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Short Communication

An optimized SDS-based protocol for high-quality genomic DNA extraction from brown midrib (BMR) pearl millet

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Abstract

Extraction of high-quality DNA from brown-midrib (BMR) pearl millet (Cenchrus americanus (L.) Morrone) is challenging due to high levels of phenolics, lignin derivatives, and polysaccharides that interfere with cell lysis and downstream molecular analyses. In this study, four SDS-based lysis buffers (SLB-1 to SLB-4) and a standard CTAB method were evaluated for DNA isolation from BMR leaf tissues. Among these, SLB-3 (2% SDS, 3% PVP-40, 900 mM NaCl, 100 mM Tris-HCl, 25 mM EDTA, and 0.5% β-mercaptoethanol) produced significantly higher DNA yield (649.1 ± 123.2 ng/µL), improved purity (A260/280 = 1.87 ± 0.03; A260/230 = 2.13 ± 0.10), and intact high-molecular-weight DNA. Successful amplification of the extracted DNA with the SSR marker confirmed the integrity and amplifiability of the DNA, indicating its suitability for downstream molecular analyses. The SLB-3 protocol demonstrated advantages over CTAB and other SDS buffers in terms of simplicity, cost efficiency, and reproducibility. This optimized method may be suitable for routine molecular breeding and genetic studies in phenolic-rich and recalcitrant pearl millet tissues.

Keywords: Pearl millet, Brown midrib (BMR), CTAB, DNA extraction, SDS.

Introduction

Isolating high-quality DNA from plant tissues rich in polysaccharides and secondary metabolites remains a persistent challenge in molecular biology. These compounds frequently co-precipitate with DNA or inhibit downstream applications such as restriction digestion, PCR, library preparation, and genome sequencing (Do and Adams, 1991; Fang *et al.*, 1992). To address these limitations, numerous extraction buffers, precipitation strategies, purification additives, and cleanup methods have been developed over the past decades. The cetyltrimethylammonium bromide (CTAB) protocol is one of the most widely used DNA isolation approaches (Doyle and Doyle, 1987). Modifications to the CTAB method often include increasing NaCl concentration to 1.4–2.0 M to prevent polysaccharide co-precipitation (Abubakar *et al.*, 2018), adding β-mercaptoethanol (1–2%) to reduce oxidative damage (Schenk *et al.*, 2023), and incorporating PVP or PVPP (1–5%) to bind polyphenols. Alternatively, SDS-based extraction methods or combinations of cationic (CTAB) and anionic (SDS) detergents have been employed to enhance lysis efficiency and impurity removal. Additional additives such as activated charcoal, chaotropic agents, Tris-HCl, and EDTA further improve DNA integrity by removing inhibitory compounds and stabilizing nucleic acids.

DNA precipitation in most plant protocols is achieved using ethanol or isopropanol in the presence of salts such as NaCl or sodium acetate to selectively recover DNA while excluding polysaccharides. Depending on tissue type, precipitation may be carried out at room temperature or at -20°C to maximize yield. Purification steps may include chloroform extraction, phenol–chloroform extraction, or the use of silica columns, magnetic beads, or resin-based matrices for improved performance in plant species (Wang *et al.*, 2024). RNase

Table 1. Composition of lysis buffers evaluated for genomic DNA extraction from BMR Pearl-millet

Buffer Component	CTAB Protocol	SLB-1*	SLB-2*	SLB-3*	SLB-4*
CTAB	2%	-	-	-	-
SDS	-	1%	2%	2%	3%
PVP	2%	1.5%	2%	3%	2.5%
β-Mercaptoethanol (v/v)	0.2	0.5	0.3	0.5	0.8
Tris-HCl (mM)	100	100	100	100	100
EDTA (mM)	25	25	25	25	25
NaCl	1.4 M	1.2 M	1 M	900 mM	500 mM

*SLB-1 to 4: Sodium dodecyl sulphate lysis buffer

and proteinase K digestion are typically incorporated to remove RNA and protein contaminants, and some studies combine commercial kits (e.g., DNeasy Plant Mini Kit) with CTAB or SDS protocols for further improvement. Although several protocols exist for genomic DNA extraction from pearl millet (Gulia *et al.*, 2010; Mace *et al.*, 2003; Nweke *et al.*, 2014), none specifically address the challenges associated with brown midrib (BMR) mutants (John, 1992; Porebski *et al.*, 1997). Our attempts using existing methods yielded degraded, brown-colored, or low-purity DNA from BMR leaves. The difficulty arises from the high levels of phenolic compounds, lignin precursors, alkaloids, and polysaccharides that accumulate in BMR tissues (Sattler *et al.*, 2010; Saballos *et al.*, 2012). Oxidized polyphenols bind covalently to nucleic acids, causing browning and degradation, while alkaloids and pigments co-precipitate with DNA. Polysaccharides increase lysate viscosity, entrap DNA, and reduce yield and purity. To overcome these limitations, we developed a simple and inexpensive SDS-based protocol specifically optimized for BMR pearl millet. The method incorporates sequential purification steps with optimized concentrations of PVP and β-mercaptoethanol, followed by chloroform-based phase separation and high-salt precipitation. DNA quality and suitability were validated through SSR analysis, demonstrating that the extracted DNA possesses the purity and integrity required for downstream molecular applications.

Leaf samples were collected 30 days after sowing from two low-lignin BMR genotypes, ICBMR07 (lignin: 3.1%) and ICBMR09 (lignin: 3.3%), and two high-lignin non-BMR genotypes, ICBP19 (lignin: 6.4%) and ICBP01 (lignin: 5.8%), grown at the Crop Improvement Farm, ICAR-IGFRI, Jhansi. The collected samples were subsequently stored at −80°C until further use. Among the twelve SDS-based extraction buffers evaluated, four of them yielded successful results, while the standard CTAB method was used as a control (Table 1). Approximately 200 mg of leaf tissue was ground in liquid nitrogen using a chilled mortar and pestle. About 1-mL of pre-warmed (65°C) extraction buffer was added, mixed thoroughly, transferred to a 2 mL tube, incubated at 65°C for 30 minutes, and centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was transferred to a fresh tube, mixed with an equal volume of chloroform:isoamyl alcohol (24:1), and centrifuged at 10,000 rpm for 10 minutes at 14°C. The aqueous phase was combined with 200 µL of 3 M sodium acetate and 2/3 volume of ice-cold isopropanol, then incubated at -20°C for 1 hour. DNA was pelleted (10,000 rpm, 10 minutes), washed with 70% ethanol, air-dried, resuspended in 100 µL nuclease-free water, and treated with RNase A (20 µg mL^{-1}) at 45°C for 30 minutes. DNA integrity was assessed on 0.8% agarose gels stained with ethidium bromide. Concentration and purity (A260/A280 and A260/A230) were measured using a NanoDrop 1000 spectrophotometer. PCR validation was performed using primer *Xibmsp*26 in a 10 µL reaction, following a touchdown protocol for amplification. Statistical analyses (one-way ANOVA and post-hoc comparisons at $\alpha = 0.05$) were conducted in Python v3.9 using SciPy v1.7.1 (Virtanen *et al.*, 2020) and Stats models v0.12 to compare DNA yield and purity across buffers. Differences among different extraction buffers were analyzed using a two-factor ANOVA with sample type (BMR and non-BMR) and buffer as fixed effects. Post hoc comparisons were performed using Dunnett's test (Dunnett, 1955), with CTAB as the reference buffer. Results for yield of DNA, A260/280, and A260/230 are

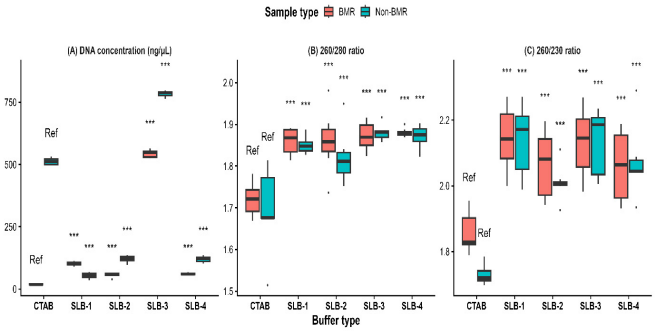

Fig 1. Quantitative assessment of DNA yield and purity from five extraction buffers (CTAB (Ref.), SLB-1, 2, 3 ,4); Boxplots illustrate the distribution of results from three biological replicates (n=3) for each buffer type; (A) Genomic DNA yield (ng/µL), (B) A260/A280 ratio and (C) A260/A230 ratio

Table 2. Comparative analysis of genomic DNA yield and purity using different lysis buffers

Buffer System	DNA Concentration (ng/µL)		260/280 Ratio		260/230 Ratio	
	BMR	Non-BMR	BMR	Non-BMR	BMR	Non-BMR
SLB-1	101.6 ± 6.8	53.3 ± 11.2	1.85 ± 0.02	1.86 ± 0.03	2.14 ± 0.1	2.14 ± 0.1
SLB-2	57.4 ± 9.2	120.5 ± 15.3	1.86 ± 0.07	1.82 ± 0.06	2.07 ± 0.1	2.01± 0.05
SLB-3	649.1 ± 123.2	870.6 ± 112.5	1.87 ± 0.03	1.88 ± 0.02	2.13 ± 0.1	2.13 ± 0.1
SLB-4	60.9 ± 3.4	119.9 ± 11.8	1.88 ± 0.01	1.87 ± 0.03	2.06 ± 0.1	2.07 ± 0.1
CTAB	19.07 ± 2.8	512.5 ± 14.7	1.72± 0.04	1.70 ± 0.1	1.86 ± 0.06	1.73± 0.03

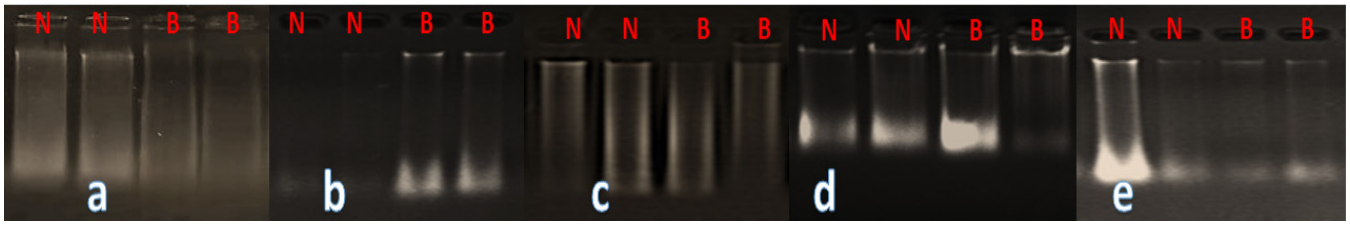

Fig 2. Qualitative analysis of genomic DNA integrity from BMR and non-BMR pearl millet samples isolated by using (a) CTAB, (b) SLB-1, (c) SLB-2, (d) SLB-3 and (e) SLB-4 buffers; Samples were labeled as N (non-BMR) and B (BMR), 3 µL of each sample loaded onto a 0.8% agarose gel; Images were captured using a gel documentation system

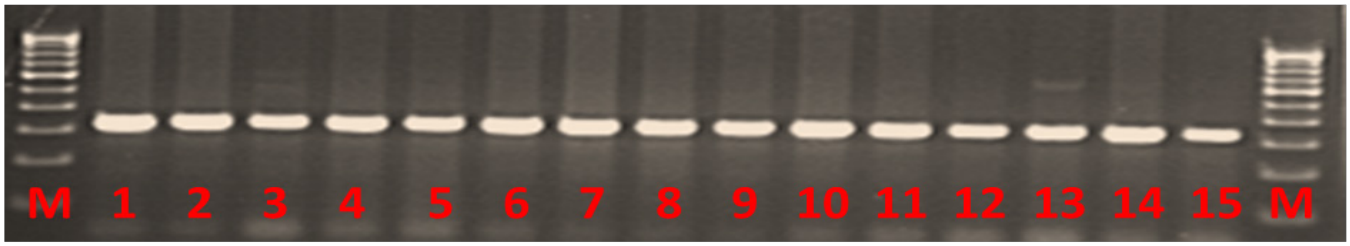

Fig 3. Functional validation of genomic DNA extracted with the optimal SLB-3 protocol. 3% agarose gel showing the results of SSR marker analysis with Xibmsp26 marker; Template DNA for all reactions was extracted from 15 different BMR pearl millet plants using the optimized SLB-3 protocol; M: 100 bp DNA ladder; Lanes 1-15: Amplification products from individual BMR DNA samples

presented as boxplot (Fig 1) and the mean concentration of different parameters from three independent biological replicates are presented in Table 2. A comparative analysis of five DNA extraction buffers (CTAB, SLB-1, SLB-2, SLB-3, SLB-4) demonstrated significant differences in DNA yield and purity parameters. Two-factor ANOVA confirmed that buffer type had a highly significant effect on DNA concentration (p <0.001) as well as purity indices, A260/A280 ratio (p <0.001), and the A260/A230 ratio (p <0.001). Among the SDS buffers, SLB-3 modification produced the highest DNA concentration (~649.1 ± 123.2 ng/µL), significantly higher DNA yield than other methods, whereas the SLB-1, SLB-2, and SLB-4 protocols yielded much less DNA (~53–60 ng/µL). DNA quality parameter, A260/A280 ratio, was significantly lower for CTAB buffer (1.72 ± 0.04) compared to all SLB-based methods (~1.85–1.88). A similar trend was observed for the A260/A230 ratio, CTAB buffer exhibited the lowest 260/230 values (mean 1.86 ± 0.06), whereas all SLB methods maintained higher 260/230 ratios (~2.06–2.14).

Electrophoretic separation of extracted DNA from different protocols shows that the CTAB buffer fails to produce any detectable bands with BMR plants, while the SDS-based buffer, notably SLB-3, produces intact, bright bands near the well (Fig 2d). High purity and intact DNA is suitable for PCR. Further, Figure 3 shows a clear, scorable, and highly reproducible SSR amplification profile with marker *Xibmsp*26 generated using DNA extracted from SLB-3.

In this study, new SDS-based DNA extraction protocols were developed to overcome the challenges posed by high secondary metabolites and polysaccharides in BMR plant tissues. The specific CTAB formulation tested in this study produced highly viscous, brown pellets with poor dissolution in TE buffer and RNA smearing in all BMR samples, whereas non-BMR tissue showed an intact band with CTAB buffer (Fig 2a), indicating its inadequacy for BMR plants. Co-precipitation of polysaccharides, oxidized polyphenols, proteins and related secondary metabolites can reduce DNA recovery and lower purity

ratios by promoting DNA loss, viscous co-precipitates, and inhibition of downstream enzymatic reactions. Four effective SDS-based lysis buffers (SLB-1 to SLB-4) were evaluated alongside the CTAB buffer. The CTAB buffer tested in the study was less effective than SDS-based buffers. A similar performance of the CTAB buffer has been reported in cotton (Ali *et al.,* 2019), okra (Kidane *et al.,* 2020), and soybean (Xia *et al.,* 2019). SDS is an anionic surfactant that interacts with cell membrane proteins, promoting cell lysis to release DNA in solution (Kasem *et al.,* 2008), its interaction with DNA can inhibit subsequent downstream applications (Xia *et al.,* 2019); thus, an optimal concentration is critical. In this study, 2% SDS yielded high-purity DNA, while both lower and higher concentrations reduced yields, similar to observations in soybean (Xia *et al.,* 2019). Polyphenolic compounds interfere with DNA extraction, and PVP effectively removes them. In the present study, a higher concentration of PVP (3%) was found to be the most effective, while other lower concentrations often failed to remove polyphenolics, resulting in poor yield of genomic DNA (Kim *et al.,* 1997). β-mercaptoethanol (0.5%) efficiently prevented oxidation and protein interference, supporting higher DNA yields, consistent with previous studies emphasizing its antioxidant role (Puchooa, 2004). Polysaccharides tend to co-precipitate with DNA; salt helps to minimize this co-precipitation. Although many protocols used 1.4 M NaCl, 900 mM was sufficient for BMR tissues in this study. Reports of effective DNA extraction with lower salt concentrations in SDS-based buffers further support this result (Xia *et al.,* 2019). SDS-based buffers produced higher yields and superior purity, with A260/280 ratios of 1.84-1.88 and A260/230 ratios of 2.04-2.14, outperforming CTAB. Despite the overall superiority of SLB buffers, DNA yield varied widely (57.4-649.01 ng/μL), reflecting that extraction efficiency depends on balanced interactions among buffer components rather than individual optimization alone.

This study systematically compares multiple SDS-based buffers for plant DNA extraction and identifies SLB-3 as the optimal buffer. The optimality of SLB-3 stems from its balanced composition, with 2% SDS for efficient membrane disruption, 3% PVP for polyphenol removal, 0.5% β-mercaptoethanol to prevent DNA degradation, and 900 mM NaCl to effectively inhibit co-precipitation of polysaccharides. This yielded the highest DNA concentration (649.1±123.2 ng/μL) as well as purity indices (A260/280= 1.88 ± 0.04; A260/230 = 2.14±0.1). The results indicate that SDS-based buffers can provide an efficient and cost-effective approach for isolating high-quality DNA from BMR tissue.

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