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Original Research

Development of dual priming oligonucleotide-polymerase chain reaction (DPO-PCR) for detection of wheat component in foods

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Abstract: This research is aimed to establish a DPO-PCR method for rapid detection of wheat component in foods. A pair of highly specific DPO primers was designed using the wheat housekeeping gene GAG56D as the target gene. By optimizing the PCR reaction system, the DPO-PCR detection method for wheat component in food was established, and the specificity and sensitivity of the method were determined. The established DPO-PCR method was highly specific, and there was no cross-reactivity to 22 control samples. The sensitivity was high, and the minimum detection limit was 1 ng/uL. Tests on commercially available product samples showed that the method can effectively detect wheat component of foods. The DPO-PCR method established in this study is simple and accurate, and provides an effective detection method for wheat component in foods.

Key words: Dual priming oligonucleotide polymerase chain reaction; Wheat component; Foods.

Introduction

Food allergy refers to the adverse immune response caused by certain foods in some people with allergies (1, 2). The specific symptoms of allergy include acute gastroenteritis, skin allergies, and respiratory allergies. In severe cases, life-threatening anaphylactic shock may occur. A study in the United States in 2007 showed that 3.9 % of 18-year-olds reported food allergies. The prevalence of food allergy reported between 1997 and 2007 increased by 18% (3). Allergens in foods cause abnormalities in the immune system, and have become an important food safety issue. There are eight main categories of food allergens regulated by the Codex Alimentarius Commission: cereals containing gluten such as wheat, rye, barley and oat; crustaceans and their products such as crabs and shrimps; eggs and egg products; fish and fish products; peanuts, soybeans and their products; milk and dairy products (including lactose); nuts and their products such as almonds, cashew and walnuts; and sulfites at levels higher than or equal to 10 mg/kg (4). Wheat is one of the most common food allergens (5). Thus, wheat allergy is a food safety issue that cannot be ignored. Wheat allergies cause infant eczema, urticaria, dermatitis, rhinitis, angioedema, asthma, and gastrointestinal symptoms (6, 7). Therefore, it is essential to develop wheat allergen detection technology, and create low allergenic wheat foods. It is also necessary to ensure the safety of people who are allergic to wheat. At present, the methods used for detection of allergens in foods involve methods for detection of allergen proteins and molecular biological detection methods based on allergen DNA (8). Traditional protein-based detection methods include electrophoresis, chromatography, enzyme-linked immunosorbent assay (ELISA), and immunosensors. The specificity of DNA-based detection is higher than that of protein. Therefore, detection methods using DNA as a test object are increasingly applied for the detection of food allergens. Compared to proteins, PCR detection technology is specific, and sensitive to the detection of specific allergens in food. At present, PCR detection techniques for established food allergens include PCR (9, 10); real-time PCR (11-13), and loop-mediated isothermal amplification techniques (14, 15).

The dual-start oligonucleotide primer contains two independent specific primer regions and an oligomeric hypoxanthine base bridging structure. The South Korea Seegene Company proposed a polymerase reaction primer design method for the first time in 2007 (16). This special structure ensures that it is difficult to form a secondary structure within the primer itself, thereby ensuring the specificity of amplification and improving the amplification efficiency. This study established a DPO-PCR method for the rapid determination of wheat allergens in foods.

Materials and Methods

Materials

A total of 22 plant and animal foods such as wheat, almonds, walnuts, pecans, peanuts, cashews, almonds, pistachios, sunflower seeds, sesame seeds, pork, beef, fish, soybeans, rice, oats, rye, and rice were purchased from local supermarkets for analysis of the specificity of wheat components. More than ten types of processed

products containing, or possibly containing wheat, such as ten brands of bread, cakes, noodles and flour were used as test materials for this experiment, so as to test the feasibility of the analytical method.

Instruments and reagents

The reagents and kits used were RNase (5 U/μL), PCR Master Mix (Tiangen Biochemical Technology Co., Ltd.); NaCL, Tris, EDTA, CTAB, chloroform and isopropanol (commercially available and analytically pure); tissue/cell genomic DNA rapid extraction kit, and a broad-spectrum plant genomic DNA rapid extraction kit (Bomed Biotechnology Co., Ltd).

The major instruments used were Grinder IKA A11 (Germany), Eppendorf 5810R high speed refrigerated centrifuge (Germany), Millipore Simfilter ultrapure water meter (USA), SANYO mov212F constant temperature drying oven (Japan), Applied Biosysterms 9700 PCR instrument (USA), Tianneng EPS200 electrophoresis instrument (Shanghai), Biotop FluorShot condensing imaging system (Suzhou), and 12-capillary Fragment AnalyzerTM system (Advanced Analytical, USA).

Methods

Preparation of samples

Fresh plant tissue (100 mg) or 20 mg of dry plant tissue, and animal tissues (about 500 mg) were thoroughly milled and mixed with liquid nitrogen in a mortar.

Template DNA Extraction

The DNA extraction was carried out using the CTAB method (17) .

Primer design

The wheat endogenous gene GAG56D was selected as the detection target gene through literature search. The PCR primers were designed using VectorNTI10.0 software, and the DPO primers were designed based on this (table1).

Optimization DPO-PCR amplification system

The PCR reaction system is shown in Table 2.

The PCR conditions were: pre-denaturation at 96 ° C for 3 min; denaturation at 96 ° C for 20 sec; annealing at 58 ° C for 29 sec, extended for 20 sec at 72 ° C. After 35 cycles, the reaction was extended at 72 ° C for 1 min, and was stopped at 4 ° C. The amplified product was detected using gel electrophoresis. The gel was stained with ethidium bromide and photographed under ultraviolet light.

Specificity test

The DNA samples from the 22 animal and plant materials were tested with DPO-PCR and verified for specificity.

Sensitivity test

Ultraviolet spectrophotometer was used to determine the concentration of extracted wheat DNA. The concentration of extracted wheat DNA template was about 760 ng/ul. The wheat DNA template was serially diluted to obtain concentration gradient of 200 and 100 ng/ul. The serially diluted DNA template was subjected to DPO-PCR amplification at 50, 20, 10, 1 and 0.1 ng/ul, and the sensitivity was tested. The reaction system was the same as depicted in Table 2. The amplification products were analyzed with capillary electrophoresis using a 12-capillary Fragment AnalyzerTM systerm from Advanced Analytical (USA).

Screening and analysis of commercial samples

The DPO-PCR was performed on commercially available processed products, and the test results were analyzed to verify the feasibility of the method.

Results

Establishment of DPO-PCR detection method for wheat components

The DPO primer was designed with the wheat

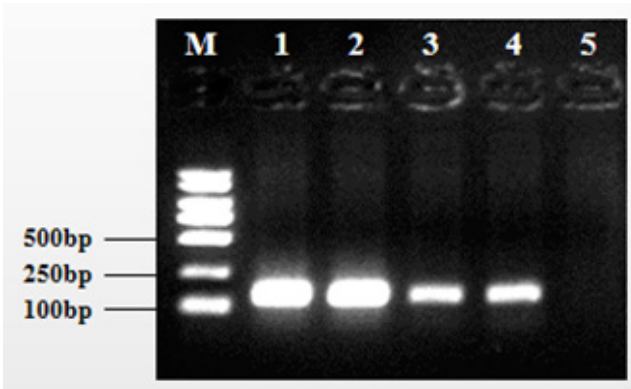

Figure 1. Establishment of DPO-PCR detection method for wheat**.** Notes: M: D L2000 DNA Marker; 1-2: positive results of common PCR; 3-4: positive results of DPO-PCR; 5: negative control.

Table 2. DPO-PCR amplification system.

Reaction system	Volume/amount
dNTP(10mM)	1uL
10×Buffer	5uL
Forward primer (10 um)	1uL
Reverse primer (10 um)	1uL
Genome DNA	50ng
EVO/GPV8	1uL
ddH_2O	-
Total volume	50uL

Table 1. Primer sequences.

Type	Name	Primer sequence
Common primer	Forward	CCGTTCATTCAGCCATCTCTCGACTTACCAGGTGAAC
	Reverse	CCGCATCACTTGGCAATCGCTAACTTGTGATCATTGA
Primer DPO	Forward	CCGTTCATTCAGCCATCTCTCGACTTACIIIIICAGGTGAAC
	Reverse	CCGCATCACTTGGCAATCGCTAACTTGTIIIIIGATCATTGA

endogenous gene GAG56D as the target gene. The DPO-PCR method was established and the reaction system was optimized. The specific electrophoresis band was detected with agarose gel electrophoresis at about 152 bp (Figure 1).

DPO-PCR specific analysis

The DNAs of the extracted animal and plant materials were detected using the established DPO-PCR method. The results showed that only wheat was positive for DPO-PCR, while the others were negative (Figure 2).

DPO-PCR sensitivity analysis

The extracted wheat DNA was subjected to serial dilutions to yield concentrations of 200, 100, 50, 20, 10, 1 and 0.1 ng/ul, using established DPO-PCR. The method effectively amplified the target gene at a DNA concentration of 1 ng/ul (Figure 3), indicating that the sensitivity of the established DPO-PCR detection method was 1 ng/ul.

Commercial sample screening and analysis

Commercially available deep-processed foods were tested using the established DPO-PCR detection method. The results were consistent with product identification (Figure 4), indicating that the method has a good practical application.

Discussion

Food safety issues are one of the important issues in public health. Traditional protein-based allergen detection methods lead to false-negative results due to protein variability and degradation during food production and processing. This has made nucleic acid-dependent detection methods, especially PCR technology, a more acceptable method in current food safety testing. A variety of allergen detection techniques have been established, each of which has its own advantages. In the field of nucleic acid detection of allergens, new techniques and new methods are continuously applied to detect allergen components in nucleic acids. Enrichment and development are of great significance.

The dual-priming oligonucleotide primer technology effectively blocks non-specific amplification and improves amplification efficiency. At present, the technology is widely used in pathogenic microorganisms and clinical tests. For example, Chung *et al.* used DPO-PCR technology to diagnose 170 patients with peptic ulcer bleeding (18). The results indicated that DPO-PCR technology has certain auxiliary diagnostic significance for the re-examination of samples with negative urease test. A multiplex PCR technique based on DPO primers has been established for detecting and distinguishing between *Mycobacterium tuberculosis* and *Mycobacterium bovis* (19). In addition, a multiplex PCR technique based on double-primed oligonucleotide primers has been developed for the detection of six sexually transmitted disease pathogens, including *Chlamydia trachomatis* (20). Four diarrhea-producing *E. coli* in foods can now be identified using a multiplex PCR technique based on DPO technology (21).

The present study has established a DPO-PCR detection method for wheat components in foods. The results show that the test method has good specificity and stability, and the sensitivity reaches 1 ng. The whole detection process is fast, high in efficiency and easy to operate. Combined with rapid DNA extraction technology, it has a wide application prospect. In subsequent studies, the detection system will be further optimized, and the number and types of test samples will be expanded. It is hoped that new ideas and methods for food allergen testing will be provided using more advanced molecular biology testing techniques.

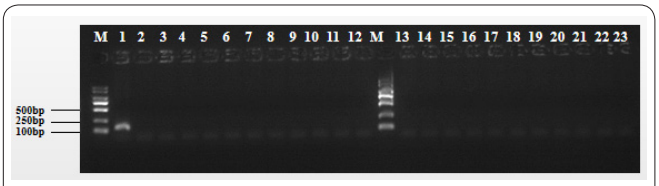

Figure 2. Specificity of wheat DPO-PCR detection method. Notes: M: D L2000 DNA Marker; 1: wheat; 2-22: other species; 23: negative control.

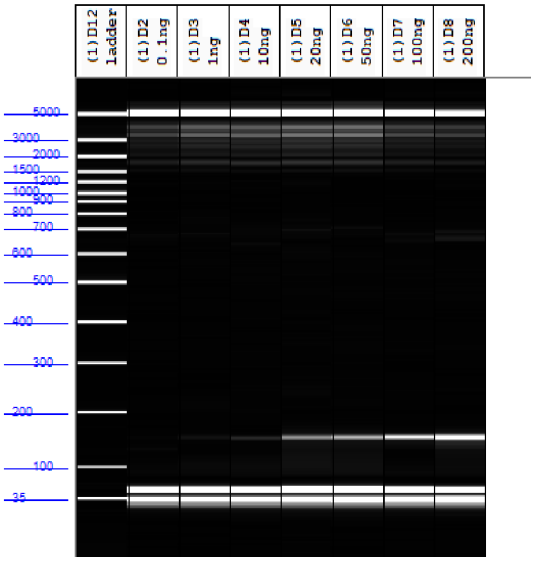

Figure 3. Sensitivity of wheat DPO-PCR detection method.

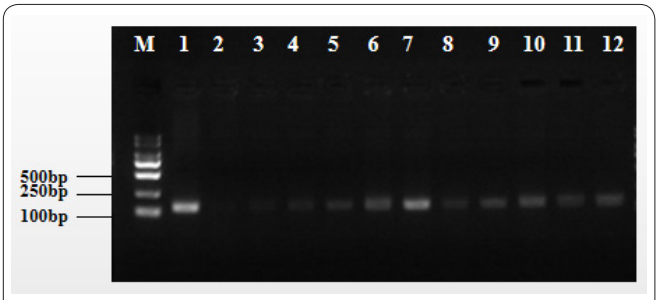

Figure 4. Results of application of DPO-PCR detection method on commercial samples.

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Conflict of Interest

There are no conflict of interest in this study.

Author's contribution

All work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Rong Li. Yan Su, Rong Li collected and analyzed the data. Yan Su wrote the manuscript. All authors read and approved the manuscript prior to publication.

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