DNA extraction from resin producing Boswellia tree

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Method Article

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Abstract

Wild and medicinal plants producing resin and ladle with phenolic and polysaccharides have been found a major hurdle to extract high quality of genomic DNA. These contaminants co -precipitate with DNA and inhibit enzymatic modification of DNA. Previously reported protocols yielded highly viscous DNA regimen that were not amicable to down-stream analysis. We tried several commonly used protocols but were unable to isolate high quality DNA from frankincense producing *Boswellia sacra*. We extensively optimized the Cetyl Trimethyl Ammonium Bromide (CTAB) based protocol used for *Commiphora wightii* and *Passiflora foetida* previously. The current methos involves changes in buffer conditions, handling methods, and cycles of steps to remove resin at earlier stages of extracton. The obtained gDNA can be used for genetic diversity, DNA barcoding and next generation sequencing approaches. To our knowledge, this is the first optimized protocol which a rapid and less laborious for the extraction of DNA from *Boswellia* species.

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).

Refrigerated Centrifuge (OHAUS, Model: FC5718R)

Gel electrophoresis system

Qubit 3 Fluorometer (Invitrogen, Serial No. 2321609764)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

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Consumable

Gloves

50 ml falcon tubes

Liquid nitrogen

Polyvinylpyrrolidone (PVP) (Sigma Aldrich, Cat No. 9003398)

Chloroform: isoamyl alcohol (Sigma Aldrich, Cat No. STBF5776V)

Ethylenediaminetetraacetic acid (EDTA) (DEAJUNG, Cat No. 40004405)

Tris base (Fisher Bioreagent, Cat No. 77861)

Tris Hydrochloride (Fisher Bioreagent, Cat No. 1185531)

Sodium Chloride (NaCl) (DEAJUNG, Cat No. 75484400)

L-Ascorbic acid (Fisher Bioreagent, Cat No. 50817)

Hexadecyltrimethylammonium bromide (CTAB) (Sigma Aldrich, Cat No. 101989078)

β- mercaptoethanol (βME) (DEAJUNG, Cat No. 55364404)

Agarose A (Bio Basic Canada INC., Cat NO. J7017150PA)

RNAse A (Sigma Aldrich, Cat No. 1002428135)

Bovin Serum Albumin (BSA; Sigma Aldrich, Cat No. A2058-1G)

Procedure

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Sample preparation

The fresh young leaf samples with lesser signs of necrosis and chlorophyll accumulation must be collected. Depending upon the distance from field to Lab, either liquid nitrogen or ice packs (4°C) must be used for shipping. In both the cases, the leaves are chopped into smaller pieces ($5mm^2$) removing the major veins and mid-ribs with a sharp sterilized blade (autoclave 121°C, 20 min) on ice in a glass petri-dish ($90x100mm^2$). Use autoclaved distilled water (ADW; chilled at 2°C) during chopping and keep it at 4°C for 3 hrs. The ADW must be changed after every 1 hrs to remove the resin oozed out from leaf parts. Maintain this process in dark to consume the starch inside leaf as well. This is followed by drying the chopped leaves on sterilized filter paper and immediate grinding in liquid nitrogen to make a fine powder. Notice a change in color from green to off-white during grinding in 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 gram of frozen finely powdered leaf tissues in a new 50 mL Falcon tube and mixed with the pre-heated extraction buffer (10 ml) for one sample. Breifly, vertex for 30sec to ensure leaf material is fully mixed with buffer. Fine grind is a key to obtaining high DNA quantity with lesser artifacts of resin.

2. If in step one the sample is not grinded with mortar and pestle then vortex the falcon tube for 5 min otherwise proceed to step 3.

3. The falcon tube was kept into the 65°C incubator or water bath and mix gently by inversion after every 10 min till 45 min**.** After incubation, place the tube at room temperature for five min to reach to room temperature environment. Centrifuge the 50ml falcon tube for 5 min at 3000×g on room temperature. For next generation sequencing, the greater the genome size, lower is speed of initial centrifugation.

4. Transfer 1ml of supernatant to each 2ml Eppendorf tubes already containing 1 ml of chloroform: isoamyl alcohol (24:1). Mix supernatant and chloroform: isoamyl alcohol by gentle inversions for 10 min

and subsequently place the tube on ice for 10 min.

5. Centrifuge the tube for 10 min at 5000 × g at 4°C. Transfer the upper aqueous phase into new 2ml tubes and add 5µl RNAse A (10 mg/mL). Place the tube for 30 min at 37°C.

6. After RNAse A treatment, add one volume of chloroform: isoamyl alcohol again and mix by inversions for 5 min. The tube is centrifuged for 10 min 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 inversions.

7. Add 2 volumes of cold 95% ethanol and mix gently by inversion. The tubes are incubated at for 45 min in -20°C freezer. It should not be for more time as some remaining phenolics and resin may also precipitate with DNA.

8. After incubation, the tubes were centrifuged at 5000 × g for 10 min at 4°C and the supernatant was gently removed. The pellet is washed two times with 1ml of 70% ethanol and the DNA is pellet by 5000 × g at 4°C for only 5 min. The supernatant is discarded and the pellet is air-dried (10 min). The pellet are 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 protocol

The comparison of the extracted genomic DNA by the current protocol with already published protocols as mentioned in abstract section was performed. For analyzing, the quality and quantity agarose gel electrophoresis (0.1% agarose) and Qubit 3 Fluorometer were used, respectively (**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 samples, 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 on 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 not 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 occurs with regard of the concentration of the DNA, there are many a number of possible reasons. The ratio of the extraction buffer and sample is very important, by increasing the sample the yield is not increased but the resin so it is recommended to use maximum up 1.0 gram of sample and 10ml buffer.

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, contamination of DNA with resin is a possible reason. This is also visible through viscous DNA pallet that is difficult to pipette and mix. 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 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 technique will contribute to the molecular biology of resin producing plants.

References

Reference

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Lade, B. D., Patil, A. S., & Paikrao, H. M. (2014). Efficient genomic DNA extraction protocol from medicinal rich Passiflora foetida containing high level of polysaccharide and polyphenol. SpringerPlus, *3*(1), 457.

Figures

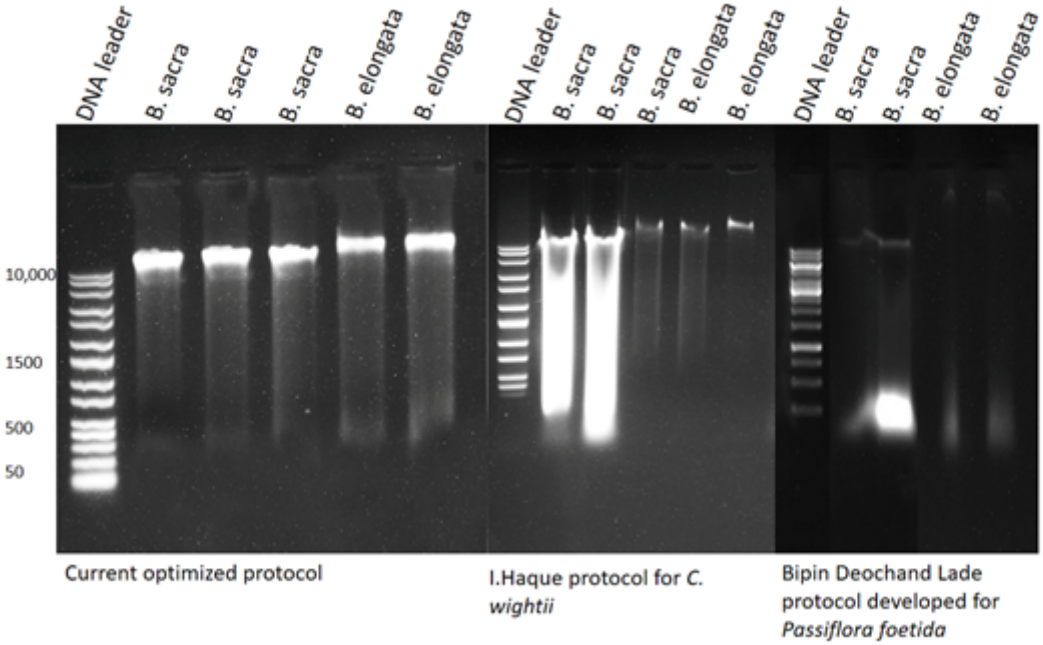

Figure 1

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

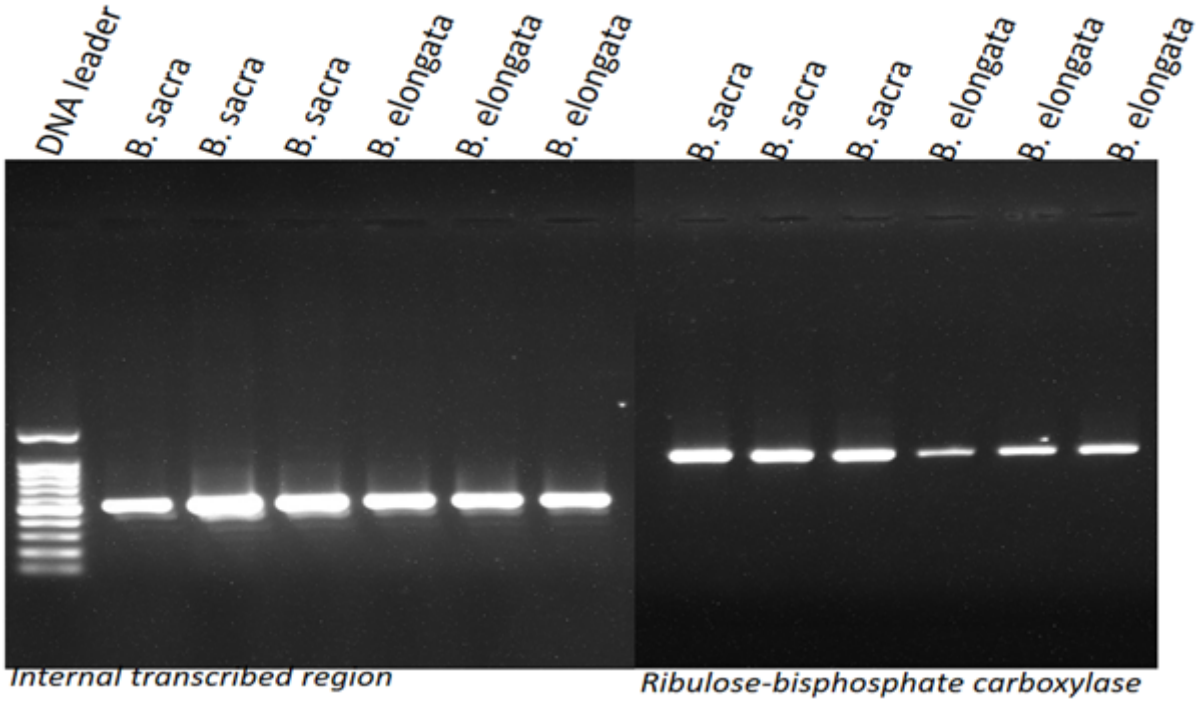

Figure 2

PCR amplification of the extracted DNA

Supplementary Files

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