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

Supriya Ambawat, Subaran Singh, C. Tara Satyavathi, Rajbala Meena, R. C. Meena and Vikas Khandelwal

ICAR-AICRP on Pearl Millet, Mandor, Agriculture University, Jodhpur -342 304, Rajasthan, India.

Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar -125004, Haryana, India.

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.

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.

*Corresponding author: E-mail: supriya_bmb@yahoo.co.in
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 A260/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 (Ethylene diamine Tetracetic Acid)  
Sodium Chloride  
β-Mercaptoethanol  
Phenol  
Chloroform  
Iso-amyl alcohol  
3 M Sodium Acetate (pH 5.8)  
Isopropanol  
RNase A (10 mg/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 MgCl₂, 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.    | Gadhwali 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.   | HHB 197   | 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.   | DHBB 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 30 s 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 from 1.74 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].

| 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.   | Chana Bajra-3 | 256.2            | 1.82         |
| 9.    | MPMH 21      | 166.4             | 1.81         | 33.   | Sulkhianiya 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.   | HHB 197      | 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         |
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 (N₂) 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

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

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