International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

A Facile & Cost-Effective DNA Isolation Method for *Abelmoschus Esculentus L.*

Hossain Sohrawardy

Lal Teer Seed Limited, Biotech Lab., R&D, Anchor Tower, 108 Bir Uttam C. R. Dutta Road, Dhaka-1205, Bangladesh

Abstract: ***Modified NaOH method is the easiest and cheapest genomic DNA isolation from Okra leaf samples for PCR amplification. DNA isolation from Okra is quite difficult job due to polysaccharides and polyphenols. Even isolated DNA is not compatible with thermal reaction. A standard protocol of Wang et al. (1993) was reviewed and modified for DNA extraction from Okra leaf tissues within a short time in an effective way which is quite cheap. This method is also effective for molecular characterization of Okra and could be used for molecular characterization in other mucilaginous crops.***

Keywords: genomic DNA, okra, cost-effective, easy, polysaccharides, compatible for PCR

1. Introduction

Okra (*Abelmoschus esculentus L.*) is one of the most widely known species of the Malvaceae family which is cultivated in tropical and sub-tropical region of the world. Now, it is grouped in Abelmoschus that differentiated from the genus Hibiscus. Okra Originated in Ethiopia. In 12th century, it widely spread in North Africa, Mediterranean, and Arabia. Okra is cultivated in the garden as well as grown on large commercial farms. Nowadays Okra plants are cultivated plethora of countries like India, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, Brazil, Ethiopia and the United States [1]. 99% Okra is cultivated mainly in the developing countries of Asia and Africa. The productivity of Okra is low especially in African countries (2.25 MT ha−1) compare to any other region. Okra cultivation area is 1.83 million ha and yield is 9.62 million metric tons (MT) annually and the average yield is 5.26 MT ha−1. India is the largest Okra producer in the world. India produced 6.3 million MT from 0.5 million ha area [2]. Okra is an important food due to its dietary fibers and amino-acid. Okra fruits are eaten commonly as salads, soups, and stews. The roots and stems are used for cleaning the cane-juice during brown-sugar preparation. The Okra seeds contain oil (30–40%), protein (15–20%) and considerable amounts of iron, calcium, manganese, magnesium and vitamins. It has various ethnopharmacological and medicinal properties against cancer, high cholesterol, and Diabetes [5]. Okra has limited work on molecular breeding due to difficulties in DNA extraction, availability of polymorphic molecular markers and absence of a molecular genetic map. Linkage groups are not yet known. Okra has a large number of chromosomes (2n = 56–196) and complex polyploidy nature of the okra genome [3]. The Malvaceae family contains secondary metabolites (Polysaccharides) like *Malachra capitata*, *Hibiscus rosa-sinensis*, (*Abelmoschus esculentus*). DNA isolation from a mucilaginous plant is difficult due to the high content of polyphenols and polysaccharides. The polysaccharides associated with DNA and inhibit PCR reaction. There are many DNA isolation protocols have been published over the last few decades from different parts of the plants [4]. Intensive genetic biodiversity studies are required globally for Okra including wild relatives for its effective utilization in breeding programs across the world. In this circumstance, the research objective is to establish suitable DNA isolation method which is prerequisites of molecular work in plants.

2. Materials and methods

"Green Finger", 'Fighter" and their parents seeds are collected from Processing Packaging and Quality Control (PPQC) Department, Lal Teer Seed limited. The research conducted at Biotech Lab., R&D, Lal Teer Seed Limited from May to November 2018.

2.1 DNA extraction

Fresh and tender primary leaves are collected from the plants. A few milligrams of young leaf sample are placed into 1.5 ml Eppendorf tube. For every mg of tissue should add 10 µl of 0.5 N NaOH in the Eppendorf tube. Samples are placed on water-bath at 85℃ for 20 minutes. After heating, the samples are spined for 30 seconds. Then transferred quickly 5-10 µl of supernatant to a new tube contained 495 µl of 100 mM Tris pH 8.0. The samples are mixed properly by finger tapping. Two µl DNA used for polymerase chain reaction directly.

2.2 Primers for PCR amplification

10 µl reaction mixtures contained 5 µl master mixes (Promega), 2 µl of nuclease-free water, 1 µl of primer and 2 µl of DNA. The PCR reaction condition was selected at 95℃ for 4 minutes for initial denaturation, followed by 32 amplification cycles, each cycle consisting of 30 seconds at 95℃ (denaturation steps), 30 seconds at 55℃ (annealing step) and 40 seconds at 72℃ extension step with a final extension of 10 minutes at 72℃. The PCR amplified products were run on agarose gel.

3. Results and discussion

In this study, simple and cheap method for Okra genomic DNA isolation was introduced. This genomic DNA used

directly for PCR. The PCR products showed polymorphic bands in both agarose gel and polyacrylamide gel. The quality and quantity of DNA obtained by this method was sufficient for genetic analysis using molecular markers. On the other hand, Others DNA extraction protocols are costly, time-consuming and laborious. Sometimes, the protocol may not be suitable for extracting good quality DNA from the plant due to the presence of secondary metabolites. Even renowned CTAB method [6] sometimes is not working properly. Mucilage comes out and inhibits DNA isolation during leave crushing by mortar and pestle in CTAB method. Liquid nitrogen is also costly for crushing leaves. Besides, commercially available extraction kits provide successful extraction of high-quality DNA but it is too much expensive.

Primer Okra Mosaic virus (OMV), ITS 4 & 5, UBC-848, UBC-856, UBC-840, UBC-855, UBC-889, UBC-890, UBC-854, UBC-888 and UBC-809 were used for polymorphism. Primer ITS 4&5, UBC-856, UBC-889, UBC-888 and UBC-809 showed polymorphism in case male of Fighter on agarose gel (Figure: 1).

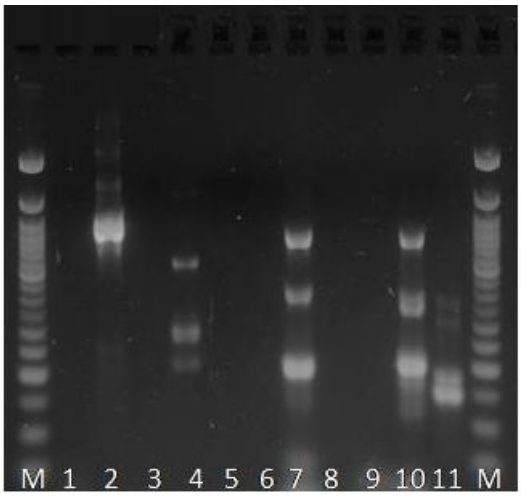

Figure 1: M=50 kb DNA ladder, 1= Primer OMV, 2= ITS 4 & 5, 3= Primer UBC-848, 4= Primer UBC-856, 5= Primer UBC-840, 6= Primer UBC-855, 7= Primer UBC-889, 8= Primer UBC-890, 9= Primer UBC-854, 10= Primer UBC-888, 11= Primer UBC-809.

Table 1: Molecular weights of bands on the agarose gel (Figure 1) are calculated by AlphaEaseFC 4.0 software

Lane number	No. of bands	Molecular weight (bp)
2	2	798, 1223
4	3	200, 277, 561
7	3	197, 406, 699
10	3	197, 361, 699
11	4	147, 175, 302, 388

Primer UBC-889, UBC-890, UBC-888 and UBC-809 were showed polymorphism in case male of Fighter and Green Finger on agarose gel (Figure: 2).

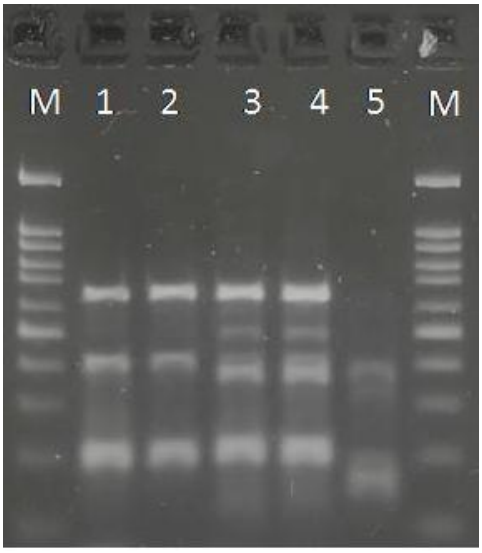

Figure 2: M=50 kb DNA ladder; 1= Fighter, Primer UBC-889; 2= Green Finger, Primer UBC-889; 3= Fighter, Primer UBC-888; 4= Green Finger, Primer UBC-888; 5= Fighter, Primer UBC-809

Table 2: Molecular weights of bands on the agarose gel (Figure 2) are calculated by AlphaEaseFC 4.0 software.

Lane number	No. of bands	Molecular weight (bp)
1	3	96, 197, 324
2	3	98, 203, 338
3	4	101, 184, 250, 338
4	4	99, 182, 245, 338
5	2	79, 186

Besides, Simple Sequence Repeat (SSR) primers (SGC 225, TM 173, Tm 824, sm 01, EP 04, EP 82, EP 133, CMTm 11, CMTm 170, CMTM 202, PP 24, PP 35, PP 54, PP 88, PP 99, PP 141, BoG 21, BoG 73, BoG 76, BoG 117, BoG 118, BoG 127, BG 27, BG 46 and UBC 848) were used. Primer TM 173, EP 04, CMTm 11, CMTm 170, PP 88, PP 99, BoG 21, BoG 73, BoG 127, BG 27, BG 46 and UBC 848 showed polymorphic band on agarose gel in Fighter variety.

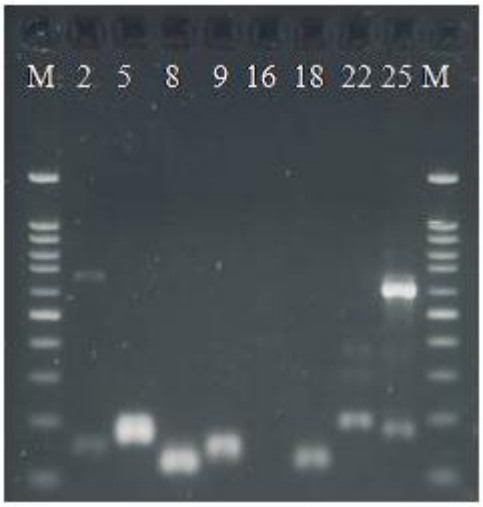

Figure 3: M=100 kb DNA ladder; 2= Primer TM 173, 5= Primer EP 04, 8= Primer CMTm, 9= Primer CMTm 170, 16= Primer PP 141, 18= Primer BoG 73, 22= Primer BoG 127, 25= Primer UBC 848

Table 3: Molecular weights of bands on the agarose gel (Figure 3) are calculated by AlphaEaseFC 4.0 software

Lane number	No. of bands	Molecular weight (bp)
2	2	149, 683
5	1	171
8	1	129
9	1	145
16	No band	-
18	1	126
22	1	176
25	2	162, 561

These PCR products also showed bands on polyacrylamide gel. On the other hand, these gel pictures are also suitable for genetic distance and dendrogram preparation by using PyElph 1.4 software.

4. Conclusion

Due to the polysaccharide, the isolation of DNA from Okra leaves is challenging job for molecular work. This is the facile & cost-effective DNA isolation method for *Abelmoschus Esculentus L.*

5. Acknowledgement

Author would like to thank Lal Teer Seed Limited to provide support for research activities. I would like to give thanks to Tanmoy Roy, Lab Assistant, Biotech Lab., Lal Teer Seed Limited.

References

[1] Mishra, P. Gyan; Singh, Bijendra; Seth, Tania; Singh, K. Achuit; Halder, Jaydeep; Krishnan, Nagendran *et al.*, "Biotechnological Advancements and Begomovirus Management in Okra (Abelmoschus esculentus L.): Status and Perspectives," In Frontiers in plant science 8, p. 360, DOI: 10.3389/fpls.2017.00360, 2017.
[2] FAOSTAT (2014), Available online at: http://faostat.fao.org/ (Accessed February 8, 2017).
[3] K.S. Sastry and T.A. Zitter, (eds.), "Management of virus and viroid diseases of crops in the tropics," in Plant Virus and Viroid Diseases in the Tropics, Vol. 2, Epidemiology and Management, Springer, Netherlands, 149–480, doi: 10.1007/978-94-007-7820-7_2, 2014.
[4] J. Jose and R. Usha, "Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*)," Plant Mol Biol Rep, 18: 349, 2000.
[5] H.F. Gemede, N. Ratta, G.D. Haki, A.Z. Woldegiorgis, F. Beyene, "Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A Review, In J Food Process Technol 06 (06), DOI: 10.4172/2157-7110.1000458, 2015.
[6] H.G. Murray and W.F. Thompson, "Rapid isolation of high molecular weight DNA," Nucleic Acids Res., 8: 4321-4325, 1980.

Author Profile

Hossain Sohrawardy acquired the B Sc in Agriculture (Honors) from Khulna University in 2005 and M S degrees in Biotechnology from Bangladesh Agricultural University in 2007. He also obtained M S in Sustainable International from Georg-August-University, Gottingen, Germany in 2014. He has plethora of knowledge in Agriculture and Biotechnology. Now, he is working as a scientist in Biotech lab, Lal Teer Seed limited. He has special competence on stress tolerance (salinity and drought), salinity screening at seedling and reproductive stages, physiology for drought tolerance, DNA extraction, PCR, markers assisted selection, molecular fingerprinting, genetic purity testing, QTL analysis, chromosome banding, ELISA, enzyme-based TILLING.