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AN EFFICIENT METHOD OF DNA EXTRACTION FROM COCONUT LEAVES

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A protocol for extraction of good quality , high molecular weight genomic DNA from young leaves of coconut was standardized using two detergents *viz.* sodium dodecyl sulphate (SDS) and hexadecyl trimethyl ammonium bromide (CTAB) at different concentrations; at pH 8.0 and pH 9.0. SDS (1%) at pH 8.0 was found to yield good quantity of high quality DNA. The extracted DNA was amenable to PCR and restriction digestion.

Key words: Coconut, DNA, protocol, PCR, RFLP

Método para extração eficiente do DNA das folhas do coqueiro. Foi padronizado um protocolo para extração de DNA de boa qualidade a partir de folhas jovens do coqueiro, usando dois detergentes: Sódio dodecil sulfato (SDS) e bromo hexadecil trimetil amonio (BHDTA) a diferentes concentrações, em pH 8.0 e pH 9.0. Encontrou-se que SDS (1%) em pH 8.0 produziu boa quantidade de extrato DNA de alta qualidade. O DNA extraído foi confirmado pelo PCR e RFLP.

Palavraş-chave: Coco, protocolo, DNA, PCR, RFLP

Introduction

Coconut (*Cocos nucifera* L.) is characterized by the presence of large number of secondary metabolites including phenols which hinder extraction of good quality DNA. During extraction of DNA these metabolites form a complex with DNA, thus, drastically affecting its quality. Efficient removal of secondary compounds, polysaccharides and proteins is important for extraction of good quality, high molecular weight DNA which is prerequisite for the success of most of the molecular biology techniques. Several methods have been described for DNA extraction from different species having high phenol content like sweet potato, oilpalm and other dicotyledonous species (Couch and Fritz, 1990; Jack *et al.*, 1995; Varadarajan *et al.*,1991). Available protocol for DNA extraction from coconut leaves (Rohde *et al.*, 1995) has many steps of purification and centrifugation resulting in low yield of DNA. The objective of this study was to develop a rapid protocol for obtaining large amounts of quality DNA. To achieve this objective we conducted experiments in which two different detergents *viz.* CTAB and SDS were tried at graded levels at two different pH *viz.* 8.0 and 9.0. The effect of these experiments on DNA yield and quality was studied. Based on these experiments, we propose modifications in existing protocols for DNA extraction from coconut leaves.

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Materials and Methods

Plant material

Spindle leaf lamina from field grown coconut cultivars, West Coast Tall (WCT), Chowghat Orange Dwarf (COD) and their reciprocal hybrids *viz.*, WCT x COD and COD x WCT were used for DNA extraction. Leaves were stored at 4°C till use.

Extraction Buffer

The composition of the DNA extraction buffer was as described by Doyle and Doyle (1990) with the difference that three different concentrations of SDS *viz.*, 0.5%, 1%, 1.5% and CTAB *viz.*, 1%, 2%, 3% were used . The pH of the buffer was adjusted to either pH 8.0 or pH 9.0. 5% w/v polyvinylpyrollidone (PVP; insoluble) and 0.2% v/v β-mercaptoethanol were added just before use.

Procedure

1. Cut 5g leaf lamina into small pieces in a mortar containing liquid nitrogen. Allow the lamina to freeze.
2. Grind the tissue to a fine powder adding liquid nitrogen as and when needed, not allowing the tissue to thaw.
3. Add PVP (5%) to the powder and mix it well.
4. Transfer the powdered tissue to 50ml polypropylene tube containing 25 ml extraction buffer.
5. Add 0.2% β - mercaptoethanol to the mixture and add either CTAB (1%, 2% or 3%) or SDS (0.5%, 1% or 1.5%). Mix thoroughly.
6. Incubate the mixture at 65°C for 1 hour with intermittent mixing.
7. Add 15 ml phenol:chloroform:isoamyl alcohol (25:24:1) and mix gently by swirling the tubes for 10 min.
8. Centrifuge the tubes at 25,000 g for 20 min.
9. Measure and transfer the supernatant to an autoclaved 50ml tubes.
10. Add equal volume of chloroform:isoamyl alcohol (24:1) and again mix gently for 15 min.
11. Centrifuge at 25,000g for 10 min and transfer the aqueous phase to a new centrifuge tube.
12. Add 2/3 volume of ice-cold isopropanol and gently mix the tube by inverting it. Incubate at 4°C for 30- 45 min.
13. Pool the precipitated DNA with the help of microtip or glass Pasteur pipettes and transfer to a microguge tube.
14. Wash the DNA twice with 76% alcohol containing 10mM ammonium acetate for 30 min.
15. Dry the pellet under vacuum.
16. Dissolve the pellet in 1 ml TE buffer (pH 8.0).

The DNA was quantified by measuring absorbance at A_{260} in UV spectrophotometer and A_{260}/A_{280} ratio was taken to evaluate the purity of DNA. The DNA yield data was analysed by computer software MSTATC. Molecular weight of the DNA was estimated by agarose electrophoresis on 0.8% agarose and visualised by ethidium bromide staining.

Results and Discussion

Effect of detergents and pH levels

A perusal of Table 1 reveals that the yield of DNA from different treatments ranged from 82 to 444 µg/g of leaf tissue. These DNA samples have A_{260}/A_{280} ratio of more than 1.7 and a molecular weight of more than 25 kb with very little RNA contamination (Fig. 1). Statistical analysis detected difference in the yield of DNA as influenced by the pH. The DNA yield obtained at pH 8.0 (mean 284.8 µg/g leaf tissue) was 230% higher than that at pH 9.0 (Mean 86.5 µg/g leaf tissue). The detergent type, although didnot significantly influenced the DNA yield, higher quantity of DNA was obtained with SDS (216 µg/g leaf tissue) as compared to CTAB (155.21 µg/g leaf tissue) thus resulting in an increase of 39%. There was a significant interaction between pH levels and the detergent. At pH 8.0, SDS gave higher yield (354.8 µg/g leaf tissue) than CTAB (214.8 µg/g leaf tissue) while at pH 9.0 CTAB yielded more DNA (95.6 µg/g leaf tissue) than SDS (77.3 µg/g leaf tissue). The maximum yield was obtained with 1% SDS at pH 8.0 (Mean 444 µg/g leaf tissue). In further extractions also 1% SDS at pH 8.0 resulted in consistently high yield (250-400 µg/g leaf tissue). Based on these results we recommend the replacement of 2% CTAB by 1% SDS in extraction buffer of Doyle and Doyle (1990) and subsequently used by other coconut workers (Rohde et al., 1995), keeping the concentration of other constituents unchanged. This protocol can be used for tall, dwarf as well as hybrids with equal efficiency as the data indicate that there is no statistically significant difference in the yield among cultivars.

Restriction analysis

The extracted high molecular weight DNA was restrictable by restriction enzymes *Pst*1, *Eco* RI and *Hin*dIII (Fig. 2). Therefore, this DNA can be used for RFLP analysis.

PCR amplification

The DNA is amenable to polymerase chain reaction using a large number of primers (Fig. 3).The amplification results in easily distinguishable bands without any background. This confirms the suitablity of DNA for PCR based analyses.

Ligation into a plasmid

The restricted genomic DNA can be ligated into a plasmid and is being used for construction of genomic library of coconut in a plasmid.

Table 1- Yield and Quality of DNA obtained with different treatments.

Sl No.	Cultivar	Detergent	pH 8.0		pH 9.0	
			Yield*	A_{260}/A_{280}	Yield*	A_{260}/A_{280}
1.	WCT	1%CTAB	156	1.88	72	1.70
2.	COD	1%CTAB	155	1.79	53	1.72
3.	TXD	1%CTAB	330	1.72	159	1.88
4.	DXT	1%CTAB	230	1.89	120	1.87
		Mean	**218**		**101**	
5.	WCT	2%CTAB	223	1.91	71	1.92
6.	COD	2%CTAB	388	1.72	79	1.90
7.	TXD	2%CTAB	65	1.87	111	1.89
8.	DXT	2%CTAB	144	1.78	108	1.91
		Mean	**205**		**92**	
9.	WCT	3%CTAB	480	1.92	84	1.78
10.	COD	3%CTAB	106	1.79	97	1.89
11.	TXD	3%CTAB	141	1.90	64	1.79
12.	DXT	3%CTAB	160	1.88	129	1,88
		Mean	**222**		**94**	
Grand Mean (CTAB)			**155.2**			
Grand Mean (pHxCTAB)			**214.8**		**95.6**	
13.	WCT	0.5%SDS	317	1.75	84	1.86
14.	COD	0.5%SDS	475	1.82	64	1.83
15.	TXD	0.5%SDS	98	1.90	47	1.89
16.	DXT	0.5%SDS	184	1.83	103	1.77
		Mean	**269**		**74.5**	
17.	WCT	1.0%SDS	250	1.79	79	1.85
18.	COD	1.0%SDS	127	1.80	63	1.87
19.	TXD	1.0%SDS	686	1.88	87	1.83
20.	DXT	1.0%SDS	713	1.78	75	1.88
		Mean	**444**		**101**	
21.	WCT	1.5%SDS	237	1.84	73	1.91
22.	COD	1.5%SDS	234	1.92	61	1.80
23.	TXD	1.5%SDS	304	1.83	95	1.73
24.	DXT	1.5%SDS	632	1.88	97	1.89
		Mean	**352**		**82**	
Grand Mean (SDS)			**216**			
Grand Mean (pHxSDS)			**354.8**		**77.3**	
Grand Mean (pH)			**284.8**		**86.5**	
SE (pH)		22.599	CD(5%)		79.69	
SE (Detergent)		27.59	NS		-	
SE (Cultivars)		39.032	NS		-	
SE (Cv. x pH)		55.20	NS		-	
SE (Cv. x Det)		55.20	NS		-	
SE (pH x Det)		39.032	CD(5%)		112.75	

*Yield -µg/g leaf tissue.

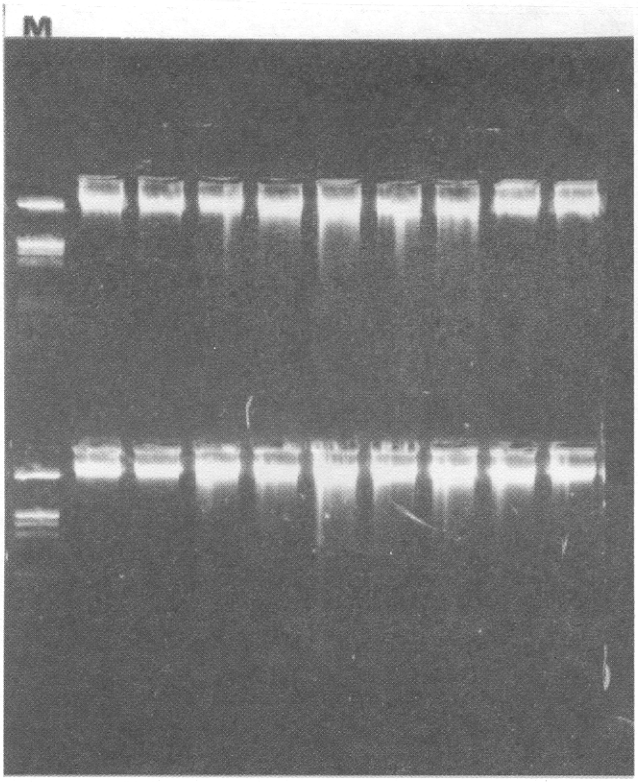

Figure 1. Agarose gel electrophoresis of coconut DNA extracted with different treatments. M: l DNA *Hin* d III/*Eco* RI double digest. 1ml of coconut DNA was loaded on 0.8% agarose gel and electrophoresed at 25 V.

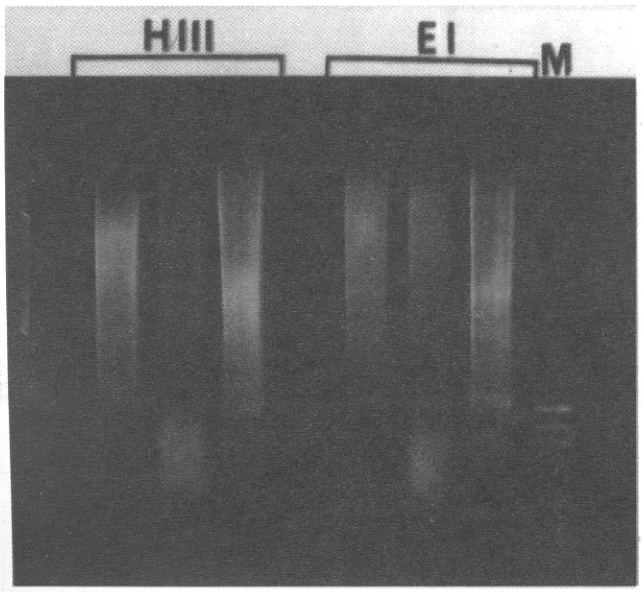

Figure 2. Restriction analysis of coconut DNA. E1 - *Eco* RI, HIII - *Hin* d III, M - f X174 DNA/*Hinf* I digest. 5mg DNA was digested with 20 units of enzyme and resolved on 0.8% agarose.

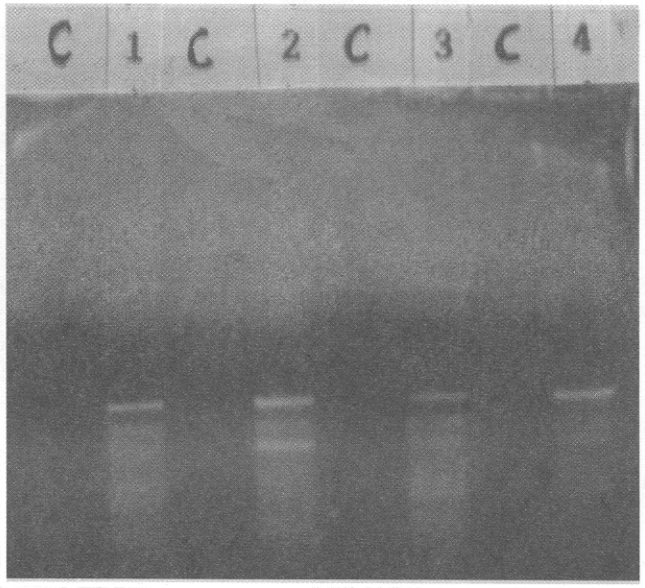

Figure3. Polymerase Chain Reaction Amplification of Coconut DNA. C - Control, 1,2,3,4 - Different WCT DNA samples. PCR amplification product was resolved on 1.2% agarose and stained with ethidium bromide.

This method has been scaled down for extracting DNA from milligram quantities of *in vitro* grown plant material. This is a rapid and efficient method and large number of samples can be processed in a day. It is a simple method and avoids the use of some complex steps of purification like phenol treatment, RNase treatment and CsCl gradient centrifugation. Elimination of expensive chemicals like CsCl, RNase and CTAB makes this method economical. This protocol may also be applied for other related palms like arecanut and oilpalm.

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