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Application News

MultiNA

Qualitative Determination of Genetically Modified Ingredients in Soybean and Corn Using the MultiNA and the PCR Method

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■ Abstract

Genomes in three groups of soybean and corn samples were extracted using a plant genome extraction reagent kit, using the soybean endogenous Lectin and corn endogenous Zein genes as internal references. Specific primers were designed for the exogenous promoter CaMV35S and exogenous terminator NOS genes commonly used in genetically modified crops for PCR amplification, and the amplified products were determined using the MultiNA to determine if any genetically modified ingredients are present. The results indicate that no genetically modified ingredients were detected in the three groups of soybean samples, while the NOS exogenous gene was detected in two groups of corn samples. This experiment indicates that the screening and qualitative determination of genetically modified crops can be realized using the MultiNA.

■ Introduction

With the development of molecular biological technology, the research and cultivation of genetically modified crops is expanding. In 2013, the cultivated area of genetically modified crops of China was 4.2 million hectares, ranking sixth in the world. In addition, since some crops in the domestic market have been expensive in recent years, such as corn, corn has been imported to China in large quantities from the U.S. and Argentina, while 80 % of corn in these two countries is genetically modified. Therefore, before a final conclusion can be drawn as to whether genetically modified products are absolutely safe for human health and the environment, it is essential to establish a reliable, sensitive and convenient means of transgene determination.

This paper performs qualitative determination on genetically modified ingredients in soybean and corn using the MultiNA by molecular biological means. The soybean endogenous Lectin and corn endogenous Zein genes were used as internal references, specific primers were designed for the promoter CaMV35S and terminator NOS exogenous genes commonly used in genetically modified crops for PCR amplification, and the amplified products were determined using the MultiNA. The results indicate that genetically modified ingredients are detected in corn. This experiment indicates that the MultiNA can realize the qualitative screening of genetically modified ingredients.

Table 1 Information on Primers Designed for the Soybean Endogenous Lectin, Corn Endogenous Zein, Exogenous Promoter CaMV35S and Exogenous Terminator NOS Genes, and PCR Amplification

Gene detected	Primer sequence	Size of theoretical PCR product/bp	Amplified gene sequence*
Soybean endogenous Lectin	5′-cctcctcgggaaagttacaa-3′	162	gtgacctcctcgggaaagttacaactca >>>>>>>>>>>>>>>>>> ataaggttgacgaaaacggcaccccaaaaccctcgtctcttggtcgcgccctctactccacc cccatccacatttgggacaaagaaaccggtagcgttgccagcttcgccgcttccttcaacttc accttctatgcc <<<<<<<<<<<<<<<< cctgacacaaaaaggc
	5′-gggcatagaaggtgaagtt-3′		
Corn endogenous Zein	5′-tgaacccatgcatgcagt-3′	190	catgaacccatgcatgcagtactgcatga >>>>>>>>>>>>>>>>> tgcaacaggggcttgccagcttgatggcgtgtccgtccctgatgctgcagcaactgttggcct taccgcttcagacgatgccagtgatgatgccacagatgatgacgcctaacatgatgtcacc attgatgatgccgagcatgatgtcaccaatggtcttgccgagcat <<<<<<<<<<<<<<<< gatgtcgc
	5′-ggcaagaccattggtga-3′		
CaMV35S exogenous	5′-gctcctacaaatgccatca-3′	195	gaaggtggctcctacaaatgccatcatt >>>>>>>>>>>>>>>>> gcgataaaggaaaggccatcgttgaagatgcctctgccgacagtggtcccaaagatgga cccccacccacgaggagcatcgtggaaaaagaagacgttccaaccacgtcttcaaagca agtggattgatgtgatatctccactgacgtaagggatgacgcacaatccca <<<<<<<<<<<<<<<< ctatccttc <<<<<
	5′-gatagtgggattgtgcgtca-3′		
NOS exogenous	5′-gaatcctgttgccggtcttg-3′	180	aagattgaatcctgttgccggtcttgca >>>>>>>>>>>>>>>>>> tgattatcatataatttctgttgaattacgttaagcatgtaataattaacatgtaatgcatgacgtt atttatgagatgggtttttatgattagagtcccgcaattatacatttaatacgcgatagaaaaca aaatatagcgcgcaa <<<<<<<<< actaggataaattat <<<<<<<<<<
	5′-ttatcctagtttgcgcgcta-3′		

*>>> and <<< are the binding sites of the upstream and downstream primers.

■ 1. Experimental Materials and Methods

1.1 Instruments

MCE-202 MultiNA, PCR instrument

1.2 Reagents

Plant gene extraction reagent kit
(Beijing Kwinbon Biotechnology Co., Ltd.) FZ-002
SYBR® Premix Ex Taq™ II (Takara Bio Inc.) RR820A
SYBR® Gold Nucleic Acid Gel Stain (Invitrogen) S-11494
1× TE Buffer
25 bp DNA Ladder (Invitrogen) 10597-011
DNA-500 Reagent Kit for MultiNA
(Shimadzu Corporation) 292-27910-91
Samples: three groups of soybean and corn samples
Primers: According to the literature and the NCBI sequence, the primers designed for amplifying the soybean endogenous Lectin, corn endogenous Zein, exogenous promoter CaMV35S and exogenous terminator NOS genes are as shown in Table 1.

1.3 Analysis Conditions

DNA-500 on chip mode

1.4 Analysis Procedure

1.4.1 DNA Extraction and Purification in Sample
1.4.1.1 Grind about 1 g of plant tissues with liquid nitrogen, and transfer the powder to a 2 mL centrifuge tube.
1.4.1.2 Add 0.5 mL of extract A, mix, and put in a 65 °C water bath for 1 hour.
1.4.1.3 After bathing, add 1 mL of extract B: extract C=1:1 mixture to the tube, mix for 30 seconds, and then centrifuge at 12,000 rpm for 5 minutes.
1.4.1.4 Pipette the upper aqueous phase into a new 2 mL centrifuge tube, add double volume of the pre-cooled anhydrous alcohol, 10 % volume of settling agent 1 and 4 μL of settling agent 2, mix, and allow to settle at -20 °C for 1 hour.
1.4.1.5 After settlement, centrifuge at 12,000 rpm at 4 °C for 15 minutes, and pour away the supernatant carefully. At this point, white sediment can be seen at the bottom of the EP tube.
1.4.1.6 Add 1 mL of pre-cooled washing solution, flip the EP tube to mix, centrifuge at 12,000 rpm at 4 °C for 5 minutes, then discard the supernatant, and invert the EP tube onto a filter paper for drying.
1.4.1.7 Add 30 μL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 °C.
1.4.2 PCR reaction system
See Tables 1 and 2 for the PCR reaction reagents and conditions.

Table 2 PCR Reaction Reagents

	Consumption	Final concentration
SYBR^{O} Premix Ex Taq II (Tli RNaseH Plus) (2′)	10.0 mL	1′
PCR Forward Primer (10 mM)	0.8 mL	0.4 mM
PCR Reverse Primer (10 mM)	0.8 mL	0.4 mM
DNA template	2.0 mL	20 ng/mL
dH_2O (sterilized distilled water)	6.4 mL	
Total volume	20.0 mL	

Table 3 PCR Reaction Parameters

Action	Time/s	Temperature/°C
Activation and pre-denaturalization of DNA active enzyme	30	95
PCR (45 cycles)		
Denaturalization	30	95
Annealing	30	55
Extension	60	72
Post-cycle retention	180	72

1.4.3 MultiNA Determination

The PCR amplified products were subject to MultiNA determination. Based on the fragment size of the theoretical product, the 500 bp reagent kit was selected for determination in the experiment. In order to verify the accuracy of measurement, this paper also includes a negative control experiment, where the DNA template was not used.

■ Discussion of Results

Fig. 1 is the electropherogram of MultiNA measurement of the three groups of the soybean endogenous Lectin gene. The resultant fragments are amplified and clearly detected, and the fragment sizes are largely consistent with the expectation, indicating that the soybean genome has been extracted successfully. CaMV35S and NOS specific primers were used to amplify the genome of the three groups of soybean samples, and the results are as shown in Fig. 2 and Fig. 3. The results indicate that the expected CaMV35S 195 bp and NOS 180 bp bands were not detected, showing that all soybean samples were free from genetically modified CaMV35S and NOS.

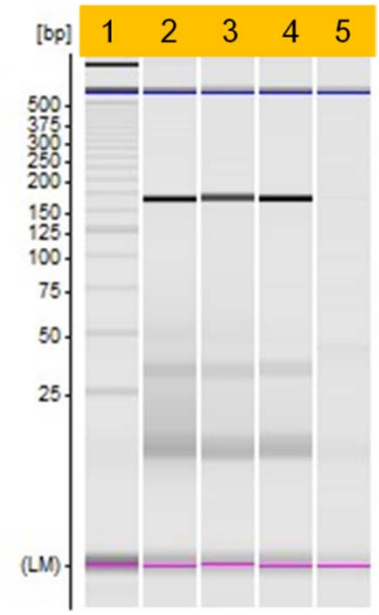

Fig. 1 Gel Determination Results of Soybean Endogenous Lectin
(1: Ladder, 2: 1# soybean sample, 3: 2# soybean sample, 4: 3# soybean sample, 5: negative control)

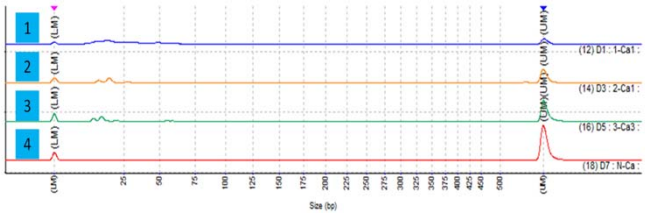

Fig. 2 Electrophoresis Determination Results of Soybean Exogenous Promoter CaMV35S
(1: 1# soybean sample, 2: 2# soybean sample, 3: 3# soybean sample, 4: negative control)

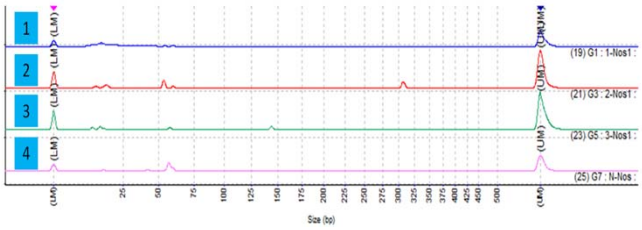

Fig. 3 Electrophoresis Determination Results of Soybean Exogenous Terminator NOS
(1: 1# soybean sample, 2: 2# soybean sample, 3: 3# soybean sample, 4: negative control)

Fig. 4 is the electropherogram of MultiNA determination of the endogenous Zein gene in the three groups of corn samples. Similar to the soybean samples, the endogenous gene is detected clearly, indicating the successful extraction of the corn genome. Fig. 5 and Fig. 6 are the electropherograms of MultiNA determination of the PCR amplified products using CaMV35S and NOS as the target genes for primer design. Fig. 5 shows that no CaMV35S band with an expected length of 195 bp is amplified from the three groups of corn samples. Fig. 6 shows that 173 bp and 174 bp bands are amplified from the 1# and 2# corn samples, close to the theoretical NOS band of 180 bp, indicating that these two groups of corn samples may contain genetically modified ingredients. The promoter CaMV35S is not detected in these two groups of corn samples, possibly because this genetically modified corn does not contain the CaMV35S promoter or the genome of the samples is damaged to varying degrees.

■ Conclusion

This paper establishes a method for the qualitative determination of genetically modified ingredients in crops using the Shimadzu MCE-202 MultiNA based on molecular biological technology. This method has high specificity and wide coverage for the determination of genetically modified ingredients, and can realize rapid transgene screening and determination, laying a good foundation for the subsequent identification of certain genes.

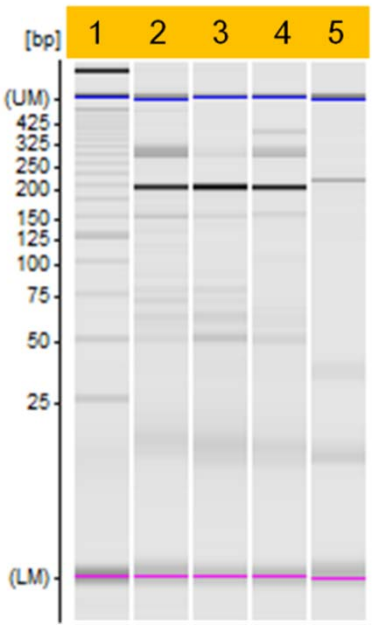

Fig. 4 Gel Determination Results of Corn Endogenous Zein
(1: Ladder, 2: 1# corn sample, 3: 2# corn sample, 4: 3# corn sample, 5: negative control)

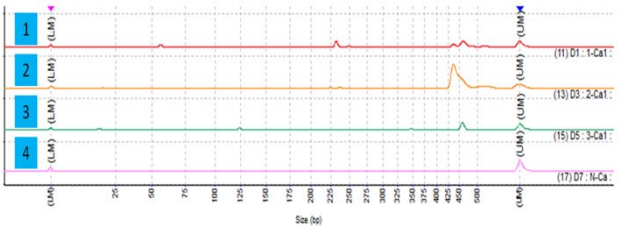

Fig. 5 Electrophoresis Determination Results of Corn Exogenous Promoter CaMV35S
(1: 1# corn sample, 2: 2# corn sample, 3: 3# corn sample, 4: negative control)

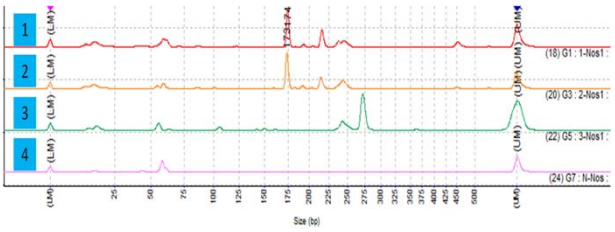

Fig. 6 Electrophoresis Determination Results of Corn Exogenous Terminator NOS
(1: 1# corn sample, 2: 2# corn sample, 3: 3# corn sample, 4: negative control)