Buletin Agroteknologi, June 2020, 1(1): 7-11
DOI: https://doi.org/10.32663/ba.v1i1.1194

BULETIN AGROTEKNOLOGI
ISSN 2722-7235 (Online media)
https://journals.unihaz.ac.id/index.php/bulgro

Extraction of high-quality genomic DNA and identification of different DNA barcoding markers for chickpea (*Cicer arietinum* L.)

Umavathi Saraswathi*[1], Lakshmanan Mullainathan[2]

[1] Adhiyaman Arts and Science College for Women, India
[2] Department of Botany, Annamalai University, India

Article Information

Received: May 12, 2020
Accepted: June 20, 2020

*Corresponding author
umas67@ymail.com

Key words:
DNA isolation,
CTAB,
DNA barcoding,
Cicer arietinum

ABSTRACT

The genetic studies of individual plants, especially self-pollinated species like chickpea need to be evaluated at the DNA level with the help of molecular markers for identifying genetic variations among the plants. High-quality DNA extraction is a prerequisite for genetic studies. Extraction of intact genomic DNA with high – molecular mass is essential for the study of many molecular biology applications like Polymerase Chain Reaction, endonuclease restriction digestion, southern blot analysis, and also for the construction of a genomic library. Several plant DNA extraction methods are available, even though the DNA isolation methods that give good yield employing both quantity and quality is quite difficult especially for self-pollinated crops like a chickpea. This work was focused on developing a standard protocol for the extraction of genomic DNA and identifying different barcoding markers. The result revealed that the CTAB extraction method with slight modification in protocol had been optimized for DNA isolation. The purified DNA, which was isolated through the CTAB method, had excellent spectral qualities and is efficiently digested by a restriction endonuclease, and is found to be more suitable for long-fragment PCR amplification. DNA barcoding is considered as a promising tool because it provides a practical and standard identification of plants. The isolated DNA sample was processed with a classical DNA barcoding approach by amplifying and sequencing with a universal primer. According to the result, among the different barcoding markers studied, the RbcL and Mat K were found to given the best result for molecular species identification in chickpea.

INTRODUCTION

DNA marker technology considered rapidly developing techniques that seemed unfeasible before are now routinely used with the development of various molecular markers based on Polymerase Chain Reaction (PCR) technique such as AFLP, RFLP, RAPD, SSR, ISSR, and STR. Molecular biology has greatly enhanced the speed and efficiency of the crop improvement program, recombinant DNA technology, and genomic DNA library construction. Isolation of DNA adequate quality and quantity becomes a prerequisite for taking advantage of these molecular techniques.

Extraction of DNA with large quantity and high quality is often a limiting factor in genetic analysis of plant traits that are important to crop

improvement programs. The isolation of pure, intact, and high-quality DNA is crucial for the success of molecular studies because many factors like the maturity of the plant, proteins, RNA, and polysaccharide composition can cause shearing of DNA during isolation. Degradation of DNA due to endonucleases is one of the problems encountered during the isolation and purification of DNA from a plant, directly or indirectly influencing the enzymatic reaction (Weishing, Nybom, Wolff, & Meyer, 1995). Polysaccharides become problematic, especially when present in DNA samples, because it may inhibit enzymatic activity. According to the previous studies, the polysaccharide has been shown to inhibit the activity of taq polymerase (Healey, Furtado, Cooper, & Henry, 2014) and restriction enzyme (Pandey, Adams, & Flournoy, 1996).

DNA barcoding is a new technique for identifying plants based on short, standardized, and universal DNA region/s (Mosa et al., 2019). A DNA sequence is generated from a small tissue sample and compared to a library of reference sequence belonging to described species providing a rapid and reproducible taxonomic recognition (Bruni et al., 2010). This method opens new perspectives for the identification of taxonomic uncertainties as well as to investigate the commercial aspects related to species traceability from field to market (De Mattia et al., 2011).

So the present study aimed at low-cost, high-yield, and high-quality method to prepare genomically and evaluate the universal applicability of DNA barcoding approach in chickpeas with different markers.

MATERIAL AND METHODS

Plant sample for DNA isolation

The locally popular variety of 'CO-4' variety of chickpeas obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, was used for this study. Fifty seeds were sown in a pot under shade net for germination. The samples of young, tender, and unbruised leaves of chickpea were collected and stored in -80^{0}C until use, but it is not recommended because DNA yield may reduce.

Extraction methods

Solutions required were extraction buffer {100 mMTris-HCl (pH 8.0), 50 mM EDTA, 1.4 M NaCl, 2% CTAB, 1% PVP (addition of PVP is optional), Chloroform-isoamylalcohol 24:1 [v/v/v]), TE buffer: 10 mMTris-HCl (pH 8), 0.1 mM EDTA, 7.5M Ammonium acetate, Isopropyl alcohol, 70% ethanol}. Genomic DNA was extracted based on the cetyltrimethylammonium bromide (CTAB) method extraction method (Doyle & Doyle, 1990).

One gram of the young seedling leaf samples was crushed in pestle and mortar and ground with 2ml of CTAB extraction buffer and 250 mg of Poly Vinyl Pyruvate. Then, the extract solution was separated and kept in a water bath at 65^0 C for 30 minutes instead of standard. After the incubation period, the sample was centrifuged at 12,000 rpm for 10 minutes and transferred the supernatant into a fresh tube. The supernatant was extracted with an equal volume of Chloroform: Isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 minutes. To the collected supernatant equal volume of isopropanol was added and incubated for 1 hour. Then centrifuged the samples for 10 minutes at 12,000 rpm. The supernatant was discarded. Ethanol was added to the residue 1 ml of 70% and centrifuged again for 10 minutes at 12,000 rpm. After discarding the supernatant, the pellet was collected and allowed for air drying. The pellet was dissolved with 0.5ml of TE Buffer and 3µl of RNase and stored at -20˚C for 30 minutes. The residue was collected and added 50 µl 0f 3M Sodium acetate, and the sample was centrifuged for 10 minutes at 12,000 rpm. After centrifugation, the pellet was collected and washed with 0.5 ml of ice-cold ethanol. The sample was centrifuged for 10 minutes at 12,000 rpm to evaporate the methanol. Finally, dissolve the pellet in 500 µl of TE buffer for further analysis.

Qualitative and Qualitative analysis of isolated DNA

The DNA yield was measured by using a Nanophotometer (IMPLEN) at 260nm. DNA purity was determined by calculating the absorbance ratio A260/280. Polysaccharide contamination was assessed by calculating the absorbance ratio

A260/230. For quality and yield assessments, electrophoresis was done for ten DNA samples in 1.0% agarose gel, stained with Ethidium bromide and bands were observed in the gel documentation system.

Table 1. List of primers used in the present study

Locus	Primer name	Sequence (5' – 3')
	Its 1F	TCCGTAGGTGAACCTGCGG
Its	Its 2R	GCTGCGTTCTTCATCGATGC
Its	Its 1F	TCCGTAGGTGAACCTGCGG
	Its 4R	TCCTCCGCTTATTGATATGC
Mat k	Mat k 2F	CCTATCCATCTGGAAATCTTAG
	Mat K 5R	GTTCTAGCACAAGAAAGTCG
Mat k	Mat k 390 F	CGATCTATTCATTCAATATTTC
	Mat k 1326 R	TCTAGCACACGAAAGTCGAAGT
Mat k	Mat k 390 F	CGATCTATTCATTCAATATTTC
	Mat k 1248 R	GCTATCATAATGAGAAAGATTTCTGC
rbcL	rbcLaF	ATGTCACCACAAACAGAGACTAAAGC
	rbcLaR	GTAAAATCAAGTCCACCACG
rbcL	rbcL 1F	ATGTCACCACAAACAGAAAC
	rbcL 724R	TCGCATGTACCTGCAGTAGC

PCR Amplification

To analyze the comparative performance of different DNA markers used, we analyzed the samples with three candidate barcoding genomic regions with seven combinations. PCR amplification was done using PCR master mix in a 25µl (2.5 µl -Taq assay buffer; 5.0 µl $MgCl_2$ (25mM); 2.5 µl-dNTPs mix (2 mM); 4.0 µl-Primer (2 µM/ µl); 1.0 µl-Template DNA(50ng); 0.25 µl- Taq DNA polymerase (5U/µl) and 9.75µl- De-ionised distilled water) reaction according to the instruction of manufacturer. PCR cycle starts with an initial denaturation followed by 35 cycles of denaturation annealing and completes its cycle with extension. The details of the primers used for amplification are described in Table 1. The amplified product was checked in 1.0% agarose gel electrophoresis, and bands were observed in the gel documentation system.

RESULTS AND DISCUSSION

We present a high throughput, high – yield, cost-effective and time consuming genomic DNA from chickpea was cultivars was isolated using CTAB methods with slight modifications including changes in temperature (65^0C in a water bath), incubation time, centrifugation time (10 min), and concentrations of chemicals used for getting a better result. The samples should be fresh and young to obtain a good quality of DNA because mature samples may contain higher quantities of polysaccharides and polyphenols (Porebski, Bailey, & Baum, 1997), which affects the quality of DNA. To find out the efficiency of the CTAB method, the DNA was isolated from both fresh leaf samples, and samples stored -80^0C were studied. The yield of DNA extracted from the sample was reported in Fig.1.The assessment of the purity of a nucleic acid sample is often performed by a procedure commonly referred to as the $OD_{260/280}$ ratio. A sample of DNA with the OD ratio at 1.8±2 has been considered as the pure sample. The DNA yield by the CTAB methods was significantly higher in the fresh sample of leaf tissue compared to the samples stored in -80^0C (Jayesh, Vikas, & Nitin, 2016). However, the mean $OD_{260/280}$ ratio was observed around 1.8±2 in both samples. The purity of extracted DNA was excellent, as evidenced by $A_{260/280}$ ratio ranging from 1.78 to 1.84, and $A_{260/230}$ was >2, suggesting that the preparations were sufficiently free of proteins and polyphenolics/ polysaccharide compounds.

Product	NanoPhotometer
Version	7122 V2.1.0
Serial Number	2155
Date	10 September 2014
Time	12:56:20
Instrument Calibration Pass 10 September 2014 12:22:02	
Lid Factor	10
Dilution Factor	1,000
Background	On
Factor	50,0
Units	ng/µl

Sample c.arietinum

Concentration 781 ng/µl

A230 A	A260 A	A280 A	A320 A
0,689	1,567	0,801	0,005

A260/A280	A260/A230
1,962	2,284

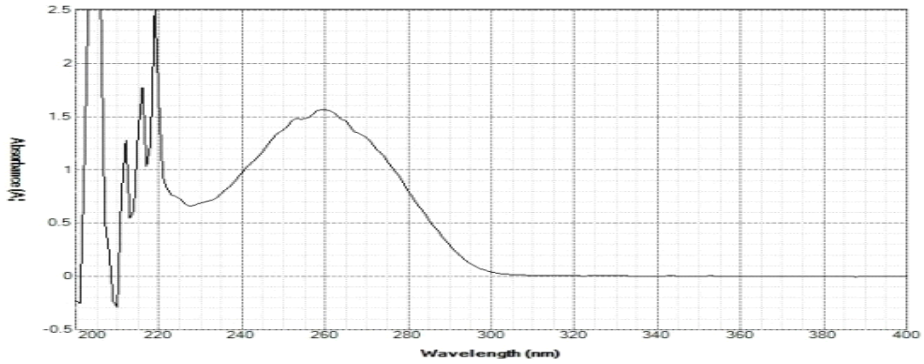
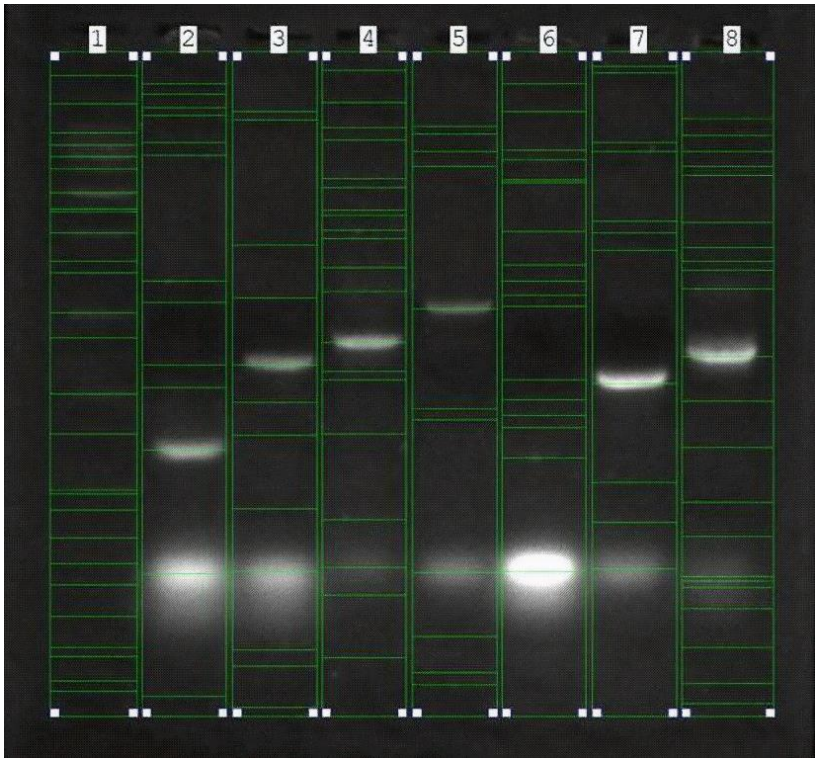


Figure 1. The yield of DNA extracted from *Cicer arietinum* L.



Lane 1 – Marker, Lane 2-Its IF:2R, Lane 3- Its 1F;4R, Lane 4 - Mat 2F; Mat k 5R,
Lane 5- Mat k 390F;Mat k 1326R, Lane 6 – Mat k 390F; Mat k 1248 R, Lane 7 – rbcLaF; rbcLaR and Lane 8 – rbcL 1F; rbcL 1F.

Figure 2. Gel electrophoresis showing PCR amplified DNA barcode markers for chickpea

The integrity, i.e., the presence of high molecular genomic DNA in the sample was analyzed by electrophoresis on 1.0% agarose gel method. The high molecular DNA bands with no smear were indicating that the DNA was intact and pure. We described a simple, efficient, and time-consuming method for a large amount of genomic DNA extraction from chickpea.

To find out the effective DNA barcode markers for chickpea, three DNA barcode markers with seven combinations were studied (Table.1). In chickpea, all the studied markers showed the sequence polymorphism and a significant sequence divergence was observed by rbcL (rbcL aF + rbcL aR) followed by Mat k and ITS (Figure 2).

Cingilli and Akcin (2005) proposed an isolation method for high-quality DNA isolation in chickpea through mini-prep and micro-prep method. The yield averaged is 150µg of DNA/ gm leaf tissue. When compared to this, in this study, the yield was about 4 – 5 times higher. Furthermore, the results revealed that this method generates DNA that produces more reproducible results in the RAPD system than the other isolation methods.

We proposed CTAB methods with slight modification for rapid and high yields of DNA for RAPD fingerprints. RAPD markers are generated by PCR amplification of random genomic DNA segments with single primers in an arbitrary sequence. Moreover, this method is comparable to any other conventional method for isolation of DNA in terms of their speed because it requires around 3 hours up to the final DNA re-suspension in the fresh sample.

CONCLUSION AND RECOMMENDATIONS

The DNA isolation from plant tissue is usually done through the classical CTAB extraction method. The quantity and quality of extracted DNA may vary from plants to plants in the CTAB method. Therefore, better yielding, slight modification based on laboratory condition, the ecology of samples, time, and others should be modified. In this study, a better isolation method was proposed with an existing method for DNA isolation from chickpea plants. The DNA barcoding easier the identification of plants easier. Hence, the present investigation summarized some useful marker for the DNA Barcoding with particular reference to Chickpea.

REFERENCES

Bruni, I., De Mattia, F., Galimberti, A., Galasso, G., Banfi, E., Casiraghi, M., & Labra, M. (2010). Identification of poisonous plants by DNA barcoding approach. *International Journal of Legal Medicine*, *124*(6), 595-603.

Cingilli, H., & AKÇİN, A. (2005). High-quality DNA isolation method for chickpea genotypes. *Turkish Journal of Biology*, *29*(1), 1-5.

De Mattia, F., Bruni, I., Galimberti, A., Cattaneo, F., Casiraghi, M., & Labra, M. (2011). A comparative study of different DNA barcoding markers for the identification of some members of Lamiaceae. *Food Research International*, *44*(3), 693-702.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*(13), 39-40.

Healey, A., Furtado, A., Cooper, T., & Henry, R. J. (2014). Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods*, *10*(1), 21.

Jayesh A., Vikas J., Nitin D. (2016). Optimization of DNA extraction methods from *Garcinia* species for ISSR-PCR, RAPD-PCR and DNA barcoding. *Asian Journal of Biotechnology*, 9:35-42.

Mosa, K. A., Gairola, S., Jamdade, R., El-Keblawy, A., Al Shaer, K. I., Al Harthi, E. K., ... & Mahmoud, T. (2019). The promise of molecular and genomic techniques for biodiversity research and DNA barcoding of the Arabian peninsula flora. *Frontiers in plant science*, *9*, 1929.

Pandey, R. N., Adams, R. P., & Flournoy, L. E. (1996). Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. *Plant Molecular Biology Reporter*, *14*(1), 17-22.

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant molecular biology reporter*, *15*(1), 8-15.

Weishing, K., Nybom, H., Wolff, K., & Meyer, W. (1995). DNA isolation and purification. *DNA fingerprinting in plants and fungi*, *2*.