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Incidence of genetically modified soyabean and maize as animal feed in Egypt

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ABSTRACT

As a consequence of rapid progress in agricultural biotechnology, increasing amounts of genetically modified (GM) crops have entered the food and feed chain in recent years. The aim of our study, which conducted during 2000-2001, was to monitor the incidence of Roundup Ready™ soyabean (RRS) and the GM maize lines Bt176, Bt11, T25, MON810 and StarLink in Egypt. With the exception of StarLink™, which was approved only for feed use, the other constructs were approved for use as food and/or feed in the US and other countries. Fifty one soyabean samples and 61 maize samples were randomly collected from different localities in Egypt. The detection techniques applied were based on Polymerase Chain Reaction (PCR) using validated, official detection methods according to Article 35 of the German Federal Foodstuffs Act. The results of our survey showed that all soyabean samples imported from Argentina, and 50% of American soyabean samples contained RRS. Of the 20 maize samples imported from USA, 16 contained Bt176, 17 Bt11, 12 MON810, 19 T25 and 9 StarLink™. In addition, of the 7 maize samples imported from Argentina, 4 contained Bt176 and MON810, 5 T25, 6 Bt11 and 2 StarLink™. In contrast, all Egyptian local varieties of soyabean and maize were non transgenic.

KEY WORDS: genetically modified feed, soyabean, maize

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INTRODUCTION

Biotechnology offers enormous potential for world agriculture. This includes the production of higher yields with decreasing use of herbicides and pesticides, the resistance to insects, diseases, abiotic stresses and improved nutritional characteristics (Flachowsky and Aulrich, 2001). Practical examples for modified feed crops from the nutritional viewpoint are soyabean or maize expressing fungal phytase (an enzyme that catalyses the release of phosphate from plants) to ameliorate the bioavailability of phosphorus (Denbow et al., 1998; Spencer et al., 2000).

To date, in the European Union, a wide regulatory framework controls multiple aspects of GM technology from the deliberate release of GM crops and seeds to the final food product. Apart from a thorough safety evaluation, Europe now requires mandatory labelling of GM foods. Furthermore, the EU established a 1% threshold for unintended contamination of unmodified foods with approved GM constructs. However, up to now no labelling is required for animal feeds. In Japan a threshold of 5% for frequently used GM crops was implemented. In the United States, GM crops or products are not labelled. However, GM plant varieties must be reviewed by a number of competent authorities including the Centre for Food Safety and Applied Nutrition (CFSAN), the Centre for Veterinary Medicine (CVM) and the Food and Drug Association (FDA). Based on information provided by the applicant the safety of the new product is evaluated prior to marketing. Today the most commonly grown transgenic crops are soyabean and maize, which represent a major source of protein and energy for livestock production. In 2001, 53 million ha of GM crops were cultivated worldwide and the trend is increasing (James, 1999). Although Egypt mainly depends on imported soyabean and maize, these crops are only evaluated for nutrient content and mycotoxin contamination, but not for the presence of GM constructs. As a result, there is no information available on the presence or absence of GM crops used as animal feed.

Our work aimed to monitor the incidence of genetically modified soyabean and maize in Egypt especially that used for animal feed. To achieve this goal, 51 soyabean samples and 61 maize samples were randomly collected from different localities in Egypt and subjected to detection techniques based on Polymerase Chain Reaction (PCR) using the official detection methods according to Article 35 of the German Federal Foodstuffs Act.

MATERIAL AND METHODS

Sampling

Fifty one soyabean samples and 61 maize samples were randomly collected from different localities in Egypt during the years 2000/2001. The soyabean samples

TABLE 1

Results of examined soyabean samples and its origin

Sample	Collection locality[1]	Origin	GMO detection (p35s-f2 / petu-r1)
27 soyabean seeds sample of different cross breeding	Giza	Local breed / Egypt	-
Soyabean meal	El-Gharbia	USA	-
Soyabean meal	Giza	Argentina	+
Soyabean meal	Bany-swef	USA	+
Soyabean full fat	Giza	Local breed	-
Soyabean meal	El-Gharbia	Argentina	+
Soyabean meal	El-Menia	Argentina	+
Soyabean meal	Kalubea	Argentina	+
Soyabean meal	Cairo	Argentina	+
Soyabean meal	Cairo	USA	+
Soyabean meal	El-Behara	USA	-
Soyabean meal	El-Sharkea	Argentina	+
Soyabean meal	El-Monofia	Argentina	+
Soyabean meal	Alexandria	Argentina	+
Soyabean meal	Kafr El-Shek	Argentina	+
Soyabean meal	Ismailia and Port-Said	Argentina	+
Soyabean seeds	El-Dakahlia	USA	-
Soyabean meal	El-Dakahlia	Argentina	+
Soyabean meal[2]	Cairo	USA	-
Soyabean meal[2]	Giza	USA	-
Soyabean meal	Giza	USA	+
Soyabean meal	Asuit	Argentina	+
Soyabean meal	El-Monofia	USA	+
Soyabean meal	Wady El-Netron	Argentina	+
Soyabean meal	El-Sadaat	USA	+

[1] locality according to Governments in Egypt

[2] soyabean meal containing 48% crude protein

(Table 1) contained 28 samples of soyabean seeds, of which 27 were local Egyptian varieties and one was of USA origin. Twenty two samples of soyabean meal which were imported from either USA or Argentina. Additionally 1 sample of full fat soyabean was obtained from the Egyptian market. The maize samples (Table 2) consisted mainly of whole grains. Thirty three samples were from different Egyptian varieties, 24 samples were imported from USA or Argentina and 4 additional samples consisted of maize gluten. One of the gluten samples was produced in Egypt whereas the other 3 samples were imported from USA.

Reference materials

Certified reference materials (CRM), produced by the Institute for Reference Material and Measurements (Geel, Belgium) were used as negative and positive

TABLE 2

Results of examined maize samples and its origin

Sample	Collection locality in Egypt[1]	Origin	GMO detection				
			Bt 176 Maize (Cry 03 / Cry 04)	Bt 11 Maize (Ivs2-2 / PAT-B)	Mon 810 Maize (VW 01 /VW 03)	T25 Maize (T25-F7 / T25-R3)	StarLink Maize Test Kit
31 maize sample of different cross breeding	Giza	Local breed / Egypt	-	-	-	-	-
Local Maize grain (white maize)	El-Monofia, Luxor and Sohag	Egypt	-	-	-	-	-
Local Maize grain (yellow maize)	Asuit, Luxor and Kiena	Egypt	-	-	-	-	-
Maize grain	Giza	USA	+	+	+	+	+
Maize grain	Cairo	USA	+	+	+	+	-
Maize grain	El-Gharbia	USA	+	-	+	-	-
Maize grain	Bany-Swef	USA	+	+	+	+	-
Maize grain	Giza	Argentina	-	+	+	+	+
Maize grain	Cairo	Argentina	+	+	+	+	+
Maize grain	El-Menia	USA	+	+	+	+	+
Maize grain	El-Gharbia	Argentina	-	+	+	+	-
Maize grain	El-Behara	USA	+	+	+	+	-
Maize grain	El-Monofia	USA	+	+	+	+	+
Maize grain	Kalubea	Argentina	+	-	+	-	-
Maize gluten	Cairo	USA	-	-	-	+	-
Maize grain	Alexandria	USA	+	+	-	+	+
Maize grain	Alexandria	Argentina	-	+	-	+	-
Maize grain	Kalubea	USA	+	+	-	+	-
Maize grain	El-Sharkea	USA	+	+	-	+	-
Maize grain	Ismailia a. Port-Said	USA	+	-	-	+	-
Maize grain	El-Dakahlia	USA	+	+	-	+	-

TABLE 2 (continued)

Sample	Collection locality in Egypt[1]	Origin	GMO detection				
			Bt 176 Maize (Cry 03 / Cry 04)	Bt 11 Maize (Ivs2-2 / PAT-B)	Mon 810 Maize (VW 01 /VW 03)	T25 Maize (T25-F7 / T25-R3)	StarLink Maize Test Kit
Maize grain	Kafr El-Shek	Argentina	+	+	-	+	-
Maize grain	Asuit	USA	+	+	-	+	-
Maize grain	El-Dakahlia	Argentina	+	-	-	-	-
Maize grain	El-Fayum	USA	+	+	-	+	+
Maize grain	Wady El-Netron	USA	+	+	-	+	+
Maize grain	Asuit	USA	+	+	+	+	+
Maize gluten	Wady El-Netron	USA	-	+	+	+	+
Maize gluten[2]	Wady El-Netron	Egypt	-	+	+	+	-
Maize grain	El-Sadaat	USA	-	+	+	-	-
Maize gluten	El-Gharbia	USA	-	+	+	+	+

[1] locality according to Governments in Egypt

[2] maize grain imported from USA

TABLE 3

Oligonucleotides primer pairs sequence and their target element

Primer	Sequence and amplification length	Target element	References
GM03 / GM04	5'- gCC CTC TAC TCC ACC CCC ATC C - 3' 5'- gCC CAT CTg CAA gCC TTT TTg Tg - 3' 118bp	Soyabean lectin gene	Meyer et al., 1996
P35s-f2 / petu-r1	5' - TgA TgT gAT ATC TCC ACT gAC g - 3' 5' - TgT ATC CCT TgA gCC ATg TTg T - 3' 172 bp	Transition site from the CaMV35S promoter sequence to the petunia hybrid chloroplast-transit-signal sequence in RRS	Wurz and Willmund, 1997
IVR1-F / IVR1 -R	5'-CCg CTg TAT CAC AAg ggC Tgg TAC C-3' 5'- ggA gCC CgT gTA gAg CAT gAC gAT C-3' 226 bp	Maize invertase gene	Ehlers et al., 1997
Cry03 / Cry04	5' - CTC TCg CCg TTC ATg TCC gT - 3' 5' - ggT CAg gCT CAg gCT gAT gT- 3' 211 bp	Transition site from the CDPK-promoter into the amino terminal sequence of synthetic cry1A(b) gene in Bt176 maize	Hupfer et al., 1998
IVS2-2 / PAT-B	5' - CTg ggA ggC CAA ggT ATC TAA T - 3' 5' - gCT gCT gTA gCT ggC CTA ATC T - 3' 189 bp	Transition site from the intron IVS2 into the PAT-gene in Bt11 maize	No. L-15.05-1, 2002
T25-F7 / T25-R3	5' - ATg gTg gAT ggC ATg ATg TTg - 3' 5' TgA gCg AAA CCC TAT AAg AAC CC 3' 209 bp	Transition site from the CaMV-terminator into the PAT gene in T25 maize	No. L-15.05-1, 2002
VW01/ VW03	5' - TCg AAg gAC gAA ggA CTC TAA Cg - 3' 5' - TCC ATC TTT ggg ACC ACT gTC g - 3' 170 bp	Transition site from the genomic maize DNA into the CaMV-Promotor in MON810 maize	No. L-15.05-1, 2002

TABLE 4

Time / temperature profiles for PCR

Process	GM03 / GM04	P35s-f2 / petu-r1	IVR1-F / IVR1 - R	Cry03 / Cry04	IVS2-2 / PAT-B	T25-F7 / T25-R3	VW01 / VW03	StarLink Kit
Initial denaturation[1]	10 min at 95°C	10 min at 95°C	12 min at 95°C	12 min at 95°C	12 min at 95°C	12 min at 95°C	12 min at 95°C	10 min at 94°C
Denaturation	30 sec at 95°C	30 sec at 95°C	30 sec at 95°C	30 sec at 95°C	30 sec at 95°C	30 sec at 95°C	30 sec at 95°C	25 sec at 94°C
Annealing	30 sec at 60°C	30 sec at 62°C	30 sec at 64°C	30 sec at 63°C	30 sec at 64°C	30 sec at 64°C	30 sec at 64°C	30 sec at 62°C
Extension	1 min at 72°C	25 sec at 72°C	30 sec at 72°C	30 sec at 72°C	30 sec at 72°C	30 sec at 72°C	30 sec at 72°C	45 sec at 72°C
Cycles	35	35 - 40	42	38	38	40	40	50
Final elongation	3 min at 72°C	10 min at 72°C	10 min at 72°C	10 min at 72°C	10 min at 72°C	10 min at 72°C	10 min at 72 °C	3 min at 72 °C

[1] time for the denaturation step is adapted to the use of Ampli Taq Gold DNA Polymerase from Perkin Elmer

controls (0.1% GMO). Because there is no CRM available for maize lines MON810 and T25, samples containing 1% GMO were prepared in the laboratory from these lines and used as positive control. For StarLink maize the positive control was provided with the commercial detection kit used.

Extraction and purification of genomic DNA

Soyabean and maize samples were ground in an electric grinder. The resulting flour (200 mg) as well as 200 mg from the CRM were used for the extraction of the genomic DNA by the cetyltrimethylammonium bromide method (CTAB) according to No. L-15.05-1 (2002). From each sample two independent extraction were performed. In addition to 200 µl of deionized water was used as a blank sample and subjected to extraction and further treated in the same way as samples to control the reagents used and the procedure of the work. The extracted DNA pellet was air dried under vacuum and was resuspended in 50 µl deionized water (Fluka, Germany). The concentration of the isolated DNA was measured fluorometerically using Dynaquant 200 system fluorimeter, according to the manufacturers instructions. The DNA concentration was adjusted by dilution using deionized water to 20-25 ng/µl prior to PCR.

Oligonucleotides primers used and PCR conditions

Primers used in this study are listed in Table 3. All primers were synthesised by TIB MOLBIOL (Berlin,Germany) and obtained in a lyophilized state. All primers were solved before using to obtain final concentration of 20 pmol/µl.

For detection of StarLink maize (Aventis) a commercial kit purchased from GeneScan Europe, Freiburg (Germany; GMO/Ident Kit StarLink™ maize) was used.

DNA amplification and PCR condition

PCR was carried out on a Gene Amp. PCR system 2400 (Perkin Elmer, Germany). For each series, a master mix was prepared. Each PCR reaction mix (25 µl total volume) contained 2.5 µl PCR buffer (10x concentrate, Perkin Elmer), 2 µl $MgCl_2$ solution (25 mM $MgCl_2$), 1 µl dNTP solution 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer, 1 Unit AmpliTaq Gold polymerase (Perkin Elmer), 2 µl of template DNA and completed to 25 µl with purified water. Table 4 explains the time/temperature profiles used in PCR. All amplicons were stored at 4°C until gel electrophoresis.

Gel electrophoresis

Amplicons together with 50 bp DNA marker (Gibco BRL, USA) were separated on 2% W/V Agarose LE (Roche) gels. The amplicons were made visible by ethidium bromide staining and documented using UV transillumination (254 nm) with a Phoretix workstation (Biostep, Germany).

RESULTS AND DISCUSSION

Amplification of extracted 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). It is detectable in transgenic as well as in conventional soyabean (soyabean specific primer pair). The primer pair Ivr1-F/Ivr1-R is specific for the invertase gene and flanks part of exon number 3 of this gene. It gives rise to a 226 bp amplicon (Ehlers et al., 1997). This product is detectable in transgenic, as well as in conventional maize (maize specific primer pair). Soyabean and maize specific primer pairs served as a control for the amplification of the isolated DNA and PCR procedure (PCR quality control). All tested samples gave positive results (not shown) with the amplification control primer pairs.

DNA target sequences detection

The primer pair p35s-f2/petu-r1 is specific for the genetic modification in Roundup Ready™ soyabean and amplifies a 172 bp segment (Wurz and Willmund, 1997). The primer pair attaches to the CaMV35 S promoter sequence and the petunia hybrid chloroplast transit-signal sequence. The amplicon is only detected in transgenic samples and GMO containing CRM as presented in the example in Figure 1.

For the specific identification of transgenic maize Event Bt176 by PCR the primer pair cry03/cry04 were used. The resulting sequence of 211 bp size is amplified from a genomic region between two adjacent genetic elements, namely the CDPK promoter and the N-terminus of the synthetic *cryIA(b)* gene (Hupfer et al., 1998). This 211 bp amplicon appears only in transgenic maize samples, as well as GMO containing CRM (Figure 2).

Primer pairs IVS2-2/PAT-B were used for the detection of the transition site from the intron IVS2 into the *PAT* gene in Bt11 maize. The bacterial *PAT* gene codes for the enzyme phosphinotricine N-acetyl transferase giving rise to the resistance of Bt11 maize to the herbicide phosphinotricine. Primer pair T25-F7/T25-R3

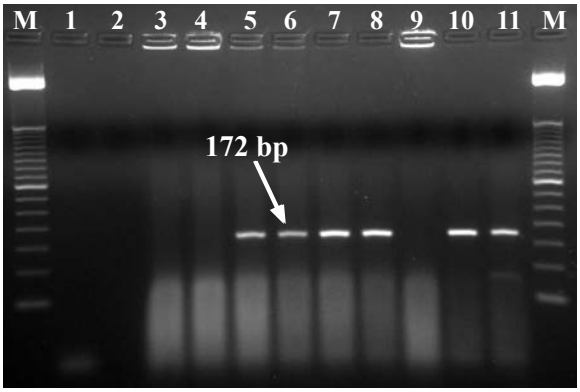

Figure 1. Detection of Roundup Ready™ soyabean (Results of PCR products of primer pair P35s-f2 / petu-r1)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: local breed soyabean seeds, 5 and 6: soyabean meal (44% CP) imported from USA, 7 and 8: Soyabean seeds imported from USA, 9: CRM negative control, 10: CRM contain 5% GMO, 11: CRM contain 0.1% GMO

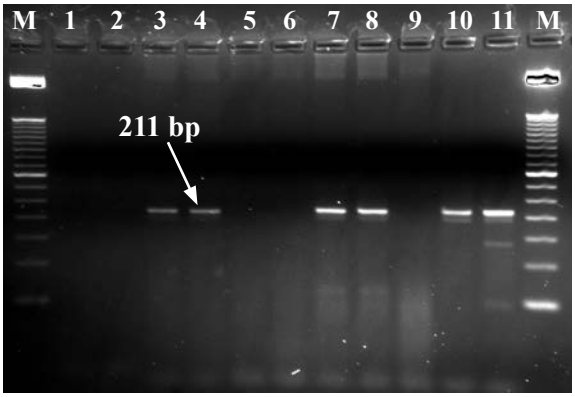

Figure 2. Detection of Bt176 maize (Results of PCR products of primer pair Cry03 / Cry04)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize gluten from USA, 5 and 6: Local breed of white maize, 7 and 8: Yellow maize grain imported from USA, 9: CRM negative control, 10: CRM contain 0.1% GMO, 11: CRM contain 5% GMO

were used for the detection of the transition site between the CaMV-terminator into the *PAT* gene in T25 maize and primer pair VW01/VW03 flanks the transition site from the genomic maize DNA into the CaMV- Promotor in MON810 maize, thus representing an event specific detection system according to No. L-15.05-1 (2002). Figures 3, 4 and 5 show results obtained for maize lines Bt11, MON810 and T25. Positive samples as well as 0.1 and 1% positive controls revealed amplicons of the the expected size of 189, 170 and 209 bp respectively, while the negative control and negative samples gave no amplification product after PCR. For the detection of StarLink maize commercial kit was used. An amplicon of 133 bp is specific for the presence of DNA from StarLink maize. It does not occur in negative samples (Figure 6).

Investigated Samples

Tables 1 and 2 summarise the results of the examined soyabean and maize samples, respectively, and the origin of the samples. All 27 locally breed soyabean samples tested negative in PCR analyses when using primer pair p35s-f2/petu-r1 and thus did not contain any genetically modified material (Table 1). In contrast, all 13 samples imported from Argentina and 5 out of the 10 samples imported from the USA tested positive for Roundup Ready™ soyabean.

Table 2 demonstrates the results of PCR for the maize samples examined. All native varieties cultivated in Egypt (33 grain samples) were negative to all primers used in this study which established that the local Egyptian maize varieties were non transgenic. In contrast, all imported maize samples tested positive for GM maize.

With respect to the 20 maize samples imported from USA, 80% contained Bt176, 85% Bt11, 60% MON810, 95% T25 and 45% StarLink maize. Furthermore, of the 7 maize samples imported from Argentina 57% contained Bt176 and MON 810, 71% T25, 85% Bt11 and 28% StarLink (Figure 7). Nearly all maize samples contained more than one GM construct. Four samples even contained a mixture of all five GM constructs investigated. Of these, one sample was from Argentina and three were from USA. The pattern of the distribution of the GM maize constructs among the imported samples was considerably and indicated different lots taken from sampling localities.

The results clearly show that imported maize and soyabean intended for animal feed in Egypt contained GM varieties to a high degree, including mixtures of several lines. In contrast, all local Egyptian varieties were free from GMO with exception of one maize gluten sample which produced in Egypt but which was probably made from imported seeds. Since no quantitative analysis was carried out, the absolute percentage of each GM line in the samples was not determined. However, the primary aim of this investigation was to present an overview on the situ-

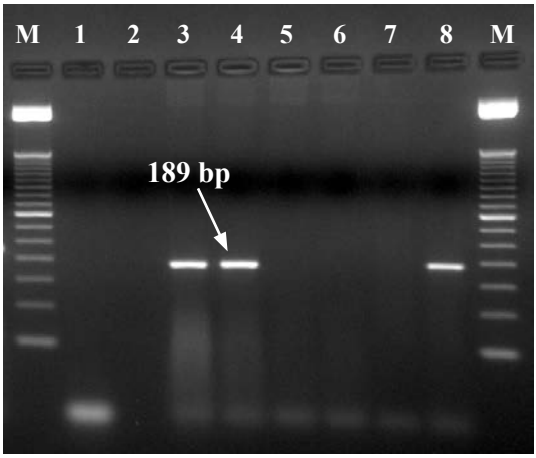

Figure 3. Detection of Bt11 maize (Results of PCR products of primer pair IVS2-2 / PAT-B)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize grain from USA, 5 and 6:imported maize grain from Argentina, 7: CRM negative control, 8: CRM 0.1% GMO

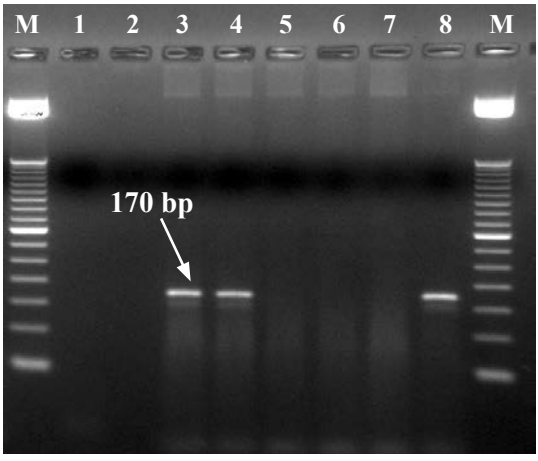

Figure 4. Detection of MON810 maize (Results of PCR products of primer pair VW01/ VW03)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize grain from USA, 5 and 6: imported maize grain from Argentina, 7: Nnegative control, 8: Prepared 1% GMO

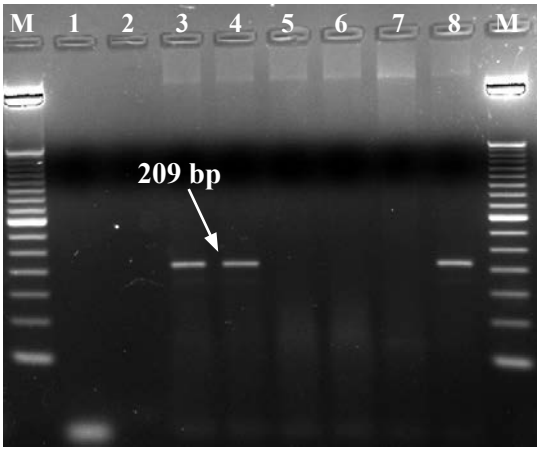

Figure 5. Detection of T25 maize (Results of PCR products of primer pair T25-F7 / T25-R3)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3 and 4: imported maize grain from USA, 5 and 6: Local white maize grain, 7: Negative control, 8: Prepared 1% GMO

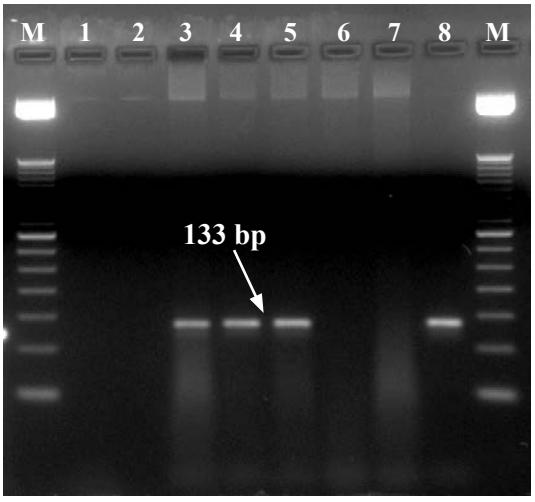

Figure 6. Detection of StarLink™ maize (Results of PCR products of StarLink kit)
M: 50 bp marker DNA, 1: blank sample (Extraction control), 2: PCR control, 3: imported maize grain from USA, 4: imported maize grain from Argentina, 5: imported maize grain from USA, 6: Local Egyptian maize grain, 7: Negative control, 8: Positive control

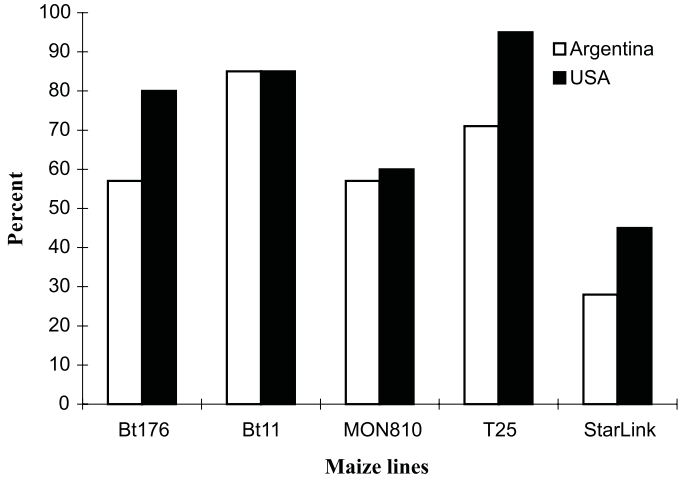

Figure 7. Percent of transgenic maize lines in the imported maize samples

ation which existed in 2000–2001 using highly sensitive, reliable methods that are capable of detecting even trace amounts. The positive controls used in this study contained 0.1% GMO, which reflect the sensitivity of the detection methods used. All GMOs examined here have been approved in other countries and have passed a safety evaluation. Nevertheless it cannot be excluded, that non-approved GM breeds may enter in uncontrolled markets. Therefore qualitative, sensitive methods, as used here, would be suited for monitoring programmes. The urgent need to monitor feed and food for the presence of GMO is underlined by the example of StarLink maize. This GM maize line, produced by Aventis Crop Inc., has been assessed for animal feed use exclusively in USA. Recently it entered the food chain unintentionally although it was suspected to produce allergenic potential for humans, but in spite of intensive investigations no allergic reactions were noted or attributed to StarLink (FDA and CDC, 2001).

In conclusion, all local Egyptian varieties of both, soyabean and maize contained no transgenic material from the constructs discussed in this study. On the other hand, the imported varieties of both, soyabean and maize contained a number of GM constructs. Therefore Egypt and other importing countries need to monitor imported feeds if they required labelling of such products.

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STRESZCZENIE

Zakres zastosowania genetycznie zmodyfikowanej soi i kukurydzy jako pasz dla zwierząt w Egipcie

W następstwie gwałtownego postępu w biotechnologii rolnej zwiększyła się ilość genetycznie zmodyfikowanych (GM) upraw, które w ostatnich latach weszły w łańcuch pokarmowy środków spożywczych dla ludzi i pasz dla zwierząt.

Celem naszych badań, przeprowadzonych w latach 2000-2001, było prześledzenie zastosowania Roundup Ready™ soi (RRS) oraz GM kukurydzy linii Bt 176, Bt 11, T25, MON 810 i StarLink™ w Egipcie, które zostały szeroko rozpowszechnione i aprobowane jako pokarm i/lub pasza w USA i innych krajach. Pięćdziesiąt jeden próbek soi i 61 próbek kukurydzy pobrano losowo z różnych miejscowości w Egipcie. Zastosowana technika oceny opierała się na Reakcji Łańcuchowej Polimerazy (PCR), przy użyciu uprawomocnionej, oficjalnej metody oceny, zgodnie z Artykułem 35 Ustawy o Paszach Federalnej Republiki Niemiec. Wyniki naszych badań wskazują, że wszystkie próby soi importowane z Argentyny i 50% prób z Ameryki zawierały RRS. Spośród 20 prób kukurydzy importowanej z USA - 16 zawierało Bt 176, 17 - Bt 11, 12 - MON810, 19 - T25 i 9 - StarLink™. Ponadto, spośród 7 próbek kukurydzy pochodzących z Argentyny, 4 zawierały Bt 176 i MON 810, 5 - T25, 6 - Btu i 2 StarLink™. W przeciwieństwie do tych prób, wszystkie egipskie lokalne odmiany soi i kukurydzy nie były transgeniczne.