Vol. 17(35), pp. 1090-1098, 29 August, 2018
DOI: 10.5897/AJB2018.16479
Article Number: 9DC999958393
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Detection of genetically modified DNA in processed maize and soybean products in Nigeria

Andrew Chibuzor Iloh[1,3*], Paul Chidozie Onyenekwe[1] and Opeolu Oyejide Ojo[2,4]

[1]Biodiversity and Climate Research Laboratory, Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, KM 32 Abuja Lokoja Express Way, Sheda Abuja, Nigeria.
[2]School of Science, University of Wolver Hampton, Wulfuruna Road, Wolver Hampton, WV1 1LY, United Kingdom.
[3]Biodiversity Education and Resource Centre (BERC), Abuja, Nigeria.
[4]Bioscience Research Education and Advisory Centre, NIHORT-Alafara Road, Ibadan, Nigeria.

Received 12 April, 2018; Accepted 20 June, 2018

Cultivation and commercial utilization of genetically modified (GM) crops has significantly increased in many parts of the world and particularly in developing countries where food security is a challenge. Despite stringent regulations requiring that food made from GM foods should be properly labelled, evidence of unlabelled foods made from GM crops sold in local markets in many of these countries is increasing. This challenge provides the justification for the development of a reliable, accurate and effective screening method. Polymerase chain reaction (PCR)-based method for detection of specific genes in GM crops is a common method used in many parts of the world. This study adapted a PCR-based technique to screen for the presence of specific DNA markers of genetic modification in finished maize and soya products collected from selected supermarkets and local markets across Nigeria. Results obtained indicated that 26.7% of samples tested contain GM specific genes. GM specific genes were also detected in some made-in-Nigeria processed food samples. The findings indicate that products made with GMO materials have entered the food chain in Nigeria at a modest scale and identifies the need for capacity building in techniques for GMO detection for regulatory agencies in Nigeria.

Key words: Genetically modified (GM) organism, GM maize, GM soy, polymerase chain reaction (PCR), CaMV35S, Nigeria.

INTRODUCTION

Over the last decade, concerns about food and environmental safety have increased considerably due to the introduction of techniques for genetic modification of organisms into agriculture and food production (Boldura and Popescu, 2016). While an increasing body of scientific evidence indicates reduction of hunger and starvation as well as resistance to pests and drought as part of the beneficial effects of genetic modification of food crops, concerns about health and environmental implications of generation of genetically modified organisms (GMOs) abound. Sateesh (2008) reported that consumption of GMOs could lead to known and unknown risks to human health and the environment, including introduction of allergens and toxins to human beings,

*Corresponding author. E-mail: chibaziloh@gmail.com, ac.iloh@shestco.org.ng. Tel: +2349091563646.

transfer of antibiotic resistance markers or unintended transgenes through cross pollination. Due to these concerns, the European Commission (2003a, b) advocates the labeling of GMO products to ensure effective monitoring and traceability. However, this requires accurate and efficient methods for detecting foreign genetic materials or other forms of genetic modification in raw materials as well as in highly processed food.

In many countries, biosafety assessment of GMOs or products made from them is required to assess the potential environmental and health impacts on consumers. This risk assessment demonstrates if unauthorized and potentially unsafe GM products are found in markets (Okpara et al*.,* 2016). GM herbicide-tolerant and insect-resistant soybeans, maize, cotton, and rape seeds are now commonly grown in many parts of the globe and this has further contributed to the global nature of the health and safety concerns of products made from GM crops (Sönmezoğlu and Keskin, 2015).

The detection of foreign DNA or identification of changes in DNA composition has been recognized as the most effective tool for GM food examination. This is because the DNA is the most stable molecule during food processing (Datukishvili et al*.,* 2015). Amplification of the promoters, terminators, or inserted transgenes by polymerase chain reaction (PCR) using specific primer sets is the gold standard method for GMO detection (Broeders et al., 2012; Milavec et al*.,* 2014). GM soybean and maize are the most widely distributed transgenic crops worldwide. Therefore, several PCR-based methods for detecting genetic modification in these crops are available in literature. Kim et al. (2013) reported qualitative multiplex PCR methods for identifying different lines of GM maize or GM soybean using event-specific primers. Matsuoka et al. (2000) also described methods which target several frequently used foreign DNA segments (including transgenes, promoter, and terminator) in a single-locus PCR-based method.

The use of three GMO-specific primer pairs directed toward the cp4-epsps transgene, 35S promoter and NOS terminator together with two soybean-specific primer pairs targeting lectin and b-actin genes has been reported by James et al. (2003). Similarly, Forte et al. (2005) developed a molecular screening method based on multiplex-PCR that involves amplification of the 35S promoter as well as the NOS terminator for the detection of GM soya and maize.

Concerns associated with increasing availability of GM plant products in many developing countries underscores the need for the development of appropriate techniques for GMO detection, which allows proper identification, monitoring and enforcement of extant regulations on labelling GM products. In a country such as Nigeria, such validated technique will become a useful tool for appropriate government agencies and help in the making of informed biosafety decisions. This study attempted the adaptation of the PCR-based technique, which uses DNA markers targeting important GMO specific sequences such as cauliflower mosaic virus CaMV 35S promoter, NOS terminator, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene for herbicide tolerance in soybeans and *Bt* resistance gene (*cry1Ab*) in maize, to screen for the presence of GM material in finished maize and soya products in selected supermarkets and local markets across Nigeria.

MATERIALS AND METHODS

Sample collection

Finished or semi-finished imported food products, made from corn or soybean, were randomly collected across markets in the six geo-political zones of Nigeria. These include products such as corn flour, soybean flour, biscuits, drinks, cooking oil, whole meals, cornflakes, cracker, corn chips, corn starch, popcorn, sweet corn, baby foods, and soy cakes. The sampling strategy targeted products which are in use by the general public. A total of 120 samples were collected in this study. Product sampling was carried out in 12 local markets and 12 major super markets across Nigeria (Table 1). All products were properly labelled for easy identification.

DNA extraction and purification

Genomic DNA extraction was carried out using the CTAB method described by Doyle and Doyle (1987) with slight modifications. Briefly, the protocol used 700 µl CTAB buffer for initial incubation, 500 µl isopropanol for DNA precipitation and 100 µl 70% ethanol for the two washing steps. Finally, extracted DNA samples were dissolved in 200 µl TE buffer supplemented with 10 mg/ml RNase (2 µl). Where necessary, DNA extraction was carried out using the commercially available Zymo Research Plant and Seed Extraction Kits following the manufacturer's recommended procedure. Purity and quantification of DNA samples were assessed using a UV spectrophotometer. The DNA samples were stored at -20°C until used for PCR.

Primer sequences

Four GMO specific DNA markers sequences, such as cauliflower mosaic virus (CaMV) 35S promoter, NOS terminator, herbicide tolerant *EPSPS* gene in soybean and insect control GM trait (*Cry1Ab* gene) were targeted in this study in line with previous studies (Datukishvili et al., 2015). Soy-specific lectin gene and maize specific zein gene were also amplified in relevant samples. Details of GMO specific sequences amplified and the list of primers used in this study are summarized in Table 2. Primers were purchased in lyophilized form from Eurofins MWG Operon (Germany) and reconstituted as directed by the manufacturer.

PCR detection of marker sequences

The PCR reaction mix (20 µl) contained 12 µl OneTaq Quick Load PCR Master Mix (New England Biolabs; 20 mM TrisHCl, 1.8 mM $MgCl_2$, 200 µM dNTPs), 1 µl Taq DNA polymerase (25 units/ml), 1 µl bovine serum albumin (10 mg/ml), 5 µl template DNA and 1 µl each of forward and reverse primers. Reactions were carried out in triplicates in order to confirm amplifications. PCR reactions were

Table 1. Distribution of sample collection.

Sample type	Location						Total
	Lagos	Kano	Edo/Delta	Rivers	Imo	Abuja	
Corn pap	1	2	2	2	3	5	15
Soy flour	4	0	0	3	0	1	8
Corn based biscuits	2	0	4	2	0	1	9
Soy based drinks	3	5	3	3	2	2	18
Soy based cooking oil	0	1	2	1	2	2	8
Corn meals	1	3	2	2	4	2	14
Cornflakes	1	2	2	1	2	1	9
Corn starch/Custard	1	2	0	1	1	2	7
Sweet corn	4	3	2	3	3	3	18
Corn based Baby foods	1	2	1	1	2	0	7
Pop corn	2	0	2	1	1	1	7

Table 2. List of PCR primers.

Target	Oligonucleotide primers		
	Primers	Sequences	Amplicon bp
Soybean lectin gene	LECT Forward	ACGGCACCCCAAAACCCTCG	101
	LECT Reverse	GGAAGCGGCGAAGCTGGCAA	
Maize zein gene	ZEIN Forward	ACACCACCGACCATGGCAGC	102
	ZEIN Reverse	TGGTGGCAAGTGCGCTGGAA	
CaMV 35S promoter	P35S Forward	CGTGCACCATGATGTGTGATTCGAC	141
	P35S Reverse	GTGGGATTGTGCGTCATCCCTT	
NOS terminator	TNOS Forward	GGTACCGGATCCAATTCCCGATCGTT	224
	TNOS Reverse	CGCGCTATATTTTGTTTTCTATCGCGT	
EPSPS gene	EPSPS Forward	ACCGGCCTCATCCTGACGCT	256
	EPSPS Reverse	CCGAGAGGCGGTCGCTTTCC	
Cry1Ab gene	Cry1 Forward	GCACCTCCGTGGTGAAGGGC	258
	Cry1 Reverse	AACCCACGGTGCGGAAGCTG	

performed using a PTC 0200 DNA Engine (Bio-Rad, USA) themocycler. For all genes except *Cry1Ab*, the PCR cycling condition involves an initial denaturation of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, elongation at 72°C for 35 s and a final extension step at 72°C for 5 min (Table 3). The PCR cycling conditions for Cry1Ab is slightly different and consists of initial denaturation at 95°C for 3 min, followed by 50 cycles of denaturation 95°C for 25 s, annealing at 62°C for 30 s, elongation at 72°C for 45 s and a final extension step at 72°C for 7 min.

Agarose gel electrophoresis

PCR products were checked for amplification of target genes using agarose gel (1%) electrophoresis at 140 V for 30 min. DNA bands were captured using the gel documentation system (Edvotek, USA). All results were analyzed visually on the gel documentation system.

RESULTS AND DISCUSSION

This study screened for the presence of DNA markers of specific GMO sequences in 120 samples made from maize or soybeans collected from different locations in Nigeria. Due to the immensely important implications of results obtained in investigations such as this, appropriate quality control to avoid false positive or false negative

Table 3. PCR conditions used for the detection of specific GM markers.

Step	CamV35S	NOS	EPSPS	Cry1Ab	Soybean Lectin	Zein
Initial denaturation	95°C/3 min	95°C/3 min	95°C/3 min	95°C/3 min	95°C/3 min	95°C/3 min
Denaturation	95°C/30 s	95°C/30 s	95°C/30 s	95°C/25 s	95°C/30 s	95°C/30 s
Annealing	65°C/30 s	65°C/30 s	65°C/30 s	62°C/30 s	65°C/30 s	65°C/30 s
Extension	72°C/35 s	72°C/35 s	72°C/35 s	72°C/45 s	72°C/35 s	72°C/35 s
Final extension	72°C/5 min	72°C/5 min	72°C/5 min	72°C/7 min	72°C/5 min	72°C/5 min
Cycles	40	40	40	50	40	40

Table 4. DNA yield and purity.

Sample type	*DNA yield (ng/µl sample)	*DNA purity (A_{260}:A_{280})
Corn pap	9.6 ± 0.36	1.84 ± 1.74
Soy flour	5.5 ± 0.41	1.81 ±1.78
Corn based biscuits	6.3 ± 0.34	1.58 ± 1.52
Soy based drinks	21.0 ± 1.00	1.81±1.50
Soy based cooking oil	0.00 ± 0.00	0.00 ± 0.00
Corn meals	6.1 ± 0.50	1.84±1.70
Cornflakes	10.2 ± 0.36	1.19 ± 1.26
Corn starch/Custard	6.7 ± 0.08	1.42 ± 1.25
Sweet corn	2.2 ± 0.17	1.64±1.63
Corn based baby foods	0.00 ± 0.00	0.00 ± 0.00
Pop corn	23.8 ± 2.20	1.88 ± 1.68

*Value ± Mean Standard Error with n = number of each sample type shown in Table 1.

results is vital (Hübner et al., 1999; Arun et al., 2013). In this study, false positive results were eliminated at two levels. In the first instance, sterile water was processed in parallel to all samples at each step of the extraction protocol in order to eliminate false positive results that may arise due to contamination during DNA extraction. Secondly, no template controls were run to eliminate contamination during the PCR amplification step of this protocol. To ensure that samples showing negative results for CaMV 35S and NOS genes contain sufficient DNA, soy-specific lectin and maize-specific were also analysed. These genes are present in both GM or non-GM soybean and maize, respectively (Zimmermann et al., 1998; Nguyen et al., 2009; Gabriadze et al., 2014).

DNA extraction

Successful screening for the presence of GMO sequences in samples, such as those used in this study, depends largely on the robustness of the DNA extraction method (Tengel et al., 2001; Ahmed, 2002). Sufficient DNA quantity was extracted from all samples used in this study except soy-based cooking oil and corn-based baby food (Table 4). DNA yield obtained ranged between 2.2±0.17 ng/µl obtained for sweet corn and 23.8±0.22 ng/µl obtained for popcorn. The A260/280 ratio obtained for all the samples ranged between 1.64 and 1.88, which indicated that they are of acceptable quality. This result is consistent with reports of previous studies which extracted DNA from processed food products (Lipp et al., 1999; Cardarelli et al., 2005; Greiner and Konietzny, 2008; Mandaci et al., 2014). According to Jasbeer et al. (2008), CTAB DNA extraction method is efficient and widely used for the extraction of pure DNA plants and plant derived food products due to its ability to provide effectively separate plant DNA from polysaccharides. However, DNA yield obtained in this study is lower as compared to values reported for similar studies (Mandaci et al., 2014). It is not yet clear if the poor yield observed results from the modification of the CTAB protocol carried out in this study or due to important food processing factors, such as temperature and pH, which affect DNA quality and quantity in processed food materials (Gryson, 2010).

The inability to extract DNA from processed soybean oil is consistent with the available (Costa et al., 2009). Moreover, it is also possible that the DNA in the corn-based baby food products has been destroyed during processing food-processing conditions such as

Table 5. General summary of results.

Sample type	Number of samples	CamV35S positive	Number positive	EPSPS/Cry1 positive	Lectin/Zein positive	No DNA	No PCR detectable band
Corn pap	15	4	4	4	15	-	11
Soy flour	8	-	-	-	8	-	-
Corn based biscuits	9	1	1	1	9	-	8
Soy based drinks	18	9	9	9	17	1	8
Soy based cooking oil	8	-	-	-	-	8	8
Corn meals	14	2	2	2	14	-	12
Cornflakes	9	3	3	3	9	-	6
Corn starch/Custard	7	2	2	2	7	-	5
Sweet corn	18	5	5	5	18	-	13
Corn based baby foods	7	-	-	-	-	7	7
Pop corn	7	2	2	2	7	-	5

temperature and pH could lead to DNA degradation which makes PCR analysis of the DNA thus rendering PCR analysis impossible (Ahmed, 2002; Gryson, 2010; Okpara et al., 2016). DNA materials extracted in this study are also visible on agarose gel (Figure 1).

PCR amplifications

In this study, zein gene was successfully amplified from the DNA samples obtained from maize-based products and a similar trend was observed for the amplification of lectin gene DNA samples isolated from soy-based products (Table 5). Agarose gel electrophoretic analysis of these samples also produced visible bands for zein and lectin gene in respective samples (Figures 2 and 3). These results indicate that isolated DNAs are amplifiable and contained maize or soy DNA, respectively. The result also validates data obtained for the PCR amplification of the GMO specific genes targeted despite the low DNA yield obtained in this study.

Positive results for the detection of GMO specific genes were observed for 28 samples, representing 26.7% of all samples screened in this study (Figure 3). This is made up of 19 maize- and 9 soy-based samples. This result indicates that 24% of all maize-based products and 34% of all soy-based samples showed positive results for the presence of GMO specific genes. The present study showed consistency in the detection of both CaMV35S and NOS terminator genes for all GMO-positive samples. The percentage of maize samples harbouring CaMV35S and NOS sequences observed in this study (24%) is lower when compared with 28% previously reported by Okpara et al. (2016). This discrepancy may be due to the fact that Okpara et al. (2016) screened 61 samples collected only from the southern part of Nigeria as compared to 120 samples collected from the six regions of Nigeria. The southern part of Nigeria hosts all the major seaports and represents the entry point for all important food products in Nigeria. This coupled with infrastructural challenges facing food transportation within the country may partly account for the higher percentage of GMO-containing food products in the southern part of Nigeria, as observed by Okpara et al. (2016). No previous studies have screened soy-based products for the presence of CaMV35S and NOS sequences and the present result represents a baseline data for future investigations.

Genetic modification in transgenic maize (corn), focused on the production of crops with agronomic desirable traits, include the incorporation of a gene that codes for the *Bacillus thuringiensis* toxin (*Cry1ab* gene) in the plant's genome. Plants harbouring this genetic modification express the *Cry1ab* protein and are resistant to insect pests. In addition, herbicide (glyphosate and glufosinate)-tolerant plants have also been produced (OECD, 2002). In this study, roundup ready soybeans, which expresses the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, is one of the first series of GM glyphosate-resistant soybeans produced and cultivated globally (Clarke et al., 2013). Screening conducted in this study showed that 35% of soy-based products harbor the EPSPS gene (Figure 4A). Interestingly, only soy-based drink samples tested positive for the presence of CaMV35S, NOS and the EPSPS genes. *Cry1ab* gene was also successfully amplified in all CaMV35S- and NOS-positive DNA samples screened (Figure 4B). This observation is consistent with the previous report by Okpara et al. (2016) which identified five products made with GM maize. GM cowpea is one of the most widely cultivated GM crop worldwide.

Consistent with other national GMO-screening studies, this study indicate that positive results or GMO-specific genes were observed mostly in products imported into Nigeria (Arun et al., 2013; Mandaci et al., 2014).

However, this study also identified GMO specific genes in some processed foods produced in Nigeria. Maize and soybean are widely cultivated in Nigeria and Nigeria has

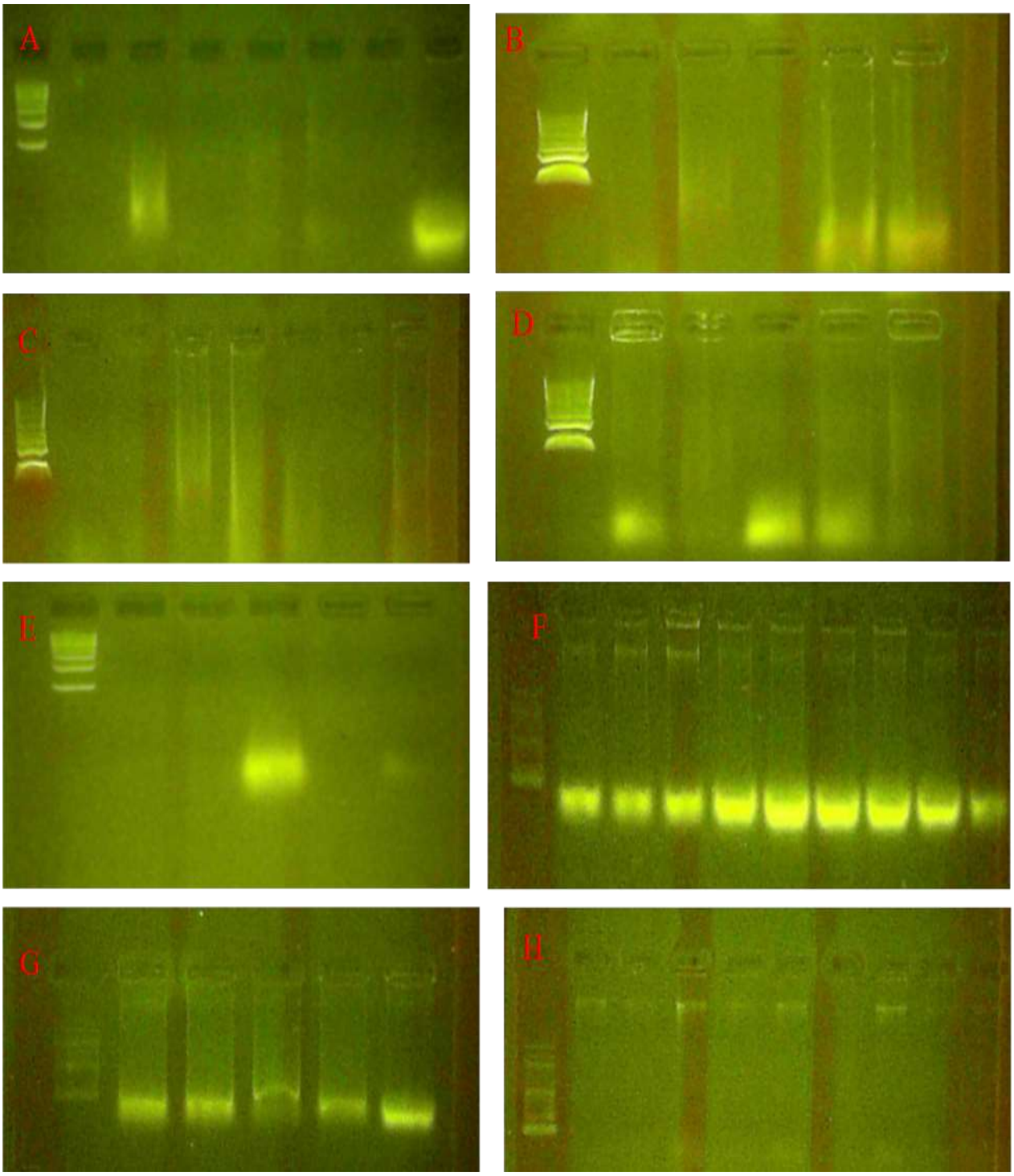

Figure 1. Total Genomic DNA extracted from food products gotten from Nigerian markets. **(A)** DNA extracted from corn based biscuits, whole meals. **(B)** DNA extracted from cornflakes, cracker. **(C)** DNA extracted corn chips. **(D)** Corn starch, corn flour. **(E)** DNA extracted from soy based Oil. **(F)** DNA from popcorn, sweet corn, baby foods, cakes. (**G and H).** DNA from soy based flour and drinks.

been reported to be the largest producer of soybean in sub-Saharan Africa (Dugje et al., 2009). Therefore, it was presupposed that raw materials for these made in Nigeria processed foods were sourced locally in Nigeria. This implies that it is possible that GM maize and soybean are also currently being cultivated in Nigeria.

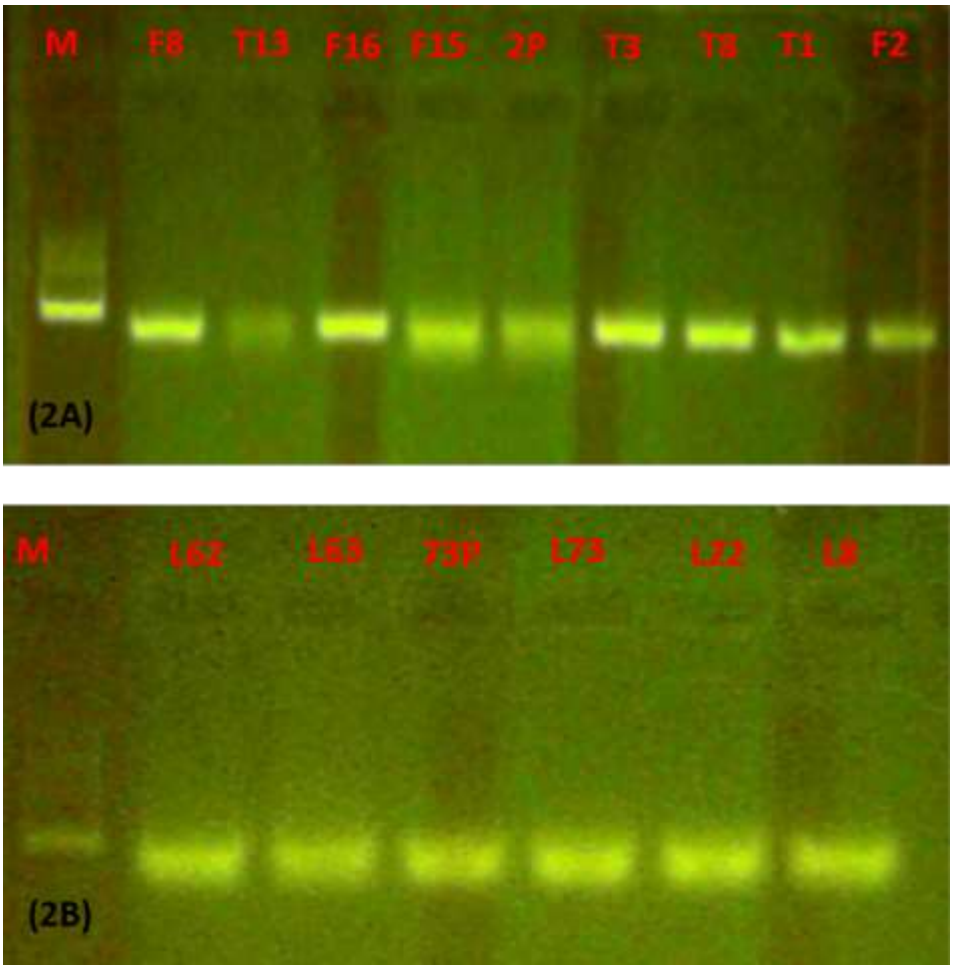

Figure 2. Representative agarose gel electrophoresis analysis of PCR products for zein (A) and soybean lectin (B) genes. 2A: M = 100 bp molecular weight marker; F8 = corn based biscuit; T13 = Corn flakes; F16 = popcorn; F15 = sweet corn; 2P = corn-based custard; T3 = Sweet corn; T8 = Sweet corn; T1 = Sweet corn and F2 = popcorn. 2B: M = 100 bp molecular weight marker; L62 = Soy drink; L63 = Soy drink; 73P = pure soy product; L73 = Soy drink; L22 = soy drink; L8 = Soy drink. Samples with similar product name and different sample codes are collected from different locations in Nigeria.

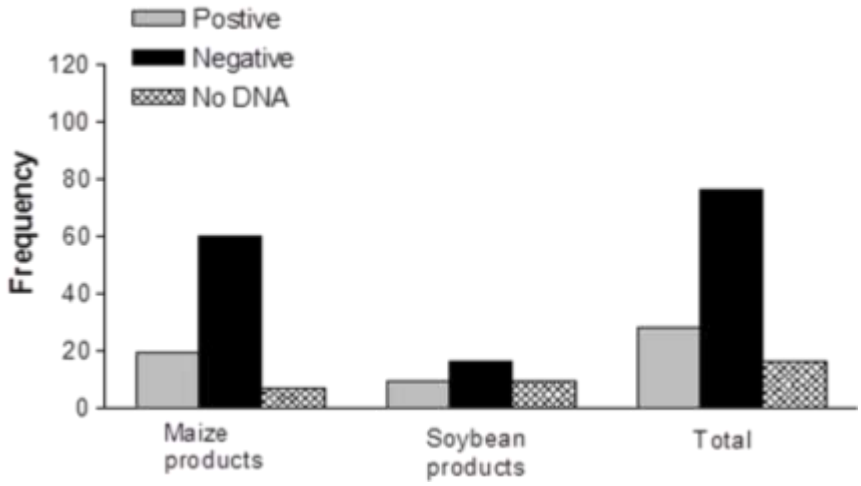

Figure 3. Distribution of samples showing positive result for GMO specific gene.

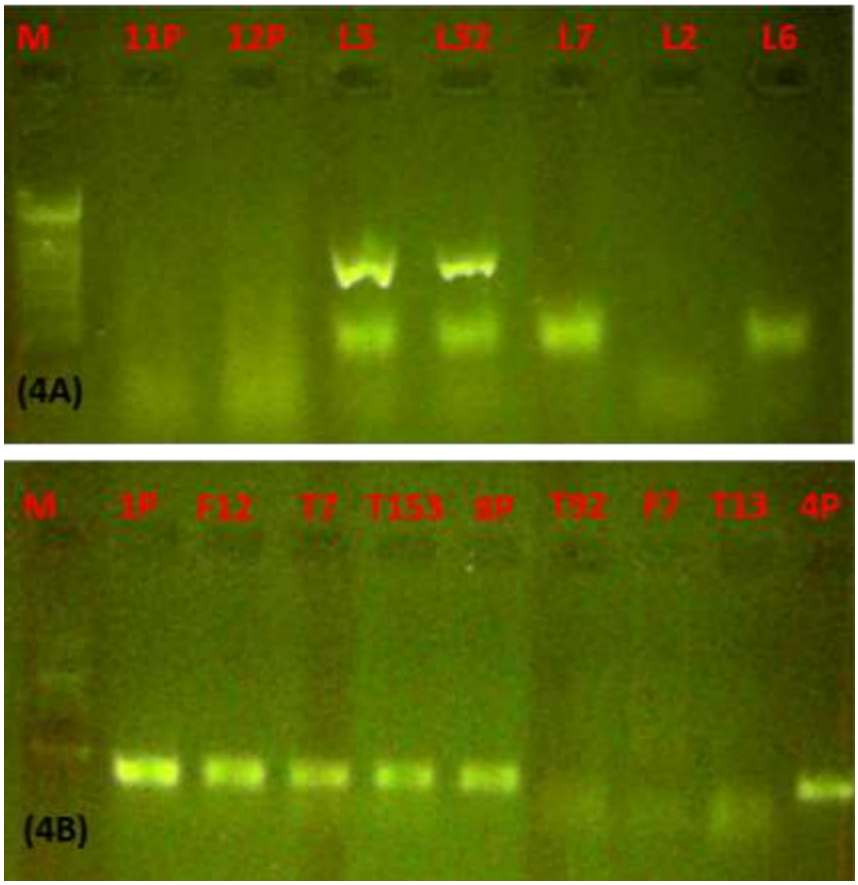

Figure 4. Representative agarose gel electrophoresis analysis of PCR products for *EPSPS* (A) and Cry1ab (B) genes. For 4A: M = 100 bp molecular weight marker; 11P = Soy powder; 112P = Soy based cake; L3 = Soy drink; L32 = Soy drink; L7 = Soy drink; L2 = Soy drink; L6 = Soy drink For 4B: M = 100 bp molecular weight marker; 1P = Corn pap; F12 = Corn based biscuit; T7 = Corn starch; T153 = Sweet corn; 8P = Corn based meal; T92 = Sweet corn; F7 = Corn based biscuit; T13 = Sweet corn; 4P = Corn base pap.. Samples with similar product name and different sample codes are collected from different locations in Nigeria.

Conclusion

This study has successfully used PCR-based techniques to screen for the presence of GM foods in the Nigerian market. PCR-based techniques for GMO detection is a highly sensitive and globally validated approach for GMO screening (Ahmed, 2002; Anklam et al., 2002; Forte et al., 2005; Meriç et al., 2014). Moreover, GMO-specific DNA markers used in this study are also the universally accepted markers for this type of screening. Therefore, based on the findings of this study, it could be concluded that products made with GMO materials have entered the food chain in Nigeria at a modest scale.

Due to ethical and biosafety concerns, GM foods are strongly regulated in many countries. Theoretically, the consumption of foods containing genetically engineered DNA is expected to be digested without any adverse consequences and there is currently no report of adverse reactions resulting from the consumption of food made from GM crops. Even though public concern of the unknown health risk of GMOs to human and animals abound (Lisha et al., 2017), proponents of GMO foods continue to highlight benefits, including the ability of the technology to tackle the problem of food scarcity in many developing countries. However, the need to have proper labelling through appropriate identification techniques to protect consumers' right and to ensure food safety has been suggested (Azadi and Ho, 2010). It is also important that regulatory bodies, particularly in countries such as Nigeria, have the capacity to check materials imported for food manufacturing purposes for the presence of GMO specific sequences. In 2015, the Nigerian Government established the National Biosafety Management Agency (NBMA) via the enactment of the National Biosafety Management Agency Act 2015. The agency provides regulatory framework for the introduction of GMOs into Nigeria, recently approved the commercial release of GM cotton and gave permission for confined field trial of GM

maize. However, findings of this study together with previous reports by Okpara et al. (2016) presuppose that products made from GM crops have been in the Nigerian market before, prior to the establishment of the NBMA.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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