

AN EASY AND SIMPLE METHOD OF ISOLATION AND PURIFICATION OF GENOMIC DNA FROM THE LEAVES OF *GYMNEMA SYLVESTRE*-AN ANTI-DIABETIC PLANT

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ABSTRACT

Gymnema sylvestre is a vine-like plant and is considered as herbal remedy for high blood sugar. The important active ingredient of *Gymnema sylvestre* is an organic acid called “Gymnemic acid”. Genomic DNA is the blue print of life, which is inherited and passed on from generation to generation directing the development of cells, organelles and organisms. Isolation of DNA, RNA and proteins is essential for all molecular biology investigations. The DNA isolation from plant tissues is very difficult due the presence of various secondary plant products. A protocol works with one plant group and may fail with the others. Hence a number of DNA isolation methods have been developed for different target plant groups. In the present study genomic DNA has been isolated and purified from young leaves of 17 ecotypes of *Gymnema sylvestre* with three different extraction methods i.e., modified CTAB method (method1), phenol-chloroform method (method 2), and Dellaporta method (method 3). Among the 3 methods tried, CTAB method was found to be suitable for PCR amplification with high purity and yield of genomic DNA.

Keywords: *Gymnema sylvestre*, seventeen ecotypes, DNA isolation, CTAB method

1. INTRODUCTION

Diabetes is one of the oldest wide spread diseases of the world which is due to lack of insulin utilization or production. It is a gene-controlled disorder besides the expression is regulated by other environmental factors like food habits, life style, age etc. In this context, plant-based therapeutics are promising. The invaluable traditional anti-diabetic plants are sure to provide alternative therapeutic agents for diabetes. *Gymnema sylvestre* is a vine-

like plant (Fig. 1) and is considered as herbal remedy for high blood sugar. The medicinally active parts of the plant are the leaves and the roots. It is known as “destroyer of sugar” because, in ancient times, Ayurvedic physicians observed that chewing a few leaves of *Gymnema sylvestre* suppressed the taste of sugar i.e, sweet foods no longer tasted sweet, but rather became almost completely tasteless. In later generations, clinical

tests showed that regular use of over a period of three to four months helped to reduce glycosuria. The important active ingredient of *Gymnema sylvestre* is an organic acid called "Gymnemic acid".

The recent studies have shown that the extract of *Gymnema sylvestre* is useful in controlling blood sugar to treat type-II diabetes. It increases the insulin producing β -cells of pancreas and significantly reduces the metabolic effects of sugar by preventing the intestine from absorbing the sugar molecules during the process of digestion. It is a potential natural alternative to chemical means of blood sugar regulation (Siddiqui et al., 2000). Coincidentally, several products containing *Gymnema* alone or in combination with other anti-diabetic herbal medicines are marketed in India. In view of its importance we attempted to isolate the DNA from 17 ecotypes of *Gymnema sylvestre* to carrying out the molecular characterization.

Extraction, purification and determination of DNA content is an essential step for any plant species before initiating studies pertaining to molecular biology. So far, mulberry genomic DNA has been isolated by Machii (1989). For molecular studies, the following criteria should be met by the nucleic acids isolated from plant material: it must be pure enough for PCR amplification, the DNA must be intact enough to give accurate and reproducible migration patterns and the yield of DNA must be sufficient so that a reasonable amount of tissue can be used.

The problems encountered in the isolation and purification of DNA specially from medicinal plants include degradation of DNA due to endonucleases, coisolation of highly viscous polysaccharides, inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with the enzymatic reactions. Moreover, the contaminating RNA that participates along with DNA causes many problems including suppression of PCR amplification (Pikkart and Villeponteau, 1993), interference with DNA amplification involving random primers, e.g.

RAPD analysis (Mejjad et al., 1994), and improper priming of DNA templates during thermal cycle sequencing. Different plant taxa often may not permit optimal DNA yields from one isolation protocol. For example, some closely related species of the same genus require different isolation protocols. Thus an efficient protocol for isolation of DNA is required.

Various protocols for DNA extraction have been successfully applied to many plant species (Doyle and Doyle, 1987, Ziegenhagen and Scholz 1993), which were further modified to provide DNA suitable for several kinds of analyses (Wang and Taylor, 1993; Ziegenhagen and Scholz, 1998). We have tested various DNA isolation protocols i.e., Phenol-chloroform method (Mahyco Research Foundation 1998), Dellaporta method (Dellaporta et al. 1983) and CTAB method (Doyle and Doyle 1987). Among the 3 methods tried, CTAB method (Doyle and Doyle 1987) was found to be suitable. Therefore, we report here a total genomic DNA isolation protocol derived from a method originally developed for other plants (Doyle and Doyle 1987). Modifications were made to minimize polysaccharide, and to simplify the procedure for processing large number of samples. Thus the protocol derived for genomic DNA isolation is efficient, inexpensive, simple, rapid, high yield and pure. The isolated DNA would be suitable for further molecular studies.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Young leaves of Seventeen ecotypes of *Gymnema sylvestre* (Panchagani, Kandala, Ambavale, Housaryghat-I, Housaryghat-II, Bhubaneswar, Warangal-I, Warangal-II, Mulugu-I, Mulugu-II, Mulugu-III, Rajahmundry-I, Rajahmundry-II, OU-I, OU-II, OU-Bridge, OU-Botanical Garden) were collected from various forests in the country and grown in experimental site. After acclimatization 1g of young leaves were harvested for DNA isolation. The following buffers and other gel concentrations were used in this study

Extraction Buffer: 4M NaCl, 0.5M EDTA (pH 8.0), 1M Tris-HCl (pH 8.0), 10% CTAB, Mercapto Ethanol, Chloroform : Isoamyl alcohol (24:1), Chilled ethanol or Propanol, 70% Ethanol, RNase, TE buffer (T10 E1), Phenol :Chloroform : Isoamyl alcohol. (25:24:1)

2.2 DNA ISOLATION PROTOCOL

- 1 gram of freshly harvested leaf samples were taken from seventeen ecotypes of *Gymnema sylvestre*, washed under running tap water and then dried on filter paper
- These leaves were ground in liquid nitrogen using a mortar and pestle along with 50mg of PVP and were made to fine powder.
- The powder was quickly transferred to centrifuge tubes, and add 5 ml of freshly prepared preheated (65°C) DNA extraction buffer to each tube and shaken vigorously by inversion to form slurry.
- The tubes were incubated at 65°C in water bath for 60-90 minutes with intermittent shaking and swirling for every 30 minutes.
- An equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed properly by inversion for 15-20 minutes and centrifuged at 15000rpm for 10 minutes at room temperature to separate the phases (long term mixing of samples in Chloroform: Isoamyl alcohol approximately for 30 min, will help in removal of pigments and formation of brownish color in DNA sample can be omitted).
- The supernatant was carefully decanted and transferred to a new tube and add equal volume of chilled isopropanol or ethanol and gently mixed. A pellet of white fibrous structure of DNA was observed in tube.
- The pellet was washed with 70% Ethanol to remove the impurities, air dried and resuspended in 300µl of TE buffer. Treat with 5µl RNase and incubated overnight at 37°C (An overnight RNase treatment helped achieving in proper genomic DNA).
- Keep DNA at -70°C for long term and -20°C for short term storage.

2.3. QUANTITY AND PURITY OF DNA

The yield of DNA per gram of leaf tissue extracted was measured using a UV spectrophotometer at 260 nm. The purity of DNA was determined by calculating the ration of absorbance at 260nm to that of 280nm. DNA concentration and purity was also determined by running the samples on 0.8% agarose gel based on the intensities of band when compared with the Lambda DNA marker.

3. RESULTS AND DISCUSSION

We have obtained higher yields of pure DNA from seventeen different ecotypes of *Gymnema sylvestre* by modifying some of the steps in the original CTAB DNA isolation protocol (Doyle and Doyle, 1987). Leaves should be fresh and young. The presence of polyphenols, which are powerful oxidizing agents present in many plants species, can reduce the yield and purity of the DNA (Katterman and Shattuck 1983; Peterson et al., 1997; Porebski et al., 1997). Addition of PVP along with CTAB may bind to the polyphenolic compounds by forming a complex with hydrogen bonds and may help in removal of impurities to some extent (Maliyakal 1992). Tannins, terpenes and resins considered as secondary metabolites are also difficult to separate from DNA (Ziegenhagen and Scholz 1998). NaCl has been used in the DNA extraction buffer to remove polysaccharides (Fang et al., 1992). Long-term chloroform : isoamylalcohol treatment ensured removal of chlorophyll and other coloring substances such as pigments, dyes etc.

Additional precipitation steps, removed large amounts of precipitates (detergents, proteins etc) by modified speed and time of centrifugation. We found these modified steps necessary to standardize and increase the quality and quantity of genomic DNA. The degree of purity and quantity varies between applications (Zidani et al., 2005). The present modified method-1 yielded 311µg of DNA from 1g of young leaf tissue in ecotype OU-Bridge to 376 µg /g leaf tissue in ecotype Warangal-1. In method-2, 188 µg/g young leaf tissue in ecotype Housary Ghat-II to 276 µg/g leaf tissue in

ecotype Mulugu-I, and method-3, 221 µg/g leaf tissue in ecotype Housary Ghat-II to 302 µg/g leaf tissue in Mulugu-II (Table 1). DNA was isolated

from all the 17 ecotypes with 3 methods, high quantity and pure DNA was obtained by method-1 (Fig.2).



Figure 1: *Gymnema sylvestre*

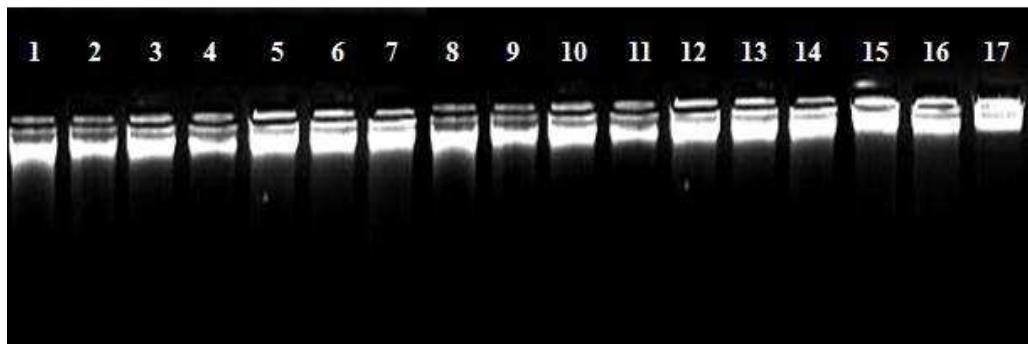


Figure 2: *Genomic DNA of 17 Different Ecotypes of Gymnema sylvestre Using Method – I (Modified Doyle and Doyle)*

1	Panchagani	9	Mulugu – I
2	Khandala	10	Mulugu – II
3	Ambavale	11	Mulugu – III
4	Housaryghat – I	12	Rajahmundry – I
5	Housaryghat – II	13	Rajahmundry – II
6	Bhubaneswar	14	OU – I
7	Warangal – I	15	OU – II
8	Warangal – II	16	OU – Bridge
		17	OU – Botanical Garden

Although, it is generally assumed that the DNA content per genome is usually constant for each species, numerous exceptions to this rule are known to exist. Chromosome variation such as aneuploidy, the presence of supernumerary

chromosomes and the loss or duplication of chromosome segments all of which can have larger effects on nuclear DNA content, may be considered as inter and intra-specific variation in DNA amount per genome (Bennet & Smith 1976).

Table 1: Comparison of DNA content with 3 methods in different ecotypes

S.No.	Ecotype	DNA content (µg/g leaf)		
		Method-1	Method-2	Method-3
1	Panchagani	349	257	269
2	Kandala	357	243	271
3	Ambavale	328	261	288
4	Housaryghat-I	339	258	236
5	Housaryghat-II	345	188 [#]	221 [#]
6	Bhubaneswar	368	232	249
7	Warangal-I	376*	238	274
8	Warangal-II	362	221	266
9	Mulugu-I	369	276*	295
10	Mulugu-II	365	249	302*
11	Mulugu-III	351	198	239
12	Rajahmundry-I	342	231	254
13	Rajahmundry-II	358	265	278
14	OU-I	329	226	242
15	OU-II	336	205	290
16	OU-Bridge	311 [#]	239	284
17	OU-Botanical garden	354	205	261

* Significantly high yield

Significantly low yield

4. CONCLUSION

In the present study revealed that this genomic DNA isolation technique in young *Gymnema* leaves can be applied for future DNA fingerprinting studies in *Gymnema sylvestre*. The procedure is fast and simple and 30-40 DNA samples may be processed in a single day.

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