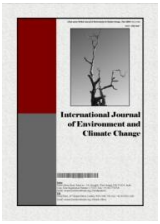

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Development of a High Quality, Rapid, Efficient and Economical DNA Extraction Protocol from Climate Resilient Pearl Millet Crop Without Liquid Nitrogen

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Authors' contributions

This work was carried out in collaboration among all authors. Author SA designed and executed the study, wrote the protocol and the whole manuscript. Authors RM, SS, VK, RCM supported in conducting the experiment. Authors SA and SS managed the literature searches and recorded feedback analyses of the study. Author CTS edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Extraction of good quality genomic deoxyribonucleic acid (DNA) from plants is a major prerequisite for molecular biology experiments. An efficient genomic DNA protocol must be simple, fast and cost effective with high yield and purity. Presence of polyphenols, polysaccharides and secondary metabolites in some plants hamper with DNA extraction making it a very laborious, difficult and time consuming procedure. Here, we portrayed a modified protocol based on the cetyl trimethyl ammonium bromide (CTAB) method to isolate DNA from climate resilient pearl millet leaf tissues having higher amount of polysaccharides. It also excludes the use of expensive chemicals and equipments like proteinase K, liquid nitrogen and tissue lyser. This method includes extraction of DNA using a buffer (pH 8.0) containing 200 mM Tris-HCl, 20 mM ethylenediamine tetracetic acid (EDTA),1.4 M NaCl, 2% CTAB, 2% sodium dodecyl sulphate (SDS) and 1.0 % β-mercaptoethanol

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followed by purification of DNA with phenol, chloroform, isoamyl alcohol and finally precipitation of DNA by sodium acetate and isopropanol. Good quality genomic DNA with sharp and clear bands was obtained from 48 pearl millet genotypes using this protocol. The yield of DNA varied from 105.2 to 328.3 ng/µl. The purity of DNA sample ranged from 1.74 to 1.95 based on the absorbance at $A_{260/A280}$ ratio indicating that it's free from ribonucleic acid (RNA) and protein contamination. PCR analysis using simple sequence repeat (SSR) primers exhibited consistent and reliable amplification products ranging from 150 to 650 bp.This study reveals a fast, simple, efficient, specific, reproducible, reliable and cost effective method for extraction of DNA from small to large number of plant samples amenable to PCR amplification and could be stored for longer duration.

Keywords: CTAB; genomic DNA extraction; Pennisetum glaucum; polyphenols; polysaccharides; secondary metabolites.

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a tall, warm season, annual grass belonging to family Poaceae. It is widely used as as a multi-purpose cereal grain crop for feed, fodder, fuel, mulch and grown on more than 26 million hectares area in semi-arid tropics of Asia and Africa. It is a highly nutritious and climate-resilient crop which can survive under harsh conditions of drought, low soil fertility, high salinity, low pH and high temperature and considered as an indispensable component of the food and nutritional security and livelihood of many million poor farmers in the changing climatic scenario [1]. Genomic DNA extraction of good quality is mainly required for various applications of biotechnology and molecular biology such as DNA restriction digestion, PCR amplification, southern blotting, gene cloning, DNA fingerprinting etc. But, extraction of high molecular weight, high quality, intact genomic DNA from plants like pearl millet having higher amounts of polysaccharides, polyphenols, secondary metabolites is a major problem and big challenge [2-4]. These are powerful oxidizing agents which interfere with genomic DNA and can further inhibit the PCR amplification.

The procedure of DNA extraction differs from crop to crop and slight modifications are required in their extraction protocol. Several methods have been used to isolate genomic DNA including cetyl trimethyl ammonium bromide (CTAB) method and its modifications, sarkosyl nitrogen method, sodium dodecyl-sulfate (SDS) method, phenol/chloroform method along with many others that are modifications of the components of these protocols [5-9]. Although, these methods have been used extensively and proved helpful in extracting DNA from different plant species but they are more time consuming, laborious and costly for various laboratories having limited resources and facilities. Many of these protocols usually use liquid nitrogen and expensive chemicals which is not easy to access due to higher cost and difficulty in procurement to remote locations [10-12]. In addition, several DNA isolation kits are also used which are based on either anion exchange chromatography or silica gel membranes. Though these kits are convenient and usually safe as they don't use hazardous reagents but their accessibility to various developing countries and higher cost make them unaffordable particularly when handling a large number of samples and performing experiments with limited financial resources. Moreover, usually commercial kits deliver lower DNA yields with variable quality [13,14]. On the other hand, some methods involve use of specific equipments designed especially for DNA isolation while others may be suitable for isolation of DNA from seed material and not for leaf tissue [15]. Thus, an efficient, fast, easy and cost effective method for DNA extraction is extremely required.

DNA extraction from any plant tissue comprises three major steps including lysis of tissue, separation of DNA from other cellular components and finally isolation of pure DNA. The cetyl trimethyl ammonium bromide (CTAB) method is the most accepted protocols for plant DNA isolation. Several modifications have been made in CTAB method to get adequate yield and intact DNA of high purity [10,16]. A good and high quality extraction protocol must be simple, quick, cheap and efficient enough giving significant levels of high quality DNA suitable for molecular studies [17,18]. Thus, the present study was conducted to develop a steadfast genomic DNA extraction protocol suitable for extracting high quality pure genomic DNA in sufficient amount from leaf tissues of pearl millet plant rich in polyphenols, polysaccharides and secondary metabolites without using expensive

chemicals and specific equipments and can be stored for a longer duration as well as can be used efficiently for PCR reactions.

2. MATERIALS AND METHODS

2.1 Plant Material

A total of 48 genotypes of pearl millet including popular released hybrids and varieties of pearl millet developed under Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India were used for this study (Table 1). Young, tender and healthy leaves of 12 days old plants grown in field conditions were collected in labeled self-sealing polyethylene bags and stored at -20°C until use.

2.2 Solutions and Reagents

CTAB (Cetyl Trimethyl Ammonium Bromide)
Tris (Tris Hydroxymethyl Aminomethane)
EDTA (Ethylenediamine Tetracetic Acid)
Sodium Chloride
β- Mercaptoethanol
Phenol
Chloroform
Iso-amyl alcohol
3 M Sodium Acetate (pH 5.8)
Isopropanol
RNase A (10mg/ml)
Absolute and 70% ethanol

CTAB Extraction Buffer: The extraction buffer consisted of 200 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 2% CTAB, 2% SDS, 1.0% (v/v) β-mercaptoethanol. The buffer should be pre-warmed at 65°C in a water bath and β-mercaptoethanol should be added just before use.

TE buffer: 10 mM Tris-HCl and 1 mM EDTA (pH 8.0)

Phenol Chloroform Isoamyl alcohol (PCI): (25:24:1)

Chloroform: Isoamyl alcohol (CI): (24:1)

2.3 DNA Extraction Protocol

2-3 young and tender leaves were ground in a pestle and mortar without liquid nitrogen adding 900 µl of CTAB extraction buffer. The mixture was transferred into 2 ml eppendorf tubes with a spatula and 600 µl of extraction buffer was added further. It was incubated at 65°C for 1.5 hr with occasional mixing by inverting the tubes. After incubation, the tubes were centrifuged at 12,000 rpm for 10 min and the supernatant was transferred into new labeled eppendorf tubes. 600 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed gently by inverting the tubes for 5 mins followed by centrifugation at 12,000 rpm for 10 mins. The aqueous phase having DNA was carefully transferred to a fresh centrifuge tube avoiding the interphase. 5 µl of RNase A (10 mg/ml) was added to it and incubated at 40°C for 45 mins in water bath. Equal volume of CI (24:1) was added to the tube after incubation and mixed gently. It was further centrifuged at 12,000 rpm for 10 mins and aqueous layer was collected in a fresh tube and this step was repeated twice. Finally, DNA was precipitated by adding 1/10 volume of 3M sodium acetate and equal volume of chilled isopropanol followed by gentle mixing and kept at -20°C overnight. Next day, the samples were centrifuged at 12,000 rpm for 10 mins and pellet was collected after gently discarding the supernatant. The DNA pellet was washed twice with 70% ethanol and centrifuged at 12,000 rpm for 10 mins. The pellet was air dried and dissolved in 100 µl TE buffer and stored at -20°C for future use.

2.4 Qualitative and Quantitative DNA Estimation

The isolated DNA was analyzed on 0.8% agarose gel electrophoresis by loading a mixture of 1µl DNA sample and 4 µl of 6X loading dye along with 50 bp ladder [19]. The gel was run at 80V, till the tracking dye migrated to the bottom of the gel. The DNA samples were visualized using a UV gel documentation system (Benchtop Lab Equipment) and photographed and documented. The concentration of DNA was also determined by taking absorbance at 260 nm using a UV spectrophotometer (Biophotometer, Eppendorf). The purity of DNA was determined by calculating the ratio of absorbance at 260/280 nm.

2.5 PCR Analysis

The suitability of extracted DNA for molecular techniques was assessed through PCR amplification using SSR markers as reported in our earlier study [1]. DNA was diluted to a final concentration of 10 ng/µl and amplification reactions were carried out in a 10 µl reaction mixture containing 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM each dNTP, 0.4

Table 1. List of 48 genotypes of pearl millet used for DNA isolation

S. No.	Genotype	Source	S. No.	Genotype	Source
1.	HHB 299	AICRP on Pearl Millet, CCS HAU, Hisar	25.	86M 88	Pioneer Overseas Corp., Hyderabad
2.	AHB 1200	AICRP on Pearl Millet, NARP, Aurangabad	26.	NBH 5061	Nuziveedu Seeds Pvt. Ltd., Hyderabad
3.	Dhanshakti	AICRP, MPKV, Dhule	27.	ABV 04	ANGRAU, Ananthpuramu
4.	RHB 233	AICRP on Pearl Millet, RARI, Jaipur	28.	MBC 2	AU, Jodhpur
5.	RHB 234	AICRP on Pearl Millet, RARI, Jaipur	29.	Pusa Comp. 612	IARI, New Delhi
6.	HHB 311	AICRP on Pearl Millet, CCS HAU, Hisar	30.	Pusa Comp.701	IARI, New Delhi
7.	AHB 1269	AICRP on Pearl Millet, NARP, Aurangabad	31.	Gadhwal ki Dhani-3	L.C. Sikar, ICAR-AICRP on Pearl Millet, Mandor
8.	HHB 272	AICRP on Pearl Millet, CCS HAU, Hisar	32.	Chanana Bajra3	L.C. Churu,ICAR-AICRP on Pearl Millet, Mandor
9.	MPMH 21	AICRP on Pearl Millet, Jodhpur	33.	Sulkhaniya bajra	Ratangarh, Churu ICAR-AICRP on Pearl Millet, Mandor
10.	BHB 1202	AICRP on Pearl Millet, SKRAU, Bikaner	34.	Chadi bajri	L.C. Jodhpur ICAR-AICRP on Pearl Millet, Mandor
11.	RHB 223	AICRP on Pearl Millet, RARI, Jaipur	35.	Dhodhsar local	L.C Sikar ICAR-AICRP on Pearl Millet, Mandor
12.	RHB 177	AICRP on Pearl Millet, RARI, Jaipur	36.	Damodara Bajri	L.C. Jaisalmer ICAR-AICRP on Pearl Millet, Mandor
13.	HHB 67 Imp.	AICRP on Pearl Millet, CCS HAU, Hisar	37.	7686	Pioneer Hyderabad
14.	PB 1756	Bayer Bio Science, Hyderabad	38.	GHB 538	AICPMIP, MRS, Jamnagar
15.	PB 1705	Bayer Bio Science, Hyderabad	39.	GHB 558	AICPMIP, MRS, Jamnagar
16.	XMT 1497	Bayer Bio Science, Hyderabad	40.	HHB 146	AICPMIP, CCS HAU, Hisar
17.	NBH 5767	Nuziveedu Seeds Pvt. Ltd., Hyderabad	41.	Nandi 72	New Nandi Seeds Corporation, Ahmedabad
18.	GHB 905	AICRP on Pearl Millet, MRS, Jamnagar	42.	MP 7171	Metahelix Life Science, Bangalore
19.	MPMH 17	AICRP on Pearl Millet, Jodhpur	43.	HHB 226	AICPMIP, CCS HAU, Hisar
20.	JKBH 1326	JK Agri Genetics, Hyderabad	44.	MP 7878	Metahelix Life Science, Bangalore
21.	HHB197	AICRP on Pearl Millet, CCS HAU, Hisar	45.	PB 180	ProAgro, Hyderabad
22.	PB 1720	Bayer Bio Science, Hyderabad	46.	Nandi 52	New Nandi Seeds Corporation, Ahmedabad
23.	DHBH 1397	AICRP on Pearl Millet, MPKV, Dhule	47.	Pusa 23	IARI, New Delhi
24.	Nandi 75	New Nandi Seeds Corporation, Ahmedabad	48.	HHB 223	AICPMIP, CCS HAU, Hisar

µM 10-mer primer, 1 unit *Taq* DNA polymerase (Bangalore GeNei, India) and 10 ng of DNA. Amplifications were carried out in a 96-well thermal cycler (Agilent Technologies). PCR programme constituted of 1 cycle of 5 min at 94°C for initial denaturation followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 55°C for annealing and 1 min at 72°C for primer extension. At last, 1 cycle of final extension was carried out for 10 mins at 72°C followed by hold at 4°C. The amplified products were analyzed on 3.5% agarose gel and visualized under UV transilluminator and photographed using gel documentation system.

3. RESULTS AND DISCUSSION

The extraction of DNA is very important in molecular biology as it is vital for various applications. But, it becomes more difficult in the plant species like pearl millet possessing higher amount of polysaccharides, secondary metabolites and polyphenols as contaminants. These contaminants are co-extracted with the DNA and form tight complexes creating a gelatinous pellet thus interfering with polymerases, ligases and restriction enzymes [20,3]. Thus, here we developed an efficient protocol for DNA extraction in pearl millet which gave good quantity and quality of DNA without the use of liquid nitrogen. The yield of extracted DNA ranged from 105.2 to 328.3 ng/µl and the ratio of $A_{260/280}$ varied from1.74 to 1.95 specifying good quality DNA which is free from protein and RNA contamination (Table 2). Similar kind of yield and quality of DNA was also reported in other protocols [2,4,19,21,22]. A ratio of absorbance of 260/280 is used to judge the purity of nucleic acid and DNA quality with an $OD_{260/}$ OD_{280} ratio between 1.7 and 2.0 is supposed to be good [23]. In addition, on analysis on agarose gel electrophoresis, sharp and distinct bands were observed with no visible DNA degradation or RNA contamination indicating good quality and quantity of extracted genomic DNA (Fig.1). The good quality of DNA extracted in our study is comparable to various other studies where it was reported that good quality DNA can be isolated without using liquid nitrogen [19,21,22,24].

Table 2. Concentration and A_{260}/A_{280} ratio of DNA isolated from 48 pearl millet genotypes

S.No.	Genotype	DNA Conc. (ng/µl)	$A_{260/280}$	S.No.	Genotype	DNA Conc. (ng/µl)	$A_{260/280}$
1.	HHB 299	105.2	1.84	25.	86M 88	250.5	1.81
2.	AHB 1200	152.3	1.77	26.	NBH 5061	247.4	1.75
3.	Dhanshakti	308.6	1.89	27.	ABV 04	223.2	1.86
4.	RHB 233	132.2	1.82	28.	MBC 2	211.3	1.82
5.	RHB 234	240.3	1.81	29.	Pusa Comp. 612	116.6	1.81
6.	HHB 311	190.3	1.78	30.	Pusa Comp.701	241.4	1.77
7.	AHB 1269	246.2	1.95	31.	Gadhwal ki Dhani-3	226.3	1.83
8.	HHB 272	156.2	1.76	32.	Chanana Bajra-3	256.2	1.82
9.	MPMH 21	166.4	1.81	33.	Sulkhaniya bajra	285.4	1.78
10.	BHB 1202	238.3	1.80	34.	Chadi bajri	256.3	1.90
11.	RHB 223	152.1	1.75	35.	Dhodhsar local	284.3	1.86
12.	RHB 177	328.3	1.86	36.	Damodara Bajri	292.4	1.91
13.	HHB 67 Imp.	143.3	1.76	37.	M7686	125.2	1.82
14.	PB 1756	127.2	1.82	38.	GHB 538	146.5	1.92
15.	PB 1705	173.4	1.74	39.	GHB 558	204.3	1.88
16.	XMT 1497	125.2	1.83	40.	HHB 146	198.4	1.83
17.	NBH 5767	192.5	1.84	41.	Nandi 72	208.5	1.84
18.	GHB 905	105.7	1.80	42.	MP 7171	127.2	1.85
19.	MPMH 17	202.3	1.86	43.	HHB 226	210.3	1.84
20.	JKBH 1326	138.3	1.77	44.	MP 7878	218.6	1.83
21.	HHB197	113.4	1.82	45.	PB 180	221.4	1.81
22.	PB 1720	120.2	1.76	46.	Nandi 52	169.5	1.75
23.	DHBH 1397	238.1	1.81	47.	Pusa 23	156.2	1.89
24.	Nandi 75	205.2	1.78	48.	HHB 223	192.3	1.86

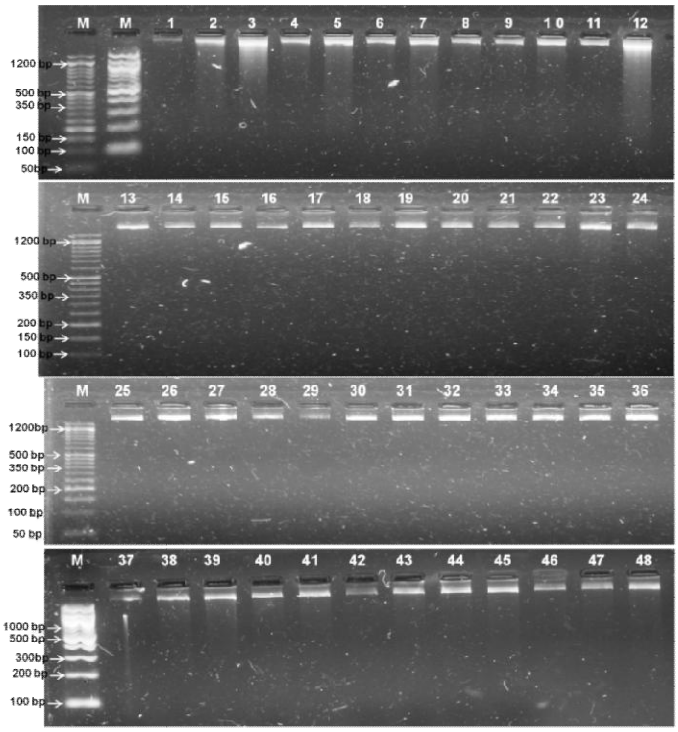

Fig. 1. Good quality genomic DNA of 48 pearl millet hybrids/varieties. Lane M-ladder, Lane 1 to 48 - Pearl millet hybrids/varieties

Most DNA extraction methods are time-consuming, involve multiple steps, expensive chemicals and enzymes and use hazardous procedures of grinding plant tissue in liquid nitrogen (N_2) to break down the cell wall of plants [21] or freeze-drying (lyophilization) [25]. Thus, any method which doesn't need liquid nitrogen can be more helpful. The method we developed is simple, fast, reproducible, inexpensive, less time consuming eliminating the use of expensive reagents like proteinase K and liquid nitrogen for extraction of high quality genomic DNA from pearl millet. Liquid nitrogen is expensive and difficult to procure in remote locations and developing countries due to unpredictable purchasing time from overseas. On the other hand, magnetic beads/tissue lyser which are used to grind the samples are mostly unavailable to some undergraduate laboratories leading to obstacles for extraction. The need of liquid nitrogen during grinding step has been eliminated by many workers using soft tissues such as flower petals or young leaves [26] cold and heat shock treatments [27] alcohol fixation [21,28].

Fresh, young and tender tissues are good source for DNA isolation than mature tissues and are the first alternative to obtain good quality DNA because mature leaves contain higher quantities of polyphenols and polysaccharides making good quality DNA isolation a difficult task. Contrary to it, we isolated good quality DNA from leaf tissues even stored at -20°C from 1-2 years. The method described here is very useful and efficient as the DNA obtained through this protocol was reasonably pure which gave amplifying products in the PCR ranging from 150 to 650 bp (Fig. 2). We made several modifications to basic CTAB DNA extraction procedure like changes in constituents and concentration of extraction buffer. We increased concentration of NaCl, CTAB, Tris-HCl, β- mercaptoethanol to reduce polysaccharide contamination and effective DNA extraction. Similar reports were observed by many workers [8,29,30]. Addition of a higher concentration of Tris-HCl facilitates constant pH during extraction with CTAB buffer. Further, we used 900 µl of CTAB extraction buffer instead of 750 µl for the lysis of cell membrane which yield more aqueous phase after first centrifugation step (~700 µl). Here, we incorporated a higher concentration of NaCl (1.4 M) in CTAB extraction buffer that improved the DNA yield and quality by preventing the sample from becoming viscous in nature during sample grinding. NaCl at

concentrations of > 0.5 M along with CTAB helps in removing polysaccharides effectively [5,31]. The concentration of NaCl varies between 0.7 M [32] and 6 M [33] and depends on the different plant species under the study.

During extraction process, cell wall is broken down first of all followed by digestion and disruption of cell membrane to release the cellular constituents and DNA into the extraction buffer using detergents like sodium dodecyl sulphate (SDS) or cetyl-methylammonium bromide (CTAB) [34]. Here, we used combination of both detergents, CTAB and SDS to make extraction more efficient in absence of liquid nitrogen. EDTA is generally added in the extraction buffer to protect the released DNA from endogenous nucleases because it chelates magnesium ions which is a necessary co-factor for nucleases. We used increased amount of EDTA (0.2 M) that efficiently neutralizes the divalent cations required for DNAse activity and thus protects the DNA from degradation. Initially, large quantity of proteins, polysaccharides, RNA, tannins and pigments are usually present along with DNA in the extract which are difficult to separate and can interfere with the extracted DNA [35]. It is more difficult to remove some contaminants like polysaccharides and polyphenols which generally inhibit the activity of certain DNA modifying enzymes thus interfering in the quantification of nucleic acids using spectrophotometer. Antioxidants like β-mercaptoethanol, PVP, bovine serum albumin and sodium azide are mainly used to get rid of the problems associated with phenolics [17,32]. In the present study, the extracted DNA was free of phenols as the pellet obtained was clean, clear and translucent which is comparable to other such recent studies [2,18,19,36,37]. β-mercaptoethanol acts as a reducing agent and removes the tannins and polyphenols from the crude extract by denaturing proteins and breaking the disulfide bonds between the cysteine residues.

Proteins are mainly removed by denaturation and precipitation of the extract following multiple extraction steps with organic solvents like phenol and chloroform [17,38]. In addition to saturated phenol, enzyme proteinase K is also used to remove proteins which however are again denatured by phenol via phenol chloroform extraction and moreover it is costly. However, in the present study we avoided use of proteinase K thus reducing cost. The chloroform extraction step was performed before precipitating the DNA to improve the DNA quality and yield by efficient removal of polysaccharides, lipids and other non-polar substances. Repeated chloroform: isoamyl alcohol treatment helps in getting rid of chlorophyll, pigments and dyes completely.

The DNA is precipitated using equal volume of isopropanol and diluting the nucleic acid with a monovalent salt. Later, the pellet is collected by centrifugation and washed with 70% alcohol to remove the salts. Sodium chloride (0.2M), ammonium acetate (2-2.5M), sodium acetate (0.3M, pH 5.2), lithium chloride (0.8M) and potassium chloride are some of the most commonly used salts for washing the pellet. On the other hand, RNAs are usually removed by treating the extract with heat-treated RNase A. Here, we enhanced the time-period of RNase A treatment for DNA from 30 to 40 min facilitating proper removal of RNA from the sample. Further, addition of chloroform and iso-amyl alcohol (24:1, v/v) after RNase A treatment of DNA improves the DNA quality by efficiently removing proteins from the DNA sample. Most people usually give RNase treatment after precipitation of DNA followed by purification steps by performing CI extractions which makes procedure lengthy and can reduce DNA quantity. But, here we eliminated repeated steps of extraction which are

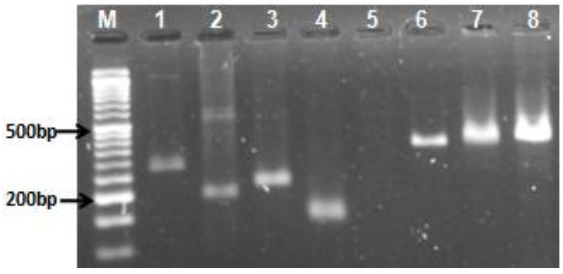

Fig. 2. PCR amplification with extracted DNA using SSR marker on 3.5% agarose gel. Lane M-50 bp ladder, Lane 1-8 – Pearl millet genotypes used for PCR

performed when RNase treatment is given at last after isolation of DNA pellet and performed it along with protein removal steps with CI to reduce time and chemicals. Thus, incorporating the above-mentioned modifications led to the extraction of high quality genomic DNA without contaminants like RNA and protein.

4. CONCLUSION

We developed an efficient protocol which is extremely suitable for extracting high quality DNA from climate resilient plant like pearl millet and other such plants having high levels of polysaccharides and polyphenolics and for processing large number of plant samples. This method resolved the issues of DNA degradation, contamination, low yield due to binding and/or co-precipitation with starches and polysaccharides. The isolated DNA also proved acquiescent to PCR amplification. Thus, we successfully established a protocol which is simple, specific, efficient and fast compared to other methods without the need of liquid nitrogen. Only low-priced chemicals and ordinary laboratory equipments are sufficient for DNA extraction and it is not very expensive as it is independent of the use of costly commercial kits. The extracted DNA is stable and even can be used and stored for longer durations. It can be used for several applications of crop improvement including marker assisted selection, DNA fingerprinting, quantitative traits loci analysis, screening of transformants and enzymatic digestion. Hence, this method is recommended even to be used in low-technology laboratories for processing large number of samples in an effective way without using liquid nitrogen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ambawat S, Satyavathi CT, Meena R, Khandelwal V, Meena RC. Molecular characterization and genetic diversity analysis of released hybrids and varieties of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Curr J Appl Sci Tech. 2020;39(31):92-104.
2. Kale SS, Kadu TP, Chavan NR, Chavan NS. Rapid and efficient method of genomic DNA extraction from sweet sorghum [*Sorghum bicolor* (L.)] using leaf tissue. International J Chem Studies 2020;8(1):166-1169.
3. Sahu SK, Thangaraj M, Kathiresan K. DNA Extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. ISRN Molecular Biology. 2012;6(4):1-6.
4. Xin Z, Chen J. A high throughput DNA extraction method with high yield and quality. Plant Methods 2012;8(1):1-7.
5. Murray MG, Thompson WF. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 1980;8:4321-4325.
6. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: version II. Plant Mol Biol Rep. 1983;1:19-21.
7. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA. 1984;81:8014-8018.
8. Doyle JJ, Doyle JL. Isolation of Plant DNA from fresh tissue. Focus. 1990;12:13-15.
9. Huang J, Ge X, Sun M. Modified CTAB protocol using a silica matrix for isolation of plant genomic DNA. Bio Techniques. 2000;28:432-434.
10. Allen GC, Vergara MAF, Krasnyanski S, Kumar S, Thompson WF. A modified protocol for rapid DNA isolation from plant tissue using cetyltrimethyl ammonium bromide. Nature Protocols. 2006;1:2320-2325.
11. Ahmed I, Islam M, Arshad W, Mannan A, Ahmad W, Mirza B. High-quality plant DNA extraction for PCR: An easy approach. Journal of Applied Genetics. 2009;50:105-107.
Available:
http://dx.doi.org/10.1007/BF03195661
12. Amani J, Kazemi R, Abbasi A, Salmanian A. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. Iranian Journal of Biotechnology. 2011;9:69-71.
13. Chen WY, Cui HR, Bao JS, Zhou XS, Shu QY. A simplified rice DNA extraction

protocol for PCR analysis. Rice Sci. 2006;13 (1):67-70.
14. Thomas J, Dominic VJ. Isolation of high quality DNA from pulverized rice grain samples by tri-phase method. Int J Sci Res 2015;4(11):524-527.
15. McCarthy PL, Berger PH. Rapid identification of transformed wheat using a half-seed PCR assay prior to germination. Biotechniques. 2002;32:560-564.
16. Kang HW, Yong GC, Unghan Y, Moo YE. A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. Plant Mol Biol Rep. 1998;16:1-9.
17. Zidani S, Ferchichi A, Chaieb M. Genomic DNA extraction method from pearl millet (*Pennisetum glaucum*) leaves. African Journal of Biotechnology. 2005;4(8):862-866.
18. Sathelly KM, Govindannagari R, Pandey S, Kompelli SK, Visarada KBRS, Kaul T. A Simple and Efficient method for high quality DNA extraction from sweet sorghum [*Sorghum bicolor* (L.) Moench]. Annals of Plant Sciences. 2014;3(7):761-764.
19. Ambawat S, Kumar R, Singh S, Yadav R. An Easy, Quick and cost effective method of high quality DNA extraction from mungbean [*Vigna radiata* (L.) Wilczek] Without Liquid Nitrogen. Int J Curr Microbiol App Sci. 2017;6(9):2695-2703.
20. Ogunkanmi AL, Oboh B, Onifade B, Ogunjobi AA, Taiwo IA, Ogundipe OT. An improved method of extracting genomic DNA from preserved tissues of *Capsicum annuum* for PCR amplification. Eur Asian Journal of BioSciences. 2008;2(14):115-119.
21. Sharma P, Joshi N, Sharma A. Isolation of genomic DNA from medicinal plants without liquid nitrogen. Indian J Exp Bio 2010;48:610-614.
22. Ferdous J, Hanafi MM, Rafii MY, Muhammad K. A quick DNA extraction protocol: Without liquid nitrogen in ambient temperature. African Journal of Biotechnology. 2012;11(27):6956-6964.
23. Maniatis T, Fritsch ER, Sambrook J. Quantification of DNA and RNA. In: Molecular cloning: A laboratory manual cold spring harbor laboratory press: NewYork. 1982;448-469.
24. Chandra A, Tewari S. Isoaltion of genomic DNA from Stylo species without liquid nitrogen suitable for RAPD and STS analysis. Cytologia. 2007;72:287.
25. Sperisen CF, Gugerli Büchler U, Mátyas G. Comparison of two rapid DNA extraction protocols for gymnosperms for application in population genetic and phylogenetic studies. Genetics. 2000;7:133-136.
26. Khan IA, Awan FS, Ahmad A, Khan AA. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Mol Biol Rep. 2004;22:89a-89e.
27. Biswas K, Biswas, R. A modified method to isolate genomic DNA from plants without liquid nitrogen Current Sci. 2011;100:1622-1624.
28. Duan YB, Zhao FL, Chen HD, Li H, Ni DH, Wei PC et al. A simplified genomic DNA extraction protocol for pre-germination genotyping in rice. Genet Mol Res. 2015;14(2):6369-6375.
29. Cheng FS, Brown SK, Weeden NF. A DNA extraction protocol from various tissues in woody species. Hortscience. 1997;32(5):921-922.
30. Sari VK, Murti RH. An effective method for DNA extraction of mature leaf of sapodilla (*Manilkara zapota* (L.) van Royen). Agrivita. 2015;37(1):18-23. DOI:10.17503/Agrivita-2015-37-1-p018-023.
31. Paterson AH, Brubaker CL, Wendel JF. A rapid method for extraction of Cotton (*Gossypium spp*) genomic DNA suitable for RFLP or PCR analysis. Plant Mol Biol Rep. 1993;11:122-127.
32. Clark MS. In: Plant molecular biology-A laboratory manual; springer-verlog berlin Heidelberg. New York.1997;305-328.
33. Aljanabi SM, Forget L, Dookun A. An improved rapid protocol for the isolation of polysaccharide and polyphenol-free sugarcane DNA. Plant Mol Biol Rep. 1999;17:1-8.
34. Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA et al. Short protocols in molecular biology. Wiley,New York. 1997;2.9:2-11.
35. Puchooa DA. Simple, rapid and efficient method for the extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.). African Journal of Biotechnology. 2004;3:253-255.
36. Rawat S, Joshi G, Annapurna D, Kumar AN, Karaba NN. Standardization of DNA extraction method from mature dried leaves and ISSR-PCR conditions for

melia dubia Cav.-A fast growing multipurpose tree species. American J Plant Sci. 2016;7:437-445.

37. Sajib AA, Bhuiya MAI, Huque R. A Simple, efficient and rapid method for good quality DNA extraction from rice grains. Rice Sci. 2017;24(2):119-122

38. McKiernan HE, Danielson PB. Molecular Diagnostics. Academic Press, Cambridge. 2017;1(3):371-394.

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