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Detection of Genetic Modified Feed Component

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Abstract

There are growing concerns over the possibility of transfer genetically modified sequences from genetically modified feed component (GM feed) to animals and their products, moreover, affect these sequences on animal and human health. This study was implemented to detect P35S in modified feed by using PCR technique by detecting presence P35S promoter, which responsible for the regulation of gene expression for most of the transgenic genes. Thirty eight feed samples were collected from different sources of Baghdad markets, which have been used for feeding livestock, comprise 21 coarse mixes feed, 13 pelleted feed, and 4 expanded feed. Genomic DNA was extracted by using two methods, CTAB method and Wizard kit. In order to verify the presence (P35S) in feed samples, a pair of primer for 35S promoter was used. The results of the present study showed that 58% of tested samples contained promoter P35S this means presence genetically modified feed in the Baghdad market.

Keywords: Feeding livestock, Genetic Modified Feed, PCR, P35S.

التحري عن وجود تحوير جيني في مكونات الأعلاف

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الخلاصة

هناك مخاوف متزايدة بشأن إمكانية نقل تسلسلات الدنا المعدلة وراثيا من مكونات الأعلاف المعدلة وراثيا (GM تغذية) للحيوانات ومنتجاتها، علاوة على ذلك، تؤثر هذه االتسلسلات على الصحة الحيوانية، وكذلك صحة الإنسان. تم تنفيذ هذه الدراسة للكشف عن الأعلاف المعدلة وراثيا باستخدام تقنية PCR عن طريق الكشف عن وجود P35S ، المسئولة عن تنظيم التعبير الجيني لمعظم الجينات المعدلة وراثيا. تم جمع ثمانية وثلاثين عينة أعلاف من مصادر مختلفة من أسواق بغداد، التي كانت تستخدم لتغذية الماشية، وتشمل 21 من النوع الخشن 13 محبب، 4 ناعم. تم استخلاص الدنا الجينومي باستخدام طريقتين، طريقة CTAB والـ Wizared. من أجل التحقق من وجود (P35S) في عينات الأعلاف، تم استخدام زوج من البادئات لـ

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35S. وأظهرت نتائج الدراسة أن 58٪ من عينات الفحص تحتوي على الـ P35S وهذا يعني وجود الأعلاف المعدلة وراثيا في أسواق بغداد.

Introduction

Genetically Modified Organism (GMO) is an organism whose genetic material has been altered by removal or alteration of a particular characteristic or trait using genetic engineering techniques. This change can be done not only within the same species, but also between different types of organisms [1]. All the information required to produce the new trait and new protein should be available in the original DNA of organism [2]. Genetically Modified Crops is one of the most important Genetically Modified Organism (GMO).

The global land area of crops modified for agronomic input traits continues to grow, these crops have become an extremely significant source of feed stuffs for farm animals [3]. The relevant GM crop species that used as a source of livestock feed components include canola (rapeseed), soybean, maize (corn), cottonseed and others [1]. These crops have been modified to express some traits such as insect resistance and insecticide [4], herbicide tolerance [5] resistance to virus infection [6] and drought tolerance [7] either singly or in combination. The proteins that have been expressed in order to confer these traits are already present in GM plant products, and may be also present in animal products (meat) that feed on the GM plants or their products.

There are growing concerns about potential risks of used the products of GM feed, because of the possibility of transfer GM sequences through the food chain to animals products [8,9]. In this context, many studies have been able to identify GM sequences in DNA of milk from animals fed on GM feed [10,11].

Moreover, The genetic modification effect may transmit to human, for example, when using bacteria-resistant genes as a new trait to the sources of livestock feed, this trait (resistance) possible transmitted to harmful bacteria to humans or the normal flora of the human intestine [12]. Therefore, there is doubted over the safety of feeding GM crops to animals especially when the animal products such as meat, milk and eggs are significant sources of high-quality food for humans and represent approximately one-sixth of their food energy and one-third of their food protein on a global basis [13]. Finally, the effects of genetically modified feed on animal and human health cannot be excluded.

The study was aimed to detect the presence of a genetic modification in the components of commercial feeds in Baghdad markets.

Materials and Methods

Sampling Process

Feed samples collected randomly from different regions of the local markets of Baghdad city from February to May 2012 and according to the approved methods [14]. Thirty eight feed samples comprise 21 coarse mixes feed, 13 pelleted feed and 4 expanded feed (Table -1). Samples are taken from different locations of the feed container and placed in disposable bags and then transported to the laboratory.

Table 1-Summary of feed samples.

Number of samples	**Sample type**	**Samples processing** (Tisch, 2006)
21	Coarse Mixes	Forms of processing: cold (milling, grinding, cracking, soaking) or hot (steam rolling/flaking, extruding, pelleting).
13	Pelleted Feed	Processed using hard cylinders to compress feed ingredients and then formed by grinding, blending and compression.
4	Expanded Feed	Undergone the samples to high temperature and drying, they exposed to steam and forces of shearing and pressure.

DNA Extraction

The basic principle of the DNA extraction consists of first releasing the DNA present in the matrix into aqueous solution, further purifying the DNA from PCR inhibitors. The current method beginning with samples processed prior to extraction procedure, which includes commercial blender or pestle to

grinding (40 mg) of the feed samples. Then used solution in wizard kit (Promega, USA) to removing contaminates from DNA and then using 3 µl from RNase and precipitate by adding 600 µl from isopropanol and then adding 600 µl from 70% ethanol. At the final step, 100 µl from rehydration solution was added and incubate for 60 min at 65°C in a water bath.

CTAB Method: Homogenized samples of up to 350 mg were mixed with 500 µl CTAB buffer [2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH 8), and 20 mM EDTA] and incubated at 65°C for 30 min. The samples were centrifuged for 10 min at 13,000 (rpm) [15]. The supernatant was transferred to a new 1.5 ml tube, extracted with 200 µl chloroform and centrifuged for 10 min at 13,000 (rpm). The upper phase was transferred into a new 1.5 ml tube, precipitated with 1 volume of isopropanol and centrifuged for 10 min at 13,000 (rpm). The supernatant was discarded, and the pellet was washed once with 500 µl of 70% ethanol, centrifuged for 10 min at 13,000 (rpm) and discarded the supernatant, after that air-dried for almost 45 min. The pellet was rehydrate by TE buffer [16].

PCR Technique (Reaction and Program)

Primers were selected according to the specialist criteria in the detection of genetic modification and previous studies [17]. The primers provided by Alpha-DNA company (Canada) according to the following sequences of nitrogenous bases of the P35S primers: P35S F **(5'-ATT GAT GTG ATA TCT CCA CTG ACG T- 3')** and P35S R **(5'-CCT CTC CAA ATG AAA TGA ACT TCC T- 3')**. To prepare the primer, adding distilled water free nuclease was added to the tube containing the lyophilized primer to get the concentration 100 pmol/µl, then the final dilution action that became 10 pmol/µl.

PCR Reaction: The final reaction mixture size 25 µl of the basic mixture of interaction master mix, which contain 12.5 µl of Go *Taq* ®Green Master Mix (2X) (Promega, USA) was prepare and added to 0.5 µl of each forward and reverse primers P35S (10 pmol/µl); 2 µl of DNA sample, and 9.5 µl of D.W.

PCR Program: The amplification program was set as following: initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, repeat these steps for 40 cycles. Finally, extension at 72°C for 7 min.

Agarose Gel Electrophoresis

The integrity of genomic DNA and PCR results was examined by agarose gel electrophoresis with ethidium bromide staining. The integrity of genomic DNA was analyzed on 0.8% agarose gel while the PCR products were analyzed using 2% agarose. After that, the agarose gel was stained with (0.5 g/ml) ethidium bromide to be the bands visible under UV light.

Results and Discussion

The experimental design used in this study is thoroughly randomized design especially with simple random sampling of mutually independent and homogenous feed samples, which purchased from the local markets of the Baghdad city and included many different feed manufactures and different sources of raw crop material.

There is no ability to cover all feed varieties because of the huge number of them. In this study, three main forms of feed were randomly selected: coarse mixes feed, pelleted feed, and expanded feed. These feeds were chosen on the basis of their usage, easy availability in feed shops, as well as, levels of processing (Table -1). The coarse mix samples contained a mixture of coarsely chopped maize grain and processed cereals such as barley, oats or wheat in various proportion. The pelleted and expanded feed were both highly processed compared to coarse mixes [18].

Two main DNA extraction methods of feed were used (Genomic DNA Purification Kit Wizard® and CTAB method), the aim of these procedures is to isolate DNA of reasonable quantity, purity, integrity and quality to allow DNA amplification and is often the most time consuming step of a DNA-based detection method [19, 20]. DNA extraction experiments showed satisfactory quality of DNA obtained by using extraction kit (Wizard) and CTAB method according to the results of PCR reaction, which is one of the important indicators of the quality of the DNA .

The efficiency of the DNA extraction step can be vital for successful amplification since there are many compounds that inhibit DNA amplification that can be co-purified with the DNA such as polyphenols, lipids and polysaccharides or extraction chemicals such as CTAB [21]. On the other hand, the time, which consumed in DNA extraction, is one of the key points that reveal the effectiveness of the DNA extraction method. Therefore, it is preferable to use DNA extraction kit more than the use of manual methods [22]. The development and optimization of protocols for

extraction of DNA for the detection of GMOs in food and feed are getting more and more critical as the numbers of GM crops that arrive at the market are increasing rapidly to [23].

PCR-based technique for GMO detection appears to be the method of choice because of their high sensitivity and specificity . Total of (38) samples of feed were analyzed qualitatively for the presence GM materials (P35S); 22 samples (58%) were found to be positive for GM materials. Figure -1 shows the presence of genetically modified feed in the samples (1 , 3) of coarse mixes feed and samples (1, 3, 5) of pelleted feed, which gave PCR positive results for P35S according to the expected size of band (110 bp) and compared with positive control samples (Pc). In figure -2, all pelleted feed samples[7, 8, 9, 10, 11, 12, 13] were GM, also the coarse mixes feed samples (9 - 21) were GM, except samples (16, 17 , 21).

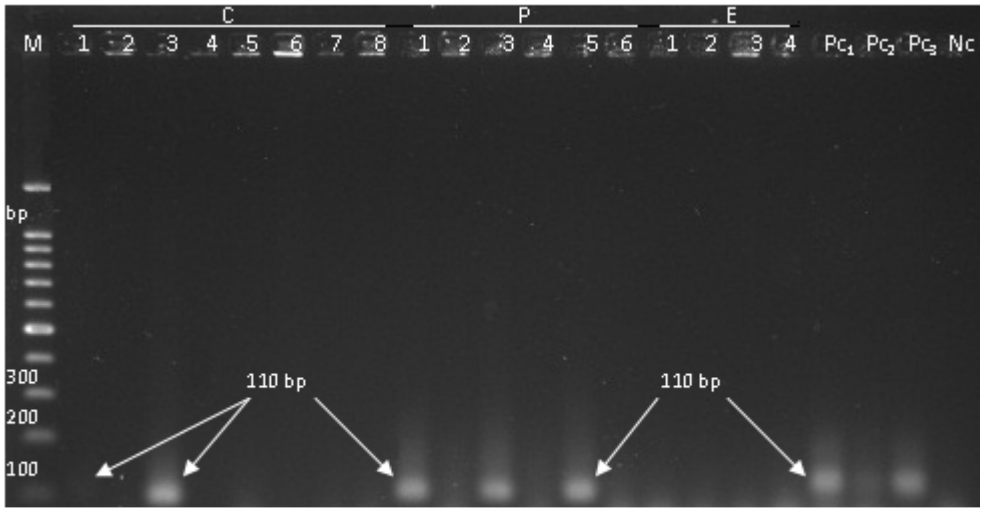

Figure 1-PCR results of primer P35S. Bands fractionated by electrophoresis on a 2% agarose gel (2hr, 5V/cm, 0.5XTris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. Lines M: 100 bp DNA ladder (Promiga, USA); (1-8) C: Coarse mixes feed; (1-6) P: Pelleted feed; (1-4) E: Expanded feed; $Pc_{1, 2, 3}$: Positive control; Nc: Negative control.

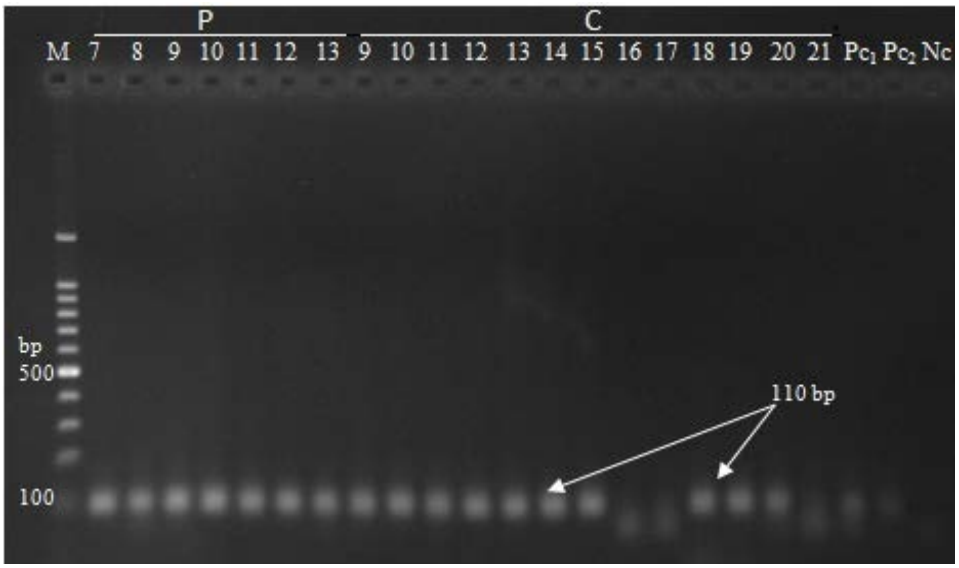

Figure 2-PCR results of primer P35S. Bands fractionated by electrophoresis on a 2% agarose gel (2hr, 5V/cm, 0.5XTris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. Lines M: 100 bp DNA ladder (Promiga, USA); (7-13) P: Pelleted feed; (9-21) C: Coarse mixes feed; $Pc_{1, 2}$: Positive control Nc: Negative control.

These positive results, which were positive for the 35S promoter amplification in feed samples, are similar to other previous studies. For instance, the results of some studies demonstrated the presence of P35S promoter in either Malaysian or Vietnamese feed with high frequency (20 positive samples out of 24 analyzed samples). And another studies, common GM sequence indicator of 35S promoter detected in 55% unlabeled GM soy and maize used as feed products from Jordanian markets [17].

An essential requirement for marketing GMO is the presence the labeling that refers to the existence of food containing GM ingredients, these labeling regulated under food organizations such as Food and Drug Administration (FDA) in USA, which regulates the approval and release to the market and develops standards for GM food labeling [24]. Overall, 58% of the testing samples were unlabeled as GM feed, although there is decision issued by the Board Advisory for Food that prevent the importation of GM crops to Iraq, but there are no available tests relating to disclosure of genetic modification that are unlabeled, especially in the government sector. Therefore, there is a need for apply of labeling regulations for GM feed, similar to the regulations for labeling of GM food products, as a first step to detect of GM feed [25,26, 27] while the next step is enhancing the detection of GM through the implementation of the national strategy for GM monitoring. Recently, Ministry of Environment tried legislation of laws that prevent the entry or trading of genetically modified organisms (GMOs) as part of the Cartagena Protocol.

From This study it can be concluded that the presence of unlabeled genetically modified feed in Iraqi markets because of lack of capabilities and capacities to control the entry of genetically modified organisms to the country despite the presence of restrictions and laws that prevent the entry or trading of genetically modified organisms (GMOs).

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