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Extraction of Genomic DNA from *Gossypium sps.* Without Detergent Suitable for PCR

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Abstract Extraction of DNA is the prerequisite for many biotechnological works like marker assisted selection particularly in commercial crops such as cotton. Here we report a simple, rapid and inexpensive procedure for the isolation of DNA from cotton seeds for laboratory purpose. This procedure avoids the usage of detergents like CTAB/SDS which are inconvenient in preparation and time consuming. In addition, this procedure involves the usage of various reagents in different combinations for the effective removal of polyphenols, proteins, salts and polysaccharides making DNA suitable for PCR. Therefore, this method can be employed for various applications even in low equipped laboratories.
Keywords *Cotton, DNA, SDS, Microsatellites*

1. Introduction

The cotton, the white gold, a variety of plants of the genus *Gossypium*, includes 50 species, four of which are cultivated, 44 are wild diploids and two are wild tetraploids [1] and is the major commercial crop in many developing countries. With the advent of molecular biology techniques, there is a large need of rapid DNA extraction procedures for cotton. Isolation of plant nucleic acids for use for southern blot analysis, polymerase chain reaction (PCR) amplifications, restriction fragment length polymorphisms (RFLPs), arbitrary primed DNA amplifications (RAPD, APPCR, DAF), and genomic library construction, is one of the most important and time-consuming steps. Even though many commercial plant genomic extraction kits available, but their availability to certain developing countries and high cost can be limiting, especially when handing a large number of samples and considering experiments with limited financial resources [2].

Traditional methods for cotton genomic DNA involves the prior preparation of detergents like sodium dodecyl sulphate which is time consuming and inconvenient. In addition, the extracted DNA contains many contaminants like polyphenols, proteins and polysaccharides which may interfere with the PCR amplification. Hence, our objective is to develop a simple, rapid and inexpensive method for the

isolation of high quality genomic DNA from cotton seeds even in low equipped laboratory. An array of DNA isolation protocols have been optimized and were used in various combinations to isolate quality DNA from cotton seeds as well in leafs for analyses [2, 3, 4, 5, 6, 7]. This modified method avoids the usage of detergents and also the use of reagents like 2–mercaptoethanol, Phenol: chloroform: Isoamylalcohol can effectively remove contaminants of extracted DNA solution making it suitable for PCR.

2. Materials and Methods

Different commercially available segregating F1 hybrid cotton seeds and their respective parents were taken and incubated overnight in dark individually with distilled water. After soaking seed coat was removed and 30mg of inner mass was crushed gently using mortar and pestle. Total DNA from seeds of cotton was extracted with extraction buffer (1M Tris HCl, 0.5M EDTA, 5.0M NaCl and 1% beta mercaptoethanol) and kept for incubation at 67^{0}C for 1 hour and cooled on ice. In this study all the centrifugation steps were done at 13,000 rpm using microfuge (Eppendorf Inc.) at 4^{0}C. Then the clear supernatant was precipitated with equal amount of isopropanol and 10M ammonium acetate mixture and again centrifuged at 13,000g to get nuclei precipitate. This was dissolved in 100ul of TE and purified with RNase A, Phenol:Chloroform:Isoamylalcohol (25:24:1), Chloroform:Isoamylalcohol (24:1), 5M sodium acetate, absolute alcohol respectively to remove RNA, proteins and salts. High quality DNA obtained was dissolved in autoclaved 50ul TE buffer. The yield of DNA extracted was measured using a UV-VIS Spectronic Genesys 5 (Biorad Inc.) spectrophotometer at 260nm. The quantity and purity of the extracted DNA was given in the Table 1.

Table 1: *Quantity and Purity of DNA extracted from Gossypium sps*

VARIETY	A 260		Purity of DNA		QUANTITY (ug/ml)	
	Without SDS	With SDS	A 280	A260/A280	Without SDS	With SDS
TCHH 144	0.025	0.016	0.022	1.136364	250	160
TCHH 252	0.011	0.010	0.006	1.833333	110	100
TCHH 004	0.019	0.018	0.010	1.9	190	180
TCS 226	0.005	0.004	0.004	1.25	50	40
TCHH 009	0.025	0.017	0.023	1.086957	250	170
TCS 173	0.023	0.017	0.016	1.4375	230	170
TCS 2	0.015	0.019	0.011	1.363636	150	190
TCHH 118	0.027	0.024	0.022	1.227273	270	240
TCS 160	0.006	0.008	0.004	1.5	60	80
TCS 33	0.031	0.026	0.022	1.409091	310	260

The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. Concentration of DNA obtained with or without detergent was compared for novelty and reliability of the procedure on a 0.8% agarose gel by comparing band intensity with that of standard amounts of uncut lambda DNA. The DNA was checked for PCR amplification using various microsatellite primers pairs taken from cotton marker database and were obtained from Bioserve Inc. The details of primer pairs used for the study were given in Table 2. PCR reactions were performed with the Eppendorf PCR System 2400. The PCR conditions must be optimized for other thermo-cyclers and annealing temperatures must be optimized for each primer set. Each 15ul reaction volume contains 2X PCR buffer, 200uM of each dNTPs, 1U Taq polymerase, 15pm/ul each of forward and reverse primers for Microsatellites. (all reagents were obtained from Qiagen Inc.) and 30ng genomic DNA. PCR consists of one cycle of 94°C, 7 min, which was followed by 27 cycles of 94°C, 45 secs; 55°C, 1 min; 72°C, 2 min, and finally one cycle of 72°C, 10 min. The PCR products were analyzed by electrophoresis using

a 3% agarose gel in TBE buffer. DNA was stained by soaking the gel in a 10 mg/ml ethidium bromide solution.

Table 2: *Details of Microsatellite Primer Pairs Used for the Study*

Primer pair	Repeat type	Forward Primer	Reverse Primer
JESPR7	$(GAA)_{14}$	GCTGACGGAAGTGACAGGACCCT	GTCTCCTTCCCCTTCCTCTTCTTC
JESPR10	$(GAA)_{20}$	GAGGCAATGTCGGATGTGGGC	GCAAGTAGGTGGTGGCCGGAG
JESPR14	$(CTT)_{17}$	GGGAGGGGGTGAATAAACGGTG	GGTCAGGTAAACTTGCCATAGTGGG
JESPR21	$(GAA)_{15}$	GAGGGGGTGAATAAACGGTGAGG	CGGCTTCTCTTGCTTAGATCTGGAC
JESPR34	$(CTT)_{11}$	TGGTACCGGGGATTCAGTGTGCCAC	ATGTGGCGCATCAGATCCGGTC
JESPR43	$(CAA)_{15}$	CGGCTTACAACAACAACAAC	GCTTCTCTTGCTTAGATCTGGAC
JESPR52	$(GAA)_{12}$	GCCGTACAATCACAGATTGGGAC	GCGCTTCTCATTGAGTCATCCTG
JESPR58	$(CTT)_{10}$	CCGCCCTTCTCTTGCTTAGATCTGG	GGAGCCAATTGAGAAGTGAATCCAA
JESPR72	$(CTT)_{10}$	CGCCCTTCTCTTGCTTAGATCTGG	GGGCAAGCTGACGATGAGGAATG
JESPR84	$(CTT)_{20}$	GACTCCCGGAGGCAATCAGAG	CCAGGGCTCATACTATCGCTGC
JESPR289	$(GAA)_{8}$	CATTGCATTTTGCCCC	AATCTAGCGCACAAGGGC
JESPR101	$(TA)_{5}(GT)_{15}$	CCAAGTCAAGGTGAGTTATATG	GCTCTTTGTTACTGAAATGGG

3. Results and Discussion

The physical characteristics of the final DNA pellet were white with no visible discoloration. The genomic DNA isolated with the given protocol yielded 50-250ng/ul of DNA which could be useful for minimum of 1500 PCR reactions. The quantity and purity of the extracted DNA was given in the Table 1. In general, the quality and quantity of extracted DNA depends on reagents used for extraction, precipitation temperature and duration. Particularly, a detergent is the essential component of DNA extraction buffer for breaking down cell and nuclear membrane to make extraction possible. Though, the usage of detergent like SDS yields good quantity DNA, simple extraction procedures yielding high quality DNA without SDS were not reported yet in cotton. The successful extraction of useful DNA from plants is associated with all extraction steps for molecular techniques used in the next steps such as PCR amplification, digestion and DNA sequencing. Particularly, the phenolic contents of the plants as well as other substances such as polysaccharides and proteins make difficult DNA extraction result in low quality and low quantity DNA [8, 9]. Current methods produce degraded and denatured DNA or give extremely poor yields. However, ccommercial kits are available from many biotech companies yielding good quality DNA, but they are expensive when hundreds or thousands of samples are extracted for DNA. To overcome all these difficulties, we modified the available genomic DNA extraction methods to get good quantity DNA with high quality. Representative photograph of the extracted DNA compared with that of DNA extracted with SDS was given in Figure 1.

The suitability of the DNA isolated as a template in PCR amplification reactions such as RAPD and Microsatellite was analyzed. The SSR-PCR amplification product profiles using DNA as template from F_1 plants and their corresponding parents was analyzed. The DNA isolated from all the plants yielded consistently amplified products. As an example the amplified products obtained from the parents and some F_1 seeds after PCR using primer pairs JESPR101 and JESPR289 are shown in Figure 2. The use of Phenol: Chloroform: Isoamylalcohol removes the proteins and lipids present in the DNA solution. Moreover, Sodium chloride and β-mercaptoethanol were added in the extraction buffer to take care of the polysaccharides and the polyphenols associated with DNA which is the compounds which could contribute to the inhibition of the DNA amplification during PCR reactions. Hence there were no additional steps needed for the removal of these compounds [10, 11, 12, 13].

Hence, this method is an attractive alternative for the extraction of plant DNA because of its efficiency and the speed of this method together with the use of inexpensive facilities and the absence of chemicals like CTAB and SDS. These results show that the DNA extracted by this simple, low cost

and safe protocol can be used in PCR-based applications, and in laboratories lacking state-of-the-art equipment and technology.

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Figures

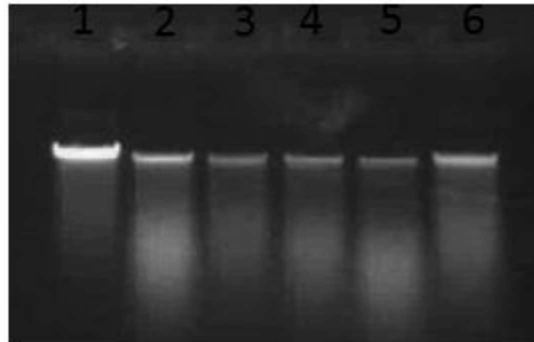

Figure 1: *DNA An Extracted from Single Cotton Seeds: Lambda DAN 150g Lanes 2-4: DNA Isolated Using the Modified Protocol Lanes 5-6: DNA Isolated with SDS.*

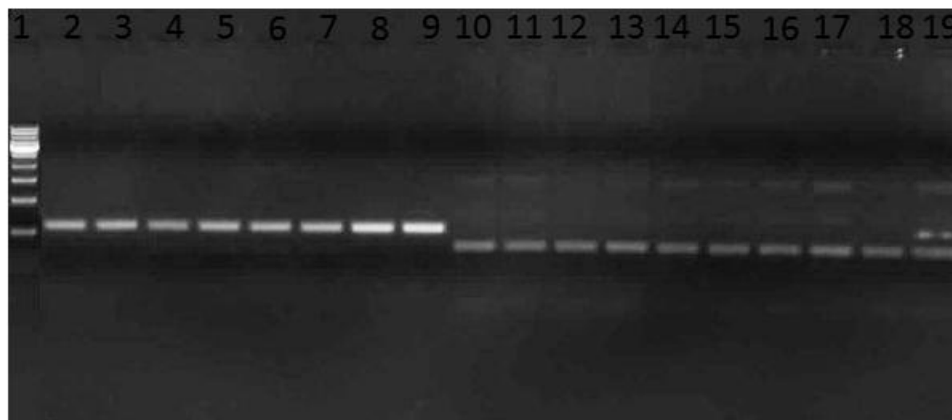

Figure 2: *Amplified PCR Product Profiles with Microsatellite Primer Pairs: 500bp Ladder, Lane 2-9: Amplified DNA Extracted with the Given Modified Protocol with Primer Pair JESPR 289. Lane 10-19: Amplified Pattern of DNA with Microsatellite Primer Pair JESPR101.*

References

[1] Percival E. et al. *Distribution, Collection and Evaluation of Gossypium. Advances in Agronomy*. 1990. 44; 225-228.

[2] Stephen L. Dellaporta et al. *A Plant DNA Minipreparation: Version II*. Plant Molecular Biology Reporter. 1983. 1 (4) 19-21.

[3] Sharma Kiran K. et al. *A Method for Isolation and Purification of Peanut Genomic DNA Suitable for Analytical Applications*. Plant Molecular Biology Reporter. *2000.*18 (4) 393.

[4] Guidet F. *A Powerful New Technique to Quickly Prepare Hundreds of Plant Extracts for PCR and RAPD Analyses*. *Nucleic Acids Res.* 1994. 22 (9) 1772-1773.

[5] Steiner J.J. et al. *A Rapid One-Tube Genomic DNA Extraction Process for PCR and RAPD Analyses*. Nucleic Acids Res. 1995. 23 (13) 569-2570.

[6] Maoteng L. et al. *A Simple DNA Extraction Method for PCR Amplification from Dry Seeds of Brassica Napus*. Pakistan Journal of Biological Sciences. 2007. 10(7) 1122-5.

[7] Prathibha S. et al. *Isolation of Genomic DNA from Medicinal Plants without Liquid Nitrogen*. Indian Journal of Experimental Biology. 2010. 48 (6) 610-614.

[8] Hanania U. et al. *An Improved Method for Isolating High-Quality DNA from Vitis vinifera Nuclei* Plant Molecular Biology Reporter. 2004. 22 (2) 173–177.

[9] Puchooa D. et al. Genomic DNA Extraction from *Victoria amazonica.* Plant Molecular Biology Reporter. 2004. 22 (2) 195-196.

[10] McCarthy P.L. *Rapid Identification of Transformed Wheat Using a Half-Seed PCR Assay Prior to Germination.* BioTechniques- The International Journal of Life Science Methods.2002. 32 (3) 560-564.

[11] Maliyakal J.E. *An Efficient Method for Isolation of RNA and DNA from Plants Containing Polyphenolics*. Nucleic Acids Res. 1992. 20 (9) 2381.

[12] Oard J.H. et al. *Rapid Isolation of Rice and Maize DNA for Analysis by Random-Primer PCR*. Plant Molecular Biology Reporter. 1992. 10 (3) 236-241.

[13] Shirzadegan M. *An Efficient Method for Isolation of RNA from Tissue Cultured Plant Cells.* Nucleic Acids Res. 1991. 19 (21) 6055.