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PZR WITH THE HELP OF MARKERS IN THE COTTON PLANT DNA

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ABSTRACT

This article provides information on the mechanism of polymer chain reaction in plant DNK using markers. In addition, we provide detailed information on the sequence of PZR analysis using DNK samples and markers, and the examination of DNK samples by electrophoresis.

KEYWORDS: *PZR, DNA, Amplification, Plant, Genotype, Agarose, Electrophoresis, Particle, Charge, Microsatellite.*

INTRODUCTION

Mechanism of polymerase chain reaction

The polymerase chain reaction is a method used to increase the amount of DNK samples and test them with markers, and this method is now widely used around the world.

The polymerase chain reaction is based on the denaturation and polymerization of DNK, in which the main stages of PCR are carried out by raising and lowering the temperature.

The advantage of PZR is that the equipment required to perform PZR analysis is relatively inexpensive and requires very little space [1].

Polymerase chain reaction

The polymerase chain reaction is performed after the genome DNK isolated from each sample has been examined in 0.9% agarose gel by electrophoresis. SSR markers were selected from the library of microsatellite markers for genotyping. SSR primer pairs are obtained from International DNK Technologies (IDT, USA).

The PZR reaction (Hot-start program) was performed in a volume of 10 µl in the following order.

10 x PZR buffer (magnesium chloride)	1 mkl
BZA	0.2 mkl

dNTP mixture 25 mM (dATP, dGTP, dTTP, and dCTP)	0.1 mkl
Properly directed primer	0.5 mkl
Reverse primer	0.5 mkl
TAQ polymerase -5 birl./mkl	0.2 mkl
Genome DNK	1 mkl
Distilled water	6.5 mkl

We perform amplification in a 45-cycle Hot-start program at the following temperature modes;

The first denaturation	95 °C	3 minutes
Denaturation	94 °C	1 minutes
Melting point of primers	±50 °C	1 minutes
Elongation	72°C	2 minutes
Closing elongation	72°C	5 minutes

I carry verification of the polymorphism of microsatellite amplification of the finished PCR product out in a 3.5% Hi-Res agarose gel at a voltage of 0.5 x TVE under a voltage of 6 v / cm in 0.5 x TVE buffer using the above electrophoresis method. Inc., USA).

Electrophoresis process.

Biologically large molecules are proteins, nucleic acids, and polysaccharides as liquid particles that correspond in shape to colloidal particles. They have a certain electric charge because they have groups that have electrolytic decomposition properties. As nucleic acids, their charges are because of the dissociation of phosphorus groups. Therefore, DNK is negatively charged in a neutral and alkaline environment.

The interaction of charged particles with the cathode or anode under the action of an electric field depends on the sign of the total charged particles (+, -). This phenomenon is called electrophoresis. Electrophoresis motion occurs because of the influence of the electric field on the velocity of particles (cm / s), 1v / cm. Its size is 2 cm c-1 v-1, and the sign a corresponds to the sign of the total charged particles. The difference in the motion of the particles serves to separate the compounds for analytical or preparative purposes. Electrophoresis motion detection is also used to determine the characteristics of substances.

During electrophoresis, the velocity of the particles being analyzed is determined by observing the movement of the dye.

Electrophoresis devices of different types should be placed between 2 electronic containers and the backing device (paper, starch, agarose and acrylamide gel) as far as possible between the two containers. Plate wires are usually used as electrodes [2].

PZR analysis using DNK samples and markers

We performed PZR experiments with DNK samples isolated from Namangan-77 and Tashkent-6 varieties and 10 primers with SSR markers.

In the control variant, DNK samples of Namangan-77 and Tashkent-6 varieties and 10 primers were amplified in a separate PZR amplifier for 45 cycles in the following PCR temperature scheme [3].

The first denaturation	95 °C	3 minutes
Denaturation	94 °C	1 minutes
Melting point of primers		
BNL-1721	53 °C	1 minutes
BNL-2646	55 °C	1 minutes
JESPR-232	58 °C	1 minutes
JESPR-252	56 °C	1 minutes
NAU-1042	60 °C	1 minutes
NAU-2119	56 °C	1 minutes
NAU-2679	58 °C	1 minutes
NAU-2687	59 °C	1 minutes
NAU-3519	55 °C	1 minutes
TMB-1660	57 °C	1 minutes
Elongation	72°C	2minutes
Closing elongation	72°C	5minutes

In this process, over 3 hours was spent on PZR with 1 primer for 2 cotton varieties.
In the experimental variant, DNK samples of Namangan-77 and Tashkent-6 varieties and 10 primers were performed in the following PZR temperature scheme.

The first denaturation	95 °C	3 minutes
Denaturation	94 °C	1 minutes
Melting point of primers	62 °C	
	20 cycle	50 second
	55 °C	
	15 cycle	50 second
Elongation	72°C	2 minutes
Closing elongation	72°C	5 minutes

In this process, they spent less than 3 hours on PZR with 10 primers for 2 cotton varieties.

Examination of DNK samples by electrophoresis.

DNK electrophoresis in agarose gel is a standard method used for the purification and identification of DNK fragments.

I widely used the method of electrophoresis of DNK on horizontal agarose gel plates in molecular genetics and biochemistry. Because the reagents of this method are easy to find, the simplicity of the method and the low cost of the equipment are because they can get very little information from tiny amounts of untreated material.

Therefore, in our experiments, we used the method of electrophoresis on horizontal agarose gel plates [2].

The product of PZR amplification was isolated from the plants and primed by electrophoresis in 3.5% agarose gel. We stained the electrophoresis gel using Etidium Bromide and photographed it on UV Tran illuminator (Innotech Inc., USA). When PCR amplification was performed using a modified scheme with a standard scheme, it was found that successful DNA amplification occurred in both schemes and there was no difference between them.

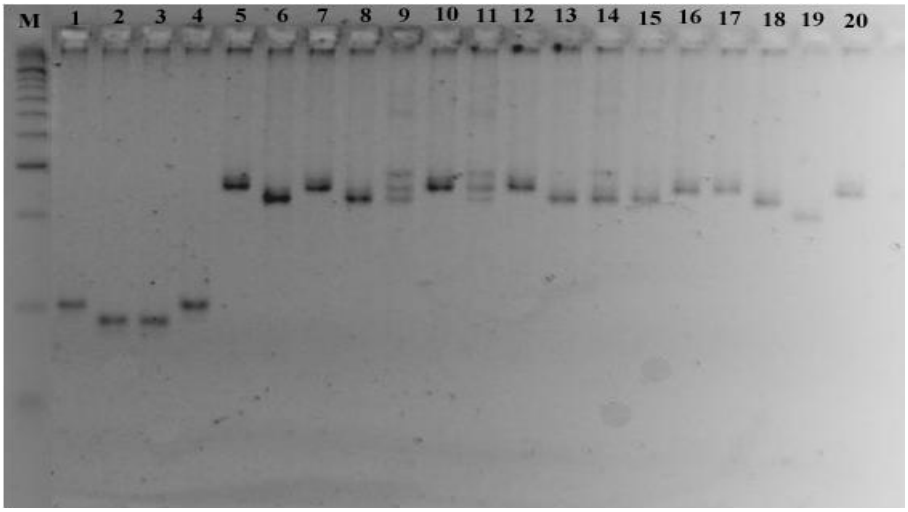

Image of electrophoresis gel of PZR product formed from the modified scheme of PZR amplification.

Optimization of PZR in plant DNK DNK markers, their types, mechanism of polymerase chain reaction, method of its implementation and methods of testing DNK samples and PZR products by agarose gelelectrophoresis play an important role in the study of modern molecular genetics.

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