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Qualitative detection of genetically modified material in crops and food products containing maize and soybean in Algeria

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The present study was carried out to ascertain the genetic status of soybean and maize crops introduced in Algeria and to test local and imported products containing maize and soybean for the presence of genetically modified organisms (GMO into the food and feed chain). Samples from crops and processed food products were selected, randomly sampled and screened by polymerase chain reaction (PCR) for the presence of the 35S cauliflower mosaic virus promoter (P35S CaMV) and the *Agrobacterium tumefaciens* nopaline synthase (nos) terminator, commonly found in transgene cassettes. Results showed that GMOs were absent in all crop samples, but present in 29 food and feed samples. These analyses are discussed for the efficiency of the Algerian regulation and the absence of labelling and traceability mechanisms within the country.

Key words: Genetically modified organisms (GMOs), detection, maize, soybean, seeds, food and feed products.

INTRODUCTION

In Algeria, the discussion around genetically modified organisms (GMO) regulation and other related issues appeared with the adoption of the Cartagena Protocol on biosafety, although the country had only little experience in modern biotechnology and biosafety regulation (Louanchi, 2009). Agricultural development in the country, a fossil fuel producer, has not been prioritized for several years. For that reason, most basic food and feed, such as cereals (wheat, maize) and oil-yielding plants (canola, soy) are imported as unprocessed or transformed products. The concern of becoming a passive consumer is even greater in light of recent studies on animal health (Séralini et al., 2014).

Moreover, introduction of the first hybrids in the 1990s failed increasing yields and provoked a genetic erosion of landraces and during the last decade, the agricultural sector showed an important development mainly based on massive importation of agricultural inputs like seeds

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Table 1. Presentation of maize and soy products analysed for GMO PCR detection.

Species	Product	Number	Origins
Maize	Food grain	18	USA, Argentina and unknown
	Canned	21	Spain, France, Italy, Brazil, Thailand, Syria, China and unknown
	Flour	5	Algeria, Tunisia
	Pop corn	12	Argentina
	Ear	43	Algiers, Tipaza, Boumerdes, Ain Defla, Béjaia, Blida, TiziOuzou, Jijel, Mostaganem, Ain Temouchent, Oran
	Seeds	7	Centre National de Certification et de contrôle
Soybean	Flour	11	Spain, Argentina, USA and unknown
	Food grain	2	Italy and unknown
	Seeds	1	Italy

with no specific control or regulation system. The country has ratified the Cartagena Protocol and promulgated a regulation prohibiting importation, production, distribution, marketing and use of genetically modified plant material (Ministerial Order No. 910 of December, 2000). However, there is no specific regulation to control the presence or use of GMOs in food crops and feed, as well as no regulation for food labelling. This situation prevents us from getting insights on the presence or absence of GMOs in agriculture, food and feed chains.

Earlier studies on GMOs identification have been carried out by using serological and polymerase chain reaction (PCR) detection methods and the results confirmed the presence of GMOs in several analyzed samples (Ghazi, 2007; Louanchi et al., 2008). Screening methods for qualitative detection (that is, presence or absence of a transgenic event) allow rapid detection of a great number of GMOs. It is a simple and fast method based on differential amplification of specific sequences commonly found in many transgenic events (Wolf et al., 2000), but does not allow differentiation of the involved GMO. In general, the screening of the 35S promoter from Cauliflower Mosaic Virus (P35S CaMV) and terminator of the *Agrobacterium tumefaciens* nopaline synthase gene (T-Nos) allows the detection of most of the authorized GMOs (Hemmer, 1997).

The aim of this study is (i) to ensure that soybean and maize seeds introduced in Algeria are not genetically modified and (ii) to test local and imported maize and soybean for the presence of GMOs in the food and feed chain in Algeria.

MATERIALS AND METHODS

Sampling

One hundred and twenty (120) samples with known or unknown origins were randomly collected from markets, harbours or institutions from several regions in Algeria during the years 2014 and 2015 (Table 1). The samples comprised 106 types of maize-grain food (18), canned maize (21), flour (5), popcorn (12), ears (43), seeds (7)- and 14 types of soybean-grain food (2), flour (11), and seed (1).

DNA extraction and purification

All the procedures were carried out under sterile conditions. Soybean and maize samples were ground with an electric grinder. 40 mg of the resulting flour were used for the extraction of the genomic DNA according to the method described in the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The extracted DNA pellet was air dried and dissolved in 50 µl of sterile distilled water. The concentration and purity of the extracted DNA were measured by absorbance under UV at 260 and 280 nm, using a spectrophotometer (NanoDrop 2000® Thermoscientific). The DNA concentration was adjusted to 25 ng/µl prior to PCR by diluting into sterile distilled water.

Oligonucleotides primers used

Primers were used to amplify DNA sequences of specific genes from maize (Studer et al., 1998), soybean (Meyer et al., 1996), the promoter 35S of Cauliflower Mosaic Virus (CaMV) (Wurz and Willmund, 1997), and were used to detect Roundup Ready GM crops (Elsanhoty, 2013) and also several Bt Maize, and the Terminator Nopaline Synthase (T-Nos) (Lipp et al., 2001) mostly observed on transgenic events (Table 2). All primers were synthesized by DIAG-GENE (Angers Technopole, France). Before their use, lyophilized primers were diluted in purified water to obtain a final concentration of 20 pmol/µl.

DNA amplification and PCR conditions

PCR amplification was performed on a C1000 Thermal Cycler (BIORAD). Amplification conditions for each set of target sequences, as described by Querci et al. (2006), are presented in Table 3. Each PCR reaction mix (25 µl total volume) contained 2.5 µl PCR buffer (10x concentrate, Promega), 2 µl $MgCl_2$ solution (25 mM $MgCl_2$), 1 µl dNTP solution 0.2 mM of each of dATP, dCTP, dGTP and dTTP, 0.5 µl of each primer, 1 Unit AmpliTaq Gold

Table 2. DNA primers used and corresponding target DNA sequence.

Primers	Sequence (5´ to 3´)	Target DNA sequence	Sequence length
GMO3 GMO4	GCCCTCTACTCCACCCCCATCC GCCCATCTGCAAGCCTTTTTGTG	Lectin gene	118 bp
ZEIN3 ZEIN4	AGTGCGACCCATATTCCAG GACATTGTGGCATCATCATTT	Zein gene	277 bp
p35s-f2 petu-r1	TGATGTGATATCTCCACTGACG TGT ATCCCTTGAGCCATGTTGT	P35 S CaMV	172 bp
HA-nos 118-f HA-nos 118-r	GCATGACGTTATTTATGAGATGGG GACACCGCGCGCGATAATTTATCC	*NOS*-terminator	118 bp

Table 3. PCR conditions for all target DNA sequences.

Process	GM03/GM04	ZEIN3/ZEIN4	P35s-f2/Petu-r1	HA-nos 118f/HA-nos 118r
Initial denaturation	3 min; 95°C	3 min; 95°C	10 min; 95°C	3 min; 95°C
Denaturation	30 s; 95°C	1 min; 96°C	30 s; 95°C	25 s; 95°C
Annealing	30 s; 63°C	1 min; 60°C	30 s; 62°C	30 s; 62°C
Extension	30 s; 72°C	3 min; 72°C	25 s; 72°C	45 s; 72°C
Cycles	40	40	35	50
Final elongation	3 min; 72°C	3 min; 72°C	10 min; 72°C	7 min; 72°C

polymerase (Promega), 5 µl of template DNA and completed to 25 µl with purified water. Negative control presented the same PCR reaction mix with water instead of extracted DNA. All amplified products were stored at 4°C until the gel electrophoresis.

DNA amplicons were separated on 2% w/v agarose gels (Promega) using TBE buffer solution at pH 8.0 (0.89 M Tris, pH 8.0; 0.89 M Boric acid; 2 mM Na_2EDTA) and 0.5 $\mu g.ml^{-1}$ of ethidium bromide at 150 v for 45 min. In addition, amplified target fragments of transgenic P35S CaMV and T-Nos sequences (DIAG-GENE, Angers Technopole, France) used as positive controls, were loaded on the gel. Target DNA sequences were observed with UV transilluminator Gel Doc Molecular Imager (BIORAD).

RESULTS AND DISCUSSION

Amplification of extracted DNA

In order to assess the presence of transgenic elements, DNA extraction using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) allows us to obtain a DNA of a good quality to perform PCR amplifications. PCR quality control, as recommended by the EU procedures for GMO detection (Querci et al., 2006), was performed with the specific primers for maize and soybean DNA. The primer pair GM 03/GM 04 is specific for the single copy lectin gene LE1 and yields a PCR product of 118 bp size (Meyer et al., 1996) detectable in transgenic as well as in conventional soybean. The primer pair ZEIN3/ZEIN4 is specific for the zein gene and yields a PCR product of 277 bp size (Studer et al., 1998) detectable in transgenic, as well as in conventional maize. Soybean and maize specific primer pairs served as a control for the amplification of the isolated DNA and PCR procedure (PCR quality control). All the tested samples gave positive results for the amplification of the control primer pairs. These results confirmed that the samples from processed food, like flour or meal, are based on maize or soybean.

DNA target sequences detection

Primers p35s-f2 and petu-r1, specific for the detection of the 35S promoter of CaMV, amplified a 172 bp fragment. These fragments are only detected in GMO samples. A specific band was amplified for the 35S promoter in 29 out of the 120 samples tested (20 from maize and 9 from soybean) suggesting that these samples contain GMO (Figure 1). Similarly, primers HA-nos118-f and HA-nos 118-r, specific for the detection of terminator nos, amplify a 118 bp fragment, detected only in transgenic samples. Here also, a specific band was amplified for the nos terminator in the same 29 samples, therefore corroborating the presence of a transgenic event (Figure

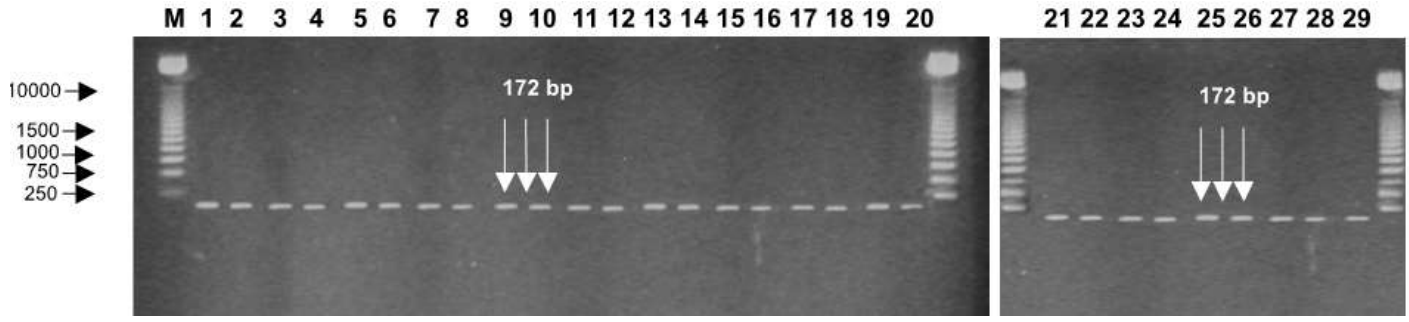

Figure 1. Detection in maize and soybean samples of the expected DNA fragment of the Promotor 35S CaMV using primer pair P35S-f2/petu-r1. M: 1 Kb ladder marker DNA; 1-20: DNA samples of maize; 21-29: DNA samples of soybean (loaded according to Table 4).

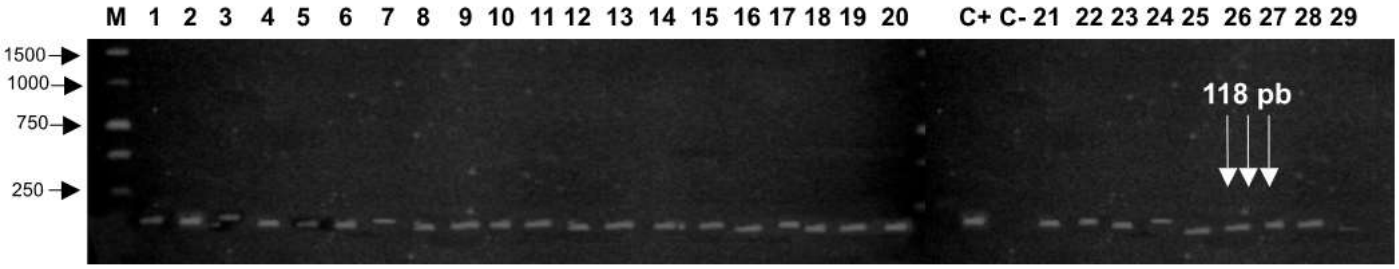

Figure 2. Detection in maize and soybean samples of the expected DNA fragment of the Terminator T-Nos using primer pair HA-nos 118-f/HA-nos 118-r. M: 1 Kb ladder marker DNA; 1-20: DNA samples of maize; 21-29: DNA samples of soybean (loaded according to table 4); C+: positive control; C-: negative control.

2).

Investigated transgenic samples

During this experiment, 120 samples of maize and soy were analysed for the presence/absence of GMOs. For crops, samples were represented by ears (43) of maize originating from several fields in eleven different localities, maize seeds (7 varieties), and one seed variety of soybean. For food products collected from markets and harbours, samples were represented by maize as food grain (18), soy as food grain (2), canned maize (21), corn flour (5), popcorn (12) and soybean flour (11). Twenty nine samples (20 from Maize and 9 from Soybean) showed the specific DNA fragment either for P35S CaMV fragment or T-Nos fragment.

Results show that transgenic elements are not present in the agricultural products (ears and seeds) investigated. This reveals that the Ministerial Decree of 2000 prohibiting the imports of GMO containing material for agricultural uses is effective. Although these crops are not widespread in Algeria, their use as animal feed and processed food goods is wide spread, therefore, we believe that it is important to strengthen this decree by setting up laboratories of control and validation of standard methods for detection as it is done in several countries, especially for UE legislation (Trapmann et al., 2001, 2002).

Indeed, it was found that four samples from grain food (3 of maize and 1 of soybean), which are close to seeds in terms of propagation, were detected positive for GMOs in our studies. Although, they are designated for food transformation, these grain feed had more than 90% of germination rate (laboratory data). This result highlights a crucial issue concerning the denomination of GMO or LMO (living modified organisms) and suggests that the latter should be included in the risk assessment, because of their possible dissemination.

Over 50% of the GMO positive samples (16 out of 29) were imported from Latin America (mainly from Argentina) as food grain, popcorn or canned food (Table 4). Six samples were imported from Asia (Thailand and China) as canned maize. Two were from USA as food grain, while five were of unknown origin.

DISCUSSION

The present studies reveal that GMOs are not present in seeds in Algeria. This result is important regarding the Algerian regulation restricting the use of GM material. However, the regulatory system should evolve towards a strengthening of the control mechanisms, including a

Table 4. Presentation of GMO samples detected and their origin.

Species	Samples	Nature	Origin
Maize	1	Canned (Royal Umbrella)	Thailand
	2	Canned (Boonsith)	Thailand
	3	Canned (Del Monte)	Thailand
	4	Canned (Mon Jardin)	China
	5	Canned (Safari Food)	China
	6	Canned (Keema)	Thailand
	7	Popcorn (Primore)	Argentina
	8	Popcorn (Ploopie)	Argentina
	9	Popcorn (Premium)	Latino America
	10	Popcorn (Snack Crops)	Argentina
	11	Popcorn (Three Stars)	Argentina
	12	Popcorn (Three Stars)	Argentina
	13	Popcorn (Three Stars)	Argentina
	14	Popcorn (Primore)	Argentina
	15	Popcorn (Boom Magic)	Argentina
	16	Popcorn (Circus)	Argentina
	17	Popcorn (Allicoupe)	Argentina
	18	Food grain	Unknown
	19	Food grain	Argentina
	20	Food grain	USA
Soybean	21	Flour	Unknown
	22	Flour	Argentina
	23	Flour	Argentina
	24	Flour	Unknown
	25	Flour	Unknown
	26	Flour	USA
	27	Flour	Argentina
	28	Flour	Unknown
	29	Food grain	Unknown

better regulation on GM related administrative procedures, risk assessment guidelines, implementation of efficient laboratories for GM control using more sensitive methods such as semi-quantitative detection (Tozzini et al., 2000), and real-time quantitative PCR detection (Poitras and Houde, 2002; Yang et al., 2007) with validated and standardized methodologies.

Our results showed that several food products which contained GMOs are mostly imported from Argentina, Thailand and China and this situation raises the problem of labelling and traceability (Miraglia et al., 2004). Indeed, the lack of labelling regulation is a concern for numerous countries, which could be restrictive countries like Saudi Arabia (Abdel-Mawgood et al., 2010) or Tunisia (Chaouachi et al., 2013) or permissive countries regarding GMOs content like South Africa (Viljoen et al., 2006) and Egypt (Tony et al., 2003).

This work is the first contribution in Algeria on the presence of GMO in crops and food products. Results show the absence of GMO in crops and seeds but their presence in 29 samples of foodstuffs. Further studies are needed to quantify the amount of GMOs that are present in the food products throughout the Algerian market. For that purpose, genetically modified products in food commodities should be checked and controlled to comply with the EU and international regulations that enforce labelling of food when GMO presence is higher than 0.9%. Although some countries might be tolerant with GMOs presence in food, the precaution principle is thought to be applied for as long the risk for human health on the long run is not known.

Our results suggest that, to comply as well with the national regulation, genetically modified material detection and monitoring plans are necessary to control the distribution of unlabelled GM- containing foods and feed in the Algerian market.

For an effective management of the genetically modified organisms and in order to set up a national

policy which can face the transnational movement of both agricultural and food product, Algeria must set up a regulation which include labelling and traceability and a detection methodology with homogenized protocols and adapted techniques used in routine.

Conflicts of interests

The authors have not declared any conflict of interests.

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