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Original Paper

The effect of DNA isolation and its applicability in the the PCR analysis of Lettuce (*Lactuca sativa* L.)

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The aim of the presented study was to compare the effect of selected DNA isolation kits – DNeasy Plant Mini Kit (QIAGEN), PowerPlant DNA Isolation Kit (MO-BIO), Isolate Plant DNA Mini Kit (BIOLINE) and the method by Rogers and Bendich on quality and purity of the final PCR products. The DNeasy Plant Mini Kit (QIAGEN) and PowerPlant DNA Isolation Kit (MO-BIO) were selected as the most suitable ones for DNA isolation, because of the high yield of genomic DNA. Isolated DNA was tested in PCR reactions and two types of DNA sequences were amplified - microsatellite DNA sequences and ITS (Internal Transcribed Spacers) regions. The PCR products of the highest quality amplified by the ISSR primer and primers for the ITS regions were detected in the samples containing DNA isolated by protocol Rogers and Bendich and by isolation kit Isolate Plant DNA Mini Kit.

Keywords: lettuce, *Lactuca sativa* L., DNA isolation, polymerase chain reaction

1. Introduction

Lettuce (*Lactuca sativa* L.) is grown throughout the world. Cultivation of lettuce is classified according to its vegetation morphology into six different groups: ice, crisp, romaine (cos), butterhead, stem, latin and lettuces. The differences among the various types of lettuce were demonstrated using morphological analysis and molecular and biochemical markers. Morphological classification is expensive, time-consuming and sometimes difficult, because many species have the same morphological characteristics (Hu et al., 2005).

Isolation of genomic DNA is the first step in most molecular biology experiments. The decisive factors when selecting an extraction method are quantity, quality and purity of isolated DNA (Valenzuela et al., 2005). Techniques of molecular biology require DNA of varying purity and quality. Currently, there are many methodologies and isolation kits for an extraction of genomic DNA with optimal properties (Oza et al., 2008). The buffer containing nonionic detergents such as cetyltrimethylammoniumbromid (CTAB) is often used for DNA isolation, and then followed by a series of steps for the purification of DNA from contaminants using organic solvents or salt precipitation (Bossinger et al., 2006). Basic principles of isolation methods are based on the chemical properties of DNA, including:

- phosphate esters are strong acids and have the characteristics of anions at neutral pH,
- precipitation of DNA using ethanol or isopropanol may be sometimes complicated,
- nitrogen bases are only weakly alkaline and uncharged,
- hydrogen bounds between NH_2 and OH groups are stable at a pH in the range 4 – 9 (Kokinčáková et al., 2009).

Isolation of DNA from plant tissues is problematic in comparison with DNA isolation from animal tissues because of the rigid cell wall that surrounds the plant cells. For DNA extraction from cells of animal tissues, only buffer containing detergents and proteinase K are necessary. For DNA isolation from plant tissues, participation of carbohydrates and enzymes ensuring lysis of cell wall is necessary (Manen et al., 2005). The presence of polysacharides, polyphenols and other organic compounds may pose problem in DNA isolation process (Cota-Sánchez et al., 2006). In techniques of nucleic acids isolation there are two basic steps – degradation of cell membranes and purification of nucleic acids from contaminants of cellular content (Kokinčáková et al., 2009).

The aim of the experimental work was to compare the effect of selected DNA isolation kits on quality and purity of the final PCR products. Subsequently, the amount of isolated genomic DNA was determined. The quality and applicability of DNA isolated from lettuce in further analysis of molecular biology was tested by polymerase chain reaction (PCR). In this study, two types of DNA sequences were amplified in PCR reactions – microsatellite sequences and ITS (Internal Transcribed Spacers) regions. The DNA yield using various

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isolation kits and PCR products were electrophoretically evaluated.

2. Material and methods

Genomic DNA was isolated from fresh leaves of lettuce (*Lactuca sativa* L.) which were obtained sowing the seeds of different genotypes – Ceandrapids, La brillante, Parade, Salinas 88, VC9 US 23, Pavane, Iceberg, Salinas (United States Department of Agriculture). The genotypes of lettuce were cultivated in a soil substrate. After 14 days of germination, leaves from the eight lettuce genotypes were collected and the surface was sterilized in 70 % ethanol. The biological material was homogenized in liquid nitrogen. The sample represented a blend of leaf tissue of the eight genotypes.

The yield of genomic DNA was compared, using three DNA isolation kits – DNeasy Plant Mini Kit (QIAGEN), PowerPlant DNA Isolation Kit (MO-BIO), Isolate Plant DNA Mini Kit (BIOLINE) and a method by Rogers and Bendich (1994). Each isolation kit was tested in triplicates. The samples of the isolated DNA were electrophoretically separated in 1 % agarose gel (Agarose Basic, AppliChem) together with 1× TBE and Gel Red 10 000× (Biotium). The amount of DNA obtained from the different isolation methods was determined using the molecular weight marker of known concentration (human genomic DNA) applied on the agarose gel in the concentrations 400, 200, 100 and 50 ng. The isolated DNA was tested in subsequent PCR reactions. Microsatellite DNA sequences and ITS (Internal Transcribed Spacers) regions were amplified.

The microsatellite DNA sequences were amplified using ISSR (Inter Simple Sequence Repeats) method. Amplification was performed in the reaction mixture containing DreamTaq™ DNA polymerase (Fermentas), 0.4 nmol dm^{-3} dNTP (each), 4 nmol dm^{-3} $MgCl_2$ together with 400 nmol dm^{-3} of ISSR primer $(CTG)_3GC$. The 12 samples containing isolated genomic DNA of lettuce (*Lactuca sativa* L.) in the concentrations of 15 ng and the sample of negative control without the addition of DNA were evaluated. Temperature profile of ISSR reactions was comprised of initial denaturation at 94 °C for 2 min; followed by 44 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min and then ended by 62 °C for 7 min.

The ITS regions were amplified using PCR reactions in the reaction mixture containing DreamTaq™ DNA polymerase (Fermentas), 0.4 nmol dm^{-3} dNTP (each), 4 nmol dm^{-3} $MgCl_2$ together with 400 nmol dm^{-3} of forward primer and reverse primer. The sequences of forward primer are 5‘ tccgtaggtgaacctgcgg 3‘ and the sequences of reverse primer are 5‘ tcctccgcttattgatatgc 3‘. The 12 samples containing the isolated genomic DNA of lettuce in the concentrations 15 ng and the sample of negative control without the addition of DNA were used. Temperature profile for ITS amplification was followed: initial denaturation at 95 °C for 4 min; denaturation at 95 °C for 1 min, annealing 52 °C for 1 min, polymerisation at 72 °C for 2 min, 34 cycles; then polymerisation at 72 °C for 10 min.

The final PCR products were electrophoretically separated on the 2 % agarose gel. Electrophoresis was conducted at a voltage of 65 V for 2 h. Electrophoreograms were processed with documentation system G:Box in GeneSnap program – Product version: 7.09 (Syngene) and GeneTools – Product version: 4.01 (Syngene).

3. Results and discussion

The average yield of the isolated genomic DNA from fresh leaves of lettuce (*Lactuca sativa* L.) varied based on the different isolation methods (figure 1). The amounts of genomic DNA obtained from various isolation methods are shown in the table 1.

Table 1 The average yields of the isolated DNA using the selected isolation methods

Method	DNA yield in ng μl^{-1}
Protocol Rogers, Bendich (1994)	20
DNeasy Plant Mini Kit (Qiagen)	40
PowerPlant DNA Isol. Kit (MO-BIO)	40
Isolate Plant DNA Mini Kit (Bioline)	20

The efficiency of the DNA isolation kits was compared and the results were evaluated. The highest yield of DNA from lettuce fresh leaves was ensured using DNeasy Plant Mini Kit (Qiagen) and PowerPlant DNA Isolation Kit (MO-BIO). Conversely, the low yield of the isolated DNA was ensured using Isolate Plant DNA Mini Kit (Bioline) and using the method by Rogers, Bendich (1994).

The Qiagen (DNeasy Plant Mini Kit) Buffer AW1 contains guanidine hydrochloride. Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves

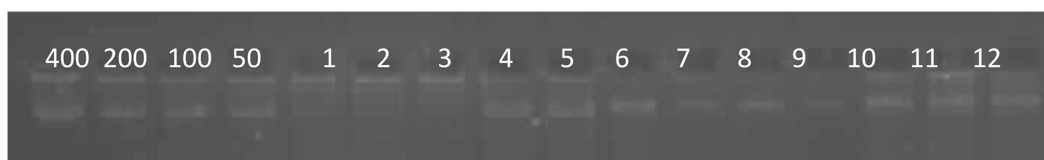

Figure 1 The amount of genomic DNA isolated from *Lactuca sativa* L. by Rogers, Bendich, (1984) method, lines (1, 2, 3), DNeasy Plant Mini Kit (Qiagen), lines (4, 5, 6), Isolate Plant DNA Mini Kit (Bioline), lines (7, 8, 9), PowerPlant DNA Isolation Kit (MO-BIO), lines (10, 11, 12) compared to DNA of known concentration (400, 200, 100, 50 ng)

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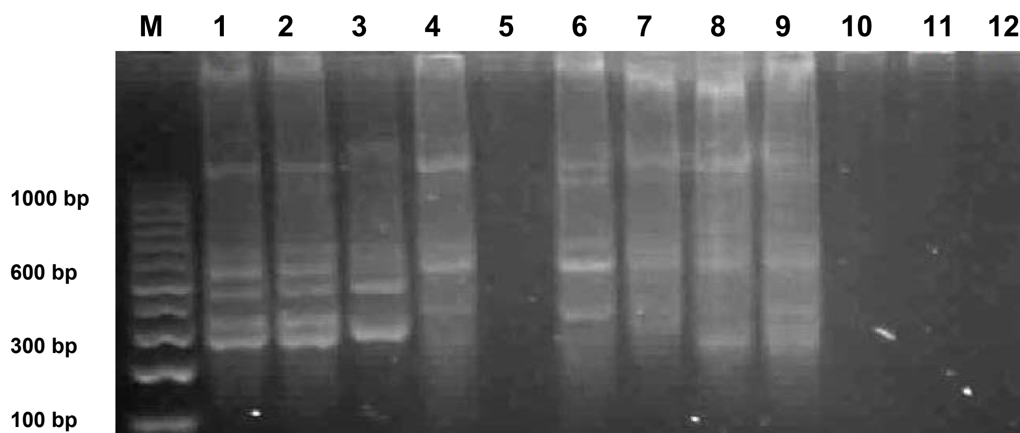

Figure 2 The amplification of microsatellite DNA sequences from DNA isolated by the Rogers and Bendich (1994) method (lines 1, 2, 3) and by isolation kits DNeasy Plant Mini Kit (Qiagen) (lines 4, 5, 6), Isolate Plant DNA Mini Kit (Bioline) (lines 7, 8, 9), PowerPlant DNA Isolation Kit (MO-BIO) (lines 10, 11, 12), M – molecular marker

minimal handling. The purified DNA is eluted in a low-salt buffer or water, ready for use in downstream applications. The DNeasy membrane ensures complete removal of all inhibitors of PCR and other enzymatic reactions.

The PowerPlant DNA Isolation Kit (MO-BIO) utilizes patented Inhibitor Removal Technology® (IRT) for removal of PCR inhibitors from plant extracts during the isolation process. Plant samples are added to a bead tube along with a kit supplied buffer for rapid homogenization. Cell lysis and DNA release occurs by mechanical and chemical methods. Released genomic DNA is cleared of PCR inhibitors using IRT, and then DNA is captured on a silica membrane in a spin column format and washed and eluted from the membrane.

The protocol of ISOLATE Plant DNA Mini Kit (Bioline) does not require the use of Proteinase K. The DNA is extracted with chaotropic salts, denaturing agents and detergents. The DNA is then bound to a silica membrane, washed and the pure genomic DNA is eluted. The kit contains two optimized lysis buffers based on the established CTAB and SDS methods. RNase A is included to remove RNA.

The method by Rogers, Bendich (1994) applies detergents as cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS). The DNA is protected from the endogenous nucleases by EDTA (ethylenediaminetetraacetic acid). The buffer/tissue homogenate is emulsified with chloroform and/or phenol to denature and separate the proteins from the DNA.

Most of the available isolation kits and published protocols use detergents which can ensure lysis of cell wall as a first step in DNA extraction from plant materials. The DNA contaminants as RNA and proteins are removed using the RNase and proteinase (Yaffe et al., 2012). Some isolation methods use the DNA binding to silicon matrix or magnetic beads with subsequent release of DNA. These methods allow avoiding exposure to organic solvents, such as chloroform on DNA (Ivanova et al., 2008). Several commercial isolation kits may be used for DNA isolation, and also for DNA purification. The limited amount of sample (20 – 200 mg) can be processed using available isolation kits. Successful DNA isolation depends on the type of plant material (Demeke, Jenkins, 2009).

In the presented study, the DNA isolated from lettuce was amplified using PCR reactions, and subsequently the molecular weight of the amplified PCR products was

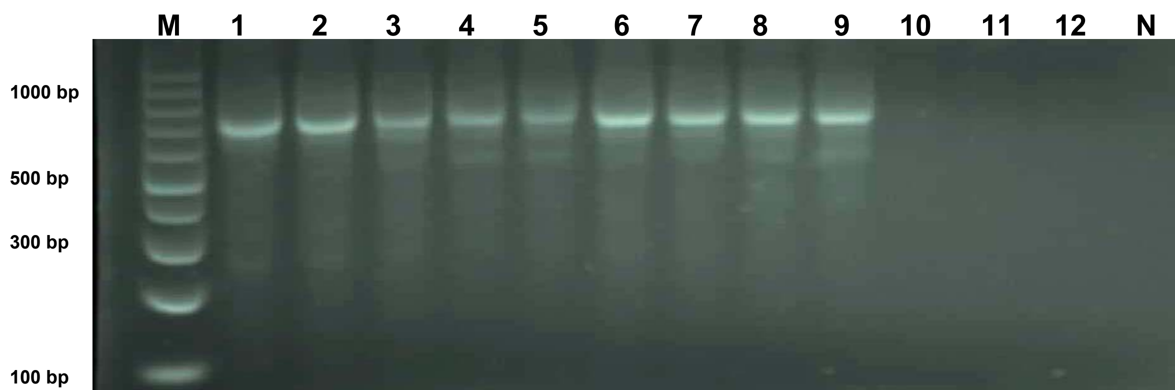

Figure 3 The amplification of ITS regions from DNA isolated using the method by Rogers, Bendich (1994) (lines 1, 2, 3) and isolation kits DNeasy Plant Mini Kit (Qiagen) (lines 4, 5, 6), Isolate Plant DNA Mini Kit (Bioline) (lines 7, 8, 9), PowerPlant DNA Isolation Kit (MO-BIO) (lines 10, 11, 12), M – molecular marker, N – negative control

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determined. The ISSR primer $(CTG)_3GC$ and the primers for ITS regions (primer 1, primer 2) were applied. Most amplified microsatellite DNA sequences were observed in the DNA samples isolated by the Rogers and Bendich (1994) method. Although the yield of DNA samples isolated by DNeasy Plant Mini Kit (Qiagen) and Isolate Plant DNA Mini Kit (Bioline) was high, the DNA samples were not suitable for amplification of microsatellite sequences. In the DNA samples isolated by PowerPlant DNA Mini Kit (MO-BIO), microsatellite DNA sequences were not amplified (figure 2).

Moreover, the ITS regions of DNA isolated from lettuce were amplified by the PCR reactions. The successful amplification of PCR products was detected in the samples containing DNA isolated using the method by Rogers, Bendich (1994) and isolation kit Isolate Plant DNA Mini Kit (Bioline). The amplification of DNA samples isolated using PowerPlant DNA Isolation Kit (MO-BIO) was not recorded, and due to the lower quality, these DNA samples are unsuitable for downstream analysis of molecular biology (figure 3).

4. Conclusions

In the presented study, the efficiency of selected DNA isolation kits – DNeasy Plant Mini Kit (Qiagen), PowerPlant DNA Isolation Kit (MO-BIO), Isolate Plant Mini Kit (Bioline) and the method by Rogers and Bendich (1994) was tested. The yield of the isolated DNA and purity of final PCR products were evaluated. The aim of the experimental work was to select an isolation kit or a method that can provide the best yield and quality of isolated genomic DNA from fresh leaves of lettuce (*Lactuca sativa* L.). The isolated DNA was electrophoretically separated and DNA concentrations were determined. High yield of isolated DNA was recorded using the isolation kits DNeasy Plant Mini Kit (QIAGEN) and PowerPlant DNA Isolation Kit (MO-BIO). Further, the DNA samples were tested using PCR amplification. Two types of DNA sequences were amplified – microsatellite DNA sequences and ITS (Internal Transcribed Spacers) regions. The most amplified microsatellite DNA sequences were recorded in the DNA samples isolated by Rogers and Bendich (1994). The successful amplification of ITS regions was recorded in the DNA samples isolated by Rogers and Bendich (1994) and isolation kit Isolate Plant DNA Mini Kit (BIOLINE). The DNA samples isolated by PowerPlant DNA Isolation Kit were not amplified, and therefore this DNA sample was evaluated as inappropriate for further analysis.

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