

Full Length Research Paper

A new DNA band display technology of microsatellite DNA

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This study proposes a new DNA band display technology of microsatellite DNA called Fluorescent Imaging Technology. In comparison with Silver Stain Technology, this technology is worth popularizing in the laboratory because of its high resolution, efficiency, simplicity and clear background.

Key words: Microsatellite DNA, polyacrylamide gel electrophoresis, fluorescent imaging technology, silver staining technology.

INTRODUCTION

Microsatellite DNA comprises nucleotide sequences that are made up of 1 to 6 tandem and repeatable bases, widely distributed in genome. It is applied successfully in species genetic diversity (Maguire et al., 2002), genetic mapping (Piquemal et al., 2005; Song et al., 2004), pedigree analysis (Kota et al., 2001; Dreisigacker et al., 2004), genetics and breeding (Susan et al., 2004; Ruiz et al., 2004), etc. It has advantages such as codominant, good repeatability, high polymorphism, stable amplification results and simple detection. Its bands identification usually adopts polyacrylamide gel electrophoresis with Silver Staining Technology. The steps are trivial, time consuming, background fuzzy and unclear strip, and they always cause faulty DNA bands.

Many researchers aiming at these problems only improve but not solve them fundamentally. This study proposed a new DNA band display technology called Fluorescent Imaging Technology, and tried to solve the problems from a new dimension.

MATERIALS AND METHODS

Plant materials and DNA isolation

A total of 34 *Armeniaca Scop.* experimental materials were used in this study, including *Armeniaca cathayana*, *A. sibirica* (L.) Dam. and *A.*

vulgaris Lam. which were collected from Luoyang in Henan province and Zhangjiakou in Hebei province.

Total genomic DNA was extracted using the CTAB procedure (Doyle and Doyle, 1990) with minor modifications. The DNA concentration was measured using a spectrophotometer and checked on 1% (w/v) TAE agarose gels.

PCR amplification

Extracted genomic DNA was PCR-amplified using SSR primers UDP98-405, UDP98-406, Aprigms18 and BPPCT030. PCR reactions were performed in a 20 μ L volume containing 10 \times PCR Buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs Mix, 1.5 unit *Taq* DNA Polymerase, 0.25 μ M for each primer, and 50 ng genomic DNA. The cycling parameters were: one cycle of 94°C for 5 min, 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 90 s, followed by 72°C for 10 min. PCR reactions were carried out in a 96-well block Bio-Rad C1000™ Thermal Cycler.

PCR products electrophoresis

Fluorescent Imaging Technology

PCR products were separated using 8% polyacrylamide gel electrophoresis in 1 \times TBE buffer, whose film size was 195 mm (long) x 120 mm (wide) x 1 mm (thick). Subsequently, the PCR products (20 μ L) were divided into two products.

The procedures of Fluorescent Imaging Technology are as follows: one product was mixed with 2 μ L 6 \times loading buffer containing GelRed/GelGreen dye in a PCR tube, after which 3 μ L of it was sampled and electrophoresed for 3 h at 120v constant voltage. After washing the double-sided glasses, the gels were peeled rinsed and placed in an ultraviolet analysis device (background,

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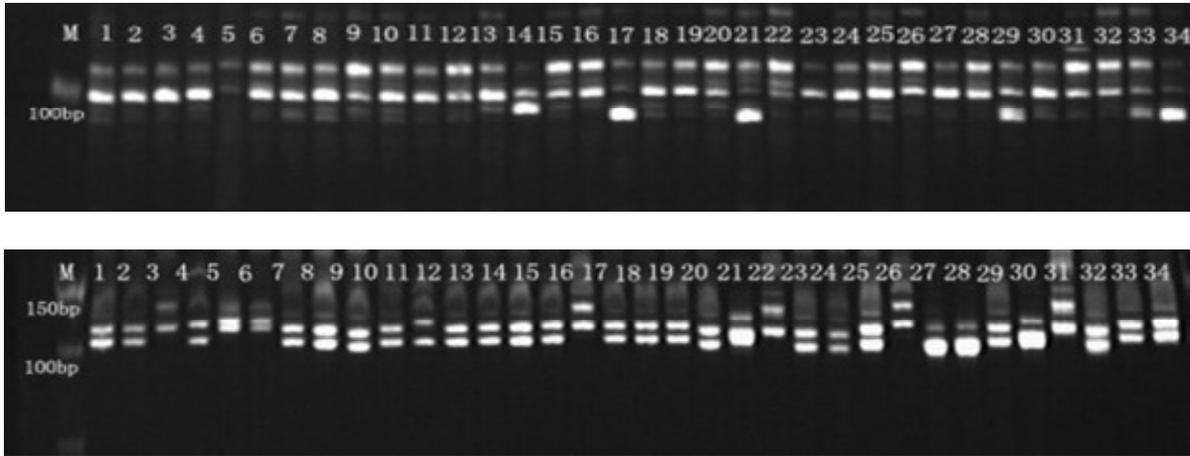


Figure 1. Results of Fluorescent Imaging Technology using primers UDP 98-405 (up) and BPPCT030 (down) with GelRed dye.

with glass board) for photo analysis.

The principle of Fluorescent Imaging Technology was that GelRed/GelGreen dye was combined with DNA/RNA in a covalent bond, following issuance of bright orange fluorescence under ultraviolet ray and displaying DNA bands.

Silver staining technology

The procedures of Silver Staining Technology are as follows: the other product was mixed with 2 μ L 6 \times loading buffer in a PCR tube, after which 3 μ L of it was sampled and electrophoresed for 3h at 120v constant voltage. After washing the double-sided glasses, the gels were peeled and taken into fix fluid (0.5% CH₃COOH, 10% C₂H₆O) for 20 min. It was washed twice, and then placed in penetration liquid (0.15% AgNO₃) for 15 min. Finally, it was washed twice again and then left in the developing solution (1.5% NaOH, 1% HCHO) until the DNA strips appeared clearly. After washing the gels, they were placed in an ultraviolet analysis device immediately (background with white board) for photo analysis.

The principle of Silver Staining Technology was that nucleotide combined with Ag⁺ and Ag⁺ formed reductase under alkaline environment, which enabled the display of DNA bands.

The effect of fluorescent imaging technology analysis

Except for the advantage of Fluorescent Imaging Technology which shows that its steps are simple, it cannot be stated that it has other advantages such as visible bands, high resolution and clear background. When compared with the effect of Silver Staining Technology, it is observed that it has more advantages.

RESULTS

Fluorescent imaging technology analysis

The results of Fluorescent Imaging Technology are shown in Figures 1 (GelRed dye) and 2 (GelGreen dye). In these figures, we can see that the background of the DNA bands is clear, its resolution is high and the specific bands are

identified easily. The differences between varieties could be separated effectively by the Fingerprint Faint nonspecific bands which display completely that the specificity bands are obviously clear. The most important of the Fluorescent Imaging Technology was that its operating steps were so simple that they did not cause hyperchromatic or coloring failure phenomenon.

Comparison of silver staining technology analysis with fluorescent imaging technology analysis

The results of Silver Staining Technology are shown in Figures 3 (GelRed dye) and 4 (GelGreen dye). The results of Fluorescent Imaging Technology were completely consistent with those of Silver Staining Technology, though they were higher than those of Silver Staining Technology in background, resolution and specific bands. Because silver staining steps were trivial and it was difficult to control, fix, penetrate and develop time, some species presented hyperchromatic and background fuzzy phenomena such as: varieties 1 and 5 fingerprints using primer UDP98-405; varieties 32, 33 and 34 fingerprints using primer BPPCT030; varieties 18 and 19 fingerprints using primer UDP98-406; and varieties 11, 12, 15 and 16 fingerprints using primer Aprigms18. For some species, it appeared that the phenomena of the distinction between specific and nonspecific bands were not obvious, such as varieties 22 and 29 fingerprints using primer UDP98-405; varieties 31 fingerprints using primer BPPCT030; varieties 9 and 26 fingerprints using primer UDP98-406; and varieties 11, 12, 15 and 16 fingerprints using primer Aprigms18.

DISCUSSION

In recent years, a lot of work have been done on Silver

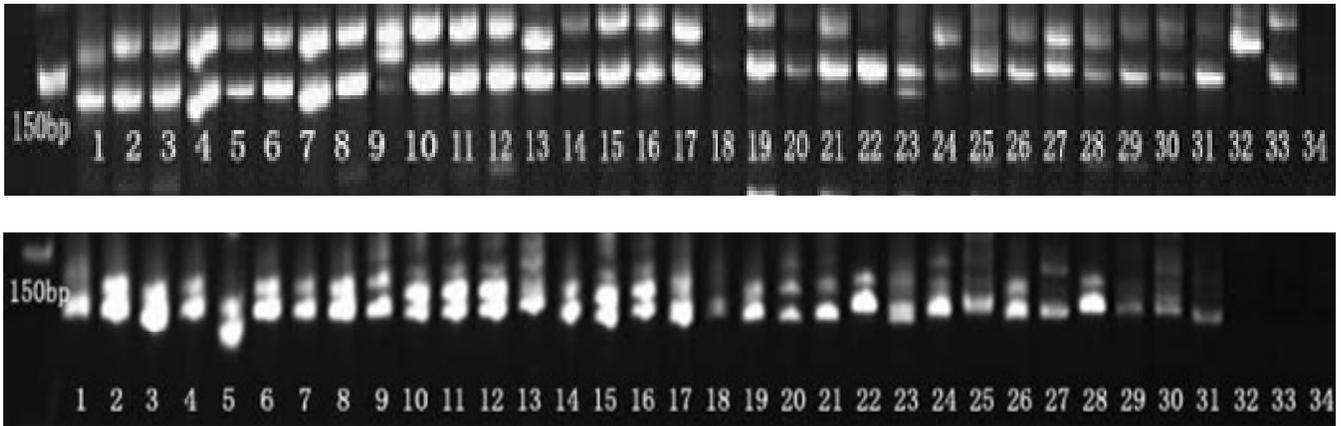


Figure 2. Results of Fluorescent Imaging Technology using primers UDP 98-406 (up) and Aprigms18 (down) with GelGreen dye.

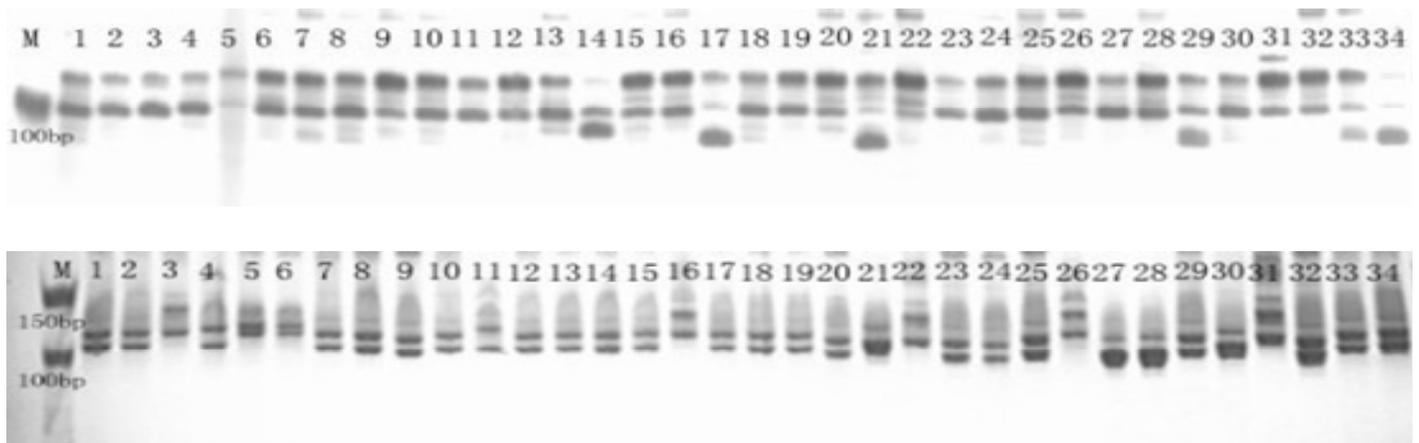


Figure 3. Results of Silver Staining Technology using primers UDP 98-405 (up) and BPPCT030 (down) with GelRed dye.



Figure 4. Results of Silver Staining Technology using primers Aprigms18 (down) with GelGreen dye.

Staining Technology, such as comparing the effect of Silver Staining Technology of Bassam and Sanguinett (Guan et al., 2006), studying how to reduce the Silver Staining Technology time (Liang et al., 2008), optimizing the type and concentration of polyacrylamide gel electrophoresis (Du and Qiu., 2008), and researching the temperature of penetration and development (Xu et al.,

2007). The principle of Silver Staining Technology was that nucleotide combined with Ag⁺ and Ag⁺ formed reductase under alkaline environment, which enabled the display of DNA bands. So it could not avoid trivial steps, unclear background and HCHO damages. In addition, in the operating process, rinsing excessively after penetration might cause unclear DNA bands, or without belts and

rough shaking might cause uneven development. It might make gel background yellow and the color deep because of high AgNO_3 density, long penetration time, short rinsing time, long developing time, etc. Moreover, it is hard to avoid these defects for Silver Staining Technology.

The three main ways of DNA bands shown are southern hybridization, agarose gel electrophoresis and polyacrylamide gel electrophoresis (Zhang et al., 2008). Southern hybridization is seldom used because of the use of radioactive isotopes. Agarose gel electrophoresis can identify and evaluate DNA bands rapidly, but cannot distinguish microsatellite DNA bands on account of its low resolution. Polyacrylamide gel electrophoresis is applied widely in microsatellite molecular marker because its high resolution (theoretically, it can separate 0.1% length difference of DNA molecules) is followed with Silver Staining Technology instead of radioactive isotopes technology. However, some defects, such as its steps are trivial, time-consuming, background fuzzy, etc., exist in Silver Staining Technology. Ordinarily, the Silver Staining Technology takes 2 to 3 h, but this experiment took 30 to 60 min to adopt the improved Silver Staining Technology.

In contrast, the Fluorescent Imaging Technology concluded the experiment for only 2 to 3 min using fluorescent GelRed/GelGreen. It has the following advantages: Simple and feasible procedure, high resolution and a clear background. It not only has rapid advantage of agarose gel electrophoresis, but also has high resolution of polyacrylamide gel electrophoresis. Generally speaking, it is worth popularizing in the laboratory for DNA bands display.

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