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Application News

MultiNA

Qualitative Determination of Genetically Modified 356043 Soybean Using the MultiNA

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■ Abstract

A plant genome extraction reagent kit was used to extract the genome of the genetically modified soybean standard, using the soybean endogenous Lectin gene as an internal reference. Specific primers were designed for the 356043 transgene sequence for PCR amplification, and MultiNA determination of the size of the amplified product. The results indicate that the fragment length of the PCR amplified product is largely consistent with the theoretical length, indicating that the 356043 transgene is detected. This experiment indicates that the identification of the 356043 variety of genetically modified soybean can be realized using the MultiNA.

■ Introduction

In the past decade, an increasing number of genetically modified crops have been approved for global cultivation and commercialization. Soybean is a very important genetically modified crop. In 2007, the cultivation area of genetically modified soybean accounted for over half of the total soybean cultivation area. Currently, eight varieties of genetically modified soybean are approved by China for import as raw materials for processing, which are Roundup-resistant GTS40-3-2 soybean, herbicide-resistant A2704-12 soybean, herbicide-resistant MON89788 soybean, herbicide-resistant 356043 soybean, quality improved 305423 soybean, herbicide-resistant CV127 soybean, insect-resistant MON87701 soybean, and insect-resistant and herbicide-tolerant MON87701×MON89788 soybean. Genetically modified 356043 soybean contains isolated glyphosate acetyltransferase (GAT) gene fragments with an improved function to make the soybean herbicide-tolerant. This fragment originates from the Bacillus licheniformis bacteria and a modification of the original synthetic enzyme gene of soybean. This paper proposes the design of specific primers for the 356043 specific sequence of genetically modified soybean for the PCR amplification of samples and the MultiNA determination of the amplified product. If the expected 145 bp specific fragment is obtained from amplification, the genetically modified 356043 ingredient is determined as being contained. This experiment determined the genetically modified 356043 soybean standard. A 153 bp specific fragment was detected from the sample using the MultiNA, largely consistent with the expected fragment length, indicating that this experimental method can realize the qualitative determination of the genetically modified 356043 ingredient in soybean.

■ 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, P/N 292-27910-91)
Samples: European standard substance, soybean seed powder, with genetically modified 356043 soybean content of 10 % (ERM-BF425d)
Primers: According to the literature and the NCBI sequence, the primers designed for the soybean endogenous Lectin and 356043 transgene are as shown in Table 1.

1.3 Analysis Conditions

MultiNA Marker mixing mode: on chip mixing

Table 1 Information on Primers Designed for Soybean Endogenous Lectin, 356043 Transgene, and PCR Amplification

Gene detected	Primer sequence	Theoretical size of PCR product/bp	Amplified gene sequence*
Soybean endogenous Lectin	5′-cctcctcgggaaagttacaa-3′	162	gtgacctcctcgggaaagttacaactcaataaggttgacgaaaacggcac >>>>>>>>>>>>>>>>>> cccaaaaccctcgtctcttggtcgcgccctctactccacccccatccacatttgggac aaagaaaccggtagcgttgccagcttcgccgcttccttcaacttca <<<<<<< ccttctatgcccctgacacaaaaaggc <<<<<<<<<<<
	5′-gggcatagaaggtgaagtt-3′		
356043 transgene	5′-cttttgcccgaggtcgttag-3′	145	cttttgcccgaggtcgttaggtcgaataggctaggtttacgaaaaagagact >>>>>>>>>>>>>>>>>> aaggccgctctagagatccgtcaacatggtggagcacgacactctcgtctactcca agaatatcaaagatacagtctcagaagaccaaagggc <<<<<<<<<<<<<<<<<<<<
	5′-gccctttggtcttctgagactg-3′		

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

1.4 DNA Extraction and Purification in Sample

1.4.1 Grind about 0.5 g of plant tissues with liquid nitrogen, and transfer the powder to a 2 mL centrifuge tube.
1.4.2 Add 0.5 mL of extract A, mix, and put in a 65 °C water bath for 1 hour.
1.4.3 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.
1.4.4 Pipette the upper aqueous phase to 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.
1.4.5 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.
1.4.6 Add 1 mL of the pre-cooled washing solution, flip the EP tube to mix, centrifuge at 12,000 rpm at 4 °C for 5 minutes, discard the supernatant, and invert the EP tube onto a filter paper for drying.
1.4.7 Add 30 μL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 °C.

1.5 PCR Reaction System

See Tables 2 and 3 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

1.6 MultiNA Determination

The PCR amplified products were subject to MultiNA determination. Based on the theoretical size of the fragment, 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 and Fig. 2 are the gel diagram and electropherogram of MultiNA determination of genetically modified 356043 soybean, respectively. The experimental results indicate that the fragment length of the soybean endogenous Lectin gene was 166 bp, largely consistent with the theoretical fragment length of 162 bp, indicating that the soybean genome has been extracted successfully and the PCR process has been implemented successfully. For the 356043 transgene, the results indicate that a 153 bp gene fragment was detected, largely consistent with the theoretical fragment length of 145 bp, indicating that the 356043 transgene has been detected successfully. No relevant fragment was detected in the negative control experiment, indicating no false positive detection.

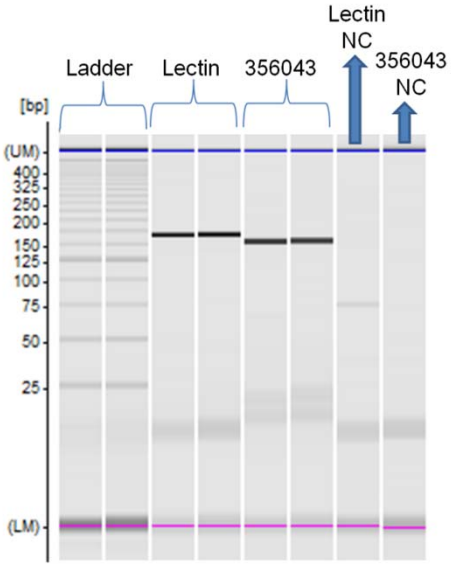

Fig. 1 Gel Diagram of MultiNA Determination
of Genetically Modified 356043 Soybean (NC: negative control)

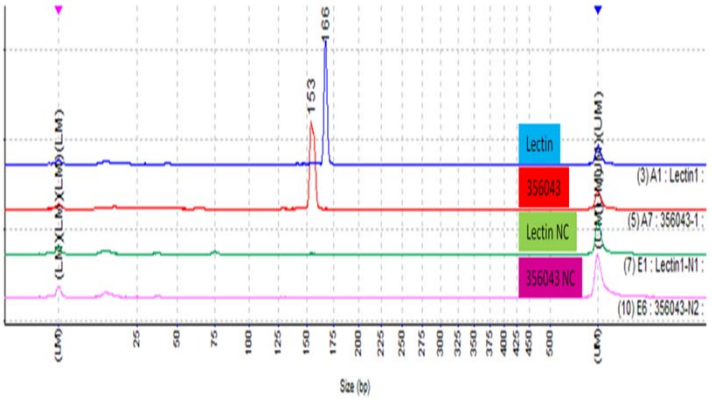

Fig. 2 Electropherogram of MultiNA Determination
of Genetically Modified 356043 Soybean (NC: negative control)

■ Conclusion

This paper establishes a method for the qualitative determination of genetically modified 356043 soybean using the Shimadzu MCE-202 MultiNA based on molecular biological technology. This method is sensitive, easy to operate and accurate for the determination of the 356043 variety of genetically modified soybean.

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