Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 3418-3420
Received: 16-05-2020
Accepted: 20-06-2020

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# A modified and efficient CTAB genomic DNA extraction method from maize leaf for PCR analysis

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**Abstract**
High throughput DNA isolation process is time consuming and laborious steps in advanced molecular genetic analysis process *viz.* gene mapping, DNA fingerprinting, marker assisted selection and purity analysis etc. In present study described a simple, cost-effective modified CTAB-DNA isolation from the leaf of maize, excluding liquid nitrogen and 2-mercaptoethanol that can be performed in a laboratory with basic facilities. Presence of non-cellulosic components and starch in cell-wall of leaves reduce the quality and also hinders the DNA isolation process. This method is simple, rapid, low cost, and reliable for PCR analysis.

**Keywords:** CTAB genomic, DNA extraction method, maize leaf, PCR analysis

## Introduction
Pure, rapid and high throughput genomic DNA extraction is prerequisite steps in molecular genetic studies such as gene mapping, DNA fingerprinting, marker assisted selection and purity analysis [1]. CTAB genomic DNA isolation methods are used in extraction of DNA from plant and widely used for PCR analysis [2, 3, 4]. The quality and quantity of DNA extraction is hindered by various biomolecules such as Proteins, Polysaccharides and DNA polymerase inhibitors etc. present within and between the cells [5]. Many researchers [6, 7, 8] develop their DNA isolation protocol, they have time consuming steps, requires liquid nitrogen which is hard to purchase, 2-Mercaptoehanol, highly toxic reducing agent with extremely pungent with unpleasant aroma. Extraction of DNA by grinding the leaf sample inside extraction buffer, without using liquid nitrogen and 2-Mercaptoehanol, make this protocol less hazardous, more cost effective as compared to mechanical grinding using liquid [9, 10]. Many DNA extraction and purification kits are available in market but it is not economically viable for marker assisted selection for laboratory with basic facilities and large sample size.

## Materials and methods
### Plant materials
Maize (*Zea mays* L.) seeds were obtained from centre for AICRP Maize, TCA, Dholi, Muzaffarpur.

**Table 1:** List of experimental materials used in the present study

| Sl. No. | Pedigree | Kernel color |
|---|---|---|
| 1 | CLQRCYQ60-B-B | Yellow |
| 2 | CLQ2540Q-B-B-B-B | Yellow |
| 3 | VL1110240/VL108722-B-14-1-BB | Yellow |
| 4 | (CG18SeqC$_5$F76-2-1-2-1-2-BBB/LN/EM-46-31XCML311-2-1-3)-B-F303--B)/G18SeqC5F76-2-2-1-2BBB-D-B$_3$-B)-B-8-BBB | Yellow |
| 5 | WNCDMR11R4093 | Yellow |
| 6 | WNCDMR11R6429 | Yellow |
| 7 | WNCDMR10YFWS8481 | Yellow |

### Genomic DNA extraction
Seeds of seven inbred lines (Table 1) were grown in separate pots. After ten days the maize plants were in 4-5 leaves stage, genomic DNA was extracted by washing 0.3 g fresh leaves with distilled water followed by crushing leaf sample using mortar and pestle inside 1500 µl CTAB extraction (0.2M Tris-HCl, 0.02M EDTA, 2% (w/v) CTAB and 1.4M NaCl) buffer till

the color of chlorophyll appeared in extraction buffer. The homogenized greenish solution was transferred into fresh 2 ml centrifuge tube. Incubate the homogenized material inside the water bath for one hour at 65 °C. After incubation, remove the centrifuge tube from water bath and spin at 4000 rpm for 5 minutes. Transfer the 1 ml homogenized sample into fresh 2 ml centrifuge tube and add 400 µl chloroform: isoamyl alcohol (24:1), mix well till its colour faint and centrifuged for 15 minutes at 4000 rpm. After centrifugation upper aqueous layer transferred into fresh 2 ml centrifuge tube and again add 400 µl chloroform isoamyl alcohol and spin at 4000 rpm for 15 minutes. Repeat the steps till the upper aqueous layer became transparent. If upper layer became transparent then this layer was transferred by using pipette to fresh 1.5 ml centrifuge tube, add 300 µl ice-chilled iso-propyl alcohol through the wall of centrifuge tube and mix by gentle inversion. The DNA precipitate appeared like bubble after adding chilled isopropyl alcohol. The DNA molecules clumped and precipitated. These precipitated DNA was transferred into fresh 1.5 ml tube by using wide bore pipette tips. Dispense gently aqueous phase without discarding DNA clump by tilting centrifuge tube. The clumped DNA molecules were washed with 76% ethanol containing 0.2M $CH_3COONa$ followed by using 76% ethanol containing 10mM $CH_3COONH_4$. After washing DNA pellet was air dried by inverting the tube on filter paper till the smell of ethanol disappear. Dissolve the DNA pellet using 50 µl 1X TE (10 mM Tris-HCl, 1 mM EDTA) buffer and add 2 µl of RNAse solution having concentration of 10 mg/ ml RNAse. Incubate the DNA containing solution at 37 °C for one hour to degrade the RNA present as impurity in the isolated DNA solution. The dissolved DNA was stored at -20 °C until the DNA was utilized.

**Assay of DNA yield, purity and quality**

The concentration of isolated DNA (ng/µl) was determined on wavelength of 260 nm and 280 nm using Nanodrop (Benchtop, model number BT-Nano-200). Purity of DNA was estimated by ratio of absorbance at wavelength 260 nm and 280 nm. The quality of the extracted DNA samples were determined by electrophoretic separation by loading 3 µl sample along with 1 µl loading dye on 0.8% (w/v) agarose gel contained with ethidium bromide (1 µg/ml) inside 0.5X TBE buffer for 45 minutes by applying a constant potential difference of 80 V. After electrophoresis gel was photographed using the gel documentation system (Benchtop lab systems).

**DNA amplification**

PCR reaction was carried out in thermal cycler (Benchtop, model K960) using 10 µl reaction mixture containing 6.3 µl nuclease free water, 1.0 µl 10X green taq buffer (Thermo scientific), 1.0 µl 2mM dNTPs (Thermo scientific), 0.5 µl of each forward and reverse maize specific microsatellite primers (G-Bioscience) having concentration 5pmol/ µl, 0.2 µl 5U/ µl Dream Taq Green DNA Polymerase (Thermo scientific) and 0.5 µl isolated genomic DNA. The programme set during the process of thermo cycling was initial denaturation for 3 minutes at 94 °C, followed by 30 cycles of denaturation at 94 °C, annealing temperature 57 °C for 1 minute, extension at 72 °C for 1 minute and at the end of 30 cycle reaction, a final extension at 72 °C for 7 minutes was set. To determine the authenticity and applicability of this method was determined by using two microsatellite primer pairs umc1165 and umc1545 (Table 2) were selected to amplify the flanking region of genomic DNA.

**Table 2:** Details of microsatellite primer pairs used for validation of amplification by Polymerase Chain Reaction using genomic DNA extracted from seven inbred lines of maize.

| SN | Primer | Chromosome number | Primer Sequence 5'-3' | Repeat Sequence | Annealing temperature (°C) |
|---|---|---|---|---|---|
| 1. | umc1545 | 7 | (F) GAAAACTGCATCAACAACAAGCTG<br>(R) ATTGGTTGGTTCTTGCTTCCATTA | $(AAGA)_4$ | 57 |
| 2. | umc1161 | 8 | (F) GGTACCGCTACTGCTTGTTACTGC<br>(R) GCTCGCTGTTGGTAGCAAGTTTTA | $(GCTGGG)_5$ | 57 |

**Results and discussions**

Several methods are available for the isolation of plant genomic DNA, however, the better results were observed with those utilized CTAB [2, 3, 4, 11, 12, 13, 14]. Isolation process involves three major steps: (A) sample grinding (B) chloroform: isoamyl alcohol extraction and (3) DNA precipitation. The choice depends on various factors; the required quantity and size of DNA, the purity required for downstream applications, the time and the expense. The protocol described here was an adaptation from the DNA isolation method suggested by [2, 4, 15]. The present modified CTAB method of DNA extraction by using maize leaves excluding liquid nitrogen and 2-mercaptoethanol. The quality of extracted DNA was ascertained by calculating the ratio of absorbance at 260 and 280 nm. The ratio of absorbance at 260 and 280 nm varied from 1.818 in case of inbred lines CLQRCYQ60-B-B and WNCDMR11R6429 to 1.909 in case of WNCDMR10YFWS8481. The values were in the range of 1.8-2.0, indicating good quality of the isolated DNA (Table 3). The amplified product intensity showed excellent amplification (Fig. 1) and it was compatible by using different microsatellite primers.

**Table 3:** Quantitative estimates of seven maize genomic DNA concentrations revealed by spectrophotometer.

| Sl. No | Pedigree | A260 | A280 | A260/A280 Ratio | DNA Concentration (ng/ml) |
|---|---|---|---|---|---|
| 1. | CLQRCYQ60-B-B | 0.040 | 0.022 | 1.818 | 100 |
| 2. | CLQ2540Q-B-B-B-B | 0.030 | 0.016 | 1.875 | 75 |
| 3. | VL1110240/VL108722-B-14-1-BB | 0.043 | 0.023 | 1.869 | 107.5 |
| 4. | (CG18SeqC$_5$F76-2-1-2-1-2-BBB/LN/EM-46-31XCML311-2-1-3)-B-F303---B)/G18SeqC5F76-2-2-1-2BBB-D-B$_3$-B)-B-8-BBB | 0.039 | 0.021 | 1.857 | 97.5 |
| 5. | WNCDMR11R4093 | 0.039 | 0.021 | 1.857 | 97.5 |
| 6. | WNCDMR11R6429 | 0.040 | 0.022 | 1.818 | 100 |
| 7. | WNCDMR10YFWS8481 | 0.042 | 0.022 | 1.909 | 105 |

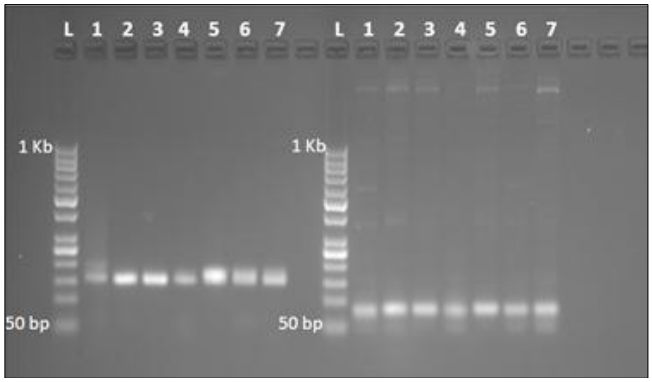


**Fig 1:** Genomic DNA amplification pattern of seven inbred lines using the primer pairs umc1545 and umc1161

## Conclusions

In summary, the procedure for maize DNA extraction described here could be a reliable and consistent protocol to work well for analysis without using liquid nitrogen and 2-mercaptoethanol.

## Acknowledgement

The Authors acknowledge Department of Agricultural Biotechnology and Molecular Biology and Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar for supporting this research.

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