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Extraction of High-quality Genomic DNA Using Species Tailored Protocol for *Prosopis cineraria (L.)* Druce (Khejri)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

To effectively conserve genetic diversity in forestry species, molecular methods are essential. These methods necessitate high-quality genomic DNA as a starting material which contains the complete genetic information of an organism, and by extracting it, scientists can access and analyze this information. This information is essential for developing effective conservation strategies and understanding the complex processes of evolution. The objective of this study was to develop a protocol for extracting High-Quality genomic DNA from the arid tree species *Prosopis cineraria*. The current extraction protocol is based upon the conventional cetyl trimethyl ammonium bromide (CTAB) method with further modifications for the extraction of DNA. The principle modifications currently employed for DNA extraction involved the use of higher concentration of

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CTAB and sodium chloride. Additionally, L-ascorbic acid and Polyvinylpyrrolidone (PVP) was also added. The incubation time was doubled and DNA was precepted in 100% ice-cold ethanol. Further a RNAse treatment was also included for 30 mins. The yield of DNA ranged from 630-4200ng/µl with average yield of 2041ng/µl, and the absorbance lied between 1.6-2.15 for A260/280 and 1.64-2.31 for A260/230, indicating minimal levels of contaminating metabolites. This method offers a simple, efficient, and cost-effective way to extract high-quality genomic DNA, making it ideal for large-scale genetic studies.

Keywords: Prosopis cineraria; DNA extraction; modified CTAB method; fresh leaf.

1. INTRODUCTION

Prosopis cineraria (family Fabaceae) constitute a very important natural resource for dry zones due to its multi-purpose nature (Pasiecznik et al., 2001). Locally known as Khejri, (Rani *et al* 2013) it is the lifeline of the Indian desert. (Prakash and Hocking, 1985) It is a source of pod, leaf fodder as well as fuel wood. In addition, the tree augments soil fertility through biological nitrogen fixation (Toky *et al* 2004). Multiple factors ranging from excessive lopping practice, receding ground-water levels, tractorization and a root borer – *Acanthophorus serraticornis* and *Ganoderma lucidum* (root rot) have led to reported mortality in the last two decades in its native habitat (Bhatnagar *et al* 2017). Understanding the current diversity status of forest genetic resources is essential before forest gene conservation program can be designed (Boshier and Young. 2000). Susceptibility to pressures towards extinction increases in a population or species which has many individuals but little genetic variation among them, than with genetically diverse species or population. Isolation of high-quality DNA is a crucial step in DNA molecular techniques used in plant studies for assessing genetic diversity (Tamari and Hinkley 2016). Various DNA extraction methods have been developed for plants (Doyle and Doyle 1990; Scott and Playford 1996; Sharma et al. 2000; Pirttilä et al. 2001; Shepherd et al. 2002; Mogg and Bond 2003). However, plant tissues often contain varying amounts of compounds like polysaccharides, polyphenols and other secondary metabolites that can hinder DNA extraction, (Healy *et al* 2014) especially in arid regions where the plants are under constant stress. Therefore, a species tailored protocol for extraction of high-quality genomic DNA is necessary for *Prosopis cineraria.* This study presents a modified CTAB method for efficient DNA isolation compared to the classic Doyle and Doyle (1990) protocol to assess DNA quality.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaf samples of *Prosopis cineraria* were collected from natural population of Jodhpur district with 30 genotypes from 5 different sites. The trees selected were healthy and free of any pests or diseases with a distance of at least 300m between each tree, the selection was random. The complete information regarding the geographical locations of sampling sites is given in Table 1.

2.2 Reagents

- 5× extraction buffer containing: 5% CTAB (w/v), 5 M NaCl, 1 M Tris-HCl pH 8.0, 0.5 M EDTA pH 8.0 (autoclaved)
- 0.2% 2-β-Mercaptoethanol.
- Chloroform:isoamyl alcohol (24:1 v/v).
- 2% PVP
- 0.8% ascorbic acid
- Ice cold 100% ethanol
- 70% ethanol
- 1× TE buffer (1M Tris-HCl, 0.5M EDTA, pH 8.0)
- Agarose (molecular grade)

2.3 Modified DNA Extraction Protocol

To extract genomic DNA from the leaves of *Prosopis cineraria*, these modifications involved the use of higher CTAB, and sodium chloride. Additionally, L-ascorbic acid and Polyvinylpyrrolidone (PVP) was also added. The incubation time was doubled and DNA was precepted in 100% ice-cold ethanol. Further a RNAse treatment was also included for 30 mins. The optimized protocol for total genomic DNA extraction, along with the specific modifications, is outlined below:

- 0.5gm ground leaf sample was incubated with 1 ml prewarmed extraction buffer

(0.8% ascorbic acid; 2% 2-mercaptoethanol; 2% PVP and 10ml CTAB 5X) at 65˚C for 1 hour with intermittent mixing.

- About 500 µl Chloroform: Isoamyl alcohol (24:1) was added, and the tubes were gently inverted to form a uniform emulsion.
- Samples were centrifuged at 10,000 rpm for 10 min at room temperature.
- The supernatant was collected in a fresh tube and again About 500 µl Chloroform: Isoamyl alcohol (24:1) was added and the tubes were gently inverted to form a uniform emulsion.
- Samples were centrifuged at 10,000 rpm for 10 min at room temperature.
- The supernatant was collected in a fresh tube and was given RNAse treatment at 37 ˚C for 30 mins
- An equal volume of 100% ethanol was added and the samples were incubated at -20 ˚C for 2 hours to precipitate the DNA.
- The precipitated DNA was pelleted down by centrifuging at 10,000 rpm for 5 min. at 4 ˚C.
- The pellet was first washed with 70% ethanol, dislodged and centrifuged at 10,000 rpm for 5 min. at 4 ˚C.
- The DNA pellet was then dried and re-suspended in 50 µl TE Buffer.

Table 1. Geographical location of 30 genotypes and their passport data

Sample No	Site	Height (m)	Girth (Cm)	Crown (m)	Latitude and longitude	Elevation (Ft)
01	Tulesar	12.2	180	8	N'26°18.624 E'72°40.516	616
02	Tulesar	8	95	6.2	N'26°18.565 E'72°40.589	602
03	Tulesar	7	105	4.5	N'26°18.551 E'72°40.644	605
04	Tulesar	8.5	95	5	N'26°18.603 E'72°40.643	608
05	Tulesar	8.2	90	4	N'26°18.670 E'72°40.600	609
06	Jhanwar	14	140	7.5	N'26°13.495 E'72°52.864	731
07	Jhanwar	12	110	6	N'26°13.552 E'72°52.866	785
08	Jhanwar	9	105	5.5	N'26°13.579 E'72°52.901	751
09	Jhanwar	6.5	80	4	N'26°13.457 E'72°52.867	744
10	Jhanwar	7.2	95	5	N'26°13.409 E'72°52.842	759
11	Jhanwar	13.3	122	7.1	N'26°13.050 E'72°05.461	708
12	Jhanwar	14	120	6.7	N'26°13.017 E'72°05.577	694
13	Jhanwar	6.8	93	4.3	N'26°13.012 E'72°05.626	698
14	Jhanwar	7.5	103	5.5	N'26°12.931 E'72°05.629	689
15	Jhanwar	7.2	88	5.2	N'26°12.941 E'72°05.551	690
16	Bhavi	7.5	94	6.3	N'26°13.927 E'72°35.319	901
17	Bhavi	6.4	64	4.7	N'26°13.896 E'72°35.278	894
18	Bhavi	11.3	115	7.4	N'26°13.953 E'72°35.270	893
19	Bhavi	7.8	74	5.3	N'26°13.935 E'72°35.176	890
20	Bhavi	7.3	126	6.1	N'26°13.989 E'72°35.124	877
21	Bhandu	8.1	152	5.7	N'26°05.168 E'72°53.047	572
22	Bhandu	7.5	101	5.2	N'26°05.119 E'72°53.443	583
23	Bhandu	6.3	80	6	N'26°05.038 E'72°53.436	600
24	Bhandu	4.4	30	3	N'26°04.930 E'72°53.458	598
25	Bhandu	5.6	71	5.8	N'26°04.405 E'72°53.440	611
26	Ummed Nagar	8.3	92	7.8	N'26°35.187 E'72°00.067	841
27	Ummed Nagar	10.7	115	9.3	N'26°35.199 E'72°00.035	842
28	Ummed Nagar	5.7	76	4.2	N'26°35.226 E'72°00.003	841
29	Ummed Nagar	6.5	79	4.7	N'26°35.882 E'72°00.852	819
30	Ummed Nagar	5.1	75	3.9	N'26°72.858 E'72°00.802	823

Fig. 1 (A) Collection of leaf sample and passport Data, (B) DNA precepted in 100% ethanol, (C) Tear drop shaped purified DNA using modified method

Fig. 2. Modified DNA extraction protocol

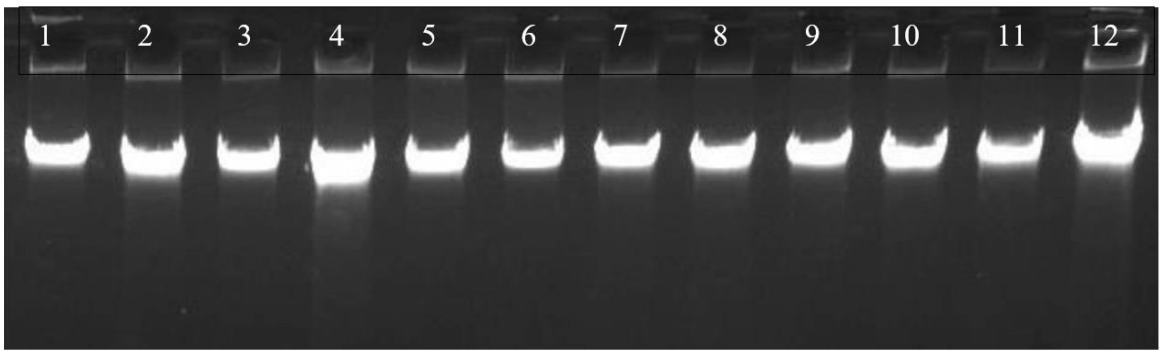

Fig. 3. Genomic DNA extracted using modified protocol

2.4 Quantitative and Qualitative Analysis of DNA Extracted by the Modified Protocol

Quality and quantity of DNA was estimated on 0.8% agarose gel and the gel was stained using 0.25 µg/ml ethidium bromide (Fig. 3). As well as, spectrophotometrically on a nano-liter scale spectrophotometer (DeNovix DS-11, USA)

3. RESULTS

Applying the present standardized method, the extracted DNA concentrations and its purity are given in (Table 2.). The yield of isolated DNA ranged from 630 ηg/ml to 4200ng/µl with average yield of 2041ng/µl, and the absorbance lied between 1.6-2.15 for A260/280 and 1.64-2.31 for A260/230, and the absorbance lied between 1.6-2.3, indicating minimal levels of contaminating metabolites.

Table 2. Purity and concentration of DNA extracted by modified protocol

Sample no	Site	A260/280	A260/230	DNA Concentration (ng/ml)
01	Tulesar	1.72	2.00	2180
02	Tulesar	1.8	1.98	3995
03	Tulesar	1.68	2.08	4105
04	Tulesar	1.74	2.14	2095
05	Tulesar	1.62	2.10	2900
06	Jhanwar	1.75	2.13	630
07	Jhanwar	2.15	1.95	3110
08	Jhanwar	2.08	1.99	2105
09	Jhanwar	1.93	1.87	1180
10	Jhanwar	1.63	1.79	2095
11	Jhanwar	1.60	2.10	3122
12	Jhanwar	1.83	2.00	1120
13	Jhanwar	1.68	1.93	793
14	Jhanwar	1.62	2.03	2103
15	Jhanwar	1.75	1.88	988
16	Bhavi	2.15	1.94	994
17	Bhavi	2.12	1.64	764
18	Bhavi	1.93	2.15	4115
19	Bhavi	1.78	1.74	2474
20	Bhavi	1.75	2.26	2126
21	Bhandu	1.81	1.72	890
22	Bhandu	2.08	2.01	877
23	Bhandu	2.14	1.80	2572
24	Bhandu	2.10	1.90	1583
25	Bhandu	2.13	2.31	4200
26	Ummed Nagar	1.95	1.92	1598
27	Ummed Nagar	1.69	2.15	1611
28	Ummed Nagar	1.88	1.76	1841
29	Ummed Nagar	1.87	2.09	1942
30	Ummed Nagar	1.79	2.07	1141

4. DISCUSSION

The modified DNA extraction protocol incorporates several key modifications to enhance the quality and yield of extracted DNA. These modifications include, **Increased concentration of CTAB and sodium chloride:** These reagents aid in cell lysis and DNA

precipitation, respectively, **Addition of L-ascorbic acid and PVP:** These substances act as antioxidants and polyvinylpyrrolidone, respectively, to inhibit the activity of polyphenol oxidases and other contaminants that can degrade DNA, **Doubled incubation time:** Prolonged incubation allows for more efficient cell lysis and DNA release, **DNA precipitation in 100% ice-cold ethanol:** This step ensures complete DNA precipitation and minimizes degradation, **RNase treatment:** This step removes RNA contamination, improving the purity of the extracted DNA. These modifications collectively contribute to a more efficient and effective DNA extraction process.

5. CONCLUSION

The modified DNA extraction protocol offers a simple, efficient, and cost-effective method for extracting high-quality genomic DNA. The incorporation of specific reagents and optimized incubation times enhances DNA yield and purity, making it suitable for various genetic studies, including large-scale projects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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