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农业生物技术

山西市场动物饲料中转基因成分的检测

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摘 要： 为了评估转基因玉米和大豆在山西动物饲料市场的占有率和标识情况，采用改良十六烷基三甲基溴化铵法 (Hexadecyltrimethy ammonium bromide, CTAB) 提取山西市场抽取的 30 份鸡和猪饲料，通过定性 PCR 打包筛查，对检测阳性结果打包饲料拆包并检测 *CaMV* 35S 启动子、*NOS* 终止子、玉米内标 *zSSIIb*、大豆内标 *Lectin* 和 *CryIA (b)* 基因。同时检测玉米和大豆转化体事件 MON810 和 GTS40-3-2。结果表明，83.3%的饲料含有转基因成分。所抽取的玉米、大豆、猪饲料和鸡饲料转基因成分阳性率分别为 6.67%、100%、93.3%和 73.3%。实时荧光定量 PCR 检测结果与定性 PCR 一致。结果提示，鸡和猪饲料中转基因成分在山西市场的占有率较高。

关键词： 商业化鸡饲料，商业化猪饲料，转基因玉米，转基因大豆，定性 PCR，实时荧光定量 PCR，转化体事件 MON810 和 GTS40-3-2

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Detection of transgenic components in animal feeds on Shanxi markets

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Abstract: To assess the presence of genetically modified (GM) maize and soybean in a range of commercialized feed in Shanxi province of China in 2015, improved hexadecyltrimethy ammonium bromide (CTAB) method was used to extract DNA. The screening of packed feeds was carried out by qualitative PCR. Then positive feeds were unpacked and detected by the *CaMV* 35S promoter, *NOS* terminator, *zSSIIb*, *Lectin* and *CryIA (b)* genes. The identified maize and soybean events were confirmed by event-specific MON810 and GTS40-3-2. Results showed that 83.3% of the feeds was tested positive for GMOs, in which positive rates of maize, soybean, pig and layer feeds were 6.67%, 100%, 93.3% and 73.3%, respectively. The results of real-time PCR were consistent with qualitative PCR. These results indicated that commercialized GM feed had a wide positive product scope in Shanxi province of China. Further studies are necessary to study effects of feeding livestock and poultry with feed containing GM ingredients on animals and their products.

Keywords: commercialized layer feed, commercialized pig feed, genetically modified maize, genetically modified soybean, qualitative PCR, real-time PCR, event-specific MON810 and GTS40-3-2

Introduction

By gene modification technique, compositions of plants, animals or microorganisms were altered to gain new characteristics, such as insect resistance, herbicide tolerance, modified nutritional composition or an enhanced shelf life, we call them genetically modified organisms (GMOs)[1-2]. Roundup Ready^{TM} soybean (RRS) was firstly produced by the Monsanto Company in Canada in 1996, which was approved for consumption[3-4]. According to the final reports of 2014, the planting of GM crops worldwide had reached 181.5 million hectares and constantly increased 106-fold since that first crop in global area[5-6]. Although GMOs had many advances, consumer had very strong reaction[3]. In China, regulations have come into force, which are the labeling and traceability of GM food. The regulations stipulate the requirement of labeling containing GM material, such as soybean seed, soybean flour, soybean, soybean oil, soybean meal, maize seeds, maize, maize oil and maize flour etc[7]. To comply with the requirements of legislation, usually reliable and practical detection methods is required[1,8-13]. Before either GMO quantification or event identification, the starting point of GMO detection is generally evaluation of the screening method[2,14]. Most of the PCR screening methods usually are to detect either *CaMV* 35S promoter (the cauliflower mosaic virus) or *NOS* terminator (the nopaline synthase) or both, because most GM products contain two or one of these sequences[8,15]. *ZSSIIb* for maize and *Lectin* for soybean are species-specific PCR testing genes, which are

present in both GM and non GM maize and soybean. For discrimination between non-approved and approved traits, event-specific PCR methods are used to identify the GMO event (for example GTS40-3-2 and MON810). *PAT* (Phosphinothricin acetyltransferase) gene mediates tolerance to the herbicide phosphinotricin (glufosinate). A number of soil bacteria naturally possess the *PAT* gene. Transgenic plants expressing the *PAT* gene are able to degrade the herbicide agent phospinotricin (glufosinate). *BAR* gene coding for phosphinothricin acetyltransferase had been isolated from *Bacillus amylolique facions*. *PAT* and *BAR* genes are widely used as selective markers for the transformation of higher plants. *CryIA(b)* gene is a synthetic gene encoding the 648 amino acids, insecticidal-active truncated product identical to *CryIA(b)* gene of *Bacillus thuringiensis* subsp (General Administration of Qality Spervision, Ispection and Quarantine of the People's Republic of China, SN/T 1202-2003).

Because GMOs have the worldwide high production and fairly uncertain of the current status of the foods, people pay particular attention to them. Soybean and maize were chosen because they were the staple constituents of feed[8,16]. The study objective was to determine the ratio of GM-containing soybean and maize feed obtained from local feed manufacturer and retail shops in Shanxi of China in 2015.

1 Materials and methods

1.1 Feed

Thirty soybean- and maize-containing feeds were purchased from 24 random local retail shops (20 feed manufacturers) in Shanxi of China in May 2015. In the 30 feeds (serial number: 01-30, there were layer feed and pig feed in three periods. Every period there were five different feed manufacturers or brands). In different periods, 5 kinds of feed manufacturers (brand) might be overlapping or different.), 15 layer feeds (including adding concentrate), accounted for about 50.0% of the total feeds, 15 pig feeds accounted for about 50% of the total feeds (Table 1).

1.2 Qualitative PCR

1.2.1 Reference materials

Certified reference samples (CRS) (GTS40-3-2 1% soybean flour, MON810 1% maize flour and flour mixture -Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour), which were provided by the science and technology development center of China's ministry of agriculture, were used as the positive controls in the study.

1.2.2 DNA extraction

For DNA isolation from feed and CRS, an improved version of the cetyltrimethyl ammonium bromide (CTAB) method was used. The collected DNA quantification (concentration and purity) was achieved by measuring the UV absorption at 260 nm and 280 nm using a biophotometer (Eppendorf.AG, Germany) and stored at –20 ℃ until used.

1.2.3 PCR primers

The *CaMV* 35S promoter and the *NOS* terminator for amplifying the specific DNA sequences were used for the GMO screening of the products[17]. The amplification of extracted DNA was verified using plant-specific primers targeting the *zSSIIb* for maize, the *Lectin* for soybean, specific sequences present in MON 810 event and RRS (GTS40-3-2) for specific GMO detection[18-19]. Primers for amplifying *CryIA(b)*,

Table 1 The composition of feed

Pig feeds			Layer feeds		
Suckling pig feed	Mid-pig feed	Pregnancy sow feed	Early layer feed	Youth layer feed	Feed in laying period
5 (01-02, 04-06)	5 (07-11)	5 (12-15, 24)	5 (16-20)	5 (21-23, 25-26)	5(03, 27-30)

BAR and *PAT* genes were used[20-21]. The primer names, orientation, sequences and length synthesized by TAKARA BIOTECHNOLOGY (Dalian, China) in the study were summarized in Table 2.

1.3 Real-time PCR of feed samples

1.3.1 Reference materials

Certified reference material (CRM) (Soya seed powder-GTS40-3-2 Soya (10%), Catalog Number: ERM-BF410GK, IRMM).

1.3.2 PCR primers

The names, orientation, sequences and length of the primer and probe (synthesized by TAKARA BIOTECHNOLOGY, Dalian, China) were summarized in Table 3[17,22]. The amplifications

Table 2 The primers used in the study

Primers	Orientation	Sequence (5′–3′)	Fragment length (bp)
zSSIIb	Sense	CGGTGGATGCTAAGGCTGATG	88
	Anti-sense	AAAGGGCCAGGTTCATTATCCTC	
Lectin	Sense	GCCCTCTACTCCACCCCCATCC	118
	Anti-sense	GCCCATCTGCAAGCCTTTTTGTG	
CaMV 35S	Sense	GCTCCTACAAATGCCATCATTGC	195
	Anti-sense	GATAGTGGGATTGTGCGTCATCCC	
NOS	*Sense*	GAATCCTGTTGCCGGTCTTG	180
	Anti-sense	TTATCCTAGTTTGCGCGCTA	
PAT	*Sense*	GTCGACATGTCTCCGGAGAG	191
	Anti-sense	GCAACCAACCAAGGGTATC	
BAR	*Sense*	ACAAGCACGGTCAACTTCC	175
	Anti-sense	ACTCGGCCGTCCAGTCGTA	
Cry ⅠA(b)	Sense	CTGGTGGACATCATCTGGGGCATCTTCG	146
	Anti-sense	TTGGTACAGGTTGCTCAGGCCCTCC	
MON810	Sense	CAAGTGTGCCCACCACAGC	106
	Anti-sense	GCAAGCAAATTCGGAAATGAA	
GTS40-3-2	Sense	TTCAAACCCTTCAATTTAACCGAT	370
	Anti-sense	AAGGATAGTGGGATTGTGCGTC	

Table 3 Real-time PCR primer sequences

Detection genes	Sequence (5′–3′)	Amplicon size (bp)
GTS40-3-2	CAT TTG GAG AGG ACA CGC TGA	74
	GAG CCA TGT TGT TAA TTT GTG CC	
	FAM-CAA GCT GAC TCT AGC AGA TCT TTC-TAMRA	
Lectin	CCA GCT TCG CCG CTT CCT TC	74
	GAAGGCAAGCCCATCTGCAAGCC	
	FAM-CTT CAC CTT CTA TGC CCC TGA CAC-TAMRA	

were performed with using Code NO. RR390A from TAKARA BIOTECHNOL (Dalian, China).

1.3.3 Calculating transformant content

The experiment observed and analyzed standard curve and amplification curve, calculated transformant content.

2 Results and discussion

2.1 Concentration and purity of DNA

In our study, an appropriate quality and quantity of DNA could be extracted from the feed samples using the improved CTAB method (Fig. 1). The date showed that DNA concentration and purity (A_{260}/A_{280}) extracted by improved CTAB method ranged from 120.9 μg/mL to 778.3 μg/mL and 1.7 to 1.99, respectively. The results showed that improved CTAB method gave sufficient yield of DNA.

In order to isolate DNA from feeds with different components of GMOs, the improved CTAB were used (Fig. 1). According to the characteristics of the feed production and processing steps in the traditional, the CTAB method was simplified, reagents used commonly in laboratory could be done. Compared to the (high) cost of the kit (40 RMB per time), the cost of the improved CTAB method was 2 RMB per time. Simultaneously, the improved CTAB method could reduce the extraction time of the kit method by at least 50 min. With the improved CTAB method, successful species-specific PCR testing (*zSSIIb* for maize and *Lectin* for soybean) and high amplification success rate were achieved with 30 different kinds of feeds extracts just one time, which confirmed that these extracts contained a sufficient amount of amplifiable DNA. In our work, the improved CTAB method was more effective and less time-consuming in comparison with the existing kit methods for isolation of DNA from plant-derived foods and feed.

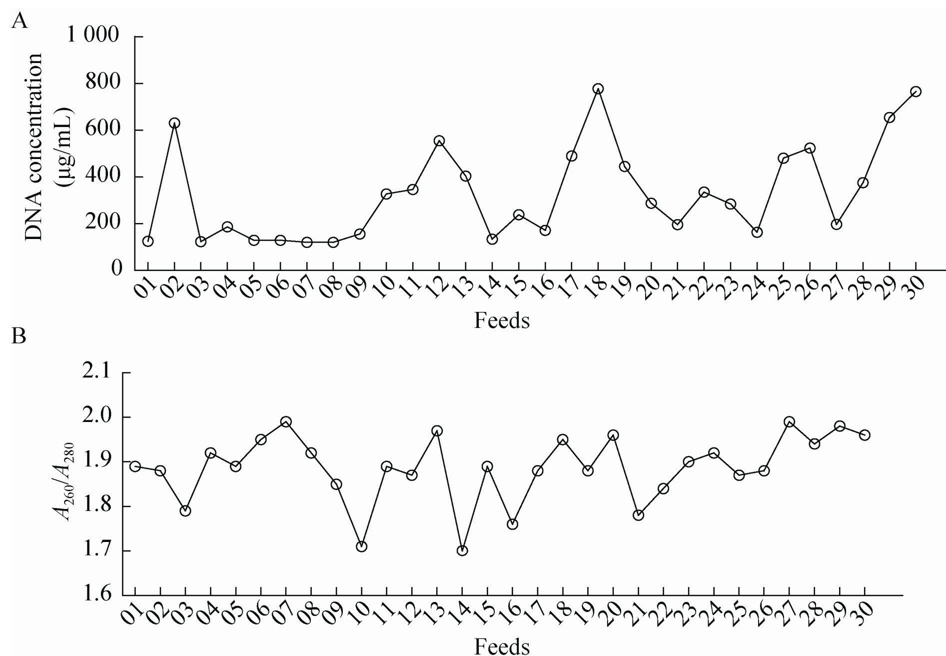

Fig. 1 The concentration and purity of DNA extracted from feeds. The graph A and B respectively on behalf of concentration and purity of DNA extracted from feeds; 01-30: 01-30 feed samples.

2.2 Screening of GMOs by packing feeds

The 30 feeds mentioned above were packaged as 5 packages (6 samples in each package) for detecting by the *CaMV* 35S promoter, *NOS* terminator, *CryIA(b), BAR* and *PAT* genes by PCR, all DNA of feeds were run in duplicate. A 195 bp fragment (*CaMV* 35S promoter) was detected in all 5 packages feeds produced through PCR amplification. Through the process of detecting, feeds of 5 packages gave 180 bp positive amplification signal of *NOS* gene. Both packed feeds 01-06, 19-24 amplified 146 bp *CryIA(b)* gene signal. The target fragments of *BAR* (175 bp) and *PAT* (191 bp) genes could not be detected in all feeds (Fig. 2).

2.3 Specific gene detection of GMOs

According to the results, the positive-packed feeds were subsequently unpacked and detected by *CaMV* 35S promoter, *NOS* terminator, *CryIA(b)*, *Lectin* and *zSS IIb* genes. All DNA of feeds were run in duplicate excepting for *zSS IIb* gene (simple sample).

2.3.1 *CaMV* 35S promoter PCR

30 feed samples were detected by *CaMV* 35S promoter. 01-23, 25-26 feed samples gave 195 bp positive amplification signal (Fig. 3).

2.3.2 *NOS* terminator PCR

01-30 feed samples were detected by *NOS* terminator. 180 bp fragment of *NOS* terminator for 01-18, 20-23 and 25-26 feed samples were produced through PCR amplification (Fig. 4).

2.3.3 *CryIA(b)* gene PCR

01-30 feed samples were detected by *CryIA(b)* gene. The target fragments of *CryIA(b)* gene could be detected in 03 and 19 feeds (Fig. 5).

2.3.4 *Lectin* gene PCR

01-30 feed samples were detected by *Lectin* gene. 01-18, 20-23, 25-26 feeds gave 118 bp positive amplification signal of *Lectin* gene (Fig. 6).

2.3.5 *ZSSIIb* gene PCR

The 30 feed samples (01-30) were unpacked for detecting by the *zSSIIb* gene. The target fragments of 88 bp *zSSIIb* gene could be detected in all feed (Fig. 7).

2.4 Event-specific qualitative PCR

According to the results, the positive feeds were unpacked and detected by specific MON810 and GTS40-3-2 events.

2.4.1 GTS40-3-2 event PCR

The 24 positive feeds (01-18, 20-23 and 25-26) containing soybean (*Lectin* gene) were detected for the presence of specific GM GTS40-3-2 event. All these feeds gave 370 bp positive amplification signal of GTS40-3-2 (Fig. 8). DNA of feeds were run in single sample.

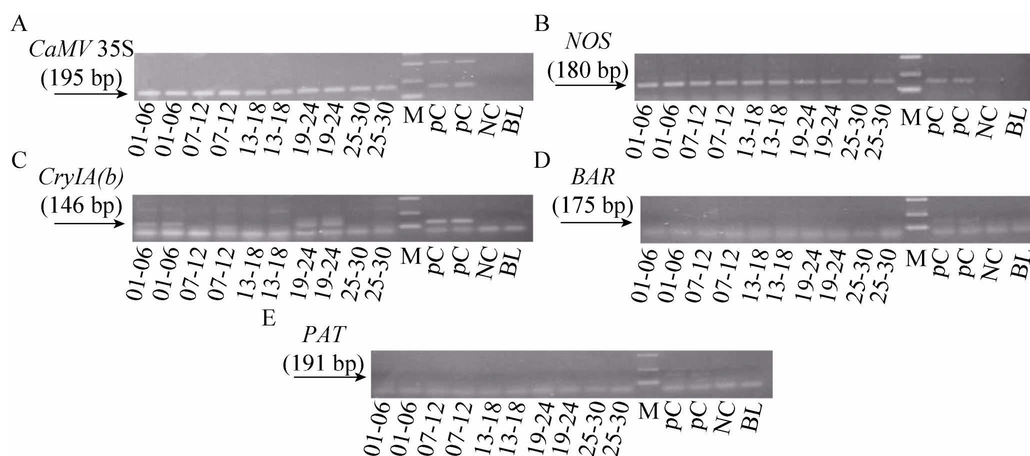

Fig. 2 The electrophoregram of *CaMV* 35S, *NOS*, *CryIA(b)*, *BAR* and *PAT* genes of packing feeds. The electrophoregram A, B, C, D and E respectively on behalf of detecting *CaMV*35S, *NOS*, *CryIA(b)*, *BAR* and *PAT* genes; 01–30: 01–30 feed samples; positive control (PC): Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour; negative control (NC): negative sample (maize flour); blank (BL): ultrapure water; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

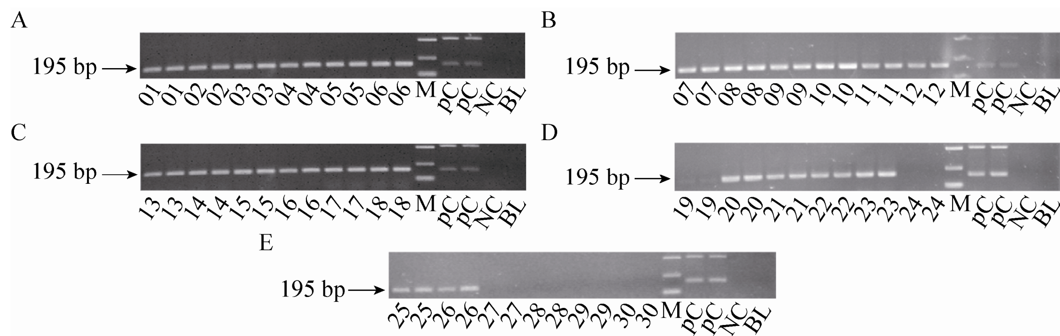

Fig. 3 The electrophoregram of *CaMV* 35S promoter. 01–30: 01–30 feed samples; positive control (PC): Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour; negative control (NC): negative sample (maize flour); blank (BL): ultrapure water, respectively; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

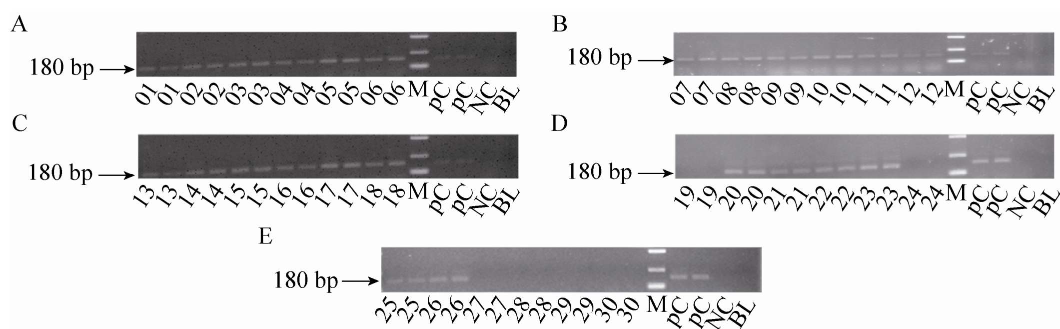

Fig. 4 The electrophoregram of *NOS* terminator. 01–30: 01–30 feed samples; positive control (PC): Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour; negative control (NC): negative sample (maize flour); blank (BL): ultrapure water, respectively; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

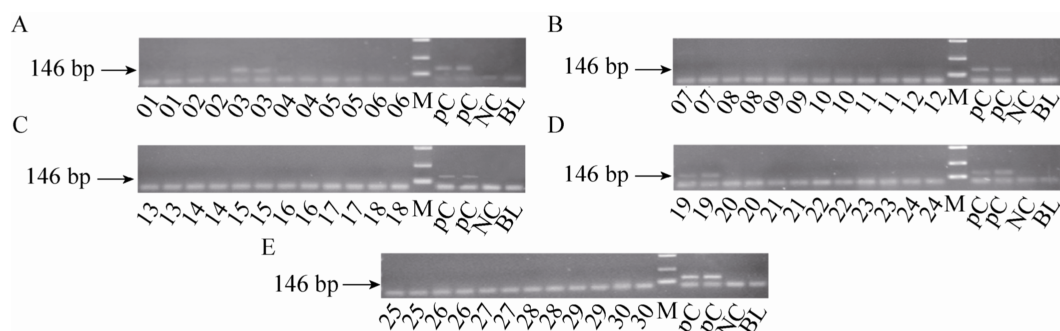

Fig. 5 The electrophoregram of *CryIA(b)* gene. 01–30: 01–30 feed samples; positive control (PC): Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour; negative control (NC): negative sample (maize flour); blank (BL): ultrapure water, respectively; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

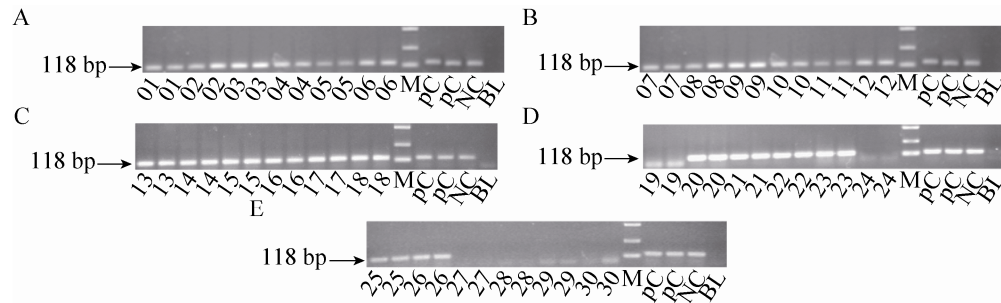

Fig. 6 The electrophoregram of *Lectin* gene of 01–30 feed samples. 01–30: 01–30 feed samples; PC: GTS40-3-2 event flour; NC: negative sample (soybean flour) and BL: ultrapure water, M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

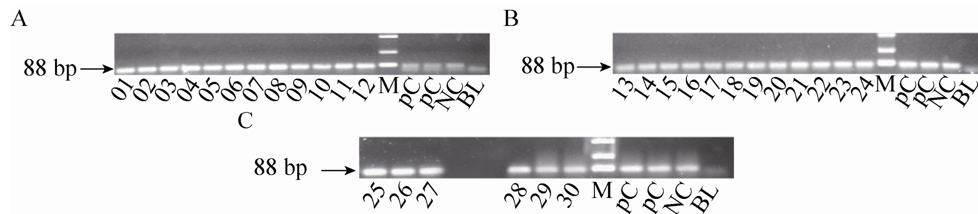

Fig. 7 The electrophoregram of *zSS IIb* gene. 01–30: 01–30 feed samples; positive control (PC): Bt176 1%, Bt11 1% and kefeng6 1% positive sample mixture flour; negative control (NC): negative sample (maize flour); blank (BL): ultrapure water, respectively; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

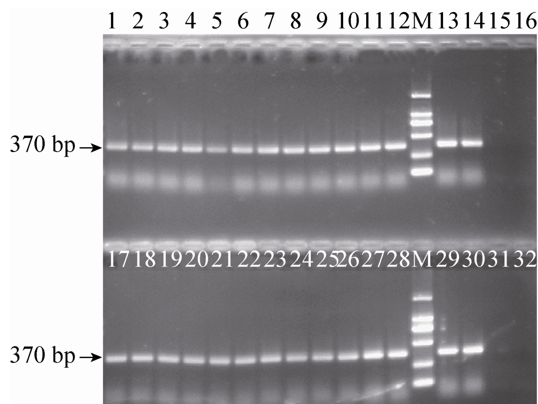

Fig. 8 The electrophoregram of GTS40-3-2. 1–12 lanes: 01–12 feed samples; 17–28 lanes: 13–18, 20–23, 25–26 feed samples; 13–14, 29–30 lanes: GTS40-3-2 positive sample flour; 15, 31 lanes: negative sample (soybean flour); 16, 32 lanes: ultrapure water; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

2.4.2 MON810 event PCR

Feeds (03 and 19) were detected by MON810 event. By detecting, 106 bp amplification signal of MON810 was produced for both feeds (Fig. 9). DNA of feeds was run in triple.

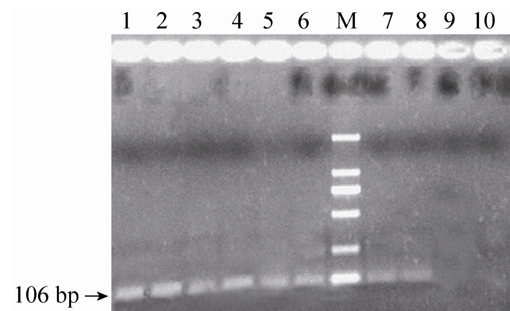

Fig. 9 The electrophoregram of MON810 of 03 and 19 feed samples. 1–3 lanes: 03 feed sample; 4–6 lanes: 19 feed sample; 7–8 lanes: MON810 1% positive sample flour; 9 lane: negative sample (maize flour); 10 lane: ultrapure water; M: DL2 000 marker (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

2.5 The summary of detecting results of feeds

In 30 feeds, 25 feeds contained GM ingredients. 01-02, 04-18, 20-23, 25-26 feeds contained *CaMV* 35S promoter, *NOS* terminator, GTS40-3-2 event, *zSSIIb* and *Lectin* genes. 03 feed contained *CaMV* 35S promoter, *NOS* terminator, MON810 event,

zSSIIb, *Lectin* and *CryIA(b)* genes. 19 feed contained *CaMV* 35S promoter, MON810 event, *zSSIIb* and *CryIA(b)* genes. 24, 27-30 feeds were non GM, only contained *zSSIIb* (Fig. 10). The overall results of GMO screening of 5 packed feeds were 100% for *CaMV* 35S promoter, 100% for *NOS* terminator, 20% for *CryIA(b)* and 0% for *BAR* gene and *PAT* gene. The detecting results of 30 feed samples showed that 83.3% of the feeds were tested positive for GMOs, in which positive rates of maize, soybean, pig and layer feeds were 6.67%, 100%, 93.3% and 73.3%, respectively. In conclusion, commercialized GM feed had a wide positive product scope in Shanxi province of China. The composition and positive rate of feeds were as shown (Fig. 11).

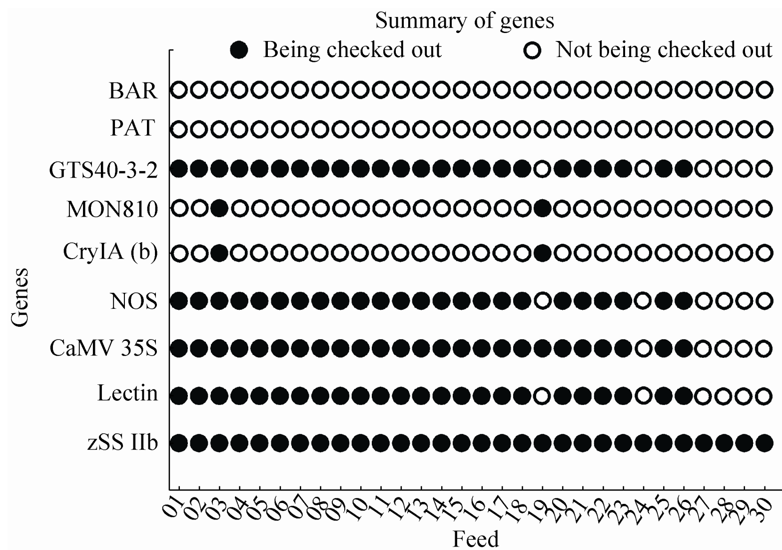

Fig. 10 The summary of detecting results of 01-30 feed samples.

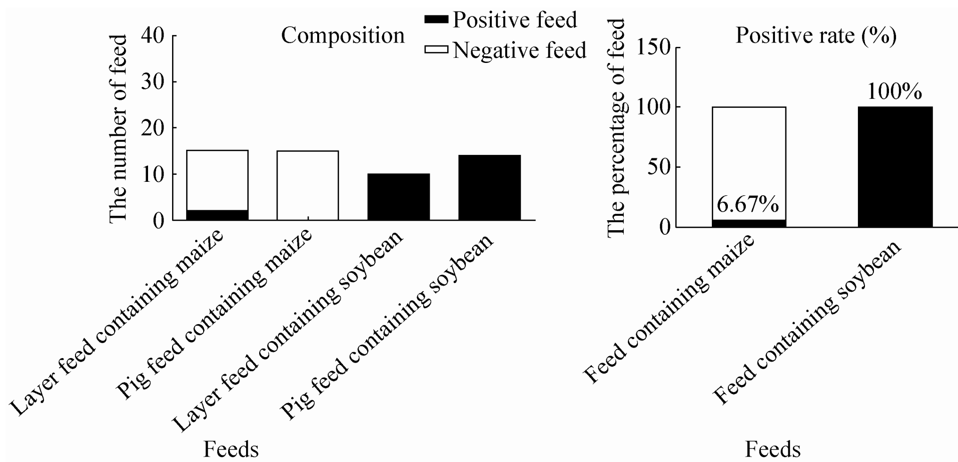

Fig. 11 The composition and positive rate of 30 feed samples.

2.6 Real-time PCR of feed samples

According to the qualitative results, 24 feed samples of containing genes of *Lectin* and GTS40-3-2 were detected by real-time PCR.

2.6.1 *Lectin* gene real-time PCR

The quantitative results of *Lectin* gene from identified 24 feeds DNA were presented in Fig. 12 and Fig. 13. The obtained real-time PCR results were according to those requirements since the correlation coefficient (R^2) of standard curves was, generally, ≥ 0.98, while PCR efficiencies ranged, on average, from 93.9% to 100.6%, indicating the adequacy of the standard curves for quantification. In Fig.12, standard of *Lectin* gene had a typical amplification curve. Amplification efficiency was 98.0%, R^2 of standard curves was 0.984>0.98, slope of standard curve was –3.370>–3.6 and <–3.1, indicating the adequacy of the standard curves for quantification. In Fig. 13, negative control (salmon sperm DNA) and blank control (water) had not typical amplification curve of *Lectin* gene, the DNA of all feed samples in *Lectin* gene amplification curve appeared typical amplification curve. In amplification results (generated by the Excel table), Ct values and copy numbers of all feed samples were in Table 4.

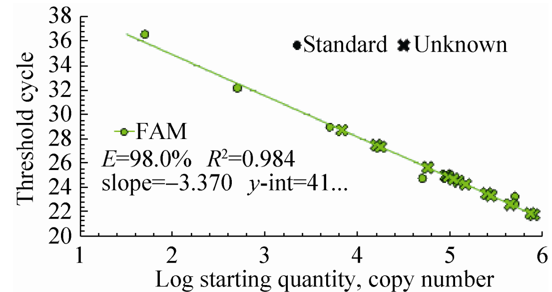

Fig. 12 *Lectin* gene standard curve.

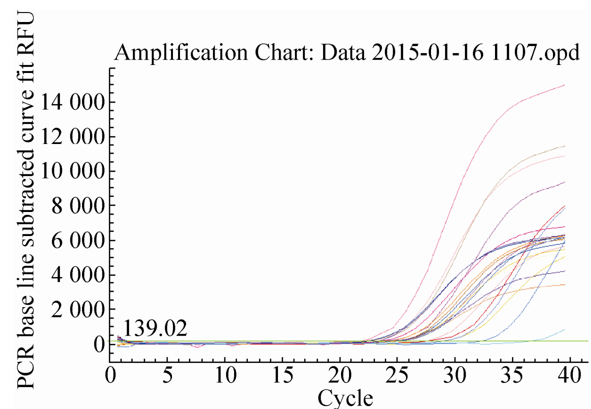

Fig. 13 *Lectin* gene amplification curve.

Table 4 The Ct value and copy number of *Lectin* gene from 24 feed samples

Sample number	Ct value	Copies/2 µL	Sample number	Ct value	Copies/2 µL
01	27.33	1.77E+04	13	24.24	1.46E+05
02	28.89	2.06E+04	14	24.68	1.08E+05
03	27.08	7.05E+04	15	24.94	9.05E+04
04	26.75	8.83E+04	16	23.26	5.00E+05
05	27.30	6.07E+04	17	22.63	5.00E+05
06	25.61	5.73E+04	18	24.73	5.00E+04
07	27.49	1.59E+04	20	23.47	2.48E+05
08	24.89	9.35E+04	21	28.74	6.76E+03
09	24.50	1.22E+05	22	21.87	7.37E+05
10	24.87	9.49E+04	23	23.30	2.77E+05
11	21.87	7.37E+05	25	24.95	8.99E+04
12	22.61	4.46E+05	26	21.75	7.99E+05

2.6.2 GTS40-3-2 gene real-time PCR

The quantitative results of special event GTS40-3-2 gene from identified 24 feeds DNA were presented in Fig. 14 and Fig. 15. In Fig. 14, standard of GTS40-3-2 gene had a typical amplification curve. Amplification efficiency was 99.9%, R^2 of standard curves was 0.988>0.98, slope of standard curve was –3.324>–3.6 and <–3.1, indicating the adequacy of the standard curves for quantification. In Fig. 15, negative control (salmon sperm DNA) and blank control (water) had not typical amplification curve of GTS40-3-2, the DNA of all feed samples in GTS40-3-2 gene amplification curve appeared typical amplification curve. In amplification results (generated by the Excel table), Ct values and copy numbers of all feed samples were in Table 5.

2.6.3 The content of the special event GTS40-3-2 in the feeds

The content of the special event GTS40-3-2 in the feeds was calculated according to the following formula:

$$C= \frac{n_{GTS40-3-2}}{n_{Lectin}} \times 100.$$

$n_{GTS40-3-2}$ was copy number of GTS40-3-2 gene, n_{Lectin} was copy number of *Lectin* gene. The GTS40-3-2 transformant content of feed samples was in Table 6.

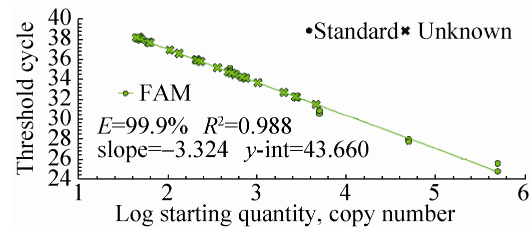

Fig. 14 GTS40-3-2 gene standard curve.

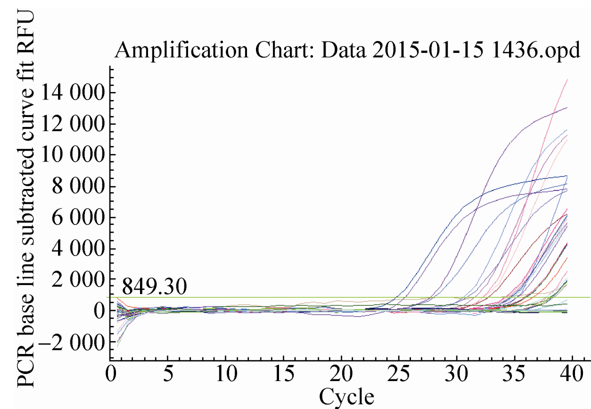

Fig. 15 GTS40-3-2 gene amplification curve.

Table 5 The Ct value and copy number of GTS40-3-2 gene from 24 feed samples

Sample number	Ct value	Copies/2 μL	Sample number	Ct value	Copies/2 μL
01	36.17	2.03E+02	13	35.16	3.61E+02
02	34.30	7.39E+02	14	34.51	5.67E+02
03	32.47	2.62E+03	15	34.11	7.48E+02
04	34.68	5.69E+02	16	32.84	2.02E+03
05	34.78	5.29E+02	17	31.65	4.60E+03
06	34.63	5.20E+02	18	34.44	6.69E+02
07	34.11	7.48E+02	20	32.68	2.01E+03
08	34.51	5.67E+02	21	32.47	2.62E+03
09	32.22	2.78E+03	22	31.65	4.60E+03
10	32.26	2.68E+03	23	34.75	4.80E+02
11	34.24	6.84E+02	25	35.96	2.07E+02
12	35.96	2.07E+02	26	31.49	4.59E+03

Table 6 The GTS40-3-2 transformant content of 24 feed samples

Sample number	Transformant content (%)	Sample number	Transformant content (%)
01	1.15	13	0.25
02	3.59	14	0.53
03	3.72	15	0.83
04	0.64	16	0.40
05	0.87	17	0.92
06	0.91	18	1.34
07	4.70	20	0.81
08	0.61	21	0.39
09	2.28	22	0.62
10	2.82	23	0.17
11	0.09	25	0.23
12	0.05	26	0.57

In our study, all 30 feeds were packed (6 feeds of each package) and screened, 5 packages (100%) were determined to be positive for two or three of the novel sequences which indicated the presence of GMOs. The dispersion of these positive packed feeds within soybean and maize were as follows: 25 of the 30 total (83.3%) detected all feeds, 11 of the 15 total (73.3%) detected layer feeds, 14 of the 15 total (93.3%) detected pig feeds were positive (Fig. 11). All 30 feeds contained maize composition, 2 of 30 feeds (6.7%) which were layer feeds were positive of MON810. 30 feeds had 24 feeds containing soybean composition, 24 feeds (10 for chicken feed, 14 for pig feed) containing soybean (100%) tested positive for GTS40-3-2 event, that came mostly from soybean meal of oil residue from imported soybean. Layer feeds had 2 positive feeds of MON810 event, pig feeds had not been detected positively for the composition of MON810 event. The data showed maize sources varied in different feed manufacturers. The ratio of positive feed containing layer feed seemed to be lower than that of the pig feed, which was a different result than was expected. In fact, there were 4 concentrate supplements of layer feeds including DDGS protein feed, dry maize lees protein feed and shoetree maize husk powder and maize feed (raw material-layer), which were also included in the layer feeds. According to the test results, a positive rate of transgenic maize in feed was very low in the market of Shanxi. The results in which we did not detect genetically modified ingredients in these concentrate supplement were also normal. In this study, positive feeds only had one feed (soybean meal) marked as “genetically modified” composition; genetically modified product identification rate was only 3.33%. Furthermore, we also found that on the identified feed products’ packaging logo, the font was very small and hard to recognize.

Amplification of the maize-specific *zSS IIb* sequence in 30 feeds and the soybean-specific *Lectin* sequence in 24 feeds confirmed that the feeds containing 5 negative feeds and 1 positive feed were negative for *Lectin*, which indicated that these feeds did not contain soybean DNA. The above mentioned 24 *CaMV* 35S and/or 23 *NOS* positive soybean feeds were analyzed for RRS and all of them gave positive amplification signal (GTS40-3-2 event) (Fig. 8). The other 2 positive feed samples containing *CryIA(b)* were screened for the presence of specific GM maize events. For this purpose, PCR detections of specific sequences of MON 810 event (containing *zSSIIb*, *CaMV* 35S, *CryIA(b)* and MON 810 genes) was performed (Fig. 9). 03 and 19 positive feeds (containing *zSSIIb*, *CaMV* 35S, *CryIA(b)* and MON810) were identified as containing MON810 event. The results of our study showed that 1 feed (19) was negative for the *NOS* terminator while positive for the *CaMV* 35S promoter (maize containing feed). In our study, the *NOS* negative feed was determined to be MON810 maize feed and thus confirmed that the feed was true positive although they did not give any amplification signal with the *NOS*. In case of maize, it could also be related to the lack of the *NOS* terminator common in

several maize events, for example MON810. Similarly, detection of both the *CaMV* 35S and the *NOS* sequences in another feed (03) confirmed the GM maize MON810 event (containing *CaMV* 35S) and GM soybean GTS40-3-2 event (containing *NOS*) presence.

By real-time PCR, 24 feed samples of containing genes of *Lectin* and GTS40-3-2 were detected. The results showed that they contained different content of GTS40-3-2 transformant (0.05%–4.70%, Table 6). The results were consistent with qualitative PCR (Fig. 10).

In this study, we demonstrated that many layer and pig feeds containing GM GTS40-3-2 event and a small number of layer feeds containing GM MON810 event were sold commercially in Shanxi of China.

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