



## ISOLATION OF GENOMIC DNA FROM TUBERS OF WHITE GUINEA YAM (*Dioscorea rotundata* Poir.) WITHOUT USING LIQUID NITROGEN

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(Received 25 February, 2025; accepted 31 August, 2025)

### ABSTRACT

Isolation of genomic DNA of high-quality is a prerequisite for molecular genetic characterisation of any plant. Genomic DNA is usually extracted from plant leaves for downstream applications such as polymerase chain reaction (PCR). Plants have to be grown in field or pots for leaves. In white Guinea yam (*Dioscorea rotundata* Poir), raising the plants in fields is a challenging task due to tuber dormancy in many genotypes. DNA can be directly extracted from yam tubers, obviating the need to raise plants in fields. However, high concentrations of secondary metabolites like polyphenols and polysaccharides in yam tubers may affect the genomic DNA extraction. A simple, rapid, inexpensive CTAB-based modified method with high PCR fidelity was standardised for DNA isolation from tubers without using liquid nitrogen. Fresh tubers and tuber samples prepared by alcohol-drying, freezing and oven-drying were employed for genomic DNA isolation. The quantity, quality and PCR-fidelity of DNA isolated from various methods were verified and compared with the DNA isolated using liquid nitrogen. Alcohol-dried tuber tissues provided consistent, high-quality genomic DNA with absorbance ratio  $A_{260/280}$  of 1.787-1.854 and a concentration of up to  $1.8 \mu\text{g } \mu\text{L}^{-1}$ . with ISSR profile comparable to that of tuber DNA isolated using liquid nitrogen.

**Keywords:** *Dioscorea rotundata*, genomic DNA, genetic characterisation, inter-simple sequence repeats, PCR, tuber, white Guinea yam

### INTRODUCTION

White Guinea yam (*Dioscorea rotundata* Poir) is a major staple food crop widely cultivated in tropical and sub-tropical regions of Africa, Asia, Latin America, the Caribbean, etc., for subterranean edible starchy tubers (Norman *et al.*, 2023). It is an economically important cash crop possessing good nutritive value, resistance to pests and diseases, long shelf-life or storage quality and has immense yield potential and socio-cultural impact in West Africa (Obidiegwu *et al.*, 2020). However, the crop is under-utilised and under-researched. White Guinea yam, belonging to the family Dioscoreaceae, is a polyploid with basic chromosome number  $x = 20$  ( $2n = 2X = 40$  for diploid and  $3X = 60$  for triploid), dioecious with male and female plants and allogamous having high heterozygosity (Agre *et al.*, 2023). The whole genome of white Guinea yam (594 Mb) has been sequenced and assembled through an international collaborative effort of Japan, Nigeria, UK and Germany, which has accelerated genetic analysis and genetic improvement programmes in the crop (Tamiru *et al.*, 2017).

White Guinea yam is propagated through whole tubers or tuber pieces, grown as annual crop and harvested 180-270 days after planting. The harvested tubers exhibit dormancy (no sprouting or

germination) for 30-150 days depending on the genotype, growth, harvesting and storage conditions. Though dormancy of tubers prolongs storability or shelf life of tubers preserving its quality, it is detrimental for crop improvement programmes since it adversely affects the number of breeding cycles or generations (Ile *et al.*, 2006; Nwogha *et al.*, 2023).

For genetic improvement and genetic characterisation of any crop, isolation of high molecular weight genomic DNA of high yield, good and consistent quality and suitable purity is a prerequisite (Chiong *et al.*, 2017). Most of the molecular techniques for genetic characterisation, identification and DNA fingerprinting of plants are based on polymerase chain reactions (PCR) such as simple sequence repeats (SSRs), inter-simple sequence repeats (ISSRs) etc. Genomic DNA is usually isolated from the leaves using liquid nitrogen. For production of leaves, plants are to be grown in field or pots. But sprouting and production of leaves are not uniform in all genotypes of white Guinea yam due to genotype-dependent tuber dormancy. Genomic DNA can be isolated from other plant parts as well, such as directly from tubers for genetic characterisation. However, the tubers of white Guinea yam contain high amounts of polysaccharides and secondary metabolites such as polyphenols, tannins, alkaloids, etc. that can combine with nucleic acids and interfere with genomic DNA isolation and inhibit DNA polymerase (Sharma *et al.*, 2008; Vohra *et al.*, 2012).

Plant tissues are ground using liquid nitrogen so that nuclease activity is prevented. However, availability, transportation and cost of liquid nitrogen are limitations in less-equipped laboratories, located especially in remote areas (Arif *et al.*, 2010; Sharma *et al.*, 2010). Collection of large and numerous plant samples from field in liquid nitrogen is also a constraint. Commercial column-based DNA extraction kits, on the other hand, yield pure DNA of higher quality free of contaminants, but are expensive and have limitation like use of specific starting material (Abdel-Latif and Osman, 2017; Mishra *et al.*, 2023). When DNA is isolated without liquid nitrogen, samples are processed by various methods like silica drying, air-drying, alcohol-drying, oven-drying, freezing or using fresh material. The present study was undertaken to isolate good-quality genomic DNA from the tubers of white Guinea yam for molecular characterisation using PCR-based molecular markers. In present study we report a simplified and rapid CTAB-based genomic DNA isolation method from tubers of yam without use of liquid nitrogen for PCR-based molecular characterisation. DNA extraction from tubers is advantageous when dormancy and non-uniform sprouting in various genotypes are constraints, and also obviates the need for raising the crop in field using expensive and laborious methods such as stakes, as support for the vines. Two buffer compositions and 4 methods for sample collection and preservation were compared for their efficiency in DNA isolation and downstream applications like genetic analysis using ISSR markers.

## MATERIALS AND METHODS

### *Plant material*

The tubers of white Guinea yam, raised in May 2023 in research fields of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, Kerala, India (8.5468° N, 76.9164° E and 11 m amsl), were harvested in January, 2024. The tubers were stored at room temperature in storage shed near the field. The yam tubers from seven genotypes *viz.*, 'DR 19', 'DR 93', 'DR 146', 'DR 161', 'DR 168', 'DR 171' and 'DR 246' were used in this study.

### *Sample preparation*

The fresh tubers were cut into small pieces of 0.8 x 0.2 cm size after removing their skin. Four different methods without using liquid nitrogen were employed. The methods used were drying tubers in absolute alcohol, oven-drying, freezing at -80°C and grinding fresh tuber pieces in extraction buffer. All the four methods were compared with the method using liquid nitrogen, to grind tuber tissues. For alcohol drying, the tuber pieces were immersed in absolute alcohol in glass petri-plates for 2 h. Then alcohol was decanted, the tuber pieces kept at room temperature for complete evaporation of alcohol,

and powdered in mortar and pestle. For oven drying, tuber pieces were kept in hot air oven at 65°C for 24 h. For frozen tubers, tuber pieces in perforated plastic bags were kept in deep freezer at -80°C for 72 h.

### **Genomic DNA isolation**

Two protocols each with two buffer compositions based on modified cetyl trimethyl ammonium bromide (CTAB) buffer in protocol 1 (Doyle and Doyle, 1990) and sodium dodecyl sulphate (SDS) buffer in protocol 2 (Xia *et al.*, 2019) were tested for DNA isolation in the samples prepared using all four methods and compared with the samples ground by using liquid nitrogen. In protocol 1, CTAB-based extraction buffer, comprising of hydroxy methyl amino methyl-hydrochloric acid (tris-HCl) (100 mM, pH 8.0), CTAB (2%), sodium chloride (NaCl) (2 M) and ethylene diamine tetra acetic acid (EDTA) (20 mM) was used (Doyle and Doyle, 1990) with slight modifications, where concentration of NaCl was increased from 1.4 to 2 M. In protocol 2, SDS-based extraction buffer, comprising of tris-HCl (100 mM), SDS (2% w/v), NaCl (150 mM), EDTA (25 mM), proteinase K (10 mg mL<sup>-1</sup>), with slight modifications from optimal buffer composition consisting of 50 mM each of tris-HCl and EDTA as per Xia *et al.* (2019). Polyvinyl pyrrolidone (PVP) (2% w/v) and  $\beta$ -mercapto-ethanol (0.1% v/v) were also included in protocol 2, which were not used in SDS-based method described by Xia *et al.* (2019). All chemicals used were purchased from HiMedia, India and Genei Labs, India.

The tuber pieces (0.1-0.2 g) were ground using a mortar and pestle in 1 mL DNA extraction buffer (CTAB extraction buffer in protocol 1, and SDS extraction buffer in protocol 2), preheated at 65°C. In protocol 1 DNA was extracted as per Doyle and Doyle (1990) and in protocol 2 as per Xia *et al.* (2019).  $\beta$ -mercaptoethanol (2  $\mu$ L) was added per mL extraction buffer in both protocol 1 and 2, to the extract in tubes after grinding. The ground sample was taken in 1.8 mL micro-centrifuge tube and incubated in water bath at 65°C for 1 h, with intermittent mixing of samples by inversion every 10 min. After this, the samples were kept at room temperature for 5 min. Equal volume (600  $\mu$ L) of freshly prepared chloroform-isoamyl alcohol mixture (in 24:1 ratio) was added, vigorously mixed and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was again extracted with chloroform-isoamyl alcohol (24:1 v/v). The steps were same till chloroform-isoamyl extraction in both protocols. In protocol 1, 600  $\mu$ L prechilled isopropanol was added for DNA precipitation, while in protocol 2, 0.1 volume of 3M sodium acetate and 2 volumes of pre-chilled 95% ethanol were added. The contents of tubes were mixed gently by regular inverting and kept at -20°C overnight. Then the samples were kept at -80°C for 1 h. The DNA was centrifuged at 12000 rpm for 20 min at 4°C. The pellets were washed twice with 70% ethanol, air-dried and dissolved in 50  $\mu$ L 1X tris-EDTA buffer (TE) containing 10 mM tris and 1 mM EDTA, in protocol 1 and 80  $\mu$ L of 1X TE in protocol 2. The isolated DNA was stored at -20°C.

### **Qualitative and quantitative estimation of DNA**

The quantity and quality of DNA was verified using agarose gel electrophoresis (1% agarose gel with ethidium bromide) in 1X tris-acetic acid-EDTA (TAE) buffer under a constant voltage of 85 V for 1.25 h. The UV-illuminated agarose gel image was recorded using a G: BOX F3 gel documentation unit (Syngene, Cambridge, UK). Genomic DNA quantification was done by measuring absorption at 260 nm and purity of DNA was determined using absorbance ratio  $A_{260/280}$  and  $A_{260/230}$  measured with 1  $\mu$ L samples using a micro-volume spectrophotometer ( $\mu^2$ NABI, Chandigarh, India).

### **Statistical analysis of data**

The significance of differences in concentration of genomic DNA,  $A_{260/280}$  and  $A_{260/230}$  ratios between two buffer compositions (CTAB and SDS) and 5 sample preparation methods (liquid nitrogen, alcohol-drying, freezing, oven-drying and fresh sample) among the various genotypes (as replications) were tested by ANOVA (analysis of variance) using AgriAnalytics@R Ver. 3.19 (<https://sreejyothi.shinyapps.io/agrianalyticr/>).

### **Inter simple sequence repeat (ISSR) marker analysis of white Guinea yam using isolated genomic DNA**

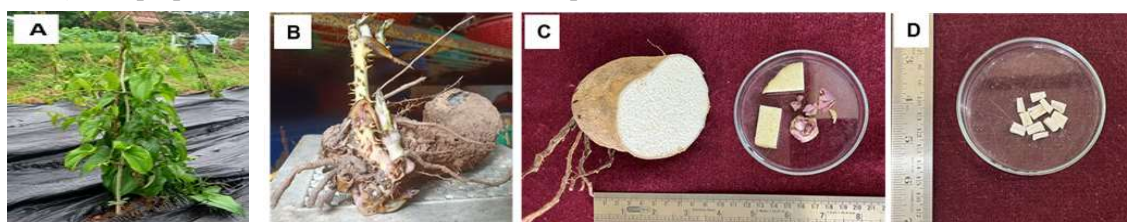
The ISSR markers UBC 807 (5' AGAGAGAGAGAGAGAGT 3') and UBC 834 (5' AGAGAGAGAGAGAGAGYT 3') were used for PCR using the extracted genomic DNA from the tubers of three genotypes 'DR 19', 'DR 168' and 'DR 171'. The UBC primer sequences (source: University of British

Columbia) available in public database were earlier synthesised (Eurofins India Pvt Ltd) in the lab as per Asha *et al.* (2019) and used. The tuber samples were prepared by using all five extraction methods (four methods without liquid nitrogen). PCR was carried out in 12  $\mu\text{L}$  total reaction volume using EmeraldAmp<sup>®</sup> GT 2X PCR master mix (DSS Takara Bio Pvt Ltd, New Delhi, India), 0.83  $\mu\text{M}$  single primer, and diluted template DNA of 100 ng concentration. Amplifications were carried out in Applied Biosystems<sup>™</sup> VeritiPro<sup>™</sup> thermal cycler (Thermo Fisher Scientific, Mumbai, India) using the programme initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec., primer annealing at 52°C for 1 min, primer extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. The amplification products were separated in 2% agarose gel, prepared in 1X TAE buffer containing 1  $\mu\text{g mL}^{-1}$  ethidium bromide. Electrophoresis was carried out at a constant voltage of 85 V for 1.25 h using 1X TAE as running buffer. The 100 bp and 1 kb ladders (DSS Takara Bio Pvt Ltd, New Delhi, India) were used as DNA markers. The agarose gel image was visualized using G:BOX F3 gel documentation unit (Syngene, Cambridge, UK).

## RESULTS AND DISCUSSION

### *Quality of genomic DNA extracted from tubers of white Guinea yam by using modified CTAB method*

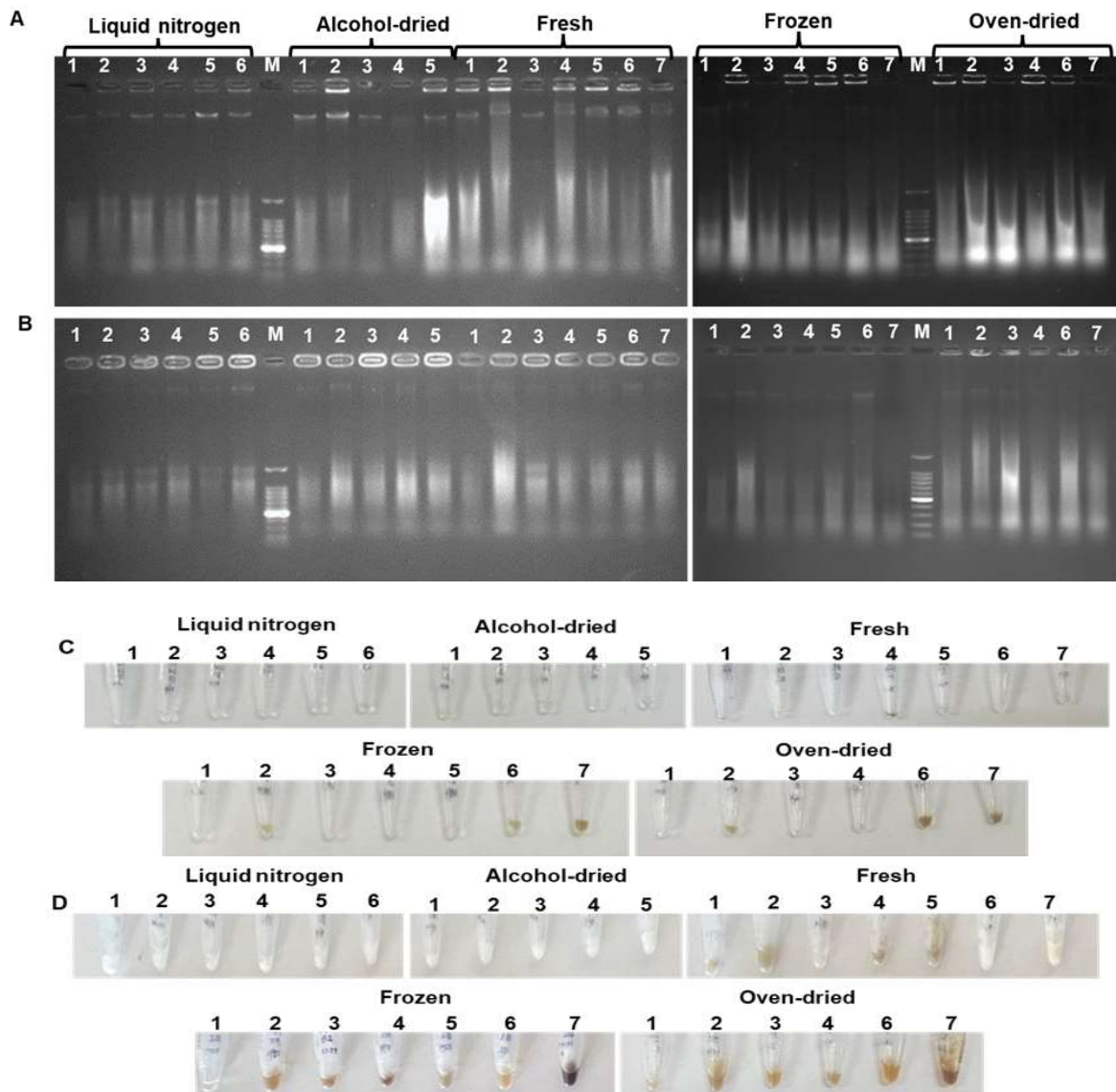
The apparently healthy whole tubers collected from the stored tubers were cut into small pieces (Fig. 1A-D), and prepared for DNA isolation. Two compositions of extraction buffers with SDS and CTAB



**Fig. 1: Sample preparation of white Guinea yam. A) White Guinea yam grown in field, B) Whole tuber stored after harvest in storage shed, C) Tuber pieces and sprouts cut out from the whole tuber, and D) Alcohol-dried tuber pieces used for DNA isolation**

four sample-preparation methods, were used to isolate genomic DNA from white Guinea yam tubers and compared with usual liquid nitrogen grind. The two methods varied significantly in DNA yield. Modified CTAB protocol gave DNA bands of higher intensity and DNA of good quality in agarose gels (Fig. 2A) when compared to SDS-based method (Fig. 2B). In CTAB-based protocol, DNA bands were visible in alcohol-dried, fresh tubers and tubers ground with liquid nitrogen, but were faint and not visible in frozen and oven-dried tuber samples (Fig. 2A). The genomic DNA extract in tris-EDTA (TE) was clear or transparent in liquid nitrogen-extracted and alcohol-dried samples, but slightly coloured and brown or black in few fresh, frozen and oven-dried samples (Fig. 2C). In SDS-based protocol, DNA pellet was not completely soluble in TE, and an opaque white precipitate-like extract was obtained in both liquid nitrogen-extracted and alcohol-dried tubers and brown or black-coloured DNA extract obtained in fresh, frozen and oven-dried samples (oven-dried > frozen > fresh), indicating the presence of high level of polyphenols and polysaccharides (Fig. 2D).

The purity of genomic DNA isolated was compared in various methods. In CTAB-based method, the  $A_{260/280}$  purity ratio ranged from 1.772 to 1.896 in four tuber sample preparation methods without using liquid nitrogen, and all tuber samples had  $A_{260/280}$  values above 1.8 when extracted using liquid nitrogen (Fig. 3A). However, in SDS-based protocol  $A_{260/280}$  was above 1.8 only in frozen samples and the values were much lower in other three methods (0.872-1.74) and liquid nitrogen extraction (0.825-1.418) [Fig. 3B]. Another purity ratio  $A_{260/230}$  values were lower than 2 (mean values: 1.265-1.633) in CTAB-based method (Fig. 3C); while in SDS-based method the  $A_{260/230}$  values were very low (mean values: 0.803-1.407), indicating high level of impurities in SDS-based protocol (Fig. 3D). A few

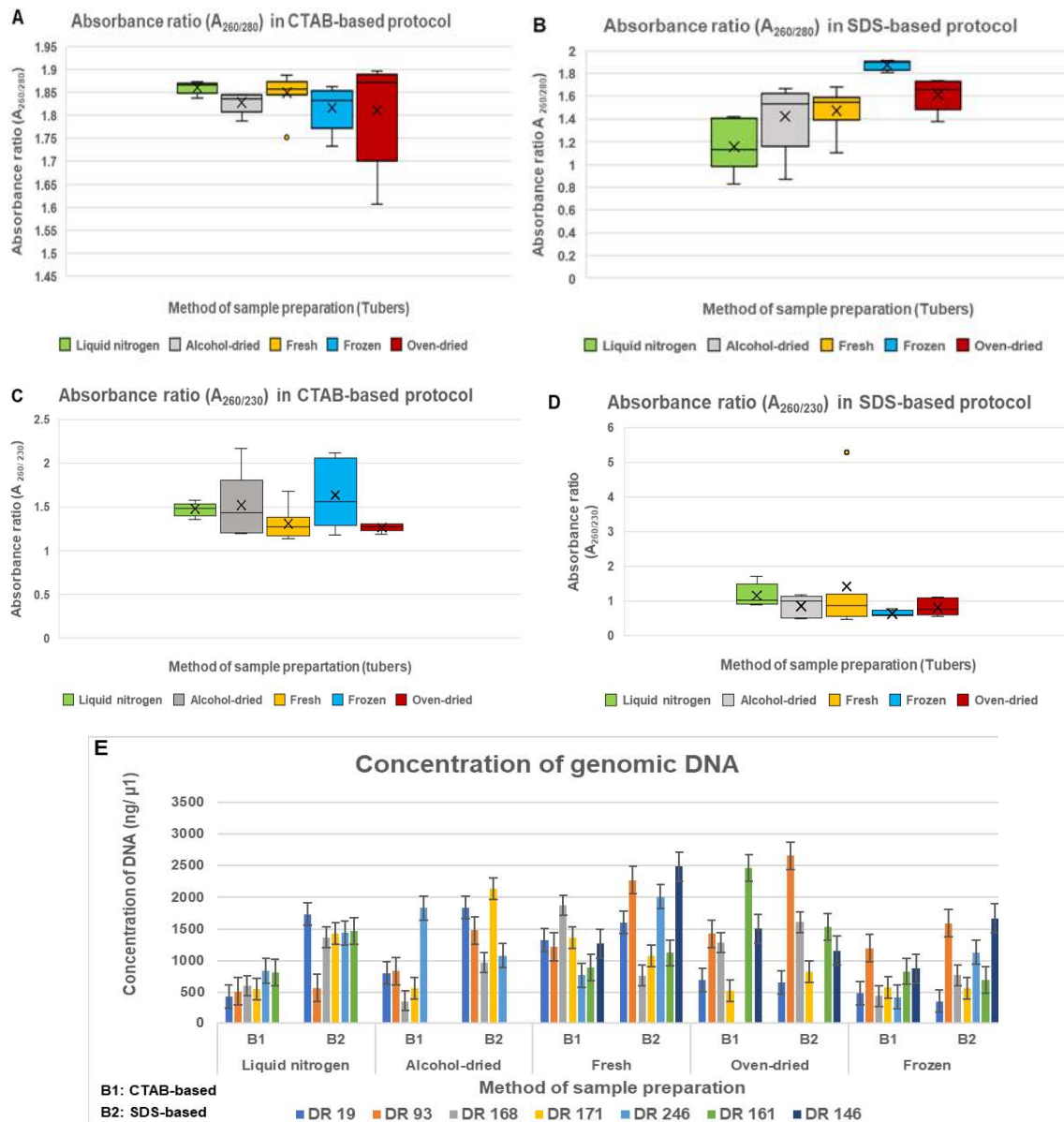


**Fig. 2:** Analysis for quantity and quality of genomic DNA extracted from tubers of white Guinea yam genotypes. Agarose gel electrophoresis (1% agarose gel) of genomic DNA from tubers extracted using A) CTAB-based protocol, and B) SDS-based protocol. Genomic DNA extracted from white Guinea yam dissolved in 1X TE showing the purity in C) CTAB-based protocol, and D) SDS-based protocol. 1-7 indicates genomic DNA isolated from white Guinea yam genotypes *viz.*, DR 19 (1), DR 93 (2), DR 168 (3), DR 171 (4), DR 246 (5), DR 161 (6) and DR 146 (7). M is 100 bp ladder.

samples in alcohol-dried and frozen samples had  $A_{260/230}$  values  $>2$ , when using CTAB-based extraction buffer. Concentration of DNA was also measured using nanodrop. DNA concentration was higher for SDS-based method than CTAB-based method (Fig. 3E), although in agarose gel, the band intensity was lower in SDS-based method.

ANOVA was used to compare the two methods based on CTAB and SDS extraction buffers and five sample preparation methods *viz.*, liquid-nitrogen, alcohol-drying, freezing, oven drying and fresh tubers. CTAB-based and SDS-based protocols differed significantly for concentration and purity ratios ( $A_{260/280}$  and  $A_{260/230}$ ) of isolated DNA (Table 1). All five methods tested were at par with respect to purity  $A_{260/280}$  of DNA extracted using CTAB-based protocol and didn't differ for purity ratio  $A_{260/230}$ .

Yams including white Guinea yam possess high amounts of polysaccharides and secondary



**Fig. 3:** A, B) Purity ratios ( $A_{260/280}$  and  $A_{260/230}$ ) and concentration of genomic DNA extracted from the tubers of white Guinea yam genotypes measured by nanodrop spectrophotometer. Box-whisker plots showing purity ratios ( $A_{260/280}$ ) of isolated genomic DNA using CTAB-based protocol (A) and SDS-based protocol (B). C, D) Box-whisker plots showing purity ratios ( $A_{260/230}$ ) of isolated genomic DNA using CTAB-based protocol (C) and SDS-based protocol (D). E) Concentration of genomic DNA extracted from the tubers of white Guinea yam genotypes using CTAB-based extraction buffer (B1) and SDS-based extraction buffer (B2)

metabolites, that may inhibit the efficient genomic DNA extraction and downstream applications of extracted genomic DNA, such as PCR, by inhibiting DNA polymerase. Commonly used method for genomic DNA isolation is to grind plant leaves using liquid nitrogen, to inactivate nucleases that may otherwise degrade DNA. The CTAB- and SDS-based extraction buffers and commercial kits were mostly employed to isolate plant genomic DNA. Commercial kits are costly with low DNA yield (Abdel-Latif and Osman, 2017). CTAB-based genomic DNA isolation in plants was modified many times, but it has limitations based on the crop, due to the varied composition of plant cell walls,

**Table 1: Comparison of the quantity and quality of genomic DNA from the tubers of white Guinea yam based on extraction buffers and sample preparation methods**

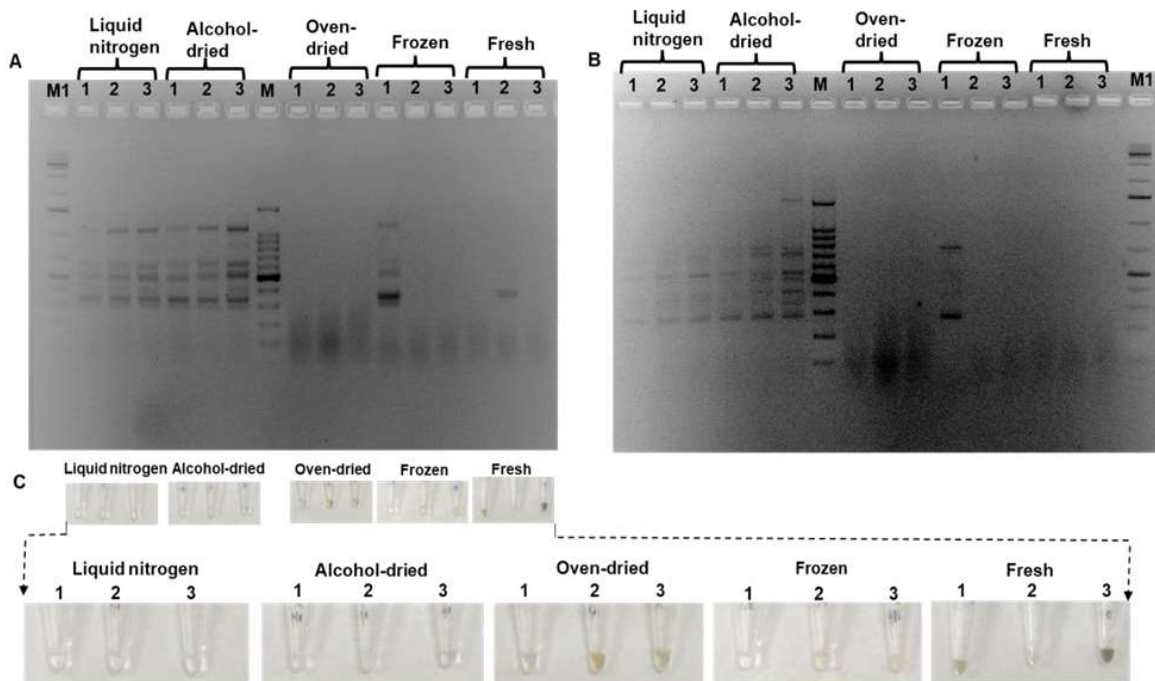
Sample preparation method	Concentration (ng $\mu\text{L}^{-1}$ )		$A_{260/280}$		$A_{260/230}$	
	B1	B2	B1	B2	B1	B2
Liquid nitrogen	628.32 <sup>d</sup>	1335.15 <sup>abc</sup>	1.860 <sup>a</sup>	1.157 <sup>d</sup>	1.480 <sup>ab</sup>	1.150 <sup>abc</sup>
Alcohol-drying	880.88 <sup>cd</sup>	1501.02 <sup>ab</sup>	1.828 <sup>a</sup>	1.423 <sup>c</sup>	1.520 <sup>ab</sup>	0.846 <sup>bc</sup>
Fresh	1243.33 <sup>abc</sup>	1618.54 <sup>a</sup>	1.849 <sup>a</sup>	1.473 <sup>bc</sup>	1.309 <sup>ab</sup>	1.407 <sup>ab</sup>
Oven-drying	1316.75 <sup>abc</sup>	1406.35 <sup>abc</sup>	1.811 <sup>a</sup>	1.615 <sup>b</sup>	1.265 <sup>abc</sup>	0.803 <sup>bc</sup>
Frozen	690.43 <sup>d</sup>	968.57 <sup>bcd</sup>	1.816 <sup>a</sup>	1.873 <sup>a</sup>	1.633 <sup>a</sup>	0.626 <sup>c</sup>

Statistical significance is denoted superscript letters a, b, c and d; B1 using CTAB-based extraction buffer, and B2 using SDS-Based extraction buffer.

cellulose, nucleus and mitochondria. Most of the modified CTAB methods employ leaf tissues for efficient large-scale extraction of genomic DNA with high-quality (Mekonnen *et al.*, 2017; Mishra *et al.*, 2023). A modified CTAB method with higher concentrations of sodium chloride and CTAB (4M NaCl, 3% CTAB, 100 mM tris, pH 8 and 20 mM EDTA) and without liquid nitrogen was used for isolating genomic DNA of high quantity and quality from diverse plant species (Mavrodiev *et al.*, 2021). High molar concentrations of NaCl in extraction buffer removes carbohydrates and proteins by increasing the polysaccharide solubility in ethanol, thus preventing polysaccharide co-precipitation along with DNA (Abdel-Latif and Osman, 2017). The oxidized form of poly-phenols binds to DNA covalently, causing brown colouration of DNA (Mishra *et al.*, 2023). PVP removes polyphenols, while lithium chloride (LiCl) removes RNA. However, DNA yield and purity did not improve by adding LiCl and PVP alone, but improved drastically using 1.4 M NaCl (Arif *et al.*, 2010).

Modified CTAB procedure was used to extract the DNA from tissues other than leaves. A modified CTAB protocol with high concentration of NaCl (2 M) and PVP (2% w/v), was used in the present study for extracting the DNA from tubers of white Guinea yam without use of liquid nitrogen. Inclusion of 1.5 M NaCl in extraction buffer facilitated the removal of polysaccharides during DNA extraction from fruit pulp of *Cyclanthera pedata* (Kumar *et al.*, 2023). DNA and RNA were extracted with a bench drill and plastic bags in modified CTAB method (100 mM tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 1.4 M NaCl, 2% CTAB, 2% PVP-40 and 1% sodium sulphite) without using liquid nitrogen from roots of banana, citrus, pineapple and cassava (Ferreira *et al.*, 2019). However, DNA isolation from maize hybrids was not effective even with 2.56 M NaCl in CTAB extraction buffer (Abdel-Latif and Osman, 2017).

DNA yield was highest from soybean seeds using SDS-based method with extraction buffer having 2% SDS, 150 mM NaCl, 50 mM tris-HCl, and 50 mM EDTA and increasing NaCl concentration in SDS lysis buffer decreased DNA yield, contrary to that of CTAB buffer where increasing concentration of NaCl in the buffer increased DNA yield (Xia *et al.*, 2019). When SDS-based extraction buffer containing 1% SDS, 100 mM tris, 50 mM EDTA, 500 mM NaCl, and 2% (v/v) 2-mercaptoethanol, was used in TENS-CO method, the proteins were bound to SDS and precipitated in DNA pellet after centrifugation. Such precipitating proteins were removed by using 3 M potassium acetate after lysis, before chloroform purification (Chiong *et al.*, 2017). In present study, SDS-based method of DNA isolation from tubers resulted in lower yields of DNA with much impurities and precipitation in all DNA samples. Tubers have higher content of polysaccharides which form a sticky gel in which DNA or RNA gets embedded, once the cells are disrupted (Chandra and Tewari, 2007). This might have caused lower DNA yield from tubers of white Guinea yam using SDS-based extraction buffer. No DNA was detected in agarose gel in SDS-based method, although nanodrop measurements showed high concentration of DNA in SDS-based method as compared to the CTAB-based modified procedure. This may be due to lower NaCl concentration (0.15 M) in SDS-based extraction buffer that would have caused DNA to co-precipitate with polysaccharides preventing even its movement in agarose gel, and fluorescence of DNA in the wells. However, such an inhibition of electrophoretic movement was not observed in case of DNA from CTAB-based method. Also, RNase treatment of DNA was not carried out and hence RNA absorbance at 260 nm also might have



**Fig. 4: Amplification of genomic DNA from the tubers of white Guinea yam genotypes using ISSR primers. A) ISSR marker UBC 807 B) ISSR marker UBC 834. M is 100 bp ladder and M1 is 1 Kb ladder. 1, 2 and 3 indicate PCR products using genomic DNA of DR 19, DR 168 and DR 171, respectively. C) Purity of genomic DNA extracted from white Guinea yam dissolved in 1X TE. 1, 2 and 3 indicate PCR genomic DNA of DR 19, DR 168 and DR 171, respectively. Different methods of sample preparation are indicated above.**

contributed to the spectrophotometric reading of  $A_{260}$  nm.

Based on the yield and purity of isolated DNA as observed using agarose electrophoresis and colouration of extracts, the DNA isolated using modified CTAB-based method possessed higher quality and higher solubility. Hence, DNA isolated with modified CTAB-based method using the five sample preparation methods of three white Guinea yam genotypes were used for verifying the PCR efficacy and fidelity, through amplification of ISSR markers. The ISSR banding patterns obtained using tuber DNA from alcohol-dried samples were only comparable to the DNA from liquid nitrogen-extracted samples, while no amplification was obtained in oven-dried samples with both UBC 807 and UBC 834 ISSR primers. Frozen and fresh samples gave inconsistent results with no amplification or partial amplifications of bands for both UBC 807 and UBC 834 ISSR primers (Fig. 4A,B). ISSR amplification was dependent on DNA purity. The samples with high level of impurities or brown colouration showed no amplification or partial amplification of a few bands, and not all samples showed amplification (Fig. 4C).

The plant genomic DNA extracted from various tissues are used for different downstream applications like PCR, restriction digestion, polymorphism studies or genetic characterisation, mutant analysis, marker-assisted selection, gene cloning, SNP genotyping and sequencing, etc. The preparation of samples is a very important step in choosing the alternative DNA extraction methods, which do not employ liquid nitrogen for tissue grinding and nuclease inactivation. Instead fixatives are used to completely disrupt the plant cells when liquid nitrogen is not used. In the present study, absolute alcohol was used as fixative and alcohol-dried tissues gave comparable results (both for isolation of high-purity DNA and ISSR marker analysis) to those obtained by using liquid nitrogen-based extraction. Absolute alcohol or ethanol was best fixative to yield high molecular weight DNA from leaves using modified CTAB method, while the addition of chloroform or EDTA did not improve the fixation of leaves nor DNA quality, but instead made the grinding of leaves difficult (Sharma *et al.*,

2003; Vohra *et al.*, 2012). In modified CTAB method, silica-dried leaf samples were incubated for 1 h at -20°C before grinding, but without using liquid nitrogen, to obtain DNA suitable for sequencing (Quiñones *et al.*, 2024). In another experiment, DNA was isolated from the sprouts and leaves also, using extraction buffer 1 (CTAB-based) and 2 (SDS-based) from alcohol-dried, oven-dried and frozen samples, and compared with those of liquid nitrogen-extracted DNA and similar results were obtained (data unpublished).

The absorbance ratio of  $A_{260}/A_{280}$  was 1.8 which indicated highly pure extracted DNA, while purity ratio  $> 1.9$  showed the presence of RNA, and  $< 1.7$  indicated protein contamination in the samples (Abdel-Latif and Osman, 2017). For pure DNA, purity ratio of  $A_{260}/A_{230}$  ranges from 2.0-2.2. Lower ratio of  $A_{260}/A_{230}$  indicated the presence of carbohydrates, EDTA, phenols, etc. (Arif *et al.*, 2010). The  $A_{260}/A_{230}$  values were  $< 2$  for both CTAB- and SDS-based protocols from tubers. RNase treatment was not carried out which will further lengthen the DNA isolation process. But still, the isolated DNA could successfully be employed for ISSR marker analysis. Hence purity of DNA was major factor affecting the PCR amplification.

**Conclusion:** Two DNA extraction buffer compositions (CTAB-based and SDS-based) and four sample preparation methods were tested for good quality genomic DNA isolation from tubers of white Guinea yam for PCR amplification. We reported a simple, rapid and less expensive modified CTAB protocol for extraction of good-quality genomic DNA from alcohol-dried tuber tissues of white Guinea yam, without the use of liquid nitrogen, that can be employed for high-fidelity PCR-based genetic characterisation. The protocol can be scaled up for extraction of good-quality DNA in large scale from tubers and also from other tissues such as sprouts and leaves without liquid nitrogen.

**Acknowledgements:** We thank Indian Council of Agricultural Research, New Delhi (India) for the financial support and Director, ICAR-CTCRI for the facilities provided for research. The first author thanks project associates Amalnath and young professional Mamatha, ICAR-CTCRI for their kind help.

**Funding:** The study was undertaken as co-Principal Investigator in Institute Project of ICAR-CTCRI (Megaproject 1: HORTCTCRISIL202000901465: Conservation and utilization of germplasm of tuber crops for sustaining production, Sub project: Yams germplasm) during 2023-24 and the project was funded by Indian Council of Agricultural Research, New Delhi (India).

**Competing interests:** The authors declare that they have no competing interests.

**Author's contributions:** STP: conceived, designed and planned the experiments, executed a part of the experiment, analysed the results, prepared the manuscript. NC executed part of genomic DNA isolation from tubers and gel electrophoresis. ES and RMV: involved with genomic DNA extraction from leaves using modified CTAB protocol and ISSR marker analysis in a few genotypes of white Guinea yam and lesser yam, as part of their M.Sc. thesis work. MNS: raised white Guinea yam genotypes in field and provided all experimental material (tubers and leaves) of yam and primers 'UBC 807' and 'UBC 834'. APV: maintained experimental field of white Guinea yam and assisted in sample collection, and SJ: conducted statistical analysis of data.

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