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Presence of *Bacillus thuringiensis* (Bt) gene in cereals and cereal-based products in local markets and supermarkets of Yaoundé, Cameroon

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The use and importation of genetically modified (GM) crops and products derived from these crops are regulated by national and international policies, which unfortunately are often not properly implemented in some countries. Given the ongoing globalization of trade and increasing availability of GM plant products, countries like Cameroon with a weak system to regulate the importation of these products face the threat of these products entering local markets. This study investigated the presence of GM cereals and cereal-based products circulating in the local markets and supermarkets in Yaoundé, Cameroon. An inventory of cereal based products from these markets was conducted and one of the products was labeled as being derived from GM cereals crops. DNA was extracted from 26 products with a protocol using SDS guanidine thiocyanate to assess the presence of the *Bacillus thuringiensis* (Bt) gene. Polymerase chain reaction (PCR) was used to amplify the DNA fragment associated with Bt gene. Majority of the products were maize-soya based and wheat-soya based. The Bt gene was present in four of the 14 maize based products tested. The presence of the Bt gene in these cereal based products suggest the need for these products to be labeled according to international regulations.

Key words: Bt gene, invertase gene, polymerase chain reaction, guanidine thiocyanate cereal, cereal based products.

INTRODUCTION

The exponential growth in human population during the last century has left various challenges, especially in the domain of feeding and health in many countries. Given this rapid growth and some environmental and agricultural challenges in many countries (Kishore et al., 1999), much effort was needed to address threats that impeded crop production. Traditional and modern biotechnology has been used to improve agronomic traits in plants. As reported, one advantage of growing genetically modified (GM) crop is the reduction in the use

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Table 1. Sequences of primers used in this study.

Primers	Length (bp)	Sequence 5'to 3'	Target gene	Annealing temperature (°C)
Ivr 1A	25	CCGCTGTATCACAAGGGCTGGTACC	Maize invertase	50
Ivr 1B	25	GGAGCCCGTGTAGAGCATGACGATC		50
Cry 1As	25	TGGGGAACAGGCTCACGATGTCCAG	Bt-maize specific	74 and 69
Cry1Ab	25	ACCATCAACAGCCGCTACAACGACC		74 and 69

Ivr 1A = Primer for the forward strand and Ivr 1B = primer for the reverse strand of the invertase gene; Cry 1As = primer for the forward strand and Cry 1Ab = primer for the reverse strand of the Bt gene.

of chemicals to control pests, leading to increased productivity and profitability (Halford et al., 2000; Engel et al., 2002; Gomez-Barbero et al., 2006).

However, many concerns have been raised on the use of GM crops. The negative consequences of the widespread use of GM plants have been divided into two categories involving: (1) adverse effects on the environment and (2) harmful effect on human and animal health (Halford et al., 2000; FAO, 2003; Qaim, 2009). The most commonly cited risks have been transfer to non-target species (Halford et al., 2000; Cartagena Protocol on Biosafety, 2000) and the possible long term undesirable consequence of biodiversity erosion through the loss of traditional crops (Firbank et al., 2006).

The negative socioeconomic effects caused by the corporate control of the food chain have also been reported. Considering the dominance of the biotech industry by large multinational corporations (MNCs), the shift in seed control from a local to a corporate level reduces farmer's involvement in seed enhancement as well as control over "food sovereignty" (FA0, 2003; Azadi et al., 2015). Considering the controversy, policies have been proposed to insure a free and informed adoption and control of genetically modified products (Hogan et al., 2001; Nair et al., 2002; MINEP, 2003; Vacher et al., 2009). Before the introduction of these products in Africa, ethical, cultural environmental, health, economic and ecological concerns were raised (MINEF/UNEP/GEF, 2003). Today, African countries have adopted GM products for commercialization (James, 2008).

Cameroon has ratified the Cartagena Protocol on Biosafety, but has not yet adopted GM crops for commercialization. Given the globalization of trade, the wide-spread adoption of GM crops, and the weak system to regulate GM food products in Cameroon, it was necessary to identify such foods in the market. This study investigated the presence of GM cereals and cereal-based products circulating in the local markets and supermarkets in Yaoundé, Cameroon.

MATERIALS AND METHODS

Sample collection and preparation

An inventory of maize, wheat and soya based products or combination from local markets and supermarkets was carried out using a structured questionnaire. Data recorded included the manufacturer, expiry date, manufacturing date, ingredients of the product, composition of the product, physical form of the product, place of manufacturing and packaging form. Processed products and seeds were collected for maize and soya-based products. The maize grain collected from the field was used to standardize the protocol for DNA extraction. Non-powdered products were separately milled and 30 g of each sample was placed in a plastic bag, sealed and stored at 25°C in a dry place.

DNA extraction

Total genomic DNA was extracted as indicated by Edwards et al. (1991) for the cell lysis steps and Chomczynski and Mackey (1995) for protein and nucleic acids precipitation steps. Note that, the cell lysis solution was added to the mixture followed by proteinase K (20 mg/ml). After an overnight incubation of this mixture, RNAse H 250 UI was added followed by incubation at 37°C, as the objective was the extraction of total DNA. The obtained DNA pellet washed using 70% ethanol with the pellet air-dried, was re-suspended in 200 µl of 1xTris-EDTA buffer. DNA samples were stored at -20°C until further analysis.

The polymerase chain reaction and optimization of reaction conditions

Amplification profiles for the invertase and Bt genes were established through two different PCR procedures; the touchdown PCR and the multiplex PCR. These methods were applied to determine the best annealing temperatures. The multiplex PCR was used to check if the two genes could be amplified under the same conditions. The method of Brinegar et al. (2004) was used with modifications. The PCR mixture consisted of 4 µl of DNA extract, 0.25 µl of 5 U/ml Taq polymerase, 0.25 µl of each primer (Table 1), 0.5 µl of 10 mM dNTPs, 2.5 µl of 10x thermopol buffer in a final volume of 16 µl. The amplifications were performed using a T3 thermal cycler (Biometra, UK) with the following conditions: 3 min at 94°C (pre-denaturation), 45 cycles of 45 s at 94°C (denaturation), 45 s at 69°C for Ivr gene and 50°C for Bt gene (annealing), and 30 s at 72°C (elongation), with a terminal step of 5 min at 72°C. The mixture was held at 4°C.

Electrophoresis of PCR products

PCR amplification products were separated by gel electrophoresis using 2.5% agarose gels (Seakem) with 1×Tris-Borate-EDTA (TBE) running buffer and stained with ethidium bromide. DNA fragments were visualized on an ultraviolet trans-illuminator and photographed with a digital camera.

Table 2. Sample distribution per manufacturer/country.

Country	Cameroon	France	Ivory Coast	Republic of South Africa	Turkey	Unknown
Percentage of products	39.29	39.29	7.14	3.57	3.57	7.14

Table 3. Sample distribution per type of cereals.

Sites of collection	Maize-based Product	Wheat-based Product	Soya-based Product	Maize-and soya based Product	Wheat- and soya-based Product	Multicereal Product	Total
Local market	3	0	1	0	0	0	4
Super-market	6	10	1	3	1	1	22

Table 4. Distribution of Ivr and Bt-genes among the study samples.

Gene Id	Number of products tested	Number of products with the gene	Number of products without the gene
Ivr gene	26	14	12
Bt gene	14	4	9

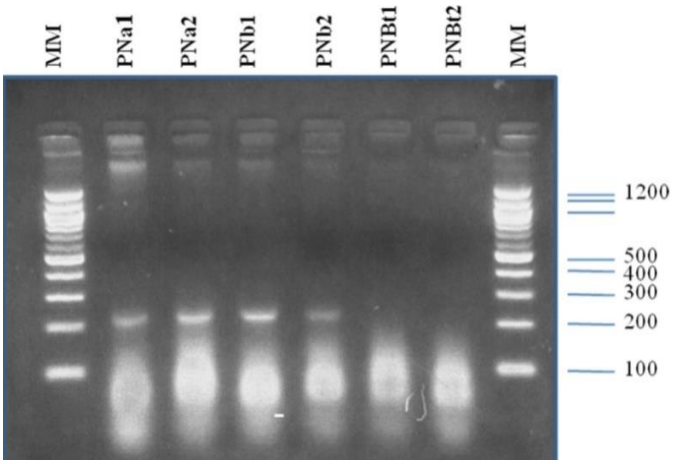

Figure 1. Bands for Ivr gene using the two different annealing temperatures (PNa=74°C – PNb=69°C). -, No bands for Bt gene with the annealing temperature of 69°C; MM = 100 base pair molecular weight marker (MM); PNa1 = PN sample code; a = annealing temperature of 69; 1 = the first tube.

RESULTS

Source and characteristics of study samples

Samples collected were from various places of manufacture (Table 2). Four products were collected from local markets and 22 from supermarkets (Tables 3 and 4).

DNA extraction and PCR assay

DNA was successfully extracted from 14 products using SDS and guanidine thiocyanate. Amplification of the Ivr gene in a sample was used as an indicator for a successful DNA extraction procedure. DNA was not successfully extracted from wheat-based products. Invertase gene was used as an internal positive control for DNA extraction and PCR conditions. Only Ivr-positive samples were tested for Bt-gene. Four of the 14 products tested were Bt-positive (Figure 1). The product used to standardize the protocol for DNA extraction was also tested for Bt gene and was found positive. Band sizes for amplified Ivr gene and Bt genes were 240 bp for Ivr gene and 200 bp respectively (Figure 2). Ivr-positive products were maize and soya based products. No amplification of Ivr gene was obtained with wheat-based products and multi-cereal products. Bt gene was mostly found in maize-based products.

DISCUSSION

Cameroon, although it has ratified the Cartagena protocol on biosafety, has not yet adopted GM crops and derived foods for commercialization. Actually, the quality control scheme of GMOs and derived products is being built. In this process, a comprehensive organization is needed. Laws have been enacted but yet few are implemented. For instance, it is accepted globally and recommended

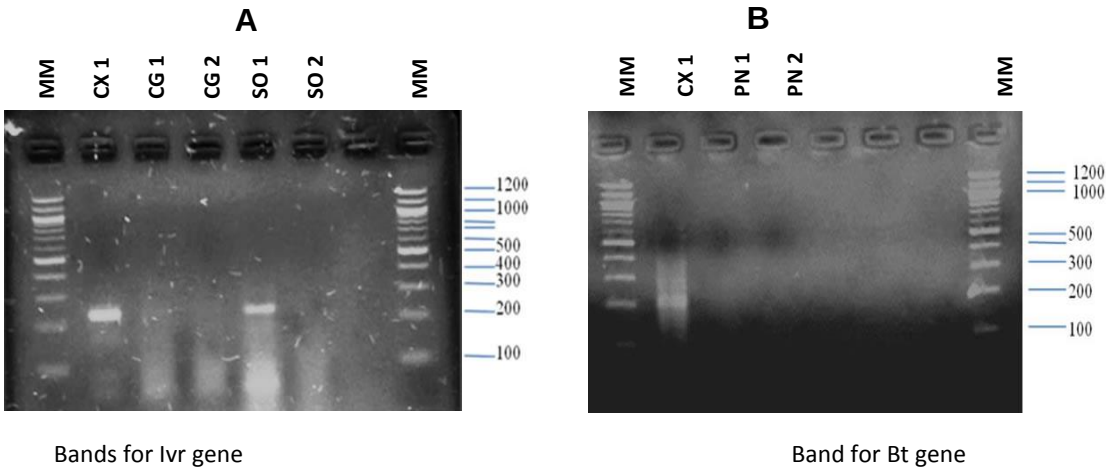

Figure 2. PCR fragments for Ivr (A) and Bt genes (B).

that GMOs and derived products should be labeled to insure a free and informed consumer choice. The presence of just one sample label that conforms to this law reveals the limited practical implementation of the regulation in use in Cameroon (Decree No. 2007/0737/PM of the 31st May 2007). Even though each country or region need to establish threshold value for GM-based product labeling application, Cameroon and even the Central region of Africa has not yet defined the percentages at which a product can be label as containing genetically modified ingredients. It might mean that no matter the percentages of ingredients present in a food product, according to the law, it should be labeled.

In the process of food control, laboratories analyses play major role as quality results are needed for good and right decisions to be taken in the regulatory processes (see "Elements for a National Biotechnology Policy Framework for Cameroon"). Using PCR techniques, the purification of nucleic acids from the sample is often the deciding factor in the production of meaningful results. The combined protocol used in this study can be used with success for DNA extraction from various food types. However, the method was best suited for isolation of DNA from less processed foodstuffs such as corn flour and corn seeds. Improvements are needed to isolate DNA from highly processed foodstuffs that might also contain PCR-inhibitory substances. This was the case of wheat-based products which consistently failed (Tengel et al., 2001). It will also be of use to evaluate the power of the method in terms of quantities and quality of DNA obtained. This will allow the establishment of standards for each product type.

Successful amplification of Bt-gene in cereals and cereal-based products made in Cameroon suggest the use of Bt maize seeds by farmers and in the industry; the source and origin are still to be identified. However, the identification of Bt-positive products was based on the qualitative-PCR analysis using species-specific primers. The obtained molecular sizes were not very different from those reported in the literature (Tengel et al., 2001; Brinegar et al., 2004). Coupled with other techniques as the quantitative-PCR analysis, Western blot, the method used in this study would allow a broad application (Roger et al., 2014). Amplification of the Ivr and Bt genes fragments demonstrated that DNA of integrity sufficient for PCR analysis can be purified using the described techniques.

The finding of this study shows the need to strengthen the national bodies in charge of control at different levels, with capacities and equipments for an efficient and effective action. Given the role of laboratory analysis in the established regulation processes for GMOs, laboratories should be equipped with appropriate trainings and equipments. A broad range assessment of various food types in their various forms would allow a global view at national scale and the standardization of laboratory techniques for GMO detection in Cameroon.

As Cameroon is considered a leading country in the Central region of Africa, the presence of National Laboratories for GMOs testing would be a valuable asset in the northern region and in the southern part of the country, as these laboratories would serve for the Central Africa zone. Quality control for GMOs testing should be added to the quality control system of the country at the various customs posts, especially at the national ports.

Conclusion

The finding of this study indicates that many of the maize-, wheat- and soya-based products present in local markets and supermarkets in Yaoundé are not labeled according to the regulation in use for genetically modified products in Cameroon. Many of which are GM-derived

food products; it will be necessary for the Cameroon government to revise this regulation to meet this challenge.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Azadi H, Samiee A, Mahmoudi H, Jouzi Z, Rafiaani Khachak P, De Maeyer P, Witlox F (2015). Genetically modified crops and small-scale farmers: main opportunities and challenges. Crit. Rev. Biotechnol. 7:1-13.

Brinegar C, Levee D (2004). A Simple Method for Detecting Genetically Modified Maize in Common Food Products. BAMBED 32:35-38.

Cartagena Protocol on Biosafety to the Convention on Biological Diversity, text and annexes, Montréal 2000.

Chomczynski P, Mackey K (1995b). Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. Biotechnology 19:942-947.

Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Engel KH, Frenzel Th, Miller A (2002). Current and future benefits from the use of GM technology in food production. Toxicol. Lett. 127:329-336.

FAO (2003). Weighing the GMO arguments: against, cited 2013-02-08, available at: http://www.fao.org/english/newsroom/focus/2003/gmo8.htm

Firbank LG, Rothery P, May MJ, Clark SJ, Scott RJ, Stuart RC, Boffey CWH, Brooks DR, Champion GT, Haughton AJ, Hawes C, Heard MS, Dewar AM, Perry JN, Squire GR (2006). Effects of genetically modified herbicide-tolerant cropping systems on weed seed banks in two years of following crops. Biol. Lett. 2:140-143.

Gomez-Barbero M, Rodriguez-cerezo E (2006). Economic Impact of Dominant GM Crops Worlwide : A review. European commission DG JRC-IPTS.

Halford NG, Shewry PR (2000). Genetically modified crops: methodology, benefits and public concerns. Br. Med. Bull. 56:62-73.

Hogan RM, Walters DS (2001). Quality control in the development of transgenic crop seed products. Crop Sci. 41:1381-1389.

James C (2008). Etat des PGM cultivées: 2008. ISAAA. 2008.

Kishore GM, Shewmaker C (1999). Biotechnology: Enhancing human nutrition in developing and developed worlds. Proc. Natl. Acad. Sci. USA 96:5968-5972.

MINEP/UNEP/GEF (2003). Proceedings of socio-economic impact of GMOs, Limbe Botanical and zoological Garden, 9-10 December 2003

Ministère de l'Environnement et de la Protection de la Nature (MINEP), Loi n° 2003/006 du 21 Avril 2003 portant Régime de sécurité en matière de Biotechnologie moderne au Cameroun.

Nair RS, Fuchs RL, Shuette SA (2002).Current methods for assessing Safety of Genetically Modified Crops as examplified by Data on Roundup Ready Soybeans. Toxicol. Pathol. 30:117-125.

Qaim M (2009). The Economics of Genetically Modified Crops*",* Ann Rev Resour Econ. 1:665-693.

Roger DD, Gone S (2014) Evidence of the Presence of Genetically Modified Foods in the Sudano-Sahelian Zones of Cameroon. Food Nutr. Sci. 5:922-928.

Tengel C, Schüßler P, Setzke1 E, Balles J, Sprenger-Haußels M (2001). PCR-Based Detection of Genetically Modified Soybean and Maize in Raw and Highly Processed Foodstuffs . BioTechniques 31:426-429.

Vacher C, Bourguet D, Marion D, Lemarié S, Ambec S, Hochberg ME (2006). Fees or refuges: which is better for the sustainable management of insect resistant to transgenic Bt corn? Biol. Lett. 2:192-202.