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Rapid and efficient method of genomic DNA extraction from sweet sorghum [*Sorghum bicolor* (L.)] using leaf tissue

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Abstract
Extraction of genomic DNA from plant leaf tissue is a basic requirement for molecular biology experiment as well as in plant breeding for crop improvement. The purity of DNA is affected by varying level of polyphenols, polysaccharides and secondary metabolites with are associated with DNA at the time of extraction. A variety of DNA extraction methods and kits are available, however they are costly, low yield and time consuming. Here, we describe a simple and efficient method of DNA extraction from sorghum leaf. The present study is based on conventional cetyl trimethyl ammonium bromide (CTAB) method with four major modifications to isolate genomic DNA from seven different accessions of sorghum and the yield of DNA was varied from1550.2 ngµl^{-1} to 1910.5ngµl^{-1} in 100 µl final volume of TE buffer. The purity of DNA sample was found to be varied from 1.53 to 2.16 based on the absorbance at *A260* and *A280* ratio. This method is very simple as it not requires liquid nitrogen or magnetic beads to grind the leaf sample which is mostly unavailable to some undergraduate laboratories.

Keywords: DNA extraction, sorghum, CTAB, ethanol

Introduction
Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a cultivated sorghum an important source for bioenergy production. Sweet sorghum breeding programs that focus on bioenergy have two main goals: to improve quantity and quality of sugars in the stem and to increase biomass productivity [1]. Another important challenge is developing advance breeding lines that can withstand and adapt to temperature fluctuations and various biotic factors is more necessary than ever. So now days a major focus of breeder is on genome analysis of sorghum for their improvement and it require a large quantity and pure DNA [2].
DNA extraction from any plant tissue comprises three major steps such as, lysis of tissue, separation of DNA from other cellular components and last is isolation of pure DNA [3]. Extraction of large quantity and quality DNA from a tissue is a challenging task in genome analysis. The method of DNA extraction will differs from crop to crop, a slight modification is required in their extraction methodology. Also the age and type of tissue greatly affect the quality and quantity of DNA [4]. Fresh, Young and tender tissues are good source for DNA isolation than mature tissue. The polysaccharides and many types of secondary metabolites present in tissue affecting the DNA purification [5]. The presence of polyphenols, a powerful oxidizing agents present in many plant species, can reduce the yield and purity by binding covalently with the extracted DNA making it useless for most genome analysis [6-10].
Here, we describe a rapid and efficient method for extraction of DNA from sorghum leaves without affecting their quality and quantity which is use in various molecular biology genome analyses techniques.

Materials and Method
Materials
Sorghum seeds of seven accessions procured from ICRISAT gene bank namely IS1041, IS1042, IS1044, IS2646, IS3419, IS3420 and IS3421 were used for the study. 15 days old leaf tissues were harvested from seven different sorghum accessions grown on experimental research field of MGM, CABT in Aurangabad, India.

Approximately 200mg of sorghum leaf (fresh weight) were sampled into labeled aluminum foil and immediately kept on ice in the field. After returning to laboratory the all samples were then placed in -20 ^{0}C deep freezer until its use.

Chemicals and Consumables

- CTAB (Cetyl Trimethyl Ammonium Bromide) (Himedia, Catalouge No. MB101)
- Tris (Himedia, Catalouge No.MB029)
- EDTA (Ethylenediamine tetraacetic acid) (Himedia, Catalouge No MB011.)
- Sodium Chloride (Himedia, Catalouge No. MB023)
- β- mercaptoethanol (Himedia, Catalouge No.MB041)
- Chloroform (Himedia, Catalouge No.MB109)
- Iso-amyl alcohol (Himedia, Catalouge No.MB091)
- Absolute and 70% ethanol
- 2.0 ml Microcentrifuge Tubes (Tarson, Catalouge No. 500020.0)
- 1.5ml Microcentrifuge Tubes (Tarson, Catalouge No. 500010.0)

Solutions

- DNA Extraction Buffer (EB):

S. No	Chemical	Stock concentration	Working concentration	Volume required for 100 ml
1	CTAB	-	2%	2 gm
2	Tris (pH 8.0)	1M	100 mM	10 ml
3	EDTA (pH 8.0)	0.5M	20 mM	4 ml
4	NaCl	5M	1.4 M	28 ml
5	β- mercaptoethanol	-	0.1 %	100 ul

β- mercaptoethanol should be added just before using the buffer.

- Chloroform: Isoamyl alcohol at 24:1 (v/v)
- TE Buffer: 10 mM Tris and 1 mM EDTA (p^H 8.0)

Equipment's

- Water Bath (Labtech, India)
- Cooling centrifuge machine (Eppendorf)
- Micropipette (Thermo Fishers Scientific)
- Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, USA)

Method

1. Around 0.2gm of leaf sample from each sorghum accession was cut into small pieces with the help of sterile scissor (wipe with 70% ethanol using cotton). Before grinding of sample wipe out the mortar and pestle with the help of 70% ethanol using cotton and transferred the sample into mortar.
2. Add 1000 µl of extraction buffer (EB) to the leaf sample and grind until homogeneous mixture is obtained.
3. Transfer the mixture to the 2.0 ml of autoclaved fresh micro centrifuge tube and incubate at 65 ^{0}C with occasional gentle swirling for 45 min.
4. Cool down the tubes at room temperature for 5 to 10 min. centrifuged the tubes at 13,000 rpm for 10 min using cooling centrifuge to separate the phase and transferred the upper phase to fresh 1.5 ml autoclaved micro centrifuge tube without disturbing cell debris.
5. To this added 700µl of chloroform: isoamyl alcohol (24:1) and gently vortex for 30 sec and incubate at room temperature for 5 min.
6. Centrifuged the tubes at 13,000 rpm for 10 min. to separate the phase and transferred the upper phase to fresh 1.5 ml micro centrifuge tube.
7. Add 700µl pre-chilled absolute ethanol to aqueous phase and incubate at room temperature for 5min for precipitating DNA.
8. Centrifuge at 10,000 rpm for 10 min and discard the supernatant.
9. Air dry DNA pellet for 10 min. by inverting the micro centrifuge tubes on tissue paper.
10. Add 100 µl of TE buffer to each isolated DNA pellet allow it to dissolve and Stored at -20˚C until use.
11. The quality of DNA was tested through agarose gel electrophoresis on 0.8% agarose gel and quantity was checked by using Nanodrop Spectrophotometer ND-1000 at OD_{260nm}/OD_{280nm}.

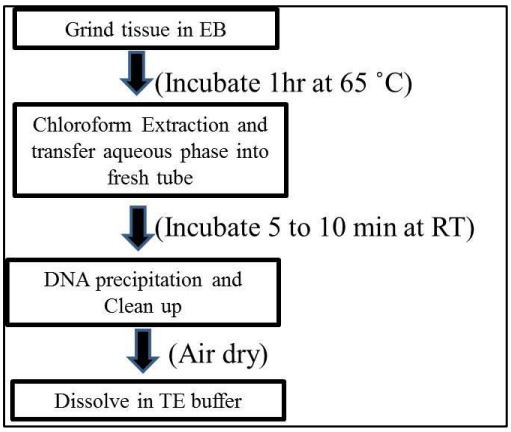

Fig 1: Flowchart of major steps of DNA extraction method.

Results

This method of genomic DNA extraction is shown in a flowchart in Figure 1. The method has four major steps required for qualitative and quantitative extraction of DNA from sorghum leaf tissue. 1. Grinding of young leaf tissue with mortar and pestle. 2. Chloroform: isoamyl alcohol extraction. This is a critical step to increase the DNA yield. 3. Precipitation of DNA with absolute ethanol and DNA cleanup. 4. Dissolve DNA pellet in TE buffer. To examine the concentration and purity of DNA, We quantify each DNA sample at OD_{260nm}/OD_{280nm} on Nanodrop Spectrophotometer ND-1000. The total seven sorghum accessions were used for DNA extraction and the yield of DNA was varied from1550.2 ngµl^{-1} to 1910.5 ngµl^{-1} in 100 µl final volume of TE buffer (Table 1). The purity of DNA sample was found to be varied from 1.53 to 2.16 based on the absorbance at *A260* and *A280* ratio. This result reflects that there is not much more RNA and protein contamination in the extracted DNA.

The quality of DNA was further tested by agarose gel electrophoresis of genomic DNA on 0.8% agarose containing 0.5 µg ml^{-1} ethidium bromide (EtBr) (Figure 2). The agarose gel electrophoresis results revealed that the high quality genomic DNA was extracted using this method. The above two results suggested that, our rapid, simple and improved

DNA extraction method produces high quantity and quality DNA from sorghum leaves that would be used for various molecular biology applications.

Table 1: Total DNA yield and purity of genomic DNA extracted from seven accessions of Sorghum investigated using Nanodrop Spectrophotometer ND-1000

S. No.	Accession No.	Total DNA yield (ng.µl-1)	OD_{260nm}/OD_{280nm}
1	IS 1041	1555.2	1.88
2	IS1042	1565.5	1.92
3	IS 1044	1655.0	2.16
4	IS 2646	1555.5	1.53
5	IS 3419	1755.7	2.07
6	IS 3420	1910.5	1.86
7	IS 3421	1555.9	2.05

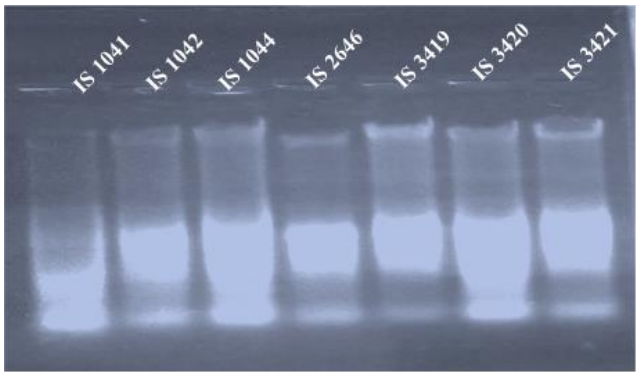

Fig 2: Genomic DNA isolated from seven sorghum accessions. Five µl of genomic DNA isolated from each of the seven plants was separated on 1% Agarose gel in 1X TAE buffer and 0.5 µg. ml-1 ethidium bromide.

Discussion

The extraction of DNA is of primarily importance in the molecular biology as it is a basic requirement those involving plant genetics [11]. The present DNA extraction method yielded good quantity and quality of pure DNA from young leaves of sorghum. But the method of DNA extraction specifically the concentration of chemicals use for DNA extraction is varied according to the cell components such as polyphenols and polysaccharides. Fresh and young leaf materials are the first choice to obtain good quality as well as quantity of DNA. However, mature leaves contain higher quantities of polyphenols and polysaccharides [9], which makes it very difficult to isolate DNA. To insure isolation of DNA with better yield and quality from leaf tissue of sorghum accessions, we optimized conventional CTAB method[12] makes it cost effective in the present modified protocol. We used 1 ml of CTAB extraction buffer instead of 750 µl for the lysis of cell membrane which yield more aqueous phase after first centrifugation step (~750 µl). Second modification we incorporate a 1.4M NaCl helps to remove proteins that are bound to the DNA and improve the purity of the extracted DNA [13]. 2-β-mercaptoethanol which successfully removed polyphenols [14, 15] giving rise a clear translucent DNA pellet. Third improvisation was the used of higher concentration of EDTA (0.5 M) that neutralizes the divalent cations required DNAse activity and thus protect DNA from degradation. The fourth modification was the precipitation of DNA with per-chilled absolute ethanol instead of isopropanol. It reduces the incubation time and DNA cleanup step during DNA extraction as there is no further necessity of DNA washing with 70% ethanol used in another DNA extraction method. Further the chloroform extraction step is performed to promote the partitioning of lipids and cellular debris into the organic phase, leaving isolated DNA in the aqueous phase [16].

The present DNA extraction method yielded good quantity and quality of pure DNA checked by using Nanodrop spectrophotometer ND-1000 and agarose gel electrophoresis. The analysis and mapping of genome using molecular markers such as, restriction fragment length polymorphisms (RFLP), simple sequence repeats (SSR), Amplified fragment length polymorphism (AFLP), Cleaved amplified polymorphic DNA (CAPS), Single nucleotide polymorphism (SNPs) and Random amplified polymorphic DNA (RAPD), etc. is the most common and basic requirement of analysis. It requires the high quality DNA for PCR amplification which would be extracted using this modified and rapid method. This method did not require liquid nitrogen (It can be difficult to procure in remote locations) or magnetic beads (tissue leaser) to crush the sample which is mostly unavailable to some undergraduate laboratories.

Conclusion

A simple, efficient and cost effective method of DNA extraction from sorghum leaf was described. The prescribed modifications in the present method establish a quick and efficient standardized protocol for DNA extraction from sorghum leaf tissue. The main advantage of this method is time saving, extracted high quality DNA and time saver. The quantification of DNA given in the table 1 showed that the, the modifications in the method consistently produced pure and high-quality DNA suitable for further molecular analysis. The DNA standardized extraction protocol presented here is important for the genetic diversity analysis, detection of genetically modified crops, association mapping, allele mining and biodiversity conservation.

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