International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 05 (C); May 2019; Page No.18710-18713
DOI: http://dx.doi.org/10.24327/ijcar.2019.18713.3585

Research Article

OPTIMIZATION AND COMPARATIVE EVALUATION OF DNA ISOLATION METHOD FOR BOSWELLIA SERRATA ROXB

Shashank Mahesh[1], Deepak Mishra[1] and Pramod Kumar[2*]

[1]AKS University, Satna (M.P.)-India
[2]Genetics and Plant Propagation Division, Tropical Forest Research Institute, Jabalpur- 482 021, India

A R T I C L E I N F O

Article History:
Received 12th February, 2019
Received in revised form 23rd March, 2019
Accepted 7th April, 2019
Published online 28th May, 2019

Key words:
Extraction, Polymerase Chain Reaction, Amplification, RAPD, ISSR

A B S T R A C T

A simple, rapid and economical method for isolating high quality DNA free from polyphenols and gum-resin is optimized for *Boswellia serrata*. The method significantly enhances the quality of DNA as a high purity ratio (1.73) was obtained. Minimized contamination of secondary metabolites yielded PCR amplification even after long duration storage. The method involves a modified SDS based extraction employing polyvinyl pyrrolidone (PVP) and require comparatively lesser time for DNA extraction from many samples per day than other published protocols. DNA isolated was used for random amplification of polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) assay. Amplification results of DNA ensure amenability of optimized method for molecular level studies in this species.

INTRODUCTION

Boswellia serrata Roxb. (Family- Burseraceae) is a tropical dry deciduous forest species once found abundantly throughout central India. Species is known for excellent quality of pulp, longer fiber length and premium olio gum resin content (AMMON *et al*. 1993). Besides, species has been over exploited because of its potential use in various pharmaceutical preparations. In present scenario, fragmented population of this plant species is available in Madhya Pradesh and Chhattisgarh in India and may be under threat of endangered if such alarming situations continues. Therefore, conservation studies are necessary and analyzing its genetic diversity is one of the prime needs of researchers. Studies at genomic level requires quantity, quality and purity of DNA but presence of high amount of polysaccharides in the form of gum and resin hampers quality and purity of DNA in this species.

The presence of polysaccharides and polyphenols in plant tissues require long and tedious extraction procedures and often do not result in good amplification products. Therefore, a rapid, economical and efficient procedure of DNA extraction for plants having high polysaccharides and polyphenols is necessary for genomic level studies based on PCR and restriction-based techniques. Although, there are large number of DNA extraction protocols but these are time consuming and require expensive reagents and equipments also.

Corresponding author:* **Pramod Kumar
Genetics and Plant Propagation Division, Tropical Forest Research Institute, Jabalpur- 482 021, India

Recently, SHARMA and PUROHIT (2012) developed a method for DNA extraction in *Boswellia serrata* which is also comparatively expensive and requires long time. Present study is a comparison of five known methods used for DNA extraction in gum/resin bearing species and among these the method M5 (DESHMUKH *et al*. 2007) was optimized with modifications for our studies. The modified method is rapid and economical in removal of gum and resin and produces good quality and purity of DNA.

MATERIAL AND METHODS

Five trees of *Boswellia serrata* of age about 20 years were randomly selected from Institute's campus. Mature leaves were collected in ice box and brought to the laboratory and kept in deep freezer at -20˚C. Three replicates each of 1g quantity from leaf samples of each tree were maintained and subjected to DNA extraction. Five methods i.e. M1 (POREBSKI *et al*. 1997), M2 (ALJANABI *et al*. 1999), M3 (HAMEED *et al*. 2004), M4 (PADMALATA, PRASAD 2006) and M5 (DESHMUKH *et al*. 2007) and modified procedure- M6 optimized in our laboratory were employed for the comparative assessment of quantity, quality and purity of the extracted DNA. Quality of the extracted DNA using all these methods was checked by measuring the ratio at two absorbance i.e. 260/280 nm and by agarose gel electrophoresis using 1% agarose. Optimized procedure is as follows:

Chemicals and Reagents

- ✓ Wash buffer: 100 mM HEPES, 0.25% (w/v) polyvinylpyrrolidone, 10% (v/v) β-mercaptoethanol.

- ✓ Extraction buffer: sucrose 15% (w/v), 50 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 500 mM NaCl.
- ✓ Resuspension buffer: 20 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0).
- ✓ TE Buffer: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0).
- ✓ RNAs: 10 µg/ml
- ✓ Proteinase K: 20 µg/ml

Modified DNA isolation method

Grinded 1 g of fresh leaves in liquid N_2. Transferred leaf powder to 30 ml centrifuge tube and added 5.0ml of wash buffer. Centrifuged at 14,000x g for 3 min after vortexing the sample. Supernatant containing polyphenols and gum-resin removed by repeated use of wash buffer for 6 to 7 times.

Added 5.0 ml of extraction buffer to the precipitant and centrifuged at 12,000x g for 5 min. Supernatant removed and added 4.0 ml of resuspension buffer to the precipitant along with 2.0 ml of 10% SDS, vortexing of the sample for 1-2 min. for proper mixing and incubated at 70ºC for 30 min.

Sample cooled at room temperature and added 1.5 ml of 7.5 M Ammonium acetate. Placed the sample on ice for 30 min.

Centrifugation at 14,000x g for 15 min. Transferred the upper clear aqueous layer to another 30 ml centrifuge tube.

Added equal amount of ice-cold isopropanol (-20˚C), mixed gently and centrifuged for 15 min at 13,000x g for 15 min.

Discarded the supernatant and washed the pellet twice with 2.0ml of 70% ethanol by centrifugation at 14,000x g for 5 min. Pellet dried after discarding ethanol and dissolved in 1.0 ml TE buffer. Added 100 µl of RNase (10 µg/ml) and incubated at 37ºC for 1 h. Added 100 µl of Proteinase K (20 µg/ml) and incubated at 37ºC for 1 h.

Transferred the aqueous solution in 1.5 ml centrifuge tube and added equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). Centrifuged at 12,000x g for 10 min.

Transferred the aqueous layer to a fresh 1.5 ml centrifuge tube and added 2 volumes of ice-cold ethanol (-20˚C) and mixed gently. Centrifuged at 12,000x g for 15 min at room temperature for sedimenting the pellet.

Washed the DNA pellet 2 to 3 times with 2.0 ml of 70% ethanol. Dried pellet dissolved in 300 µl of TE buffer and stored at -20ºC for further use.

Gel Electrophoresis

The quality of DNA was checked by 1% agarose gel electrophoresis. DNA sample (2 µl) was mixed with bromophenol blue dye in a ratio of 1:2 and subjected to electrophoresis in TBE buffer (0.5x) for 1 h at 100 V. Gels were visualized under UV light (Fig. 1) using Gel Documentation System (Alpha Innotech, USA).

DNA Quantification and PCR Amplification

DNA concentration was quantified in a UV-vis spectrophotometer (GBC, Australia) by measuring the absorbance at 260 and the purity was checked by the absorbance ratio of 260nm/280 nm. The extracted genomic DNAs of *B. serrata* trees from the modified method (M6) were allowed for the amplification using RAPD and ISSR primers (IDT-USA) after storage of more than 24 months.

RAPD reaction

Extracted genomic DNA samples were checked for their amplification using RAPD primer OPAW-04 by applying modifications in method (WILLIAMS *et al*. 1990) and followed by NARAYANAN *et al*. (2007). Each 25 µl of reaction volume contained 1x PCR buffer (Promega-Green GoTaq®-43.4% water, sucrose 25-50%, Tartrazine <1.00%, pH 8.5), 0.2 mM dNTP mix (Promega- USA), 2.5 mM $MgCl_2$ (Promega- USA), 1 unit Taq polymerase (Promega- USA), 20 picomoles of decamer primers with 20 ng of genomic DNA samples. The thermo-cycler (Eppendorf- USA) was programmed for an initial denaturation step of 4 min at 94˚C, followed by 40 cycles of denaturation for 1 min at 94˚C, annealing for 1 min at 37˚C, extension for 2 min at 72˚C and final extension for 7 min at 72 ˚C with a hold temperature of 4°C at the end. PCR products were observed on 1.5% agarose gel containing 0.5µg/ml ethidium bromide using 0.5x TBE buffer under the electrophoretic condition (SCIE-PLAS-UK) of 100 volt for 3 hrs and O'Gene Ruler 1 kb Plus DNA Ladder (Fermentas). Separated fragments were checked (Fig. 2) under gel documentation system.

ISSR reaction

DNA samples were also amplified through ISSR primer UBC-845. PCR was done by using modifications in method (WILLIAMS *et al*. 1990) and followed by NARAYANAN *et al*. (2007). Each 10 µl of reaction volume contained 1x PCR buffer (Promega-Green GoTaq®-43.4% water, sucrose 25-50%, Tartrazine <1.00%, pH 8.5), 0.1 mM dNTP mix, 2.5 mM $MgCl_2$, 1 unit Taq polymerase, 0.8 µM of primers with 20 ng of genomic DNA samples. The thermo-cycler was programmed for an initial denaturation step of 3 min at 94˚C, followed by 35 cycles of denaturation for 30 sec at 94˚C, annealing for 30 sec at 50˚C, extension for 1 min at 72˚C and final extension for 10 min at 72 ˚C with a hold temperature of 4°C at the end. PCR products were observed on 1.5% agarose gel containing 0.5µg/ml ethidium bromide using 0.5x TBE buffer under the electrophoretic condition of 100 volt for 3 hrs and O'GeneRuler 1 kb Plus DNA Ladder. Separated fragments were checked (Fig. 3) under gel documentation system.

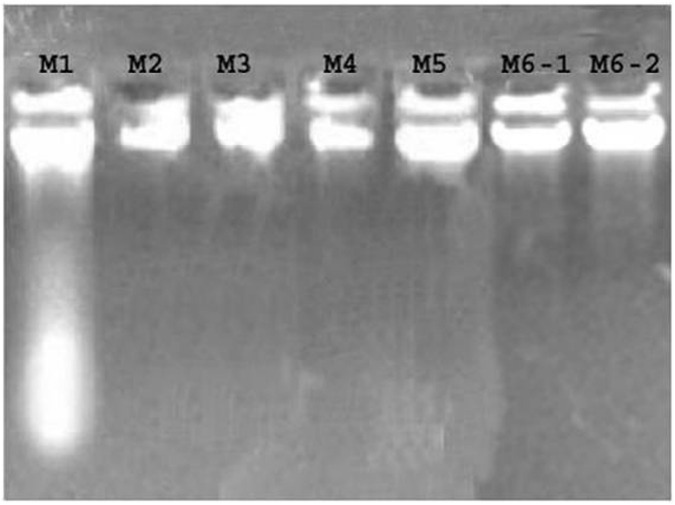

Fig 1 Isolated genomic DNA of *Boswellia serrata* using M1 to M6 (M6-1, M6-2) methods on 1% agarose gel

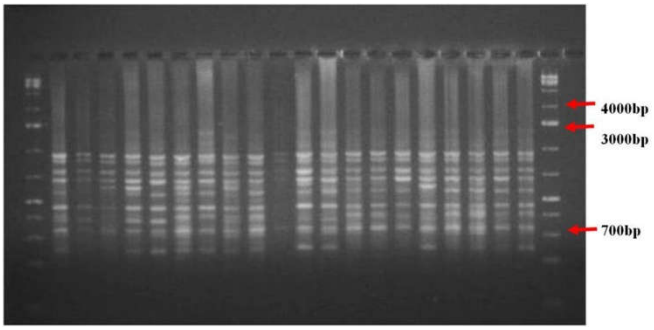

Fig 2 Amplification of 20 DNA samples of *Boswellia serrata* using RAPD primer OPAW-04

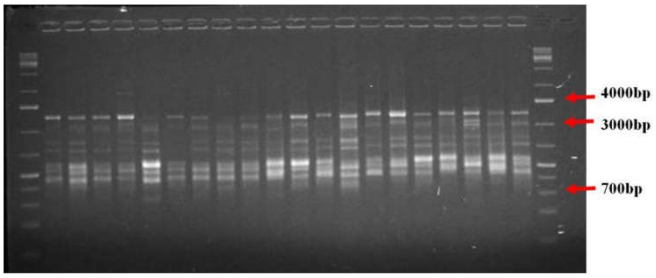

Fig 3 Amplification of 20 DNA samples of *Boswellia serrata* using ISSR primer UBC-845

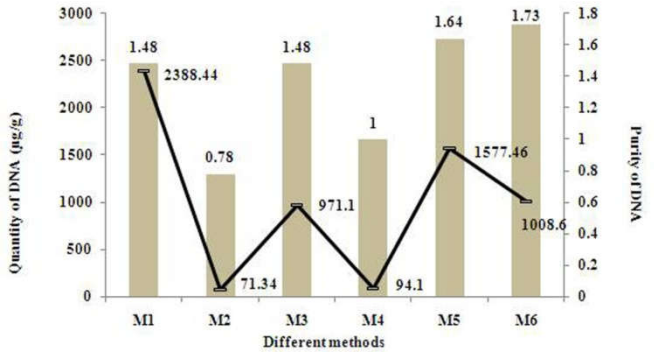

Fig 4 Quantity and quality of isolated genomic DNA using M1 to M6 methods in *B. serrata*

RESULTS AND DISCUSSION

Comparative efficiency of these methods in *Boswellia serrata* is depicted in Fig. 4. Though, the yield of genomic DNA in our optimized method (M6) is less than the two other methods (M1 and M5) but the quality and purity was exceptionally good and high than all other methods. Highest DNA yield (2388.44 µg/g) was obtained by using the CTAB method (POREBSKI *et al*. 1997) with a high concentration of NaCl followed by the unmodified method (DESHMUKH *et al*. 2007) (1577.46 µg/g) and our optimized method (1008.6 µg/g). However, the purity index was highest (1.73) in DNA obtained through our optimized method which becomes possible due to repeated washing.

Cells are usually ruptured by mechanical force during DNA extraction. Grinding of the plant tissues in the presence of liquid nitrogen freezes the tissues rapidly and thus allows fine grinding. The disrupted plant cells are lysed and extracted with a suitable buffer which contains a detergent such as CTAB (cetyl trimethyl ammonium bromide) or SDS (sodium dodecyle sulphate). Heat treatment is often given at this stage to completely lyse the plant cells. And after that it is extracted once with chloroform which dissolves most of the impurities like protein, carbohydrate, cell debris etc. Nucleic acids present in aqueous phase may be separated by centrifugation. DNA can be precipitated by addition of ethanol/isopropanol and can be spooled out using a glass rod. Spooled DNA can be taken in to fresh tube and then it is dissolved in TE buffer and quantitated (KUMAR *et al*. 2002). However, it is difficult to deliver reliable quantity and purity of DNA for molecular analysis of a forest tree species. The same is true with the *Boswellia serrata*, which contains a high level of polysaccharides, gums and resins (SHARMA, PUROHIT 2012).

Among the five methods tested for comparison, the CTAB based method M1 yielded DNA with impurities of RNA and require more time. Electrophoresis formed smear thus indicating degradation also. Another CTAB method M2 yielded low quantity viscous DNA containing gum-resin. M3 (SDS based method) also yielded yellowish viscous DNA with incomplete removal of gum-resin. CTAB based method M4 require more time and the extracted DNA was in low quantity, brownish colored and without smooth pellet formation. DNA extracted through M5, a SDS based method, exhibit incomplete removal of secondary metabolites and thus, not suitable for long storage (Fig. 1).

All these methods were selected on the basis SDS or CTAB detergent with their extraction component which differ in their concentration. These methods either incompletely remove gum-resin or require more time. Therefore, necessary modifications viz. repeated washing with wash buffer with increased concentration of PVP and β-mercaptoethanol, increased time period for incubation and ammonium acetate treatment, aqueous layer separation and treatment with changed ratio of phenol: chloroform: isoamyl alcohol (25:24:1) and treatment with Proteinase K for degradation of proteins were made and the modified method M6 developed which yielded high quality white translucent DNA free from protein, RNA and gum-resin suitable for PCR amplification. Thus, the optimized method M6 was selected and used for further DNA extraction and molecular assay in this species. Besides, the method is simple, rapid and economical so that large number of samples may be extracted for DNA in short span and the extracted DNA may be analysed for molecular studies even after long storage of 24 months without degradation of its quality.

Acknowledgement

Financial support received from the Indian Council of Forestry Research and Education, Dehradun is gratefully acknowledged.

References

Aljanabi S.M., Forget L., Dookum M. A. (1999): An improved and rapid protocol for the isolation of Polysaccharide- and Polyphenol-free sugarcane DNA. Plant Moleclar Biology Reporter, 17: 1-8.

Ammon H.P., Safayi H., Mack T., Sabieraj J. (1993): Mechanism of anti inflammatory actions of curcumine and boswellic acids. *Journal of Ethnopharmacology*, 38: 113-119.

Deshmukh V.P., Thakare P.V., Chaturvedi U.S., Gawande P.A. (2007): A simple method for isolation of genomic DNA from fresh and dry leaves of *Terminalia arjuna* (Roxb.) Wight and Argot. *Electronic Journal of Biotechnology,* 10: 468-472.

Hameed A., Malik S.A., Iqbal N., Arshad R., Farooq S. (2004): A rapid (100 min) method for isolating high yield and quality DNA from leaves, roots and coleoptile of wheat (*Triticum aestivum* L.) suitable for apoptotic and other molecular studies. *International Journal of Agriculture Biology*, 6: 383-387.

Kumar A., Pandey D., Taj G., Garg G.K. (2002): Techniques in Molecular Biology, GB Pant University of Agriculture and Technology, Pantnagar, India.

Narayanan C., Wali S.A., Shukla N., Kumar R., Mandal A.K., Ansari S.A. (2007): RAPD and ISSR markers for molecular characterization of teak (*Tectona grandis*) plus trees. *Journal of Tropical Forest Science*, 19: 218-225.

Padmalata K., Prasad M.N.V. (2006): Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal of Biotechnology*, 5: 230-234.

Porebski S., Bailey L.G., Bernard R.B. (1997): Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter, 15: 8-15.

Sharma A.P., Purohit S.D. (2012): An improved method of DNA isolation from polysaccharide rich leaves of *Boswellia serrata* Roxb. *Indian Journal of Biotechnology*, 11: 67-71.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531-6535.

How to cite this article:

Shashank Mahesh, Deepak Mishra and Pramod Kumar (2019) 'Optimization and Comparative Evaluation of Dna Isolation Method for Boswellia Serrata Roxb', *International Journal of Current Advanced Research,* 08(05), pp. 18710-18713.
DOI: http://dx.doi.org/10.24327/ijcar.2019.18713.3585

****