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A Rapid, Easier and Inexpensive Method for Isolation of Genomic DNA from Plant Tissue of Jatropha Curcus

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ABSTRACT

An inexpensive user friendly method for extraction of high molecular weight DNA from Jatropha curcus tissue was reported in the current work. Three different methods were used to isolate DNA i.e. 1. Leaves sample were chilled at -20°C for 2 hours then lyophilized in the Lyophilizer for 24 - 30 hours, 2. Dehydrated in hot air oven at 60°C for overnight and 3. fresh leaf samples were homogenized in liquid Nitrogen and subsequently DNA isolated using the method described by Doyle and Doyle (1990). The quality of the DNA and their effectiveness in molecular biology were studied through RAPD and ISSR markers. Least amount of DNA yield (1.09ng/l) was observed in the sample prepared through Hot Air oven method per gram fresh weight of sample. Though a significant difference was not found between the quantity of DNA isolated from the leaf sample prepared through Lyophilizer and liquid Nitrogen but in respect to cost effectiveness and user friendly it was observed that use of Lyophilizer is most cost effective and user suitability than that of liquid Nitrogen method.

INTRODUCTION

The utilization of current nucleic acid technologies in crop improvement and phylogenetic studies require the development and application of efficient DNA extraction procedures from plants. Early protocols for isolation of plant DNA called for the use of liquid nitrogen to assist in the grinding of plant material for DNA extraction (Murray and Thompson, 1980; Dellaporta et al., 1983; Richards, 1987). Although this method consistently yielded good quantities of high quality DNA, the use of liquid nitrogen presented some problems.

Once exposed to liquid nitrogen, it was imperative that the tissue not be allowed to thaw before extraction. If large populations of plants were to be extracted, the tissue would have to be stored frozen or processed and then stored in a freezer. Storage of tissue in freezers may lead to problems involving lack of space. The requirement of manual labor to process frozen tissue may be inefficient and inappropriate when dealing with large populations. In addition Plant materials which were only available from distant sources must be shipped frozen at high cost. Above all the availability of the liquid Nitrogen is now a very big problem for most of the laboratories. Besides there are numbers of plant where the leaf fall is a very serious problems for population study because fresh leaves were not available throughout the year. So if someone interested to study the population diversity in the molecular level, it is bound for the researcher to isolate the DNA of entire population at a stretch. Again storing of DNA is also another problem in order to avoid the use of liquid Nitrogen and make the process easier our group has developed a protocol by using *Jatropa curcus* as the standard material.

MATERIAL AND METHODS

Jatropha curcus is well known as oil yielding plant or Bio-fuel plant as it content more amount of oil and commonly used for bio-diesel production. Besides this plant contain different types of other secondary metabolites in high content. So getting purified and high yield of DNA is a very difficult task, for which we have chosen *J. curcus* as the standard plant material for the experiment. Young leaves were first collected from the IIT garden and washed with running tap water followed by ddH_2O, and then the leaves were soaked with tissue paper. After cleaning of the leaf sample, the leaves were processed in three different ways to isolate DNA, i.e.

- The leaves sample were chilled at -20^{o}C for 2 hours then lyophilized in the Lyophilizer for 24 - 30 hours. The lyophilized tissues were used for DNA isolation.
- The leaf samples were dehydrated in hot air oven at 60^{o}C for overnight. The dehydrated samples were used for the DNA isolation.
- The fresh leaf samples were homogenized in liquid Nitrogen, which were successively used for the DNA isolation.

For the above three different samples one common DNA isolation procedure is adopted i.e Genomic DNA was extracted from leaves using N-Cetyl-N,N,N-trimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990) with modifications. Ten milliliters of preheated extraction buffer [4 % (w/v) CTAB, 0.2% β-mercaptoethanol (v/v), 100 mM Tris-HCl pH 8.0, 2 mM EDTA, 1.4 M NaCl] were added per 100 mg of leaf powder material and incubated for two hours at 65 ^{0}C. The DNA pellet was resuspended in 200 µl to 300 µl of Tris-EDTA buffer. DNA quantification was performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel. After isolation and quantification of DNA, the next part of our objective is to check the quality i.e. whether the isolated DNA can be used in further works or not, which was checked by Polymerase Chain Reaction (PCR) amplification. For PCR amplification the resuspended DNA was then diluted in sterile distilled water to 8 ng/µl concentration.

The reaction mixture of final volume of 25 µl containing 20 ng template DNA, 100 µM of each deoxyribonucleotide triphosphate, 20 ng of primer 1.5 mM $MgCl_2$, 1X Taq buffer (10 mM Tris-HCl [pH-9.0], 50 mM KCl, 0.01% gelatin), and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, Bangalore, India) was used for Polymerase chain reactions (PCR). Amplification was performed in a PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94 ^{0}C, followed by 40 cycles of denaturation at 94 ^{0}C for 20 sec., annealing at 37 ^{0}C for 30 sec. and extension at 72 ^{0}C for 1 min, finally at 72 ^{0}C for 10 min amplification. Amplified products were separated by 1.2 % agarose gel electrophoresis alongside a molecular weight marker (1.0 Kb plus ladder, M/S Bangalore Genei, Bangalore, India) in 1X TAE (Tris Acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad, California, USA).

Table1. *A comparative analysis of DNA isolated from leaf sample of Jatropa curcus processed through three different methods*

Sample type	DNA quantity (ng/gm tissue fresh weight	Quality of the DNA	PCR response	
			RAPD	ISSR
Lyophilized in freeze dryer	13.09	a	**	**
Lyophilized in liquid Nitrogen	09.90	aa	**	**
Dehydrated in Hot Air Oven	01.02	b	*	*

'aa' represents good quality of DNA, 'a' represents good quality of DNA with little amount of sharing, 'b' represents shearing of DNA.

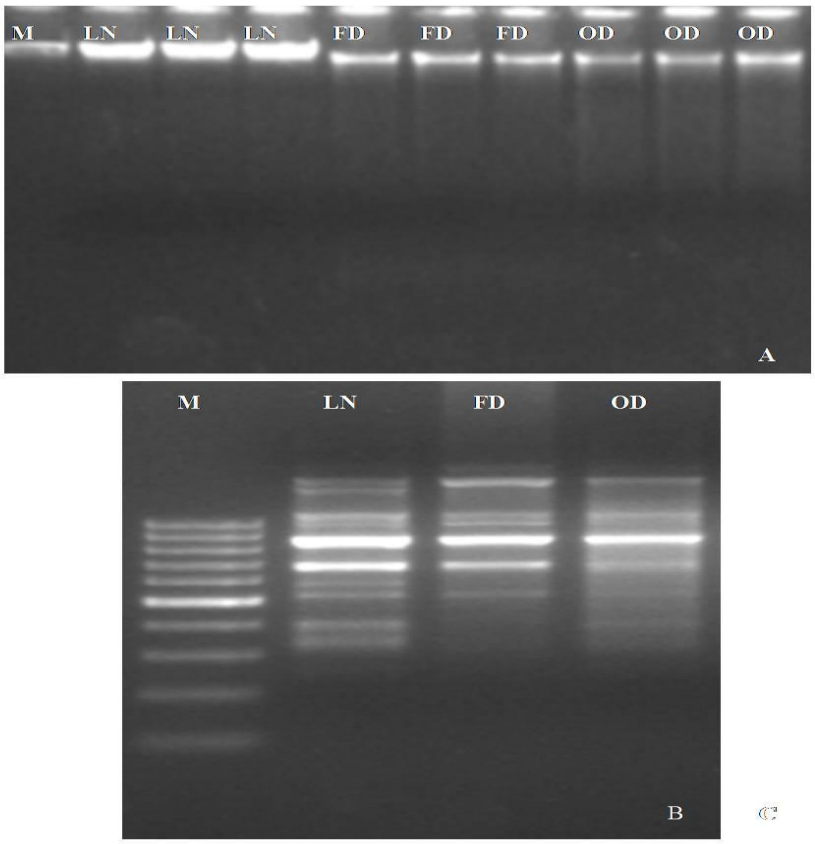
*'**' represents good response to RAPD and ISSR primers, '*' represent mild response to RAPD and ISSR primers.*

RESULT AND DISCUSSION

Among the three different methods used in preparing sample for DNA isolation i.e. lyophilization by Lyophilizer, dehydration of the sample through hot dry oven and lyophilization in liquid Nitrogen, it was found that in all the three methods DNA can be isolated from the leaf sample. The differences found among the DNA isolated from different method are in quantity, quality and it cost effective. From the quantification results it was observed that DNA isolated from the sample prepared by Lyophilizer gave a very high yield (13.09ng/l) of DNA per gram fresh weight of sample. No significant difference were observed between the quantity of DNA isolated from the leaf sample prepared through Lyophilizer and liquid Nitrogen but a significant difference was observed when compared with the DNA isolated from the sample prepared through Hot Air oven method. Least amount of DNA yield (1.09ng/l) was observed in the sample prepared through Hot Air oven method per gram fresh weight of sample (Table-1). Though a very large difference was not found between the quantity of DNA isolated from the leaf sample prepared

through Lyophilizer and liquid Nitrogen but when we compared in respect to cost effectiveness and user friendly it was found that use of Lyophilizer is most cost effective and user suitability than that of liquid Nitrogen method, similar results were also reported by Thomas et al (1993). When we compared all the three methods it was found that use of Hot Air Oven is most cost effective and user friendly than the other two methods but the yield is very less. As per our 2nd objective when we checked the quality of the isolated DNA through PCR particularly through PCR based molecular marker (RAPD and ISSR), it was observed that the DNA isolated Lyophilizer gave better amplification result by both RAPD and ISSR primer as like as the amplification of DNA isolated by using liquid Nitrogen (fig-1).

But the DNA isolated from the sample prepared through Hot Air Oven gave a smearing in the amplification both in RAPD and ISSR primers, which represent that the quality of the DNA may not be as good as the DNA isolated through the other two methods.



M: Marker, LN: Liquid Nitrogen, FD: Freeze dried, OD: Oven dried

Figure-1

Fig1 (A-B). *Representative photograph showing A-Quantification of DNA isolated from leaf sample of Jatropha curcus processed through three different methods, B PCR amplification pattern of DNA isolated from leaf sample of Jatropha curcus processed through three different methods using RAPD marker.*

CONCLUSION

From this experiment it was found that DNA can be isolated irrespective of the Liquid nitrogen, easily and effectively and can also be used for further molecular study.

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