DNA extraction from resin producing Boswellia tree

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Method Article

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Abstract

The wild nature of plant and the presence of the secondary compounds e.g., Resin and phenolic compound make DNA extraction problematic. Some of them will co-precipitate with DNA during extraction and inhibit further enzymatic modification of the DNA. Furthermore large amounts of complex polysaccharides also make extraction of usable DNA impossible. Previously reported protocols yielded highly viscous and slimy DNA preparations that were not amenable to further analysis. We tried many commonly used protocols but were unable to isolate high quality DNA from the gum containing plant Bosweliia sacra. We did extensive optimization of the Cetyl Trimethyl Ammonium Bromide \(CTAB) protocol established by Doyle in 1987 to extract DNA which is feasible for PCR amplification. In this study, we have specifically tested the previously developed CTAB based protocols developed by I Haque adapted for Commiphora wightii and Bipin Deochand Lade protocol developed for Passiflora foetida. Both are developed for plants, which contain high amount of secondary metabolites, polysaccharides and phenolic compounds. Furthermore, we investigate the possibility to extract DNA from resin producing plants which is feasible for downstream application. To our knowledge, this is the first optimized protocol which a rapid and less laborious method for the extraction of DNA from Boswellia species \(Boswellia sacra and Boswellia elongata found in Oman and Yemen respectively) which are resin \(Luban) producing plant. The isolated DNA by the currently optimized protocol were suitable for polymerase chain reaction \(PCR) mediated amplification for applications genetic diversity and DNA barcoding.

Reagents

Requirement Reagents Extraction buffer consisted of 100 mM Tris-HCL \(PH 7.5), 25 mM EDTA, 1.5 M NaCl, 2.5% \(w/v) CTAB, 1.5% PVP, 0.5% L. ascorbic acid, 0.5% BSA and 0.5% \(v/v) β-mercaptoethanol). 95% ethanol \(v/v) 70% ethanol \(v/v) TE buffer \(10 mM Tris. HCl pH 8.0; 1 mM EDTA pH 8.0). Autoclave distal water

Equipment

Equipment Mortar and pestle Water baths or incubators \(65°C and 37°C). Centrifuge \(OHAUS, Model: FC5718R) Gel electrophoresis system Qubit 3 Fluorometer \(Invitrogen, Serial No. 2321609764) -20°C freeze

Procedure

Procedure Sample preparation After collection the fresh leaves sample should be immediately frozen in liquid nitrogen and until further analysis. Before starting DNA extraction take the sample out from liquid nitrogen or -80 and by pressing the frozen leaves it well chopped into small pieces. Put all the leaves material in the beaker containing autoclave distal water around 200 ml. Shake the beaker for 1 minute and the then keep it for 2 to 3 minutes so the midribs of the leaves will set down. Transfer the leaves parts into another beaker which containing same amount of autoclave water and keep it for four hours at

4°C and change the water after each hour. After this, take the leaves out from water and make it air dry for minutes. Grind the leaves into a fine powder in cool mortar and pestle with the help of liquid nitrogen. Take all the powder in 50ml falcon tube and immediately stored at -80°C until further analysis. Preparation of DNA extraction buffer The Optimized extraction buffer consisted of 100 mM Tris-HCL \(PH 7.5), 25 mM EDTA, 1.5 M NaCl, 2.5% \(w/v) CTAB, 1.5% PVP, 0.5% L. ascorbic acid, 0.5% BSA and 0.5% \(v/v) β-mercaptoethanol). Firstly, the buffer containing 100 mM Tris-HCL \(PH 7.5), 25 mM EDTA and 1.5 M is to be autoclaved and stored at room temperature \(24°C). Just before use, 2.5% \(w/v) CTAB, 1.5% PVP, 0.5% L. ascorbic acid, 0.5% BSA and 0.5% \(v/v) β-mercaptoethanol) is added to the pre-quantified volume of the buffer \(for each 10ml Extraction should be prepared) and was warmed to 65°C in autoclaved bottles. CTAB, 2.5% w/v and PVP, 3.0% w/v were added and allowed to dissolve by gentle intermittent swirling. Steps of DNA extraction process 1. Take one 1 gram of frozen leaf tissue \(powder already prepared) in a new 50 mL Falcon tube directly and mixed in the pre-heated extraction buffer 10 ml for one sample. At this stage, if the sample is not grinded well previously in the liquid nitrogen can be grind again in mortar and pestle and recover everything again in 50 ml falcon tube. 2. If in step one the sample is not grinded with mortar and pestle then vortex the falcon tube for 5 minutes otherwise proceed to step 3. 3. The sample was put into the 65°C incubator or water bath and mix by inversion after every 10 min for 45 min. After incubation placed the tube at room temperature for five minutes. Centrifuge the 50ml falcon tube for 5 minutes at 5000 × g on room temperature. Transfer 1ml of supernatant to each 2ml tube already containing 1 ml of chloroform: isoamyl alcohol. Mix supernatant and chloroform: isoamyl alcohol by inversion for 10 minutes and subsequently placed the tube on ice for 10 minute. 4. Centrifuge the tube for 10 minute at 5000 × g at 4°C. Transfer the upper aqueous phase into new 2ml tubes and add 5µl RNAse A \(10 mg/mL). Placed the tube for 30 minutes at 37°C. After RNAse A treatment add one volume of chloroform: isoamyl alcohol again and mix by inversion for 5 minutes. The tubes is centrifuged for 10 minute at 5000 × g at 4°C. Transfer the clear supernatant into new 2ml tube and add half volume of 5 M NaCl to the sample and mix gently by inversion. Then, add 2 volumes of cold 95% ethanol and mix gently by inversion. The tubes are incubated at for 45 minutes \(should not be more than 1 hour because after long incubation the resin is precipitated with DNA) in -20°C freezer. 5. After incubation, the tubes were centrifuged at 5000 × g for 10 minute at 4°C and the supernatant was gently poured off. The pellets were washed two times with 1ml of 70% ethanol and pellet the DNA by 5000 × g at 4°C but for only 5 min. The supernatant was discarded and the pellets were air-dried \(10 minute). The pellets were allowed to re-suspend in 50 µL of TE \(10 mM Tris. HCl pH 8.0; 1 mM EDTA pH 8.0). DNA extracted by the current protocol The comparison of the extracted genomic DNA by the current protocol with already published protocols as mentioned in abstract section. For analyzing, the quality and quantity was measured using agarose gel electrophoresis \(0.1% agarose) and Qubit 3 Fluorometer, respectively in Table 1 and Fig. 1. Briefly, the concentration of extracted DNA B. sacra and B. elongata was 71.046 µg g-1 leaf and 51.05 µg g-1 leaf respectively. The extracted DNA concentration for B. sacra and B. elongata were 2.51 µg g-1 leaf and 0.62 µg g-1 and 5.09 µg g-1 leaf and 2.4 µg g-1 leaf based the previously published protocols of Bipin Deochand Lade and I. Haque respectively. As shown in Fig. 1, the analysis of the extracted DNA on 1% agarose gel electrophoresis appeared high quality due to the single and pure band.

Troubleshooting

Possible Troubleshooting: mandatory points to be considered and recommendation 1. If the quality of the extracted DNA is good or seems degraded, the possible reason is that the starting material was not handle carefully and the DNA is degraded by nucleases or due to repeated thawing of the powder sample. To avoid the problem proper handling and recommendation for sample preparation should follow. 2. The other possible reason could be during or post extraction of the DNA, DNA can be degraded by extensive pipetting for mix or transferring the supernatant and storing of DNA for long period in water after extraction. The problems can be overcome by using the cut tips and storing the DNA after extraction in in multiple tubes to avoid the repeating thawing of whole DNA for each experiment. 3. If the problem occur with regard of the concentration of the DNA, there are many a number of possible reasons. The ration of the extraction buffer and sample is very important, by increasing the sample the yield is not increase but the resin so it is recommended to use maximum up 1.5 gram of sample not more than this. 4. The other possible reason which can negatively affect the quantity of the DNA is the initial sample grinding, if the sample grinded poorly the less will be the quantity. So for high quantity DNA isolation proper grinding is mandatory. 5. If the DNA failed to proceed with downstream processing e.g., not amplifying by PCR. The possible reason may the contamination of DNA with resin. In this case the resulting is of whitish color and viscous. To avoid the problem care should be taken during taking supernatant after phase separation. Don’t take whole upper phase because there is more chance of taking the middle fatty acid and resin containing part.

Anticipated Results

Conclusion In the current study the optimized DNA extraction protocol allowed us to obtain a high quality DNA from resin producing plants. The quality of extracted DNA was sufficient to perform amplification of a specific gene using PCR technique. This techniques will contribute to the molecular biology of resin producing plants. DNA extraction method Plant species DNA concentration \(ng/µl) DNA concentration \ (µg g-1 leaf) Bipin Deochand Lade protocol developed for Passiflora foetida B.sacra 12.55 2.51 B. elongata 3.36 0.672 I. Haque protocol for C. wightii B.sacra 25.45 5.09 B. elongata 12.00 2.4 Current protocol established for Boswellia species B.sacra 355.23 71.046 B. elongata 255.25 51.05

References

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Figures

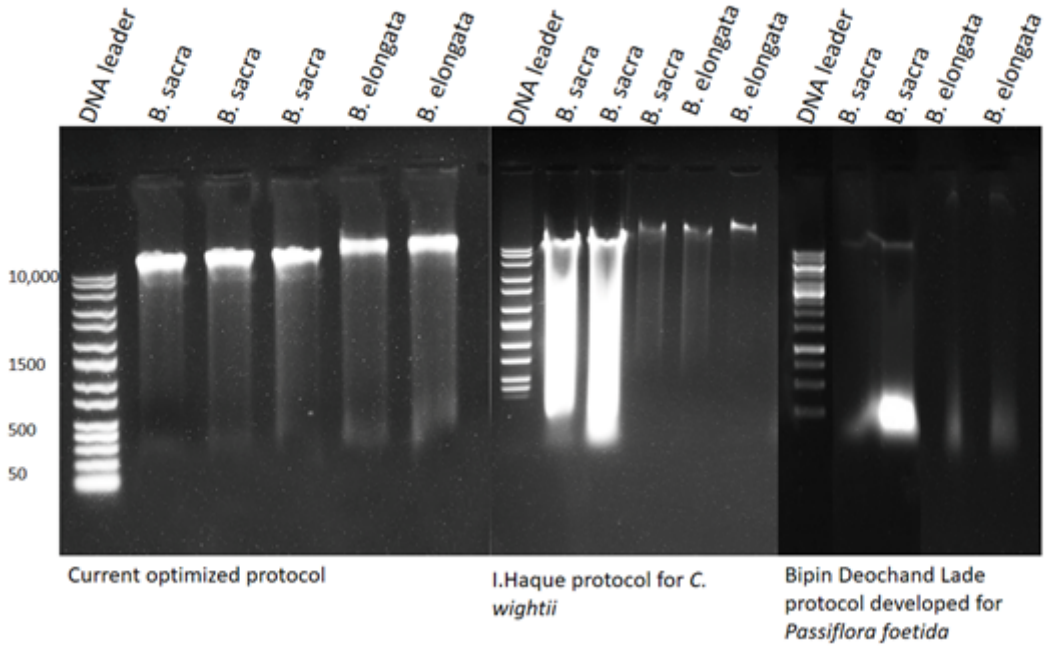

Figure 1

Gel electrophoresis of the extracted DNA by three different protocols including current optimized protocol.

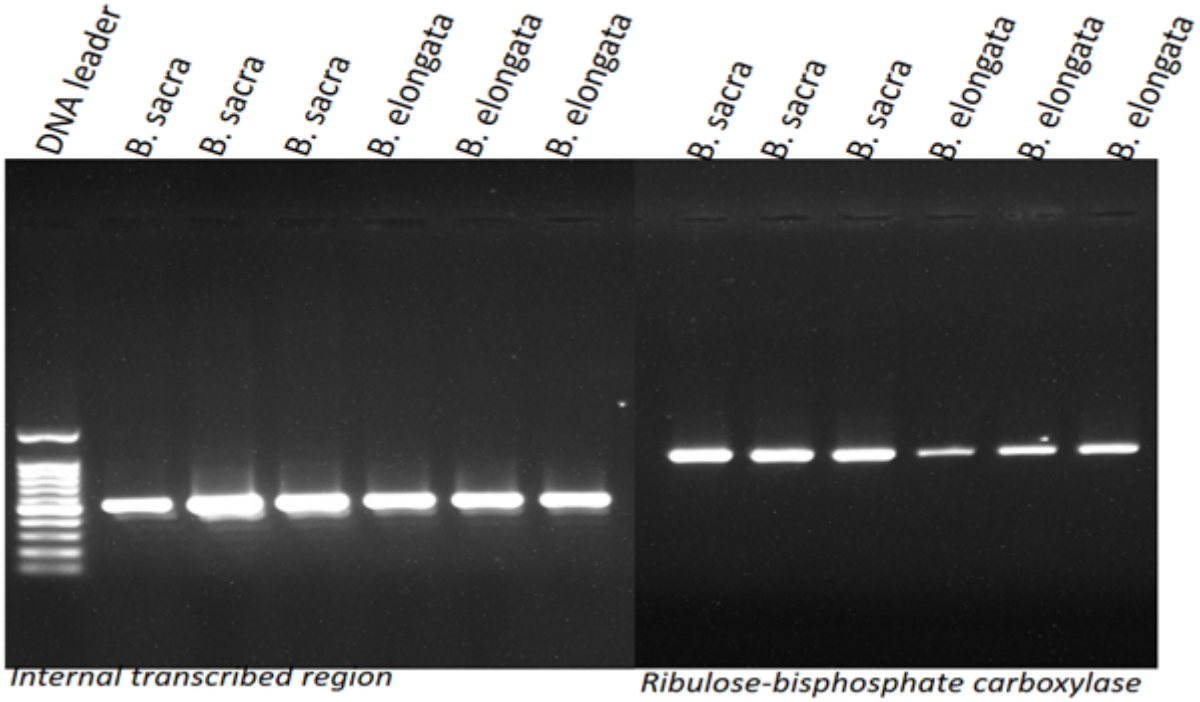

Figure 2

PCR amplification of the extracted DNA

Supplementary Files

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