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Improved method of DNA extraction from leaf and rhizome samples of black turmeric (*Curcuma caesia*) for molecular analysis

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ABSTRACT

Curcuma caesia belongs to the genus *Curcuma* and the family of Zingiberaceae, which is a very important but unexplored medicinal plant. It is locally known as black turmeric or *kali halide* and is mainly used by the local tribal community as traditional medicine for the health sector. The rhizome of black turmeric has wide applications in the economic pharma sector due to essential active ingredients. This research aims to standardize a rapid**,** simple and efficient protocol for DNA extraction in *Curcuma caesia* which can be used for another genus of Zingiberaceae to obtain DNA from leaf samples. Changes in the concentration of components of DNA extraction buffer have improved the quantity of DNA from leaf samples compared to rhizomes. Extracted DNA samples also proved more efficient in PCR amplification of DNA barcode primers. The protocol developed in the present study is more efficient for leaf samples of *Curcuma caesia* compared to rhizome samples.

Keywords: Black Turmeric, *Curcuma caesia*, DNA Isolation, Leaf, Rhizome.

INTRODUCTION

Curcuma caesia (Roxb.) is an endemic and perennial herb with a bluish-black rhizome. It is native to North-East and Central Asia. India's wealthiest hotspot zone of Black turmeric is the North-East and southern regions of India and is also found sparsely distributed in Chhattisgarh, Madhya Pradesh, Maharashtra, Bihar, and Uttar Pradesh regions. The rhizome of Black Turmeric is aromatic due to the presence of volatile oil components, and the colour is much darker blue than in *C. aeruginosa*. The leaves have a deep red bluish colour, which runs throughout the whole lamina. Usually, the upper side of the leaf is rough, shortly pubescent. Black Turmeric, one of the important species in the Curcuma genus, has been used by various tribal communities for a long before for curing multiple diseases due to its medicinal therapeutic properties like antioxidant, anti-tumour, anti-asthmatic, anti-inflammatory, hepatoprotective, blood purifier, stomachic and carminative nature [8]. Total thirty significant components found in *Curcuma caesia* plant such as representing 97.48% of the volatile oil, with camphor (28.3%), *ar*-turmerone (12.3%), (*Z*)-*β*-ocimene (8.2%), *ar*-cur cumene (6.8%), 1,8-cineole (5.3%), *β*-element (4.8%), borneol (4.4%), bornyl acetate (3.3%) and *γ*-cur cumene (2.82%) as the major constituents [7]. In this context, *Curcuma caesia* will play a significant role in the health sector. Thus, it needs more scientific research for qualitative validation for crop improvement. Molecular technology is growing fast, and adaptive techniques for identifying species and adulteration thus need novel DNA techniques. Here we have validated and standardized methodology for isolating and amplifying DNA from leaf rather than rhizome of black turmeric. It will also benefit other Zingiberaceae species to rapid molecular studies for crop improvements.

MATERIALS AND METHODS

The plant materials used in the study were healthy leaves and rhizomes of Black Turmeric collected from the field of Biotechnology Centre, JNKVV Campus Jabalpur. For DNA isolation, fresh leaf samples and rhizomes from mature plants of turmeric were washed in sterile distilled water to isolate DNA.

Genomic DNA isolation from leaf and rhizome

A standard method modification in the extraction buffer composition was used to isolate genomic DNA from leaf and rhizome samples [6,15]. In standard method, extraction buffer consisted EDTA, pH 8.0 (0.1M), NaCl (1.4M) and CTAB (2%). Extraction buffer in modified method consisted of EDTA pH 8.0

(20 mM), Tris-HCl pH 8.0 (0.1M), NaCl (1.5M), CTAB (4%) and β-mercaptothion (0.2%) was added to the extraction buffer after preheating at 65°C DNA from leaf and rhizome was initiated by disrupting tissues in liquid nitrogen using a mortar pestle. The crushed powder was mixed with extraction buffer and incubated at 65°C with intermittent shaking at the interval of 10 min. After incubation, samples were centrifuged for 10 min at 10000 rpm and supernatant was taken in another tube. Further supernatant was mixed with 5 µl RNase (20 mg/ml) and incubated for 40 min at 37°C. After 40 min, samples were mixed with chloroform: isoamyl alcohol (24:1) and mixed gently. Samples were centrifuged for 10 min at 10000 rpm, and the upper layer was transferred to another tube. Pre chilled isopropanol added in the DNA sample and incubated at -20°C. for 1 to 1.30 h. DNA samples were centrifuged for 15 min at 12000 rpm, and the pellet was washed twice with 70% ethanol. The washed pellet was dried to remove ethanol and diluted in ultrapure water. Diluted DNA was run on 0.8% agarose gel and quantified in Nanodrop (Jen way, model- Genova Nano).

PCR amplification

Isolated DNA samples were used for PCR amplification of *the rbcL* region using sequence-specific primers (Forward- 5'ATGTCACCACAAACAGAGACTAAAGC 3' and reverse 5' GAAACGGTCTCTCCAACGCAT 3'). Primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was performed in a 10 µl reaction mixture comprising of 1x PCR buffer, 1U Taq DNA polymerase, 0.2 mM dNTP, 10 p.m. of each forward and reverse primers and 20-50 ng of genomic DNA. Reaction conditions included initial denaturation at 94°C for 5 min followed by 35 cycles each of denaturation at 94°C for 2 min, annealing at 55°C for 45s and extension at 72°C for 1 min with a final extension step at 72°C for 10 min. The amplified product was separated on 1.2% agarose gel containing 0.5 µg/ml ethidium bromide at 100 V with a standard DNA marker (100 bp). The separated bands were visualized under UV light and photographed under a Gel documentation system (Viler Lour mat).

RESULTS AND DISCUSSION

Effect of modified DNA extraction method on yield of genomic DNA

During the present experiment, a standard method of DNA extraction from leaf and rhizome yielded 712 µg/ml and 587 µg/ml of genomic DNA, respectively, whereas, in the modified method, 1665 µg/ml and 1388 µg/ml of DNA were produced from leaf and rhizome samples respectively. Various secondary metabolites in leaf and black turmeric rhizomes can affect DNA yield. In the modified method of DNA extraction, the enhanced concentration of CTAB and $NaCl$ improved the yield of extracted DNA compared to the standard method of DNA isolation (Fig. 1). An increased amount of CTAB and $NaCl$ have yielded more genomic DNA from monocot as reported [12]. It has also been reported that more than 1.5 M $NaCl$ concentration removes polysaccharides and permits subsequent manipulation of DNA [5]. The increased amount of CTAB, PVP, β-mercaptothion, and $NaCl$, decreasing the concentrations of polyphenols, polysaccharides, and alkaloids from the rhizome samples of turmeric and ginger [13].

The availability of many essential oils, different types of polysaccharides, alkaloids, polyphenols, and other essential secondary metabolites in rhizomes and leaf's facing interfere with DNA isolation, PCR amplification, in molecular studies. Increased CTAB and $NaCl$ concentrations have increased the yield of total cellular DNA in different monocots species [12]. More than 1.5 M $NaCl$ concentration removes different polysaccharides and permits subsequent manipulation of DNA Isolation [5]. Similarly, in last step DNA precipitation has increased DNA yield and purity due to substituting used ethanol for isopropanol [9]. We have isolated DNA successfully from leaf and rhizome samples of *Curcuma caesia* by following a modified CTAB method [10,11,15]. The original method was modified by precipitating in ethanol instead of isopropanol and by increasing 10% CTAB, 4M $NaCl$, and 0.2% β-mercaptothion concentrations.

PCR amplification using rbcL primers

The purity of extracted DNA is a major concern for utilization in molecular techniques like restriction digestion and PCR amplification using gene-specific primers [2-4]. Degraded DNA or impure DNA with RNA or traces of protein may hinder enzyme activity required for digestion or PCR amplification. In the present study, gel electrophoresis of DNA showed that the modified method produced intact and pure DNA compared to the standard method (Fig.1). PCR-related techniques require less DNA, but purity is necessary to ensure repeatability and confidence [16,17]. During the present investigation, *rbcL* amplification using extracted DNA proved the importance of pure DNA. Isolated DNA from the modified method produced a more intense band than DNA from the standard method (Fig.2). The *rbc*L analysis using 25 ng of genomic DNA, 0.2 mM dNTP, 5 µmol of primer, 2.5 mM $MgCl_2$, and 1 U Taq DNA polymerase in a 10-µL reaction volume [14]. The intensity of the PCR product is vital in cases where sequencing is required, such as DNA barcoding. The present modified method of DNA isolation for turmeric samples is suitable for this purpose.

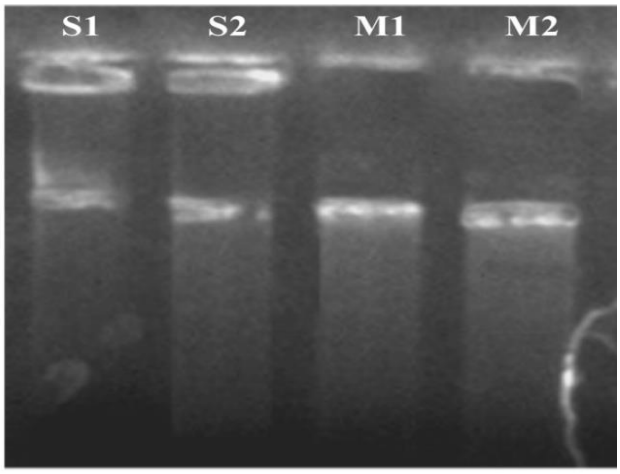

Figure 1: Gel electrophoresis of isolated DNA samples from leaf and rhizomes using standard and modified methods. (S1 and S2 - DNA sample from leaf and rhizomes respectively using standard method; M1 and M2 - DNA sample from leaf and rhizomes respectively using modified method)

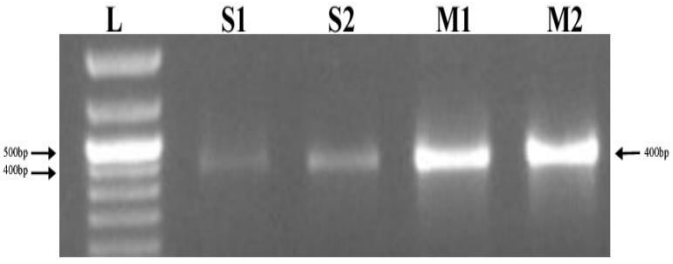

Figure 2: Amplification of *rbcL* region of *Curcuma caesia* using isolated DNA samples. (L- 100 bp DNA ladder; S1 and S2 - DNA sample from leaf and rhizomes respectively using the standard method; M1 and M2 - DNA sample from leaf and rhizomes respectively using the modified method)

CONCLUSION

The present study improved the yield of DNA from leaf and rhizome samples with an increase in CTAB and $NaCl$ concentration. The DNA isolated from the modified method was intact and high in quantity. Leaf samples have a higher amount of DNA than rhizomes. The DNA isolated from the modified method exhibited higher intensity of PCR

product than the standard method, which is a prerequisite for sequencing. Sound amplification was obtained with primers, this protocol is suitable for fast isolating good quality and quantity DNA from leaf as well as rhizome tissues of *Curcuma caesia* and also for other related to family Zingiberaceae for molecular studies.

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Conflict of Interest

None declared.

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None declared.

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