components the Eppendorf tubes are centrifuged at 6000 rpm for 10 s. The tubes were then placed on the PCR machine and set the recommended program run. The PCR program is described in table 3. Analysis of amplified products was done on agarose gel electrophoresis and visualized using Gel doc. A 50 bp DNA ladder was also run on the agarose along with the samples.

3.Results

3.1. DNA isolation

Isolation of DNA was performed using modified CTAB DNA extraction method. It was found that the isolation of DNA was difficult in the harder samples like cornflakes, fruit loops and corn snacks as compared to the powdered or softer samples. These samples were soaked in the extraction buffer for 30 to 60 min to facilitate the extraction process. The CTAB protocol had to be altered depending upon the state of the samples (solid/liquid). The efficiency of the extraction was determined by agarose gel electrophoresis and bands were observed under UV transilluminator. The presence of bright bands confirmed the presence of DNA in the sample. The observed gel is shown in figure 1.

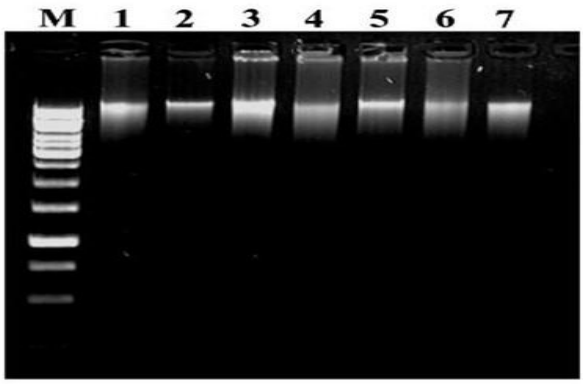

Figure 1. Agarose Gel Electrophoresis showing the bands of DNA extracted from the samples.

3.2. Evaluation of DNA quality (A260/A280 ratio)

In order to measure the purity and quality of DNA, the ratio of 260 nm to 280 nm absorption values were used. The value of pure DNA is 1.8. The A260/A280 ratios calculated in this study are shown in table 4.

Table 4. Evaluation of DNA quality

Sr. No.	Sample	A260/A280
1.	Corn kernel	1.69
2.	Instant cereal – cornflakes	1.46
3.	Instant cereal – fruit loops/rings	1.49
4.	Instant popcorn	1.42
5.	Tofu	1.6
6.	Lactose free soy instant formula milk	1.67
7.	Soymilk	1.56
8.	Soybean seeds	1.61
9.	Protein powder	1.63
10.	Soya sauce	1.49
11.	Corn snack	1.39
12.	Corn flour	1.47

3.3. PCR Amplification and DNA analysis

The DNA was then amplified by PCR using the protocol specified in the HIMEDIA'S MBPCR055 GMO detection kit and visualized under gel doc as shown in figure 2, 3 and 4.

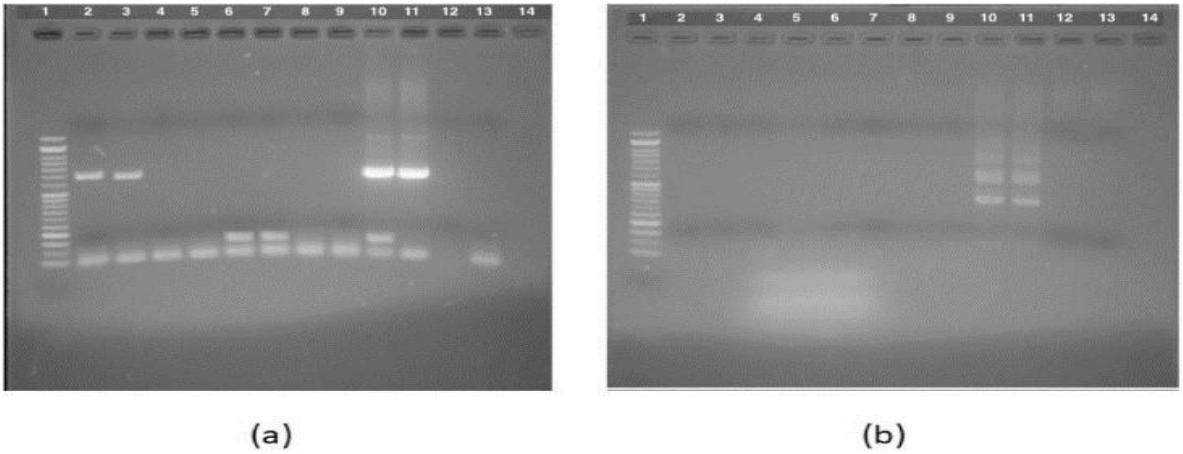

Figure 2. Agarose Gel Electrophoresis for PCR products (specifications mentioned in table 5) to detect a) NOS terminator and b) 35S promoter

Table 5. Observations made from Fig. 2

Lane	Sample No.	Sample Details (Amplified products from)	Observation for NOS gene	Observation for 35S gene
1	50 bp ladder		-	-
2,3	Sample 1	Corn kernel	685 bp amplicon of rbcl gene	No amplification of 490 bp amplicon of 35S gene
4,5	Sample 2	Instant cereal – cornflakes	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
6,7	Sample 3	Instant cereal – fruit loops/rings	118 bp amplicon of NOS gene	No amplification of 490 bp amplicon of 35S gene
8,9	Sample 4	Instant popcorn	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
10,11	Positive control		685 bp amplicon of rbcl gene (internal control) 118 bp amplicon of NOS gene	35S gene amplicon of 490 bp
12,13	Non-GMO control		No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
14`	Blank		-	-

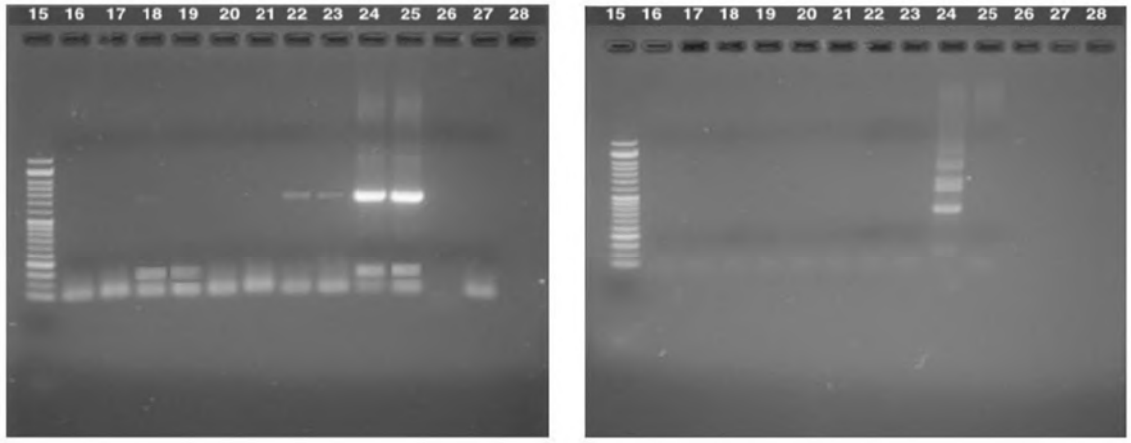

Figure 3. Agarose Gel Electrophoresis for PCR products (specifications mentioned in table 6 to detect a) NOS terminator and b) 35S promoter

Table 6. Observations made from Fig. 3

Lane	Sample No.	Sample Details (Amplified products from)	Observation for NOS gene	Observation for 35S gene
15	50 bp ladder		-	-
16, 17	Sample 5	Tofu	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
18, 19	Sample 6	Lactose free soy instant formula milk	118 bp amplicon of NOS gene	No amplification of 490 bp amplicon of 35S gene
20, 21	Sample 7	Soymilk	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
22, 23	Sample 8	Soybean seeds	685 bp amplicon of rbcl gene	No amplification of 490 bp amplicon of 35S gene
24, 25	Positive control		685 bp amplicon of rbcl gene (internal control) 118 bp amplicon of NOS gene	35S gene amplicon of 490 bp (lower bands present are due to nonspecific binding)
26, 27	Non-GMO control		No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
28	Blank		-	-

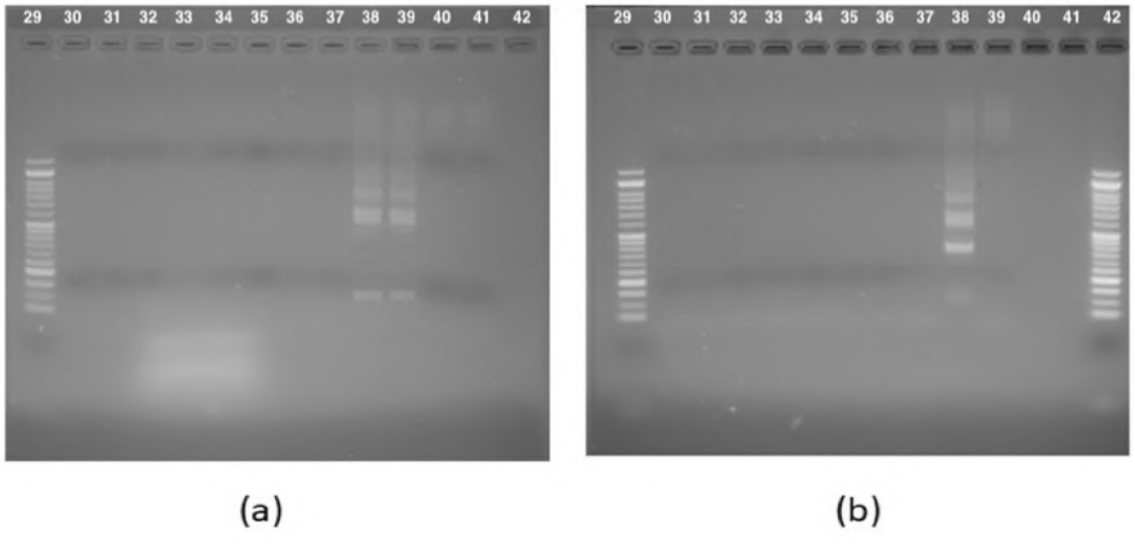

Figure 4. Agarose Gel Electrophoresis for PCR products (specifications mentioned in table 7) to detect a) NOS terminator and b) 35S promoter

Table 7. Observations made from Fig. 4

Lane	Sample No.	Sample Details (Amplified products from)	Observation for NOS gene	Observation for 35S gene
29	50 bp ladder		-	-
30, 31	Sample 9	Protein powder	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
32, 33	Sample 10	Soya sauce	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
34, 35	Sample 11	Corn snack	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
36, 37	Sample 12	Corn flour	No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene
38, 39	Positive control		685 bp amplicon of rbcl gene (internal control) 118 bp amplicon of NOS gene	35S gene amplicon of 490 bp
40, 41	Non-GMO control		No amplification of NOS gene	No amplification of 490 bp amplicon of 35S gene

Table 8. Interpretation of results

Sample No.	Type	Origin	Result
1	Corn kernel	Indian	GMO Negative
2	Instant cereal – cornflakes	US	GMO Negative
3	Instant cereal – fruit loops/rings	US	GMO Positive
4	Instant popcorn	US	GMO Negative
5	Tofu	Indian	GMO Negative
6	Lactose free soy instant formula milk	Netherlands	GMO Positive
7	Soymilk	Indian	GMO Negative
8	Soybean seeds	Indian	GMO Negative
9	Protein powder	US	GMO Negative
10	Soya sauce	Indian	GMO Negative
11	Corn snack	UAE	GMO Negative
12	Corn flour	Indian	GMO Negative

Thepresence of one of the regulatory genes ie. 35S promoter (490 bp amplicon) and NOS terminator (118bp amplicon) indicates the presence of GM DNA. The presence or absence of ribulose-bisphosphate carboxylase (RBCL) gene (internal control-685 bp amplicon) extracted from chloroplast of plants does not detect the presence of GM DNA. The results were interpreted based on the 50 bp ladder. Intense band was seen in lane 10, 11 of NOS terminator (figure 2) which indicates the presence of RBCL gene. The lower bands present in the NOS terminator are due to nonspecific binding i.e. primer dimer formation. The lane 6,7 (figure 2) and lane 18,19 (figure 3) of NOS terminator shows a band corresponding to 118 bp of DNA ladder. This indicates the presence of NOS terminator in the DNA sequence which confirms the sample as GM positive. The samples present in these lanes were sample 3 (fruit loops) and sample 6 (lactose free soy infant formula milk). No bands corresponding to 490 bp were found which indicates that 35S promoter sequence was not present in any of the samples.

Further, the interpretation of these results has been summarized in table 8.

The screening tests for GMO detection is based on detecting the presence of exogenous transgenic regulatory elements (10). The most frequently used method for detecting GMO material is screening for the CaMV-35S promoter (P-35S) from the cauliflower mosaic virus (CaMV) and the 3' non-translated region of the nopaline synthase gene (T-*nos*) from *Agrobacterium tumefaciens* using PCR (11). The kit used in the present study works on the same principles. The food sample which tested positive in the present sample showed the presence of NOS terminators.

4. Discussion

For the GMO screening by PCR method, proper DNA extraction is very important which has to be standardized for both solid and liquid samples. As suggested by Vijaykumar et al, increasing the amount of samples helps in extraction of sufficient amounts of DNA (12). Hence, in the present study also, the sample amount was considerably increased and accordingly the standardization of the protocol was done. From this pilot study, efforts were made to prove that PCR based

method which is the primary method for DNA level detection of GMOs (13) is reliable to identify GM markers from processed food samples. In the past, many investigators have tried using PCR based methods for detection of GMO in commercially sold food products from various different places like Brazil (14, 15), Turkey (16,17), Serbia (18), Syria (19), Egypt (20), India (6,21) among others. Most of the studies use CaMV 35 S and nos genes as the markers for detection of GMO (22). Other markers like FMV promoter (21), Cry3Bb, gat-tpinII, and t35S pCAMBIA can also be used (22). Other than PCR, other DNA level methods like DNA Miroaaray, Southern blotting and NGS can also be used for detecting specific GM markers (13).

Out of 12 products tested in the present study, only two products tested positive for GMO. So, overall, 16.66% of the total samples tested positive for GMO. Both the products contained both corn and soy. In the present study, samples manufactured in India were tested negative for GMO. Nevertheless, Vijayan et al reported presence of GMO in 17% of the food products manufactured in India in their study but all of them were based on cottonseed (21). As in this study the focus was only corn and soya based food products, none of the Indian origin products were found to be GMO positive. Out of the 6 imported food products, 33.33% were found to be GMO positive which were imported from the US and Netherlands which is parallel to the findings of CSE reports which showed 32 per cent of the food product samples tested were GM positive. The report also mentioned that the imported foods that had tested positive were majority from the countries as reported in this study.

Moreover, CSE report and Ateş Sonmezoglu et al. showed the presence of GMO in some of the baby foods. The present study also shows that out of the two samples that tested positive for GMO, one was baby food. This is also a concern as the potential ill effects of the genetically modified food on the health and overall well-being of the children is still unclear (23). Moreover, none of the samples that tested positive were labelled that they contained GM ingredients which misleads the consumers.

5. Conclusion

Although the number of samples tested in the present study was very less, GMO positive food products were still detected which concludes that the presence of GMO containing products in the local supermarkets and online markets. GMO testing is necessary for many producers/exporters/importers to meet specified regulatory requirements, commercial contract requirements and to ensure internal quality control. It will help in identification of illegal GM foods and creates awareness about genetically modified foods among consumers.

Conflict of interest

The authors have no conflict of interest to declare.

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