

Improve CTAB method for DNA isolation of Vanda orchids

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Improve CTAB method for DNA isolation of *Vanda* orchids

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Abstract

Background: Growing interest in molecular studies and bioinformatics increases in demand for good quality DNA extraction techniques.

Results: We demonstrate an easy and rapid DNA extraction process by modifying many existing ones. The present study focuses on isolation of clean and pure DNAs from the leave explants of *Vanda testacea*, *Vanda ampullacea* var. *auranticum* and *Vanda* PB Mazumder. Demonstration of the purity of the isolated DNAs is obtained through reproducible PCR amplification with several random primers using 25µl reaction mixture.

Conclusion: The improved CTAB DNA extraction method are practically suited for PCR analysis and this technique is simple, rapid and efficient, and can be used in analysis in situations where large numbers of samples are to be analysed.

Key words: DNA extraction, PCR, RAPD, ISSR, SSR.

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Introduction

Vanda testacea is an endangered orchid with highly medicinal value, ornamental and epiphytic forest orchid [1]. *Vanda ampullacea* var. *auranticum* is an endemic orchid of Manipur with highly ornamental values [2]. *Vanda* PB Mazumder is a hybrid orchid synthesized from *Vanda testacea* × *Vanda ampullacea* var *auranticum* with pale yellow flower. Over the last two decades the techniques of plant molecular biology such as RAPD[3], ISSR[4], SSR [5], CAPS[6], STS [7], microsatellite [8], nrITS [9], AFLP [10], etc have been extensively used for molecular mapping, identification of genotypes associated with genes of interest and genetic diversity studies. These techniques require the isolation of DNA of suitable purity for PCR but the growing number of DNA isolation protocols for specific plant species suggest that DNA extraction standard protocol are not always simple and the published protocols are not necessarily reproducible for all species [11]. The presence of high phenolic compounds can hamper the DNA isolation procedures and reactions such as in PCR amplification by inhibiting Taq polymerase activity in vandaceous orchids [12]. The presence of phenolic compounds can reduce the yield and purity of extracted DNA [11, 13]. Therefore DNA extraction is an important issue for those working with orchids where specific DNA extraction protocol has not been developed for that particular vandaceous species. Early protocols for isolation of DNA called for

the use of liquid nitrogen to assist in the grinding of plant material in mortar and pestle for DNA extraction [14-16]. Although these methods consistently yielded good quantities of high quality DNA, the use of liquid nitrogen presented some problems. In addition, growth and management of plants and storage of plant samples is often difficult. Here, we are presenting an easy and a rapid extraction process by modifying existing ones using without using liquid nitrogen, for PCR study. This report provides relatively clean DNA and can be used for large samples analysis without undergoing the lengthy purification process after isolation. The obtained DNA is consistently amplifiable in PCR using RAPD, ISSR and SSR technique for all the samples tested.

Materials and method

Young leaves from *Vanda testacea*, *Vanda ampullacea* var. *auranticum* and *Vanda* PB Mazumder were used as a source of DNA extraction and total genomic DNA were extracted by following the protocol of HiPurA[®] plant DNA isolation kit (CTAB Method) (MB502) with 1% PVP in the CTAB extraction buffer. Intactness of the genome was checked by subjecting 5 µl DNA and 5 µl loading dye to 2% ethidium bromide 0.8% agarose gel electrophoresis in TBE buffer. Amplification was performed using reagents from Bangalore Genie Pvt. Ltd., India in 25 µl reaction volume containing 1×Taq polymerase assay buffer (10 mM Tris–HCl pH 8.0, 50 mM KCl, and 2.5 mM MgCl₂), 0.2 mM of dNTPs, 1 µM primer, 1.0 unit of Taq polymerase and 2 µl of template DNA. The sequence of RAPD: 5'CTGAGGTCTC3', 5'ACCTTCGGAC3'; ISSR: 5'GAGAGAGAGAGAGAGATC3', 5'AGAGAGAGAGAGAGAGG3' and SSR: F: 5'AGCAACGATGGAGCAAGA3' R: 5'GCTGACCACGCTAACCTC3', F: 5'GTCCCGAGCCTCACATAA3' R: 5'AAAGCAGTTCCATAAAGATTG3' were used. The thermocycler was performed at initial denaturation at 94°C for 4 min, then 94°C for 1 min, 35°C (RAPD)/40°C (ISSR)/50°C(SSR) for 1 min, 72°C for 2 min (repeated 35 cycles), the last extension cycle at 72°C for 10 min and 4°C for forever. Electrophorese the amplified products on a 2% ethidium bromide stained 0.5% agarose gel in TBE buffer by loading 5 µl of the amplified products with 5 µl of loading dye and photographed using Gel Documentation System.

Results and discussion

The average of the molecular weight DNA extracted from the leave explant of three different samples of vandaceous orchid using this protocol was greater than 10,000 bp. Figure 1 represents a good quality of the extracted DNA resolved on a 1 % agarose gel. In this protocol, the addition of PVP to CTAB extraction buffer prior to grinding in the buffer was critical. PVP in CTAB extraction buffer reduce polyphenol by forming complex through hydrogen bonding allowing them to separate from the DNA [17]. In order to evaluate the efficiency and reliability of the isolated DNA in downstream application, the DNA was subjected to PCR amplification using primers of RAPD, ISSR and SSR. The PCR was successful in all the samples in a 25 µl reaction mixture. Figure 2 shows the PCR products from successful amplification of the isolated DNA samples using the primer (A) RAPD: 5'CTGAGGTCTC3', 5'ACCTTCGGAC3'; (B) ISSR: 5'GAGAGAGAGAGAGAGATC3', 5'AGAGAGAGAGAGAGAGG3' and (C) SSR: F:

5'AGCAACGATGGAGCAAGA3' R: 5'GCTGACCACGCTAACCTC3', F: 5'GTCCCGAGCCTCACATAA3' R: 5'AAAGCAGTTCCATAAAGATTG3'. It demonstrates the purity and clean nature of the DNA isolated from all the samples tested was amenable for any diagnostic purpose employing PCR as a technique. In short this method described here is, therefore, rapid, simple, efficient and labour-effective for the isolation of total DNA from leave explant. Since the method is carried out in different mortar and pestles and different microcentrifuge tubes for each samples the possibility of potential DNA cross contamination is highly reduced. This method will greatly enhance the usefulness of the PCR technique such as RAPD, ISSR and SSR by obviating the time-consuming liquid nitrogen steps. Further, the method is appropriate for simultaneous processing of large number of samples in eppendorf tubes. Thus, this method has a great potential for the development of technology for the rapid RAPD, ISSR and SSR profiling using primers for other vandaceous orchids with high polyphenol contents.

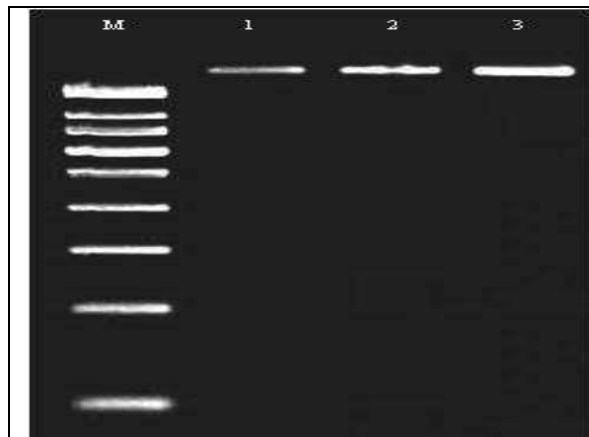


Figure 1: Genomic DNA isolated from orchid leaves for *Vanda ampullacea* var. auranticum, *Vanda testacea* and Vanda PB Mazumder in 1% agarose gel electrophoresis (Lane M- 1 kb ladder, 1- *Vanda testacea*, 2- *Vanda ampullacea* var. auranticum, 3- Vanda PB Mazumder)

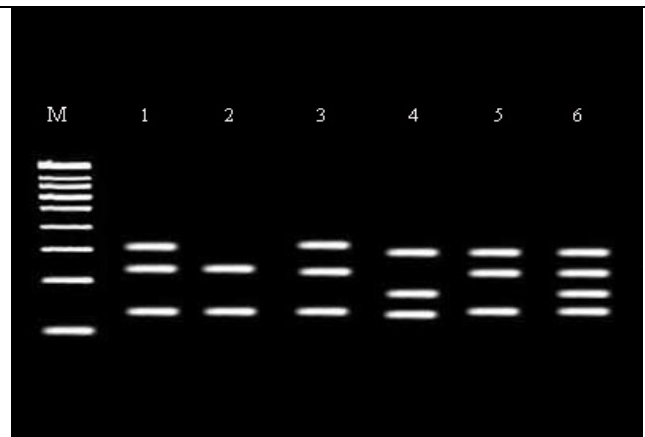
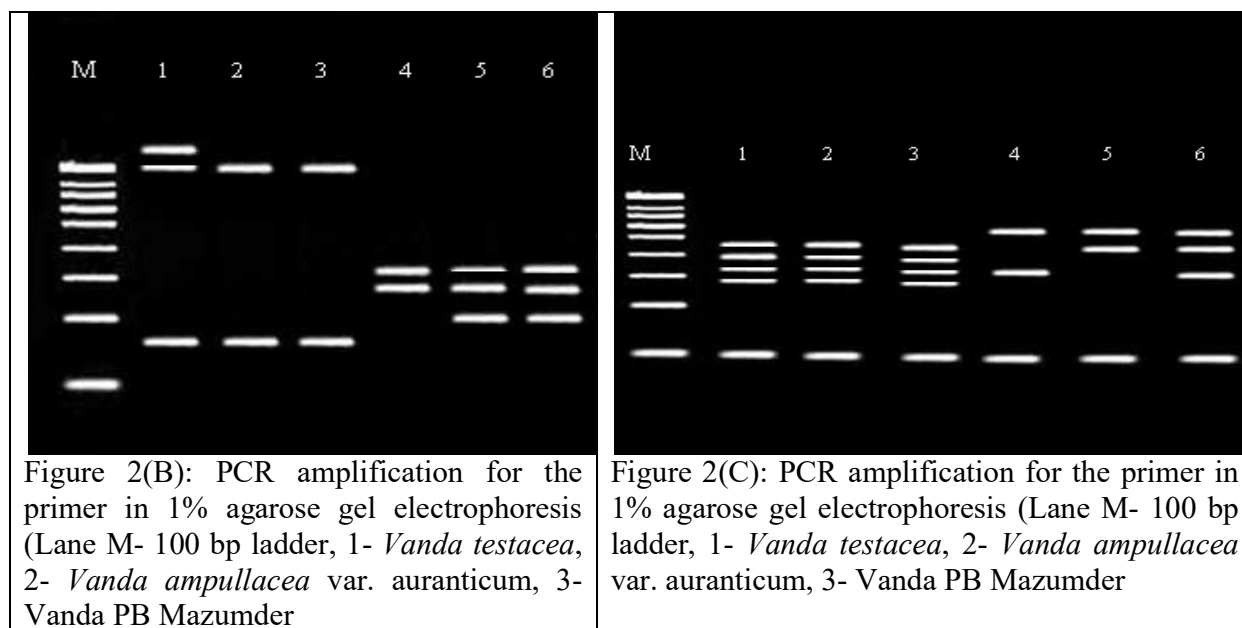


Figure 2(A): PCR amplification for the primer 5'CTGAGGTCTC3', 5'ACCTTCGGAC3' in 1% agarose gel electrophoresis (Lane M- 100 bp ladder, 1- *Vanda testacea*, 2- *Vanda ampullacea* var. auranticum, 3- Vanda PB Mazumder)



Abbreviations

RAPD: Random amplified polymorphic DNA; ISSR: Inter Simple sequence repeats; SSR: Simple sequence repeats; BP: Base pair; M: DNA ladder; PCR: Polymerase chain reaction; CAPS: Cleaved amplified polymorphic sequences; STS: Sequence tagged sites; nrITS: Nuclear ribosomal internal transcribed spacer; AFLP: Amplified fragment length polymorphism; CTAB: Cetyltrimethylammonium bromide, TBE: Tris-borate-ethylenediamine tetraacetic acid; HCl: Hydrochloric acid; KCl: Potassium Chloride; MgCl₂: Magnesium Chloride; dNTPs: Deoxynucleotide triphosphates; PVP: Polyvinylpyrrolidone; DNA: Deoxyribonucleic acid; mM: Millimolar.

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Author contributions

JH planned and performed the experiment the experiment, analysis and is the main author of the manuscript. PBM designed the overall study, is the major contributor in the present work.

Availability of data and materials

All data are provided in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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