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Application News

MultiNA

Qualitative Determination of Genetically Modified Bt-11 Corn Using the MultiNA

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■ Abstract

A plant genome extraction reagent kit was used to extract the genome of the genetically modified Bt-11 corn standard, using the corn endogenous Zein gene as an internal reference. Specific primers were designed for the Bt-11 transgene sequence for PCR amplification, and the Shimadzu MultiNA microchip electrophoresis system was used to determine the amplified product. The results indicate that the fragment length of the amplified product is largely consistent with the theoretical length, indicating that the Bt-11 transgene is contained. This experiment indicates that the qualitative determination of Bt-11 in genetically modified corn can be realized using the MultiNA.

■ Introduction

Currently, China imports corn mainly from the U.S. and Argentina, the largest corn cultivating and exporting countries in the world. Most (over 80 %) of the corn in these two countries is genetically modified, and there are at least 35 varieties, or nearly 50 including multi-gene compound varieties. However, only 15 varieties of genetically modified corn are permitted for import to China, which are Roundup-resistant NK603 corn, insect-resistant and herbicide-tolerant MON88017 corn, insect-resistant MON89034 corn, insect-resistant MIR604 corn, herbicide-resistant GA21 corn, insect-resistant and herbicide-tolerant Bt-11×GA21 corn, herbicide-resistant T25 corn, insect-resistant MON810 corn, insect-resistant MON863 corn, insect-resistant TC1507 corn, insect-resistant 59122 corn, insect-resistant Bt-11 corn, insect-resistant Bt-176 corn, drought-tolerant MON87460 corn, and an improved variety 3272. Genetically modified Bt-11 corn is an insect-resistant and herbicide-tolerant variety, where the insect-resistant Cry1Ab gene derived from the Bt bacteria is inserted, and the phosphinothricin acetyltransferase (PAT) gene is inserted to resist the herbicide phosphinothricin.

This paper proposes the design of specific primers for the Bt-11 specific sequence of genetically modified corn for the PCR amplification of samples and the MultiNA determination of the amplified product. If the expected 207 bp DNA fragment is obtained from amplification, the genetically modified Bt-11 ingredient is judged as being contained. This experiment determined the genetically modified Bt-11 corn standard. A 209 bp specific fragment was detected using the MultiNA, largely consistent with the expected fragment length, indicating that this experimental method can realize the qualitative determination of the genetically modified Bt-11 ingredient in corn.

■ 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, corn powder, with genetically modified Bt-11 corn content of 4.89 % (ERM-BF412f)
Primers: corn endogenous Zein
The primer design for the Bt-11 transgene is as shown in Table 1.

Table 1 Information on Primers Designed for Corn Endogenous Zein Bt-11 Transgene, and PCR Amplification

Gene detected	Primer sequence	Size of theoretical PCR product/bp
Corn endogenous Zein	5'-tgaacccatgcatgcagt-3' 5'-ggcaagaccattggtga-3'	190
Bt-11 transgene	5'-tatcatcgacttccatgacca-3' 5'-agccagttaccttcggaaaa-3'	207

■ 1.3 Analysis Conditions

MultiNA Marker mixing mode: on-chip mixing

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 ℃ 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 into a new 2 mL centrifuge tube, add double volume of pre-cooled anhydrous alcohol, 10 % volume of settling agent 1 and 4 mL of settling agent 2, mix, and allow to settle at -20 ℃ for 1 hour.

1.4.5 After settlement, centrifuge at 12,000 rpm at 4 ℃ 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 ℃ for 5 minutes, discard the supernatant, and invert the EP tube onto a filter paper for drying.
1.4.7 Add 30 mL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 ℃.

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 Bt-11 corn, respectively. The experimental results indicate that the fragment length of the corn endogenous Zein gene was 196 bp, largely consistent with the theoretical fragment length of 190 bp, indicating that the genome has been extracted successfully and the PCR process has been implemented successfully. For the Bt-11 transgene, the results indicate that a 209 bp gene fragment has been detected, largely consistent with the expected 207 bp, indicating that the Bt-11 genetically modified ingredient has been detected successfully. No relevant fragment was detected in the negative control experiment, indicating no false positive detection.

■ Conclusion

This paper establishes a method for the qualitative determination of genetically modified Bt-11 corn 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 Bt-11 variety of genetically modified corn, and may be used as a reference for testing agencies.

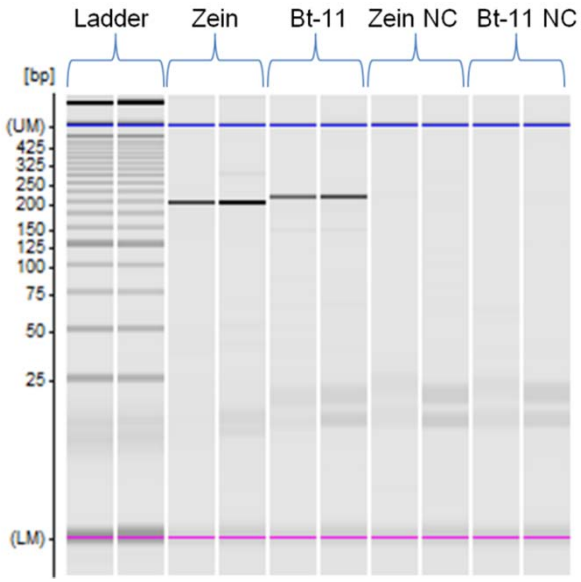

Fig. 1 Gel Diagram of MultiNA Determination of Genetically Modified Bt-11 Corn (NC: negative control)

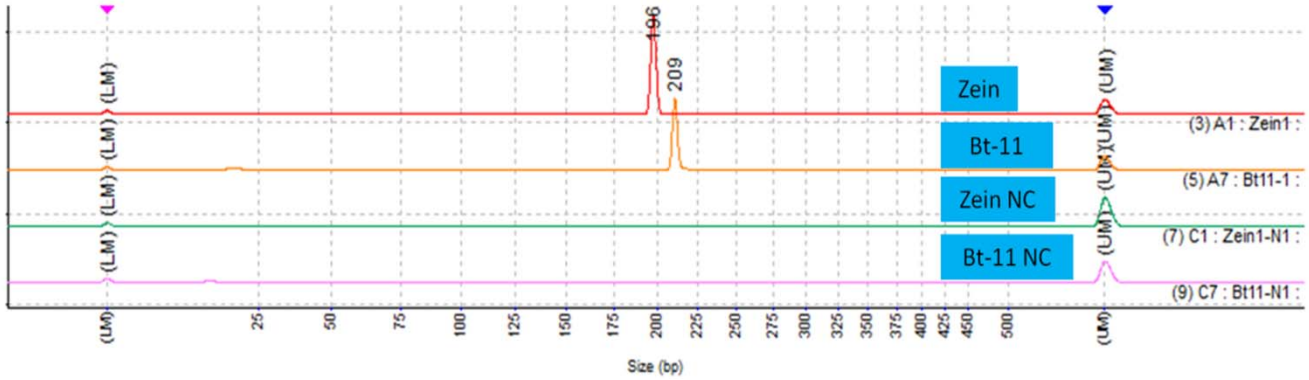

Fig. 2 Electropherogram of MultiNA Determination of Genetically Modified Bt-11 Corn (NC: negative control)

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