

Detection of DNA fragments from roundup ready soya and Bt maize in organs of broilers

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Abstract

Possible transfers of DNA fragments from genetically modified Roundup Ready soybean, containing transgene for tolerance against herbicides with glyphosate, and Bt maize MON810, with transgene for insecticide protein Cry 1A(b), from fodder into selected organs of broilers ROSS 308 were studied. Three feeding experiments were performed. Twenty samples of kidney and twenty samples of liver were randomly selected from 120 collected samples.

Analyses were divided into two parts. First, we were looking for control genes of Roundup Ready soybean (lectin) and Bt maize (*HMG* gene). Eighteen samples of liver were positive for soybean control gene - lectin. Any sample of kidney was not positive for lectin fragments. Detection of *HMG* gene was negative in both organs. The other part was focused on detection of transgenes in liver and kidney samples. Fragments of soybean transgene were identified in 3 samples of liver. Fragments of Bt maize transgene were not detected in livers. None positive detection of any transgene was in samples of kidney. These results demonstrated possible transfer of DNA fragments from fodder into organs of broilers.

Introduction

Increased usage of genetically modified crops in food- and feed-processing industry is connected with increasing of public interest about information, connecting with possible risks of GMO food and feed. Possible transfer of transgene from GMO crop and its further activity in the organism of recipients belong to the most discussed topics. Several studies, focused on this problems, were realized since yet. The aim of our work was to elaborate detection of control genes and transgenes from genetically modified crops in organs of recipients, using PCR methods, as well as to confirm possibility of transfer of transgene into organs of recipients.

Flachowsky *et al.* (2005) observe that the amount of DNA absorbed with food varies between 0,1 and 1 gram per day by humans and includes fragments of plant and animal genes, degraded to different degrees, as well as bacterial DNA. After receiving from food, DNA is digested into smaller parts – fragments. It exists a presumption, that these fragments can be retained in intestinal tract or blood for a short time (Schubert *et al.*, 1994). Because intestinal tract does not absolutely prevent from penetration of macromolecules and/or microorganisms, transfer of DNA fragments

from intestinal tract to blood is possible. Mode of transmission of DNA fragments through cell wall of intestinal tract is not known.

Doerfler (2000) also supposed, that DNA fragments are not completely digested in intestinal tract and they can probably penetrate into organism of recipient. This author mentioned about eventually incorporation of DNA fragments into cells from intestinal mucosa. Jonas *et al.* (2001) confirm that the likelihood of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells is minimal. Same conclusions were reached by Tony *et al.* (2003) and Bertoni and Marsan (2005). Einspanier (2001) describes transfer of nontransgenic plant genomic DNA into organs of broilers, whereas sequences of the transgene were not identified. Similar experiments with broilers were done by Aeschbacher *et al.* (2005). On the basis of these experiments, they confirmed, that foreign DNA received from fodder is not completely digested in intestinal tract. They deduced, that DNA fragments can penetrate from intestinal tract into blood and consequently into cells of liver and/or pancreas.

Roundup Ready soybean and Bt maize MON810 belong to the most used GMO crops. Hence, many studies are focused on questions

of their safety. Roundup Ready soybean contains transgene, coding synthesis of CP4 EPSPS protein. Stability of this transgenic DNA, found in duodenal humour of sheep, was studied in 2004. Sheep were fed by extracted scrap from Roundup Ready soybean. This work also confirm possible relasing of transgenic DNA into small intestine of sheep. Transgenic DNA can be quickly degradeted by pH 7, which occurs in intestinal tractof sheep. Possibility of transfer of transgenic DNA into other organs can be considered to be minimal (Alexander *et al.*, 2004).

Analogical study was performed by Rossi *et al.* (2005). Authors observed effect of fodder with Bt maziie on efficiency of broilers. Part of this study was focused on faith of the forign DNA, receiving by fodder, in intestinal tract. They found, that foreign DNA is stepwise degradated. They found fragments of foreign DNA in blood. Fragments were relatively short. They did not found differences in lenght and amount of the detected DNA fragments between broilers, fed by GMO-free fodder, and broilers, fed by fodder containing Bt maize. Conclusion of their work is, that fragments of transgene and fragments of nontransgenic DNA are passed through the same degradation process.

Material and methods

For studies of transfer of genes from GMO into organs of broilers Roundup Ready soybean line GTS 40 – 3 – 2 and Bt maize MON810 were used. Broilers (hybrid ROSS 308) were assigned to 4 treatment groups, fed by different diet. Diet for the first group contained Bt maize MON810, for the second group Roundup Ready soybean and for the third group both, Roundup Ready soybean and Bt maize MON 810. The fourth group was control with GMO free diet. Ten broilers were selected from each group and organs of these broilers were collected. Experiment was repeated threetimes. From all collected samples 20 liver and 20 kidney were selected randomly.

Genomic DNA was isolated from livers and kidneys following NucleoSpin Tissue (50 prep.) kit (Macherey-Nagel), according to manufacturer's protocols. Two controls of isolation were carried out - by electrophoresis and by PCR. Standard electrophoresis for genome DNA was used, in 1.5% agarose gel containing ethidium bromide. Second control was performed by PCR for chicken growth hormone gene. Primers used to control PCR were: 5'-ATC CCC AGG CAA ACA TCC TC-3'(forward) (GCH1F) and 5'-CCT CGA CAT

CCA GCT CAC AT-3'(reverse) (GCH1R). After presoaking 4 min. at 94°C, 35 PCR cycles were carried out each consisting of 30 s at 94 °C, 120 s at 60 °C, and 90 s at 72 °C (Kuhlein *et al.*, 1997).

Commercial kits for detection of GMO *GMOIdent* Roundup Ready™ Soy and *GMOIdent* MON810™ Corn (Eurofins - Gene Scan) were used for detection of foreign DNA in organs of broilers.. Each kit contains premastermix for specific transgene, RRS for Roundup Ready soybean and YG-IR for Bt maize, and premastermix for control genes, lectin by soya and HMG gene by maize. Primers for chicken growth hormone gene were used like internal control. We used multiplex PCR for analyses.

First, multiplex PCR for control genes was performed. Reaction mixture was allowing: 19,9 µl premastermix with primers for control genes, 1 µl primers GCH1R and GCH1F each, 2 µl dNTP's, 2 µl Taq and 2 µl DNA. PCR consists of 2 min. presoaking at 94 °C and 50 cycles: 25 s at 94 °C, 30 s at 62 °C and 45 s at 60 °C, following by 3 min. at 72 °C. PCR products were analysed by electrophoresis in 2% agarose gel containing ethidium bromide. Positive and negative control were carried out. Every sample was analysed threetimes. Positive control with commercial genomic DNA and negative control without DNA in mixture were carried.

After this part of work, multiplex PCR for transgene detection was performed. There was used premastermix for specific transgene in PCR reaction mixture for detection of specific transgene. All others conditions were the same like by PCR for control genes.

Results and discussion

Twenty samples of liver and twenty samples of kidney were randomly selected for detections of control genes nad transgenes.

Table 1 reported about amount of performed analyses. First problem of this work was connected with isolation DNA from chicken organs. Although we used commercial kits for isolation, quality of isolated genomic DNA was not very well. We had to isolate every sample twice. Quality of DNA isolation was confirm by electrophoresis and PCR reaction with primers for chicken grawth hormone gene. Higher number of detections was caused also by necessity of 3 repeats of every analysis. The reason of this procedure is to exclude casualness of results and possibility of human error. It is necessary to mentioned presence of

positive and negative control, which are used for exclusion of contamination of sample. We

used 1 positive and 1 negative control for every 5 samples.

Table 1 Summary of number of detections. (J – liver, L – kidney)

Samples of kidneys and livers	Number of detections			Number of isolations
	Samples	Positive and negative controls	Total	
Soybean - control	98 (J 56 + L 42)	34 (J 20 + L 14)	132 (J 76 + L 56)	80 (J 40 + L 40)
Soybean - transgene	114 (J 62 + L 52)	48 (J 26 + L 22)	162 (J 88 + L 74)	
Maize - control	123 (J 63 + L 60)	52 (J 28 + L 24)	175 (J 91 + L 84)	80 (40 J + 40 L)
Maize - transgene	120 (J 58 + L 62)	52 (J 24 + L 28)	172 (J 82 + L 90)	

Multiplex PCR was used like method for detection of control genes and transgenes. This method is based on using two pairs of primers. One pair is for detection of appropriate control gene or transgene. An other pair serve like an internal control and for elimination of false negative results. In case, that internal control is not present, reaction can be regarded as correctly performed and it is necessary to repeat the reaction. Demonstration of multiplex PCR is displayed by Fig. 1.

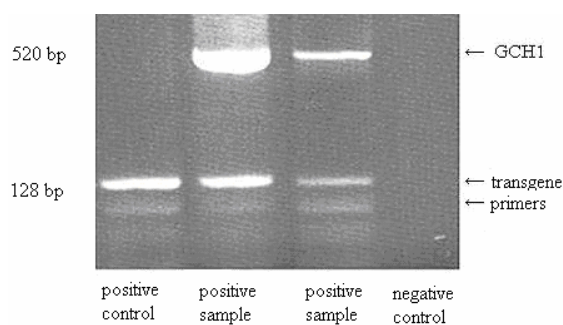


Fig. 1 Detection of transgene of Roundup Ready soybean in liver

First step was detection of control genes for soybean (lectin) and maize (*HMG* gene). Fragments of lectin were found in 18 samples of liver. Positive detection in all 3 repeats was

found only in 1 sample. The other 17 samples had positive reactions just in 1 or 2 repeats. None positive detection of *HMG* gene was found in 40 samples of liver and kidney.

An other part was focused on detection of transgenes of Roundup Ready soybean and Bt maize MON810. There were reported only 3 positive detection of transgene of Roundup Ready soybean. this detection was not confirmed in all 3 repeats. Fragment of transgene of Bt maize was not detected. We can not affirm, that transfer of transgene from fodder into organs of broilers is possible. Summary of results of both parts of experiment are summarized in Table 2.

Our results are in concordance with results, reported by other authors (Doerfler, 2000; Aeschbacher *et al.*, 2005). It can be confirmed that transfer of DNA fragments from intestinal tract into organs of recipient is possible, but not very usual. Multiplex PCR represent suitable method for detection of fragments of foreign DNA. This method is not available to detect, if a transferred fragment can be functional in the organism of recipient. Transfer of unfunctional fragment can be explain by many ways, from crosscontamination to possibility, that recipients had some infection (Petr, not published).

Table 2 Summary of results of detections of control genes and transgenes of Roundup Ready soybean and Bt maize MON810 in liver and kidney

Samples of kidney and liver	Number of positive samples			Total number of samples
	Positive samples (in all 3 repeats)	Positive samples (in 1 or 2 repeats)	Total	
Soybean - control	1	17	18	40
Soybean - transgene	0	3	3	
Maize - control	0	0	0	40
Maize - transgene	0	0	0	

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