IECF 2022
The 3rd International Electronic Conference on Forests
Exploring New Discoveries and New Directions in Forests
15–31 OCTOBER 2022 | ONLINE

Standardization of extraction of DNA from silica-gel dried leaf sample of *Garcinia indica* (Thouras) Choisy

Anila M Sunny[1], Sreedevi C. N[1], Vilas Kumar Patil[2], Tresa Hamalton[1] and N. Ravi[1]
[1]Institute of Wood science and Technology Bengaluru, India.
[2]College of Forestry, University of Agricultural Sciences, Dharwad, India.
Corresponding author: nravi@icfre.org

INTRODUCTION

- *Garcinia indica* is a fruit bearing tree with multifarious uses both traditionally and commercially.

- The leaves of *Garcinia indica* contains high quantity of secondary metabolites such as, phenolic acids, flavonoids, bioflavonoids, xanthones, benzophenones and terpenoids (Pandey *et al*.,2015).

- Quality and quantity of DNA will reduce if the leaf samples were brought without proper preservation.

- Transporting leaf sample in silica – gel is an inexpensive method of preservation.

MATERIALS AND METHODS

- Fresh mature leaf were collected from naturally distributed locations of Western ghats viz., Karwar (n = 6) and Kukke Subramanya (n =4).
- Leaves were cleaned with distilled water and air dried for 30 minutes. The samples were placed in zip lock bag containing dried silica gel and transported to laboratory in Bengaluru, Karnataka, India.
- Silica gel were removed periodically when the colour turns to white from blue.
- In laboratory leaves were dried till the whole moisture losses and stored in -20ºC until further use.

Extraction of DNA by modified Thatte et al., 2012

- **Suspension buffer**

0.5M sucrose, 120mM Tris HCl, 50mM EDTA and 1.7 M NaCl.

- **Extraction buffer**

20 mM EDTA, 100 mM Tris HCl (pH 8), 1.4 M NaCl, 4% CTAB, 4% β-met.

Extraction of DNA by Thatte et al., 2012

- **Extraction buffer**

20 mM EDTA, 100 mM Tris HCl (pH 8), 1.4 M NaCl, 2 % CTAB, 0.2 % β-met

Genomic DNA extraction

Thatte *et al.*, 2012

- Silica-gel dried leaf sample of 1.0 g were pulverised in liquid nitrogen in pestle and mortar
- To the powdered leaf sample 9 ml of extraction buffer was added
- The reaction mixture was homogenised thoroughly and incubated in water bath for 60° C for 1 hour.
- An equal amount of C:I was added and centrifuged at 10,000rpm for 25 minutes.
- Aqueous layer was collected and equal amount of CI was added. Mixture was centrifuged at 10,000 rpm for 10 min. This step was repeated twice.
- Supernatant collected was added with 0.5 volume of 5 M NaCl and 2 volume of chilled ethanol.
- The samples were centrifuged at 5000 rpm for 5 min and the pellets were washed with 80% ethanol.
- The pellet were allowed to dry overnight and was dissolved in 0.1 X TE buffer. The DNA were stored in 20°C .

Modified extraction method

- Silica -gel dried leaf samples of 1.0 g were pulverized in liquid nitrogen in ceramic pestle and mortar with 3% PVPP
- Powdered leaf samples were transferred to centrifuge tube with 4 ml of suspension buffer and 100µl of β – met
- Tubes were incubated at 65 ° C for 1 hour, centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to another tube
- Extraction buffer of 7 ml was added to supernatant and incubated in water bath at 65 ° C for 60 minutes
- Equal amount of C:I was added and centrifuged at 15, 000 rpm for 10 minutes
- Aqueous layer was collected and added 20 µl of RNase (20 mg/ml) and incubated at 37 ° C on dry bath for 2 hours. Followed by this 25 µl of proteinase (20 mg/ml) treatment was given for 1 hour in dry bath at 37 ° C
- Equal amount of C:I was added, centrifuged at 10,000 rpm for 10 minutes. This step was repeated until a clear solution was obtained
- Supernatant was transferred and added 100 µl of Ammonium acetate and ¾ th volume of chilled ethanol and kept at -20°C for overnight
- Next day, samples were centrifuged at 10,000 rpm for 10 minutes to develop DNA pellets. The pellets were then washed with 70% ethanol and air- dried at room temperature for 3 – 4 hours
- The pellet was then dissolved in 30 -50 µl of TE buffer and stored at -20°C until further use

RESULTS

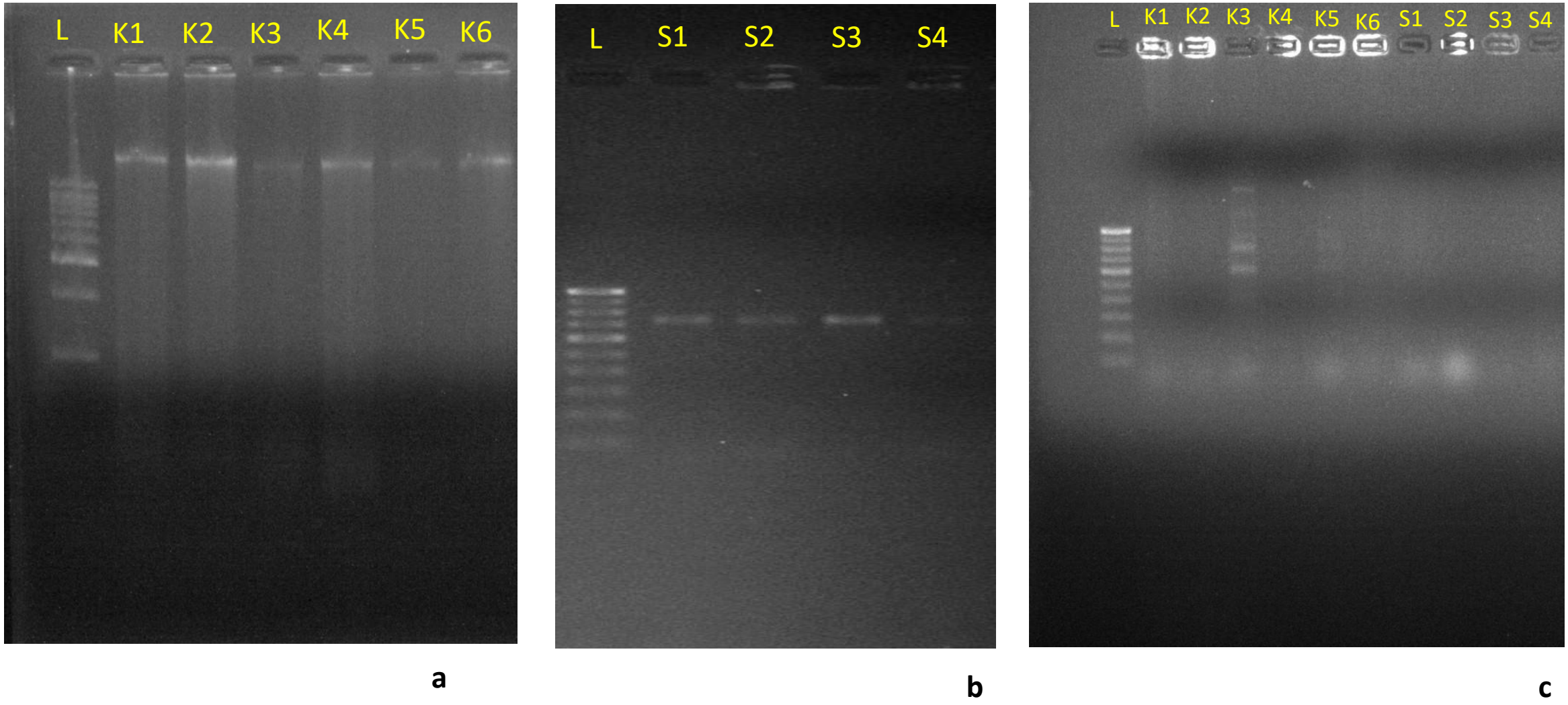

Fig 1. Genomic DNA of *Garcinia indica* silica- gel dried leaf sample extracted using modified Thatte *et al*., 2012 method - (a) Karwar (K1-K6), (b) Kukke (S1 –S 4) and Thatte *et al*., 2012 method – (c) Karwar (K1-K6) and Kukke (S1-S-6) resolved under 0.8 % agarose using 1Kb ladder (L)

Quality and Quantity of Genomic DNA

Thatte *et al.*, 2012		
SAMPLE NAME / ID	PURITY (A 260/280)	CONCENTRATION (ng/µ l)
KARWAR -1/ K-1	1.21	20.6
KARWAR -2/ K-2	1.35	55.6
KARWAR -3/ K-3	1.10	34.2
KARWAR -4/ K-4	1.00	82.3
KARWAR -5/ K-5	1.12	41.8
KARWAR -6/ K-6	1.20	15.1
KUKKE -1/ S-1	1.30	25.3
KUKKE -1/ S-1	1.10	39.3
KUKKE -1/ S-1	1.25	57.4
KUKKE -1/ S-1	0.99	32.9

Modified extraction method		
SAMPLE NAME / ID	PURITY (A 260/280)	CONCENTRATION (ng/µ l)
KARWAR -1/ K-1	1.69	127.5
KARWAR -2/ K-2	1.68	111.8
KARWAR -3/ K-3	1.58	61.2
KARWAR -4/ K-4	1.75	180.9
KARWAR -5/ K-5	1.64	150.6
KARWAR -6/ K-6	1.81	147.9
KUKKE -1/ S-1	1.81	252.5
KUKKE -1/ S-1	1.80	297.9
KUKKE -1/ S-1	1.74	249.5
KUKKE -1/ S-1	1.76	279.2

CONCLUSIONS

- The DNA of *Garcinia indica* obtained using modified extraction method was observed to have high purity and good quality.
- The purity of DNA obtained using modified method ranged between 1.58-1.81 and concentration ranging between 61 – 297 ng/µl.
- The results confirmed that modified extraction method can be used for DNA finger printing in *Garcinia indica.*

REFERENCE

Pandey,R.S, Chandra,P., Kumar, B., Srivastva, M.,Aravind, A., Shameer P.S., Ramesh kumar,K.B. (2015). Simultaneous determination of multiclass bioactive constituents of *G. indica* using UHPLC. Industrial crop products,77:861-872.

Thatte, K.S, Khandekar, R.G. and Deodhar, M.A. (2012). Assessment of diversity in Garcinia indica (Dupetit-Thouars.) Choisy. using morphological and molecular markers, Journal of tropical Agriculture, 50: 30-36.

ACKNOWLEGMENT

Financial Support by CAMPA National Authority through AICRP – FGR Project Indian Council of Forestry Research and Education, Dehradun, India.