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Molecular Test for the Detection of Residual DNA in Blood, Milk, Urine and Faeces Samples from Holstein Cattle Fed with Genetically Modified Cereal

Research Article

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ABSTRACT

In the past century, recombinant DNA technology has opened new avenues for modern agriculture with the introduction of genetically modified cereal (GMO) crops. However, contamination with GMO elements remains an open problem in the field of transgenic feed. Therefore, in order to solve this problem, we are investigating and searching the degree of degradability of the residual elements of the foreign DNA-derived cry1Ab gene in blood, milk, urine and feces samples of Holstein cattle suspected to be on GMO diet Bt fed corn to be infected. In our preliminary tests, we randomly collected 30 different samples from different dairy farmers (blood, milk, urine and faeces samples) using routinely available methods and then performed a PCR test using specific primers of the cry1Ab gene sequence in order not to distinguish between transgenic and genetically modified corn and their products. Our results showed evidence for the presence of 206 bp amplicons of the cry1Ab gene derived from stool samples. However, the result provides no contamination by GMO elements in blood, milk or urine samples. Taken together, the present results confirm the applicability of imported Bt corn in formulating diets for dairy farmers. However, it is worth discussing these interesting achievements also on the low contamination rate of GMO maize and its residual elements during the current work. Overall, our method was the one that yielded the most cost-effective test, and more work is certainly needed to decipher these complexities to ensure risk assessment and up-to-date highlights of biosecurity regulations.

KEY WORDS cry1Ab gene, degradability, transgenic plants.

INTRODUCTION

For decades, one of the most popular ideas in the genetic engineering literature has been the idea of growing genetically modified (GM) crops. A common recombinant DNA strategy used for studies produced high-yield, lower-cost grades that are genetically resistant to insects. Therefore, the issue of GM feed has received significant critical attention, and the literature claims that GM crops are more disease-resistant, nutritious, and potentially climate-friendly varieties to meet future demand (Bravo *et al.* 2007). A summary of the literature research to date shows that almost 165 genetically modified plants in 19 plant species are used extensively for animal feed (alfalfa, oilseed rape, maize, cotton, soybeans, sugar beet). In this context, extensive confirmation and approval tests are carried out in many countries before a transgenic plant is commercialized (Guttikonda *et al*. 2016; Tiwari and Singh, 2018; Boutigny

et al. 2019). As this transgenic plant matures, a plethora of well-understood methods and procedures have been used in the past to attempt to randomly insert a foreign gene into the genome of these wild-type varieties. Understanding the complexity of foreign genes and the location of insertion in the host genome is critical to assessing adverse events in residual gene fragments and their health and environmental safety (Li *et al.* 2017; Boutigny *et al.* 2019).

These transgenic plants produce crystal proteins (Cry) or toxins derived from the soil bacterium Bacillus thuringiensis (Bt). The toxin produced by proteins is amazing because during sporulation the bacterium produces crystals containing endotoxin in selectively targeted caterpillars within the order Lepidoptera (DeMaagd *et al.* 2001). The plant biologically causes death by blood poisoning in larvae of insect pests of agricultural plants and causes further damage by paralysis and loss of appetite shortly after feeding the insect with this toxin. After all, it affects the plant and the death of pests and insects (Guttikonda *et al.* 2016).

One of the major highlights of GMO crop technology is the hypothesis of targeted gene expression in tissues. Recent theoretical developments emphasized that the Bt gene encodes a toxic protein in the vegetative organs under special promoters, and scientists theoretically expect not to observe contamination with GMO elements in seeds (seed reproductive organs), so contamination of see (Guttikonda *et al.* 2016).

A known problem with GMOs is that when residual d reproductive organs is still a open issue is stays in risk assessment and biosecurity highlights bt gene elements show up in the reproductive organs of transgenic plants, it indirectly means that something is going wrong during gene transfer and expression in host varieties. Therefore, several authors have noted that small fragments of recombinant DNA were also detected in the milk sample from the Italian market, which the authors suspected was contaminated with feed and faeces during milking (Agodi *et al.* 2006; Li *et al.* 2017; Boutigny *et al.* 2019).

It is of interest to know if the presence of foreign DNA residues in bovine consumer products is still relevant and if the results of the risk assessment are relevant for the safety of society. In order to solve this problem, some approaches to risk assessment in domestic animals such as cattle fed with genetically modified plants are to determine the nucleotide sequence of the foreign gene of bacterial origin in the metabolites of the rumen, urine, faeces, blood, milk and meat of the affected identify animals (Guttikonda *et al.* 2016; Boutigny *et al.* 2019).

To our knowledge, no previous studies have examined the condition of dairy farms. As the authors previously noted, more work is needed to test the DNA-derived cry1Ab gene in livestock edible products for safe commercialization. With this in mind, we are therefore investigating and seeking the degree of degradability of the foreign DNA-derived residual elements of the cry1Ab gene in blood, milk, urine and fecal samples from Holstein cattle suspected of being fed GM Bt corn to have been fed.

MATERIALS AND METHODS

Animals

The focus of the experiments was on the injection of sources. Here we divide the originality of corn into two groups by producer: imported and native varieties. Traditionally, the import source of corn has been reported from three South African, Brazilian and Argentinian countries.

For this study, we analyzed the randomly collected data from 30 different samples from different dairy farmers (blood, milk, urine and faeces samples) using routinely available methods. As detailed information, blood, milk, urine and faecal samples were taken from candidate cows for our study at approximately 5 and 18 hours after feeding monitoring. Figure 1 indicated the map profile for the geographic location of sampling from different dairy farms.

Figure 1 Map profile showing geographic location of different dairy cows. Dairy farmes geographically from north west of iran was candidate for sample collection

DNA extraction

In order to extract DNA from faeces, due to the difficulty of the extraction procedure with general kits, a special kit manufactured by Favorgen Company (Vienna, Austria) specifically for the extraction of DNA from faeces and urine was used. The laboratory guidelines proposed by this company were used to perform the procedures. A kit (ROJE Technologies, Yaz, Iran) was used to extract DNA from blood and milk. Gel monitoring and 0.8% agarose gel electrophoresis were used to ensure DNA extraction. Samples containing high-quality DNA without fragmentation were stored in a minus 20 ˚C freezer until the beginning of the

next phases of the experiment, and the sample label and the date of its collection were accurately recorded on all Eppendorf tubes.

Primers sequence and Cry1Ab Gene amplification

The following universal primer sequence was used to amplify 206 bp of the Cry1Ab gene (GenBank: AY326434.1, Guertler *et al.* 2009):

5'-CCTGGAGAACTTCGACGGTA-3'
5'-TCGTGCCGTAGAGAGGAAAG-3'

The polymerase chain reaction was performed using a universal PCR lyophilization kit (Taq DNA Polymerase Master Mix RED, Denmark) containing Mix-PCR mixes. The final concentration of materials in 25 L was: 1 unit Taq polymerase enzyme, 1X PCR buffer, 10-20 picomoles primer mix, 50-100 ng DNA and standard PCR buffer. The PCR reaction was performed with the following thermal program for 35 cycles in a thermal cycler (Biometra T-Gradien Tm, Germany). The heating program was: 94 ˚C for DNA annealing for 45 seconds, 55 ˚C for annealing of primers for 1 minute and 72 ˚C for extension for 1 minute. The final extension was 72 ˚C for 6 min.

To observe the PCR products, 1.8% agarose gel and 70-100 volts for 2 hours were used. The gel was stained with ethidium bromide (10 mg/mL). The amplified fragment was observed under a UV document with a wavelength of 230 nm and photographed with a Biometra device. In summary, based on the electrophoresis pattern and the presence and absence of 206 bp of the Cry1Ab gene, it was reported.

To test the accuracy of PCR amplified products using candidate primers, a simple bioinformatics alignment (BLAST-n) was performed to double the confirmed accuracy of the observed PCR size during molecular analysis.

MAFFT software (MAFFT.cbrc.jp/alignment/server/) was applied for the creation of outputs. Figure 2 illustrated a bioinformatic analysis of the DNA-derived cry1Ab gene amplicon sequence and position of primers annealing for amplification of 206 bp fragments.

RESULTS AND DISCUSSION

Since GMOs have become an integral part of modern agriculture, contamination with GMO elements in the transgenic feed sector remains an open problem (Boutigny *et al.* 2019). Molecular diagnostic methods for non-transgenic and transgenic grains provide insights into key risk assessment tools and biosecurity highlights. In this study, we examined the pattern and variability of foreign DNA-derived residual elements of the cry1Ab gene in blood, milk, urine, and faecal samples from Holstein cattle suspected of being infected with GMO Bt. corn diet to have been infected. We hypothesized that the pattern and variability of the cry1Ab gene residue would derive from different animal products. The study results provided some interesting insights into the presence of 206-bp fragments in blood, milk, urine and feces samples from Holstein cattle suspected of having been fed genetically modified Bt corn, some of which were collected 5 and 18 hours after monitoring feeding time. The results showed evidence for the presence of 206 bp amplicons of the cry1Ab gene derived from stool samples. However, the result provides no contamination by GMO elements in blood, milk or urine samples. Taken together, the present results confirm the applicability of imported Bt corn in formulating diets on dairy farms. However, it is worth discussing these interesting achievements also on the low contamination rate of GMO maize and its residual elements during the current work. As shown in Figure 3, only stool samples showed the presence of cry1Ab gene fragments.

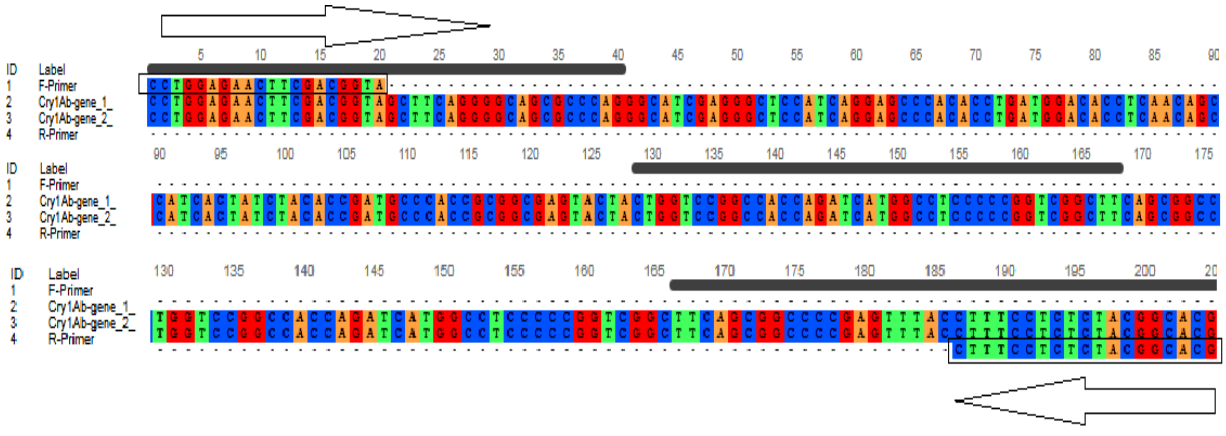

Figure 2 Results for bioinformatic analysis of DNA-derived cry1Ab gene amplicon sequence and position of primers annealing for amplification of 206 bp fragments

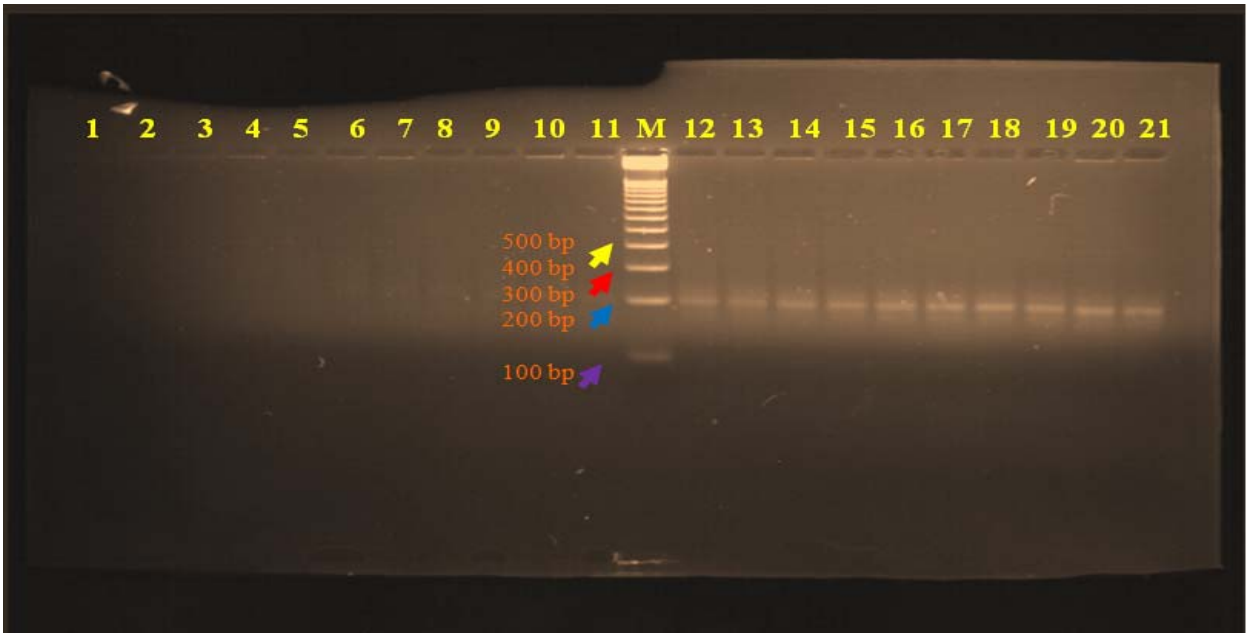

Figure 3 Overview of the detectability of molecules for degradability of the foreign DNA-derived cry1Ab gene in blood, milk, urine and feces samples (1-3: blood sample, 3-6: milk sample, 7-11: urine samples, M: 100-bp leader and 12-21: faecal sample all samples are from Holstein cattle suspected of being fed GMO diet Bt corn)

Here, we compare the results of the present report with those of similar previous literature. Several short-term studies (two to thirteen weeks) with milk from cows showed that feeding transgenic maize had no significant effect on milk production and milk composition compared to the control group (Clark and Ipharraguerre, 2001; Donkin *et al.* 2003; Ipharraguerre *et al.* 2003; Calsamiglia *et al.* 2007). Consistent with previous studies and when reviewing the sources of the research results, no recombinant DNA was detected in the milk of cows fed transgenic feed (Phipps and Park, 2002; Nemeth *et al.* 2004).

A similar pattern of results was obtained in our research, which was missing a 206 bp fragment in urine, blood, and milk. Despite these findings, foreign DNA fragments can cross the intestinal barrier and cry1Ab DNA fragments and Cry1Ab protein can be detected in the blood (Bertheau *et al.* 2009). Contrary to the results of some previous literature, some authors emphasize the presence of foreign DNA in the blood and body organs of the piglets examined (Mazza *et al.* 2005). However, as we conducted our discussion, an inconsistency of some observations with our reports emerged, the presence of transgenic DNA was observed in the contents of all parts of the intestines of mice 2 hours after feeding transgenic soybean leaves and in feces, blood and several organs of mice (Schubert *et al.* 1997; Hohlweg and Doerfler, 2001).

Similarly, foreign DNA has also been reported in animal and human circulation, but the origin and mechanism of dissemination are not fully understood (Tsang and Lo, 2007). In general, in the safety assessment process, it is extremely difficult to identify possible adverse effects and their relationship to a specific characteristic. In practice today very few foods (particularly foods for special populations such as infants and nursing mothers etc.) are subjected to the systematic safety assessment process and all are known to be nutritionally safe (Alexandrova *et al.* 2005). The Standard for Toxicology and Possible Risk Assessment Methods for Whole Foods, an alternative method, has been accepted as a framework for safety assessment of genetically modified (GM) foods. When assessing possible risks, a suitable method and solution is first sought to compare the transgenic plant and its food with its suitable non-transgenic counterpart (Guttikonda *et al.* 2016).

This comparison is products actually the starting point of the safety assessment, which continues to focus on the favorable (wanted) and unfavorable (undesirable) differences between the transgenic plant and its traditional counterpart (Alexandrova *et al.* 2005).

The logical reason why the present result deviates from other findings lies primarily in the different forms of nutrition, the lactation and reproduction period and calving, or the methods for detecting foreign DNA residues as well as

livestock species and breeds or molecular detection methods and primers. The biotechnological methods and the techniques chosen for molecular tracing should be sensitive enough to detect the gene(s) below the relevant competence threshold (e.g. 5% in the United States, 1% in Canada and 9% in the European Union) to prove. Currently, qPCR is a standard method used in reference laboratories to identify and quantify GM products (Dalmira *et al.* 2016; Li *et al.* 2017).

On the other hand, the need for bioinformatics for data analysis and more advanced equipment limits the application of this method in GM identification (Boutigny *et al.* 2019). In summary, this work argued that the presence of the 206 bp amplicons of the cry1Ab gene from stool samples and the 206 bp fragment were absent in ruminant urine, blood and milk. To our knowledge, this is the first report of detection of the foreign cry1Ab gene on Iranian dairy farms. Future research is needed to validate the conclusions that can be drawn from this study.

There are limitations to this study. A limitation of our implementation is that we have used simple PCR methods to test transgenic and non-transgenic corn and some types of literature have applied real-time PCR. Another limitation is that we only focused on dairy farms with animals in the same lactation period and on the same feed recipe, and the collection time was around 5 and 18 hours after monitoring the feeding time. It suffers from the same limitations associated with a DNA kit that does not have high sensitivity to the small amount of DNA.

CONCLUSION

To date, recombinant DNA technology has opened new avenues for modern agriculture with the introduction of GMO crops. However, contamination with GMO elements remains an open problem in the field of transgenic feed. Overall, our method was the one that yielded the most cost-effective test, and more work is certainly needed to decipher these complexities to ensure risk assessment and up-to-date highlights of biosecurity regulations. This present study demonstrated the presence of the 206 bp amplicons of the cry1Ab gene derived from stool samples and the 206 bp fragment was absent in urine, blood and milk. This study is the basis for future investigations to assess the detect ability of the foreign cry1Ab gene in Iranian dairy farms. However, future investigations of the limited research and some other research questions are needed to improve our knowledge.

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