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Evaluation of economical and rapid method of plant DNA extraction for PCR analysis of different crops

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Abstract: In the recent genomic era, polymerase chain reaction (PCR) has become a basic tool in molecular studies and the success of PCR depends upon the template DNA. PCR technique is quite robust and often unnecessary to extract high quality of DNA and hence crude DNA can be used as template for amplification. Therefore, we have evaluated NaOH-Tris DNA extraction method for PCR analysis because this is very simple, time saving and safe without the need to use expensive or rare materials and laboratory apparatus. This method only requires a small amount of leaf tissue, NaOH, Tris, micro tube and plastic pestle. The amplified PCR products showed clear, sharp and uniform bands gave similar results as compared with the modified CTAB method. The DNA obtained is crude contains impurities like protein, RNA but these impurities did not affect PCR amplification. This DNA extraction method is evaluated for brinjal (*Solanum melongena* L.), chilli (*Capsicum annuum* L.), rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.) crop. Many other crop plants could also be amplified using the same DNA extraction method for molecular analysis of large samples. Thus, the use of NaOH-Tris method will allow researchers to obtain DNA from plant quickly for use in molecular studies.

Keywords: DNA extraction, NaOH, PCR, Tris

INTRODUCTION

Polymerase chain reaction has become a basic tool in molecular biology and is employed in a variety of molecular studies including genetic diversity analysis, marker assisted selection, quantitative trait loci QTL mapping, purity analysis and varietal identification. The major limitation for these studies is time and cost for extracting DNA in analyzing large plant samples. The extraction of DNA from plant tissue is time consuming, laborious and involves the use of many expensive chemicals (Dellaporta *et al.*, 1983; Saghai-Maroof *et al.*, 1984; Bernatzky and Tansley, 1986; Lassner *et al.*, 1989; Doyle and Doyle, 1990). The conventional method for extracting DNA from plant tissue are often unnecessary for routine genotyping because it needs multiple steps require special chemicals or instruments and they are not suitable for use with a large number of plant samples. Therefore, the genomic DNA extraction procedure for large scale genotyping analysis must be very simple, inexpensive and they can be used without compromising the accuracy of results. Nevertheless, the issue prompted us to survey previously published literature for rapid extraction protocols. There are various methods of rapid DNA extraction from plant tissues, but almost all of these involve liquid nitrogen for efficient grinding of plant tissues and use of organic solvents (Edwards *et al.*, 1991; Langridge *et al.*, 1991; Berthoumieu and Meyer, 1991; Oard and Dronavalli, 1992; Cheung *et al.*, 1993; Chunwongse *et al.*, 1993; Wang *et al.*, 1993; Guidet, 1994; Thomson and Henry, 1995; Haymes, 1996; Lange *et al.*, 1998). However, NaOH-Tris extraction method (Wang *el al.*, 1993) was found to be effective for the molecular analysis in terms of success rate, cost, speed and simplicity but still not popular. Therefore, in the present study NaOH -Tris extraction method has been evaluated for rapid extraction of DNA from four crops namely brinjal (*Solanum melongena* L.), chilli (*Capsicum annuum* L.), rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.) to minimize time and the use of laboratory materials.

MATERIALS AND METHODS

Plant material: In this study, four brinjal varieties (Arka Shirish, Arka Neelkanth, Lalpari, Arka Anand), four chilli varieties (Guntur Hope, Bullet, Pusa Jawala, Pusa Sadabahar), three tomato varieties (Arka Vikash, Arka Meghali, Arka Saurabh) and three rice varieties (Rajendra Sweta, Sabour Shree, Swarna Sub1) were used for DNA extraction.

DNA extraction: Ten milligrams of fresh leaf tissue of each variety were used to extract DNA. The tissues were homogenized with 100 µl 0.5 M NaOH in 1.5 ml

sterile centrifuge tubes with a plastic pestle for 1 minute. After homogenization, 900 µl of 100 mM Tris pH 8.0 were added and vortexed for 30 seconds and then centrifuged at 10,000 g for 1 minute at 25 °C and 1 µl of the supernatant was used as the DNA template for PCR analysis. DNA was also extracted by using modified CTAB method which served as control for comparison. The time required for extracting DNA with both methods was recorded and compared using ten randomly selected leaf samples.

PCR amplification: DNA extracted were used for PCR amplifications on a Eppendorf Mastercycler (Eppendorf, USA) in a total volume of 15 µl containing 1 × PCR buffer, 0.2 mM dNTPs, 0.2 µM each forward and reverse primer (Table 1), 0.5 U Taq DNA Polymerase (Xcelris, India) and 1 µl template DNA using the following profile: a 4 minutes denaturation at 94 °C and 35 cycles of 30 seconds denaturation at 94 ° C, 60 seconds annealing at 55 °C for SSR/ gene specific markers and a 60 seconds extension at 72 °C, followed by a final extension at 72 °C for 5 minutes. The PCR products were resolved by electrophoresis in 1.5 % (R2M1S marker) and 2.5 % (CAMS351, emb01M15 and ART5 marker) agarose gels (HiMedia) using 1X TAE buffer. The amplicons were visualized by UV light and documented (Uvitec gel doc system, UK). PCR Amplifications were carried out for at least twice for each sample. A DNA ladder 100 bp (Xcelris, India) was used as molecular markers to estimate the size of the amplicons.

RESULTS AND DISCUSSION

Comparison of DNA extraction method: NaOH-Tris and modified CTAB: DNA extraction is prerequisite and most important step in molecular studies. Consequently, it is essential to use a suitable DNA extraction method which is not only rapid and simple but also use small amount of tissue and extraction solutions. Considering these facts NaOH-Tris for extracting crude plant DNA was evaluated in this study. The NaOH-Tris method is much faster than other rapid DNA extraction protocols and the complete DNA extraction procedure takes 2.30 minute. This method, involves fewer steps, required mirocentrifuge tubes, less time and therefore is more economic as cost per sample is reduced when compared to conventional methods. Compared to modified CTAB method, the NaOH-Tris method has significant advantages. First, the NaOH-Tris method does not involve the mechanical breaking of plant cell walls, incubation at 65 ºC or 37 ºC, DNA purification using phenol or chloroform and the time required for DNA extraction is therefore reduced greatly (Table 2). In a single day, one person can complete DNA extraction from more than 200 leaf samples using this method. This method has been routinely used to extract DNA from rice for marker assisted selection in our laboratory. Second, the NaOH-Tris method does not require liquid nitrogen or an ultra low temperature centrifuge, which may be unavailable to many small laboratories and the method only requires a normal centrifuge. This method is suitable for a general molecular biology laboratory and is easily learned by layman. Third, the NaOH-Tris method does not use toxic chemicals (*e.g.*, liquid nitrogen, CTAB, β-mercaptoethanol, phenol or chloroform) and so it is safe for users and does not require disposal of harmful wastes. Fourth, in this method, very common chemicals were used for DNA extraction instead of costly chemicals. Fifth, the NaOH-Tris method requires only a small quantity of plant tissue (10 mg) and there is no sample waste whereas in modified CTAB method requires large quantities of plant tissue and ground in a mortar and pestle with liquid nitrogen. Finally, contaminant DNA can cause significant problems with PCR (Kwok and Higuchi, 1989), but the NaOH-Tris method reduces the chance of cross contamination

Table 1. List of primers used in the study for PCR amplification.

S. No.	Crop	Marker name	Sequence(5'-----3')	References
1.	Chilli	CAMS351F	CGCATGAAGCAAATGTACCA	Minamiyama *et al.*, 2006
		CAMS351R	ACCTGCAGTTTGTTGTTGGA	
2.	Brinjal	emb01M15F	GCAAGGCTCAAAGTCACAAGTCAA	Nunome *et al.*, 2009
		emb01M15R	GGCTCTGCCCCTAACATCTACAAA	
3.	Tomato	R2M1SF	GGAAATCCTCCGCCTTACTT	Zhang *et al.*, 2014
		R2M1SR	CGAGTTGCAACCTCTAGACTCA	
4.	Rice	ART5F	CAGGGAAAGAGATGGTGGA	Septiningsih *et al.*, 2009
		ART5R	TTGGCCCTAGGTTGTTTCAG	

Table 2. Comparison of time required for NaOH-Tris and modified CTAB DNA extraction methods.

Steps	DNA extraction method	
	NaOH-Tris	CTAB
Cell wall distruption/ homogenization/ suspension	2.30 min	5-10 min
Incubation	-	30-60 min
Purification	-	35 min
Precipitation	-	25 min
Washing	-	10 min
Drying	-	30 min

Dash (–) means the lack of a step

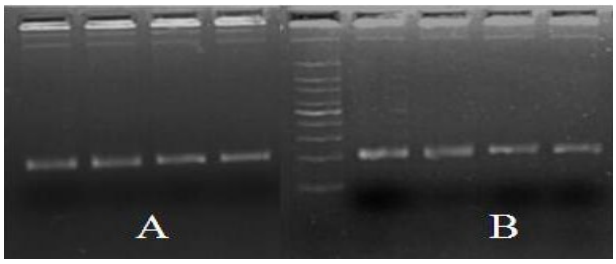

Fig. 1. *PCR amplification using CAMS-351 primer in four different chilli genotypes. DNA isolated by A) NaOH-Tris method B) CTAB method. Lane M: 100 by DNA ladder, Lane 1-4: Chilli genotypes Guntur Hope, Bullet, Pusa Jawala, Pusa Sadabahar.*

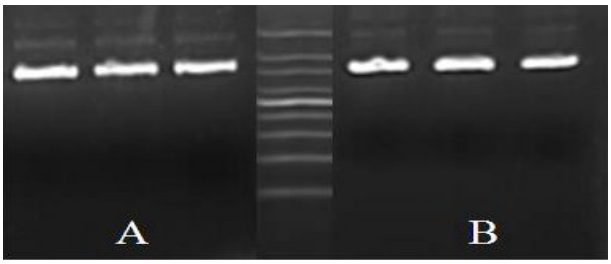

Fig. 3. *PCR amplification using R2M1S primer in three different tomato genotypes. DNA isolated by A) NaOH-Tris method B) CTAB method. Lane M: 100 by DNA ladder, Lane 1-3: Tomato genotypes Arka Vikash, Arka Meghali, Arka Saurabh*

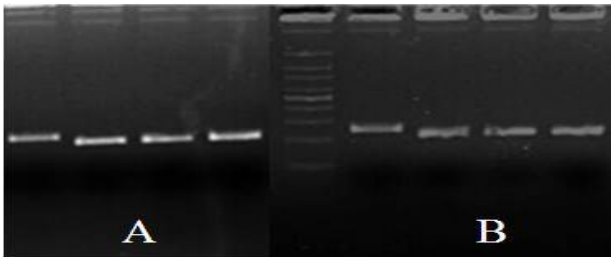

Fig. 2. *PCR amplification using emb01M15 primer in four different brinjal genotypes. DNA isolated by A) NaOH-Tris method B) CTAB method. Lane M: 100 by DNA ladder, Lane 1-4: Brinjal genotypes Arka Shirish, Arka Neelkanth, Lal-pari , Arka Anand*

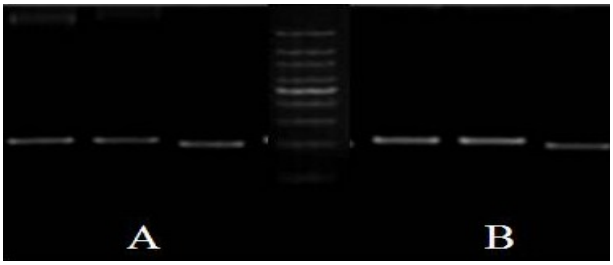

Fig. 4. *PCR amplification using ARTS primer in three different rice genotypes. DNA isolated by A) NaOH-Tris method B) CTAB method. Lane M: 100 by DNA ladder, Lane 1-3: Rice genotypes Rajendra Sweta, Sabour Shree, Swarna Subl*

because it prevent many of the surface contacts (e.g., contact between DNA and mortar, pestle, spatula, multiple pipetting and other equipment) that occur with the modified CTAB method.

Comparison of NaOH-Tris and modified CTAB extractions for PCR amplification: A single DNA extraction method cannot always be successfully applied to a broad range of crop plants. With the present NaOH-Tris method, it was possible to extract DNA from brinjal (*Solanum melongena* L.), chilli (*Capsicum annuum* L.), rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.). The crude DNA extracted from these crops was sufficient for SSR and gene specific markers. All the DNA samples produced a clear, sharp and uniform band and at the same time it gave similar results as compared with the modified CTAB method (Fig. 1, 2, 3, 4). The DNA template was suitable for amplification by *Taq* DNA polymerase. No inhibition of *Taq* DNA polymerase activity was observed, indicating no disadvantage of use of crude DNA extracted and which can be efficiently used for various types of molecular studies (Collard *et al.*, 2007). The NaOH solution allows sufficient dilution of the extract and significantly reduces the effect of potential inhibitors of PCR. Zhang *et al.* (2000) reported NaOH DNA extraction method did not reliably produce PCR amplification products of > 600 bp in length. However, the DNA extracted in our study was suitable for PCR amplification of products > 600 bp in length (Fig. 1c). Warner *et al.* (2001) reported a rapid DNA extraction method which requires NaOH were easily degraded in barley. The DNA samples extracted in the present study were also stable and could be stored for a month without degradation.

Table 3. Comparison of NaOH-Tris and modified CTAB DNA extraction cost.

Chemicals/consumables	Cost per sample (Rs)	
	NaOH-Tris	CTAB
Liquid nitrogen	-	0.85
Extraction buffer	0.01	0.20
Chloroform:isoamyl alcohol	-	3.60
Isopropanol	-	1.25
70% Ethanol	-	0.05
1.5 ml micro tube	0.83	0.83
1000 µl Tips	-	0.54
200 µl Tips	-	0.71
Total cost per sample	0.84	8.03

Dash (–) means the chemicals or consumables not required

Cost estimation: The cost required in the DNA extraction for the NaOH-Tris and modified CTAB method was estimated. A cost for chemicals (HiMedia make) and plastic consumable (Tarsons make) items like pipette tips and 1.5 ml tubes is only included in the cost estimations. The cost involved in the extraction by NaOH-Tris method is Rs. 0.84 per sample as against a cost of Rs. 8.03 involved in DNA extraction through modified CTAB method. Thus NaOH-Tris method is effective for reducing the cost of DNA extraction (cost only for NaOH, Tris and micro tube).

NaOH-Tris method is not only very simple, but is also time and cost effective. Thus, this method will be suit-

able for marker assisted breeding programs, where we can save considerable time and expense which is important criteria for marker assisted selection as the sample number for each analysis is very high. Many different plants could be amplified using the same DNA extraction method and the same PCR protocol.

Conclusion

We found that modified CTAB DNA extraction procedures are unnecessarily expensive, laborious and time consuming for routine genotyping of crop plants. The present NaOH-Tris method delivers PCR-ready DNA from different crop plants for use in molecular studies is much cheaper in terms of time, chemical use and labor input. The time required for DNA extraction is 2.30 minutes, whereas modified CTAB method took 3 hour. In a single day, one person can complete DNA extraction from more than 200 leaf samples using NaOH-Tris method. The cost involved in the extraction is approximately Rs. 0.80 per sample as against a cost of Rs. 8 involved in DNA extraction through modified CTAB method, thus reducing the cost by 10 times. Moreover, this method requires only few milligrams of leaf, no expensive equipment and chemicals and is suitable for large scale genotyping so making method economic for marker assisted breeding.

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