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Research Article

Isolation and PCR Validation of DNA from Chicory (*Cichorium intybus* L.) Leaves Using an Optimized CTAB Method to Propel Medicinal Insights

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Abstract

Isolating and purifying DNA from plants, particularly from species like *Cichorium intybus* (chicory), poses significant challenges due to the presence of rigid cell walls and high levels of polyphenols and polysaccharides. These compounds can severely hinder the efficiency of traditional DNA extraction protocols, often resulting in poor-quality DNA. Conventional methods, such as the Cetyl-trimethyl-ammonium bromide (CTAB) protocol, are often found inadequate due to these biochemical barriers. In this study, we formulated an optimized DNA isolation protocol for chicory that eliminates the need of liquid nitrogen and phenol during processing. The refined approach resulted in the extraction of high-quality DNA suitable for downstream applications. The effectiveness of the optimized method was validated by polymerase chain reaction (PCR) amplification of the sucrose: sucrose 1-fructosyl transferase (*1-SST)* gene, a key gene involved in the biosynthesis of inulin- a compound with notable medicinal and nutritional value. The PCR amplification yielded the expected 2.01 kb product, confirming the high quality and integrity of the extracted DNA. Overall, the quantity and quality of the DNA were well-suited for molecular analysis, demonstrating the success of our method. This optimized protocol provides a valuable tool for molecular research on chicory and other plants with similar biochemical challenges, facilitating more efficient genetic studies and holding potential for advancing biotechnological applications.

Keywords

Chicory, CTAB, DNA, Polyphenols, Polysaccharides, 1-SST Gene

1. Introduction

Cichorium intybus L., commonly known as chicory, is a typical perennial herb belonging to the Asteraceae family, characterized by a height of approximately 1 meter with huge basal leaves and a thick taproot up to 75 cm long. This globally distributed plant has diverse commercial applications, including forage, salad, and as a coffee substitute. Additionally, it exhibits a wide range of medicinal properties, such as antimalarial, anthelmintic, antimicrobial, hepatoprotective, antidiabetic, anti-inflammatory, analgesic, antioxidant, tumor inhibitory, and anti-allergic activities [1-4]. The sucrose-sucrose 1-fructosyl transferase (1-SST) gene of chicory plays a critical role in the biosynthesis of inulin, a dietary fibre

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beneficial for diabetic patients [5, 6]. Cetyl-trimethyl-ammonium bromide (CTAB) method is a widely used DNA isolation protocol in plant systems [7, 8]. However, the high levels of polysaccharides and polyphenolic compounds in chicory present significant challenges for DNA isolation and subsequent polymerase chain reaction (PCR) amplification [9]. The present study focuses on optimizing the CTAB method for effective DNA extraction from chicory followed by PCR amplification of the *1-SST*.

2. Materials and Methods

2.1. Study Samples

The study was conducted at the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, Pantnagar, India. Samples of fresh etiolated young leaves of the chicory plant were collected from four regions in Uttarakhand (India) - Almora, Nainital, Tanakpur, and Sariyatal.

2.2. Solutions and Reagents for Extraction of DNA

1x CTAB extraction buffer (pH 8): 2% CTAB, 100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), 0.2% β-mercaptoethanol, 2% polyvinylpyrrolidone (PVP); *1x Tris-EDTA (TE) buffer (pH 8)*: 10 mM Tris-HCl (pH 8), 1mM EDTA (pH 8); *Chloroform:isoamylalcohol (24:1 v/v) solution*; *Isopropanol*; *7.5 M ammonium acetate (pH 5.5)*; *70% ethanol*; *RNase enzyme (10 µg/ml).*

2.3. DNA Isolation and Purification

The etiolated leaves were thoroughly washed with sterilized distilled water followed by 70% alcohol and stored at -80°C before processing. Using sterile blades, the leaves were cut into approximately 3 mm pieces and ground ~0.5 gm sample on pre-chilled mortar and pestle in 2-3 ml pre-warmed fresh CTAB extraction buffer. After grinding, the mixture was transferred into a 2 ml micro-centrifuge tube and incubated at 65°C for 30 min with intermittent gentle mixing. Following incubation, the mixture was cooled at room temperature (RT) and centrifuged at 10,000 rpm for 10 min at RT. The aqueous phase was then transferred to a fresh tube for RNA degradation using 5- 10 µl of RNase enzyme for 1 ml solution, followed by gentle mixing and incubation for one hour at 37°C. Thereafter, an equal volume of the mixture of chloroform: isoamylalcohol (24:1 v/v) was added and blended by inversion to soften, followed by centrifugation at 10,000 rpm for 10 min at RT. The aqueous phase was transferred to a fresh tube using a broad pore pipette and the DNA was precipitated with an equal volume of ice-cold isopropanol and 7.5 M ammonium acetate (100 µl for 1 ml solution). This mixture was gently mixed and stored at 0°C for 1 hour. Precipitated DNA was centrifuged at 10,000 rpm for 10 min at RT. The supernatant was decanted carefully and the pellet was washed with 70% ethanol by centrifugation at 10,000 rpm for 10 min at 4°C. Again, the supernatant was decanted and the pellet was air dried for 15 min and dissolved in 100-200 µl of 1x TE buffer, and stored at -20°C.

2.4. Quality and Quantity Estimation

DNA quality and quantity were assessed with Nanodrop™ UV Visible Spectrophotometry (Thermo Fisher Scientific, United States), aiming for an optical density (260/280 nm) ratio between 1.7 and 1.9. The integrity of DNA was checked through gel electrophoresis using 0.8% agarose gel stained with ethidium bromide and imaged under the ChemiDoc XRS Gel Imaging System (Bio-Rad, United States).

2.5. PCR Validation

The *1-SST* (Accession no: JQ346799.1) was amplified using Veriti™ 96-well Fast Thermal Cycler (Thermo Fisher Scientific, United States). Primers: 5'-TCNGGCTGGTAAATGAAAAGAATGGGAAGG-3' (Forward primer) and 5'-CATGTGGGGTGGTCTCTCTACTACGCACA-3' (Reverse primer) were designed using Primer 3 software (https://primer3.ut.ee/4.1.0). PCR mix of 15 µ reaction volume (lx) contained 1 µl of template DNA, 2.5 µl of 10x Taq reaction buffer, 0.25 µl of 2.5 mM dNTPs, 0.5 µl of Taq DNA Polymerase enzyme, 1 µl of 10 pmol forward and reverse primers each and rest 8.75 µl of nuclease free water. The PCR program had 35 cycles in which initial denaturation at 95°C for 5 min, segment denaturation at 95°C for 1 min, annealing at 58°C for 30 sec, extension at 72°C for 2 min and final extension at 72°C for 5 min. PCR products were visualized on a 1.2% agarose gel stained with ethidium bromide. The expected amplicon size was 2.01 kb.

3. Results

The yield of DNA was approximately 64.56 ± 30.51 ng/µl, with an OD of about 1.75 ± 0.12 (Table 1). All extracted DNA from different locations was examined through gel electrophoresis and showed satisfactory results (Figure 1). PCR amplification of the *1-SST* gene showed expected 2.01 kb amplicons in all samples showing the credibility of the reported protocol (Figure 2).

Table 1. Quality & quantity of extracted DNA from etiolated leaves of chicory.

Location	Sample Id	Concentration (ng/µl)	OD (260/280 nm)
Nainital	S1	30.6	1.78
	S2	49.2	1.71
Almora	S3	88.3	1.56
	S4	112.1	1.66
Tanakpur	S5	23.5	1.81
	S6	78.9	1.68
Sariyatal	S7	52.8	1.86
	S8	81.1	1.92

OD: optical density

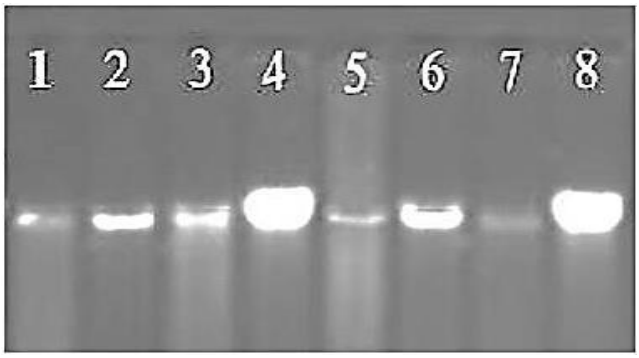

Figure 1. Agarose gel electropherogram of genomic DNA extracted by the modified CTAB method (Lanes 1-2, S1&2; lanes 3-4, S3&4; lanes 5-6, S5&6; lanes 7-8, S7&8).

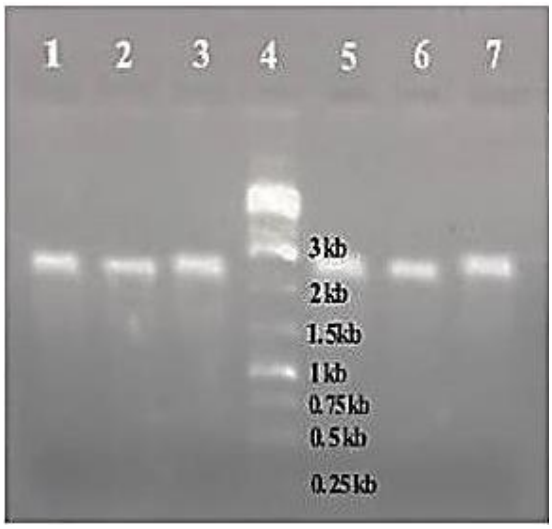

Figure 2. Agarose gel electropherogram of PCR amplified product of 1-SST with expected 2.01 kb bands in all samples (Lanes 1-2, S1&2; lane 3, S4; lane 4, 1 kb DNA ladder; lane 5, S5; lanes 6-7, S7&8).

4. Discussion

The CTAB method's efficiency was enhanced through several modifications. EDTA serves as a chelating agent for magnesium ions, inhibiting DNase activity, while Tris-HCl maintains a slightly alkaline pH for the stability of DNA. β-mercaptoethanol acts as an antioxidant, preventing the oxidation of polyphenols by disrupting di-sulphide bonds to denature proteins [10]. The pre-warmed CTAB buffer eliminates the need of liquid nitrogen, simplifying the protocol. In our study, increased concentrations of CTAB (1.5% → 2%) and PVP (1% → 2%) as compared to the traditional method, enhance the removal of polysaccharides and phenolic compounds, as PVP forms complex hydrogen bonds with latex lactones, lactucin, and other phenolics, while CTAB binds with fructans, and other polysaccharides and ultimately co-precipitate with cell-debris upon lysis. When the extract is centrifuged in the presence of chloroform: isoamylalcohol, the PVP and CTAB complexes accumulate at the interface between the organic and the aqueous phases [10-12]. The inclusion of 7.5 M ammonium acetate during DNA precipitation stabilizes the negatively charged DNA; and helps in precipitation hence improving overall yield [13]. Although commercial DNA isolation kits offer convenience, their high-cost limits accessibility [14, 15]. By eliminating phenol in the purification process, our method reduces hazards.

5. Conclusion

The extensive diversity found within the plant kingdom necessitates molecular analysis of each species, highlighting the need for the development of DNA extraction methods that are adaptable to a wide range of plant types if not all. This optimized CTAB protocol advances plant genomic research, providing a reliable method for DNA extraction, which is essential for further molecular analysis.

Abbreviations

1-SST	Sucrose: Sucrose 1-fructosyl Transferase
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
OD	Optical Density
PCR	Polymerase Chain Reaction
PVP	Polyvinyl Pyrrolidone

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Author Contributions

Conceptualization, S.A., M.A. and G.T.; Methodology, S.A. and M.A.; Validation, S.A. and G.T.; Writing-Original Draft, S.A.; Writing-Review & Editing, M.A. and G.T.; Supervision, G.T.; Project Administration, G.T.; Funding Acquisition, G.T.

Conflicts of Interest

The authors declare no conflicts of interest.

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