ISSUES OF INCREASING THE PRODUCTIVITY OF THE COTTON PLANT IN THE DEVELOPMENT OF THE ECONOMY OF UZBEKISTAN

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Abstract: *Several DNA isolation protocols and commercial kits are currently available for plants. However, in some, costs are prohibitive for small laboratories, and therefore finding a cost-effective alternative is technologically important for small biotechnology laboratories in developing countries. This study aims to provide an alternative economic, reliable and safe genomic DNA isolation method from cotton.*

Key words: *DNA extraction, Cotton, Ravnaq, CTAB method, Gel-electrophoresis, Nanodrop.*

Isolation and purification of DNA is a crucial step in DNA molecular techniques used in plant studies to determine genotypes, economic traits associated with genes of interest, and genetic diversity. Different plant species have different levels of polysaccharides, polyphenols, and other secondary metabolites that combine with nucleic acids during DNA isolation and further affect the quality of the DNA obtained. The main modifications currently used for DNA extraction include the use of high CTAB concentrations and high levels of 2-β-mercaptoethanol. Also, phenol-chloroform-based methods are widely used in DNA extraction. In addition, high concentrations of sodium chloride and potassium acetate are added simultaneously with absolute ice-cold isopropanol to precipitate polysaccharide-free DNA.

DNA molecular techniques are mainly based on polymerase chain reaction (PCR) analysis, which requires the isolation of genomic DNA of suitable purity. Various extraction protocols have been developed to extract pure and intact whole genomic DNA from plant tissues.

However, many difficulties in extracting quality DNA from plants have been reported. These difficulties were related to the different levels of polysaccharides, polyphenols and other secondary metabolites in different plant species. These components usually hinder DNA purification and its use in molecular research. These plant components have a similar structure of nucleic acids that interfere with the total DNA isolation of secondary metabolites and polysaccharides. They bind strongly to nucleic acids during DNA isolation and affect the quality of DNA obtained from higher plants [3]. These metabolites also affect the amount and purity of isolated nucleic acids. Removal of such contaminants requires complex and time-consuming protocols. A single DNA isolation protocol cannot be used for all plant tissues. Most of the cetyl

trimethylammonium bromide (CTAB)-based protocols used for DNA extraction are adapted to the internal components of each plant species.

In this work, cotton seeds were treated with physiologically active preparations. The seeds treated with drugs were planted in soil in special pots in a phytotron. As a control, seeds that were not treated with any drug were planted. 3-week-old leaves of young plants were used for DNA extraction. In order to obtain high-quality genomic DNA from plants, a modification of the CTAB method was used. Gel electrophoresis and spectrophotometric methods of genomic DNA purity were used to evaluate the efficiency of this protocol.

The experiment was conducted in 2023 at Gulistan State University "Agrobiotechnologies and Biochemistry Scientific Research Institute". As part of the protocol, 100 mg of cotton leaves were collected from 21-day seedlings grown in a phytotron. The collected leaves were washed with distilled water, dried, cut into 1 to 1-2 cm lengths and placed in sterile mortar and pestle. It was wrapped with aluminum foil and stored in a -20 ºC refrigerator for 24 hours.

The STAB method for extracting genomic DNA from plants was carried out in the following order. DNA Concentration, Purity, and Quality DNA concentration was determined spectrophotometrically at absorbance at 260 nm (A260) using a NanoDropOne C (Thermo Scientific). Purity of DNA from protein and polysaccharide contamination was assessed by evaluating the absorbance ratio at A260/A280 and A260/A230, respectively. The quality of the obtained DNA was also evaluated by electrophoresis for all DNA samples on a 0.9% agarose gel stained with ethidium bromide (1 mg/ml).

DNA was analyzed by agarose gel electrophoresis using a 0.9% agarose gel (AgaroseTM, Helicon, for genomic and amplified DNA). Electrophoresis was performed using 1 × Tris-Borate EDTA (TBE) buffer containing 1 μg/ml ethidium bromide (EtBr) and 100 V DC for 50 min. The amount of DNA was visualized and images were captured using a Transluminator Kvant C (Helicon, Russia). Electrophorogram of genomic DNA isolated from leaves of cotton plant exposed to substances with physiological activity.

According to this protocol, the quality of total DNA obtained from different plant species was evaluated by electrophoresis. The results showed intense bands very close to the gel wells. Genomic DNA from the same samples did not produce clear or intact bands by the CTAB method of Doyle and Doyle (1990).

We implemented several steps in this modified protocol to ensure DNA isolation with good yield and quality from seeds of different plants. Liquid nitrogen has been used to break the cell wall and disrupt the cell membrane, inactivating cellular enzymes and other unwanted chemicals, reducing DNA breakage and damage. Other methods used to break down plant tissue, such as digestion with pectinase and cellulose, are not as reproducible or precise as using liquid nitrogen.

These modifications successfully removed polysaccharide impurities from DNA extracted with this modified protocol from all plant samples, generating pure and high-quality DNA suitable for further molecular analysis. Proteins, most lipids and cell debris were removed by binding to anhydrous compounds and precipitated in the chloroform-isoamyl alcohol phase.

Longer incubation of extracted DNA at −20 °C also enhanced DNA precipitation. In general, the amount and quality of isolated DNA depends on the temperature and duration of precipitation. The low-temperature precipitation used in this modified protocol increased DNA yield. Extracted DNA was resuspended in a minimal volume of 1x TE buffer, as the presence of chelating agents in TE buffer may affect PCR and other molecular analysis of extracted DNA.

Electrophoretic separation of DNA extracted using this protocol showed strong bands very close to the gel wells (Figure 1), indicating high purity and intact DNA. It is known that the presence of stains can be a sign of degradation of the obtained DNA, which easily affects the quality of the results of subsequent molecular application. A260/A280 purity ratio is an important measure to evaluate the degree of polyphenol contamination of extracted DNA. If the A260/A280 ratio is below 1.8, the obtained DNA is unsuitable for molecular research.

Therefore, high A260/A280 absorption coefficients in our case may be related to a small change in the pH value of the obtained samples. Polysaccharide contamination was also assessed by

evaluating the A260/A230 absorbance ratio as a secondary measure of nucleic acid purity (Table 2). This ratio is important for estimating the level of salt residues in the purified DNA. Greater than 1.5 and close to 1.8 is recommended. The reported values of the A260/A230 ratio in most DNA plant samples obtained by the current modified protocol are higher than in DNA samples obtained by other classical methods.

Various modifications of the STAB method have been popularized by researchers. In this method, the extraction of genomic DNA in pure form without using some toxic substances is one of the problematic issues.

The modifications identified in this method provided a safe, rapid, and efficient standardized protocol for DNA extraction from cotton plant leaves. The applied modifications consistently produced pure and high-quality DNA suitable for further molecular analysis.

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