RESEARCH PAPER | Biotechnology | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

An Efficient Method of Genomic DNA Isolation from Mucilage-Rich Okra Leaves for Molecular biology studies

KEYWORDS: Genomic DNA, okra, polyphenols, SDS, NaOH, random amplified polymorphic DNA

Rakesh Kumar Meena	Tanushree Chhatterjee	Sanket Thakur
Aditya Biotech Lab and Research Pvt. Ltd., Raipur Chhattisgarh India	Raipur Institute of Technology, Raipur Chhattisgarh	Aditya Biotech Lab and Research Pvt. Ltd., Raipur Chhattisgarh India

ABSTRACT *In this study we have developed a protocol for high purity genomic DNA isolation from dried leaves of Abelmoschus esculentus and PCR analysis from the resultant DNA. Okra leaves have rich acidic polysaccharides (mucilage) and polyphenols so it create problem at the time of extraction. The isolated DNA was free from contaminating agents like polysaccharides, proteins, and polyphenols and 260/280 ratio 1.56 to 2.50 indicate that DNA isolated by this method was more pure and analyzed by PCR using RAPD and ISSR DNA primers. The extraction method is simple and does not require liquid nitrogen. The quantity and quality of the DNA are satisfactory and the method can be very useful for molecular biology related studies.*

Introduction:-

Bhendi or Lady's finger, also called okra (*Abelmoschus esculentus* (L). Moench) belongs to the family Malvaceae. It is one of the important vegetable crops grown throughout the tropical, subtropical and warm sections of the temperate zones of the world [1]. Okra is cooked with meat for flavoring and because of high mucilaginous content, the fruits are ideal for both thickening and flavoring stews and soups. Okra has a relatively good nutritional value and is a good complement in developing countries where there is often a great alimentary imbalance. Fruit contains Moisture (89.6 percent), K (103mg), Ca (90 mg), Mg (43 mg), P (56 mg), and vitamin C (18 mg) in 100 g of fresh fruit. The composition of okra leaves per 100 g edible portion is: water 81.50 g, energy 235.00 kJ (56.00kcal), protein 4.40 g, fat 0.60 g, carbohydrate11.30 g, fibre 2.10 g, Ca 532.00 mg, P 70.00 mg, Fe 0.70 mg, ascorbic acid 59.00 mg, ß-carotene 385.00 µg, thiamin 0.25 mg, riboflavin 2.80 mg, niacin 0.20 mg [2 and 3]. Fresh leaves of okra can be obtained from treatment of plant with maize Stover compost used as insecticide in organic farming system in raise okra plant [4]. Previous investigations into the composition and properties of okra mucilage have been reviewed [5]. Various workers have given different composition of mucilage [6, 7, and 8]. Okra mucilage is an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid [6]. Molecular biology techniques require isolation of genomic DNA with high quality and quantity. At the time of DNA isolation, green leaves are very difficult to crush. Due to this reason we developed or standardized DNA extraction method for further genomic analysis through different molecular related studies [9, 10]. Polysaccharide has viscous glue like texture and makes DNA unmanageable in pipetting and inhibits PCR reaction [11]. Plant contamination like polysaccharides and polypolyphenols compounds are difficult to separate from isolated DNA and these contaminants obstruct polymerases, ligases and restriction enzymes during their activity [12, 13, 14, 15, and 16]. These contaminants are abundance in the foliage of perennials and they co-exist with isolated DNA [17, 18, and 19]. The polysaccharides interfere with nucleic acids and protein isolation thereby affecting the downstream molecular analysis. So, to understand the molecular systematics of okra, high quality DNA, RNA and proteins are essential. In this study we present protocol for extracting genomic DNA from dried leaves of okra and used for RAPD, ISSR & PCR analysis.

Material Methods

Plant Material:

Young leaves of *Abelmoschus esculentus* collected from different cultivar of okra from our research filed and dried at room temperature and used for study. Okra belongs to family is available throughout the year, no difficulty for continual availability of plant material.

DNA Extraction and Isolation:

In the present study, young leaf tissues were collected into polythene begs. The tissue was dried at room temperature and after drying dried leaves was crushed in morter pestle without any buffer and without liquid nitrogen. After crushing the leaf samples add 100 µl of extraction buffer (200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS) [20] was added to the tube and mixed well. The tube was placed in a water bath at 65°C for 30 min. Subsequently, an equal volume of chloroform:iso-amyl alcohol (24:1) was added to the sample, which was then mixed and centrifuged at 12,000 rpm for 15 min, at 4°C. The aqueous supernatant was ransferred to a new eppendorf tube, to which an equal volume of isopropanol was added in with 100ul 3M sodium acetate, in second tube add 100ul 1M NaOH, in third tube 100ul 5M Nacl and in last tube add 50ul:50ul 1M NaoH and 5M NaCl then mixed and incubated at -20°C for 1hour. Following centrifugation at 12,000 rpm for 15 min, the pellet was dried and dissolved in (100 µl) TE buffer. The final samples were store at 4°C.

DNA Quantification

Quantification of DNA accomplished by Nanodrop spectrophotometer with absorbance ratio OD 260/280 gives the value between 1.7 and 1.9 for all DNA samples (Table 1). Genomics DNA also quantified by electrophoresis on a 0.8 % agarose gel (Fig1). PCR Amplification Using RAPD Primers RAPD analysis was performed in five DNA sample with one RAPD primer RPIC2 and one ISSR marker-B-17899. In 20 µL reaction mixture 10xbuffer with MgCl2, 2mM dNTP's (Sigma), 1.0U *Taq*DNA polymerase (Bangalore Genei), 2ul primers (ILS) and 50ng of template DNA to perform PCR reaction in Eppendrof Master Cycler, Germany. Amplification reaction was initiated with initial denaturation at 950C for 5 minute and followed by 40 cycles each at 94C, 54 0 C for 1 min, and 72 0 C for 2 min. A final extension step at 72 0C for 10 min was performedafter 40 cycles. PCR amplified products were separated by electrophoresis in 1.8% agarose gel.

Result and Discussion

We demonstrate a simple, efficient and new method to extract high quality and high quantity genomic DNA from dried leaves of okra with liquid nitrogen. We tried to extract genomic DNA using some traditional methods like [21, 22,

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

23 and 20] but failed to obtain high quality DNA from okra. The tissues of okra have high mucilaginous acidic polysaccharide content forming pectin and its main component is polygalactourinic acid associated with minerals. The green leaves of okra undoubtedly the most difficult for extraction of genomic DNA but this method has enabled us to prepare the DNA with good quality and quantity in dried leaves.

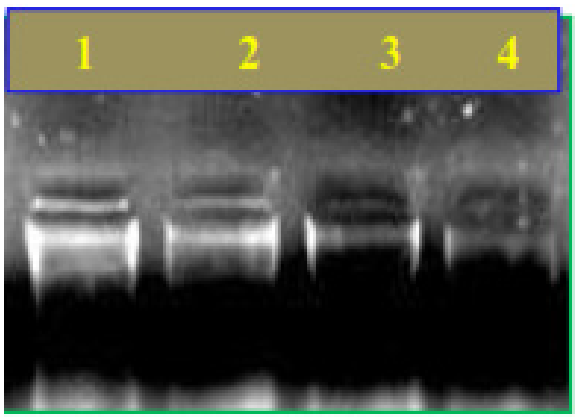

Fig1. Showing Genomic DNA Lane no.1 and 2 new method using NaOH with Isopropanol, Lane no. 3Using NaCl and Lane no. 5 CATB method

The DNA isolated by this method was tested for yield, purity and ability to be amplified by PCR.

PCR amplification of DNA obtained from our modified protocol was possible due to absence of impurities (Table 1). NaOH and sodium dodecylsulfate (SDS - a strong detergent) This causes the cell walls and membranes to burst and concentration of 5M NaCl add to the suspension in the lysis step did result in the highest yields of total genomic DNA [24, 25]. The Salt use during precipitation of DNA increases solubility of polysaccharides in ethanol thus preventing co-precipitation with DNA [24]. This is achieved by grinding dried okra leaves in a mortar without under liquid nitrogen. Complete removal of polysaccharides is essential otherwise failure of DNA amplification during PCR due to inhibition of *Taq* DNA polymerase activity [11]. This protocol provide high amount of DNA therefore higher content of DNA template for PCR reactions despite the fact that the concentration of total template DNA was slightly higher when using standard protocol. One RAPD primer RPIC2 and one ISSR B-17898 were tested on four templates DNA. The significant bands appear during amplification of DNA through PCR with RAPD primers was clear as shown in Fig.2 A and 2B.

Table: 1 Showed concentration of DNA with different treatment and CTAB method

S. No	Name of Samples	Concentration of DNA(ng/ul)	Ratio 260/280	Remarks
1	Okra	3524	1.80	Using New method
2	Okra	1500	2.50	Using old method
3	Okra	500	1.60	Using old method
4	Okra	35	1.56	Using old method

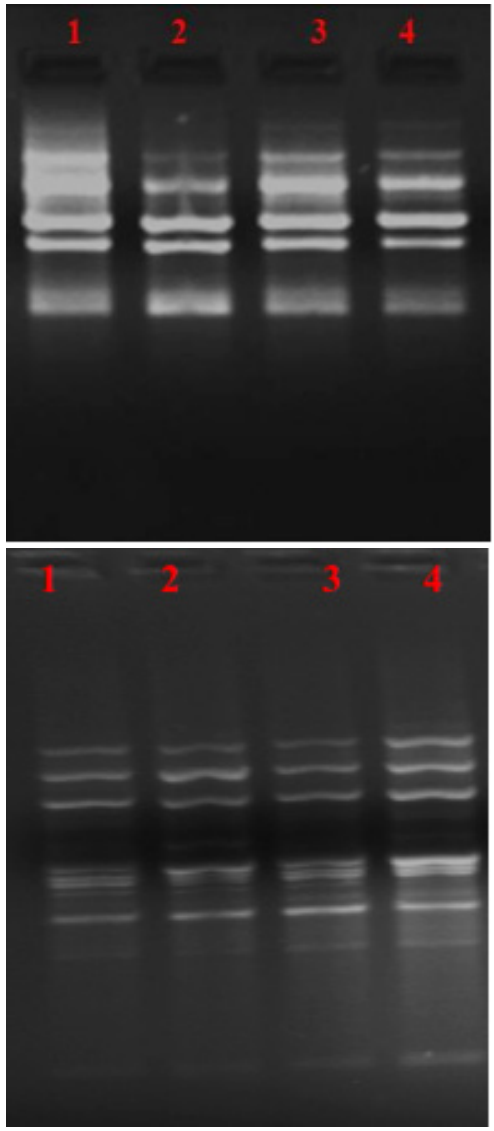

Fig2. Analysis of the DNA extracted from *Abelmoschus esculentus* **using a A. RAPD Primers (RPIC2) B: ISSR B-17899 Lane Lane 1, 2, 3, and 4 amplified DNA through PCR**

CONCLUSION

Different modification has been incorporated in DNA isolation method like adding of 5M NaCl at timing of crushing and 1M NaoH at the same time. Adding of 5M NaCl and 1M NaOH separately with iso-propanol and combined with iso-propanol. But high quality and quantity DNA was obtained with 1M NaOH and it's suitable for PCR amplification and RAPD analysis. These studies open the door for molecular characterization & genetic improvement works in this promising vegetable and medicinal okra plant.

Acknowledgement
We are thankful to Aditya biotech Lab and Research Pvt. Ltd., for providing facilities for work.

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

REFERENCE

1. Charier, A., 1984, Genetic resources of the genus Abelmoschus. International board for plant genetic resources, Rome, pp 21-41. | 2. Gopalan C, Sastri SBV, Balasubramanian S (2007): Nutritive value of Indian foods, National Institute of Nutrition (NIN), ICMR, India | 3. Varmudy V (2011): Marking survey need to boost okra exports. Department of economics, Vivekananda College, Puttur, Karnataka, India | 4. Alao FO, Adebayo TA, Olaniran OA, Akanbi WB, Preliminary Evaluation of the Insecticidal Potential of Organic Compost Extracts Against Insect Pests of Okra (Abemoschus esculentus (L.) Moench) Asian J. Plant Sci. Res., 2011, 1(3), 123-130. | 5. BeMiller JN, Industrial Gums (Whistler, R.L. and BeMiller, J.N. eds.), Academic press, London, 1973, pp360. | 6. Whistler RL, Conrad HEJ, Am. Chem. Soc., 1954, 76, 3544. | 7. Amin, El. S J, Am. Chem. Soc., 1954, 78, 828. | 8. Kelkar GM, Ingle TR, Bhide BVJ; Indian. Chem. Soc., 1962, 39, 557. | 9. Jitu B, Adv. Appl. Sci. Res., 2011, 2(6), 454-459. | 10. Noori A, Ahmadikhah A, Soughi H, Dehghan M, Adv. Appl. Sci. Res., 2010, 1(3), 153159. | 11. Fang G, Hammar S, Grumet R, BioTechniques., 1992, 13, 52-56 | 12. Michard H, Lumaret JP, Ripoll LT, Plant Mol. Bio. Rep., 1995, 2, 131-137. | 13. Porebski S, Bailey LG, Baum BR, Plant Mol. Bio. Rep., 1997, 15, 8-15. | 14. Csaikl UM, Bastian H, Brettschneider S, Gauch A, Meir M, Schauerte F, Scholz C, Sperisen B, Vornam ZB, Plant Mol. Bio. Rep., 1998, 16, 69-86. | 15. Tribounch SO, Danilenko NG, Davydenko OG, Plant Mol. Bio. Rep., 1998, 16, 183-189. | 16. Schlink K, Reski R, Plant Mol. Bio. Rep., 2002, 20, 423a-423f. | 17. Scott KD, Playford J, Biotechnique., 1996, 20, 974-978. | 18. Shepherd M, Cross M, Stokoe RL, Jones ME, Plant Mol. Bio. Rep., 2002, 20, 425a-425j. | 19. Bhattacharjee R, Maria KA, Peter A, Sunday T, Ivan I, Plant Mol. Bio. Rep., 2004, 22, 35a-435b. | 20. Edwards K, Johnstone C,Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research. 19: 1349. | 21. Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19: 11-15. | 22. Cheng YJ, Guo WW, Yi HL, Pang XM, Deng X (2003). An efficient protocol for genomic DNA extraction from citrus species. Plant Mol Biol Rep. 21: 177a-177g | 23. Couch J.A. and Fritz P.J., 1990. Isolation of genomic DNA from plants high in polyphenolics, Plant mol.Biol.Rep. 8(1):8;12 | 24. Lodhi, Muhammad A, Guang-Ning Ye, Norman F Weeden and Bruce I, Plant Mol. Bio. Rep., 1994, 12 (1), 6- 13. | 25. Pooja PP, Purvi MR, Kiran SC, Vrinda ST, Euro. J. Exp. Bio., 2012, 2(1), 1-8.