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User friendly DNA isolation protocol optimized for *Ricinus communis* L. seeds

Ajmal Iqbal[1], Waqar Ahmad[2], Asaf Khan[3], Murad Khan[3], Mohammad Nisar[1*]

[1]*Department of Botany University of Malakand, KP, Pakistan,*

[2]*Department of Pharmacy, University of Malakand, KP, Pakistan*

[3]*Department of Biotechnology University of Malakand, KP, Pakistan*

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Abstract

A protocol was developed to isolate high quality genomic DNA from the seeds of *Ricinus communis* L. (caster) without using liquid nitrogen.The DNA extraction buffer used in this novel protocol constitutes SDS (1%), Tris (1.21%), NaCl (0.58%), EDTA (0.32%), 0.12% and β–Mercaptoethanol witha pH 8.5. In the protocol 0.09g of crushed seeds of caster bean, 600ul of DNA extraction buffer and and 500ul of phenol: chloroform: iso-amylalcohol with a ratio of 25:24:1 were used. The isolated DNA was amplified in Polymerase Chain Reaction using RAPD and SSR primer sets. The primer set successfully amplified the isolated DNA. Hence, the protocol is recommended as a user friendly novel protocol for DNA isolation from the castor beans seeds.

* **Corresponding Author:** Mohammad Nisar ✉ dr.nisar@uom.edu.pk

Introduction

Ricinus communis L., commonly known as caster bean, is a member of the spurge family,Euphorbiaceae (Smith 1986). *Ricinus* is indigenous to tropical Asia and Africa, but today it is cultivated for seed oil throughout the tropical and subtropical regions of the world (Seo, 2011). The annual production of caster bean throughout the world is 460,000 tones. In Pakistan it is grown on 3204 ha and its annual production is 2089 tones, the average seed yield of caster bean in Pakistan is 652 kg ha-1 . This seed yield of Pakistan is very low (Anonymous, 2006). Oil derived from the seeds of caster has several potentials in many industries like medicine, and cosmetics (Akande, 2012). Castor bean seed oil is highly valued in several sectors of the chemical industry and is considered a bioenergy and phytoremediation resource in the subtropics (Baldanzi *et al.* 2005). Castor plant has a great drought tolerance due to deep root system with the ability to explore the deeper layers of soil, which helps increase aeration, water retention and distribution in soil (Embrapa *et al.*, 2006).

The Preliminary Phytochemical study of *Ricinus communis* revealed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides (Kang, *et al.*, 1985). The leaves of the *Ricinus communis* contain flavonoids, tannins and phenol (Yadav RNS & Agarwala M. 2011; Ilavarasan *et al.*, 2006).

The isolation of high-quality DNA is prerequisite for any molecular biology work because contaminants such as proteins, polyphenols and polysaccharides may interfere with enzymes, such as endonuclease (in blotting techniques) and *Taq* polymerase in Polymerase Chain Reaction (Ausubel *et al.*, 1994). The phenols covalently bind to proteins and DNA, giving the DNA a brown colour and making it useless for most research applications (Katterman & Shattuck, 1983; Guillemaut & Drouard, 1992, Aljanabi *et al.*, 1999). Polyphenol contamination of DNA makes it resistant to restriction enzymes (Katterman & Shattuck, 1983).

Several methods for extracting DNA for different plant are available (Doyle and Doyle *et al.*, 1990;

Khanuja *et al.*, 1999; Kumar *et al.*, 2003 Islam *et al.*, 2013). In Pakistan no work has been done on the extraction of DNA from *Ricinus* species. Therefore the aim of the present study is to develop simple DNA extraction protocol from the seeds of six different varieties of *Ricinus communis* without liquid nitrogen and for further genome characterization using RAPD and SSR primers.

Materials and methods

Plant material

Ricinus communis seeds of different lines (Fig. 1) were obtained from Gene Bank, Institute of Agriculture Biotechnology and Genetic Resources, National Agricultural Research Centre, Islamabad Pakistan.

Reagents

DNA extraction buffer, Phenol–Chloroform–Isoamylalcohol (25:24:1), 3M Sodium acetate, Isopropanol, 0.12% β–Mercaptoethanol, RNAase, TBE (Tris Borate EDTA) and Proteinase.

DNA extraction buffer

SDS (1%), Tris (1.21%), NaCl (0.58%), EDTA (0.32%). Adjust PH to 8.5.

Method for DNA extraction

About 0.09g of crushed seeds of caster bean was added into 1.5ul eppendorf tube, containing 600ul of DNA extraction buffer. A volume 1.28ul of β-Mercaptoethanol was added to disrupt the structure of proteins. The tubes were vertexed to homogenize the chemical and crushed seeds and were then incubated at 58° C for 1 hour in water bath. The tubes were vertexed for 1minute at interval of 15 minutes during incubation. After incubation 500ul of phenol: chloroform-iso-amylalcohol with a ratio of 25:24:1 was added to the tubes and were vertexed for 3 minutes. The tubes were then centrifuged at 13000 rpm for 30 minutes. The upper phase (300ul) of each tube was transferred to a fresh tube. A volume 50ul of

3M sodium acetate and 400ul of chilled isopropanol was added to the tubes and mixed the contents to precipitate DNA. The tubes were then kept in refrigerator at -4° C for 1 hour to ensure the maximum precipitation. The tubes were then centrifuged for 10 minutes at 8000 rpm. The supernatant was discarded and the pallet containing DNA was washed with 70% ethanol. The tubes were air dried and the pelleted DNA was dissolved by adding 25ul of TE to each tube. The presence of DNA was checked by running the samples on 0.5% of agarose gel.

Gel Electrophoresis

Agarose gel was prepared by adding 99.5ml of 1XTBE in 0.5g agarose powered and boiled the solution for complete homogenization of agarose powered. About 5ul of ethidium bromide was added to the gel. The gel was solidified under room temperature and put in horizontal gel electrophoresis apparatus and adjust with 100volt for 40 minutes. As 2ul of isolated genomic DNA with 1ul of 6X loading was added in each well of agarose gel. After completion of 40 minutes, the presence of DNA in each well was documented in gel documentation system (Vilber lourmat, France). The electropherogram was developed to know about DNA profiling (Fig. 1–4).

Results

Through the optimized protocol, DNA is extracted throughout the year from the seeds of *Ricinus communis*. By this protocol an acceptable quantity of high-quality DNA is extracted. The DNA isolated was checked by 0.5% agarose gel electrophoresis. Isolated DNA was observed under gel documentation system, so pure non degraded DNA was observed in the samples, but RNA was present as shown in Fig. 2. To get RNA free pure DNA, RNAase treatment was performed for degradation of RNA contents Fig. 3. The isolated DNA was used for PCR amplification by using RAPD and SSR primers. The isolated DNA was amplified successfully in Polymerase Chain Reaction with both RAPD and SSR primer sets as shown in Fig 4 and 5 respectively. with Premixed used for RAPD and SSR are represented in table 1 and 3 respectively. While PCR conditions are represented in table 4 and 5.

Table 1. Pre mix used for RAPD reaction.

S.No	Reagent	Quantity
1.	H_2O	10.8µl
2.	dNTPs	2 µl
3.	$MgCl_2$	1.2 µl
4.	10xTaq Buffer	2 µl
5.	RAPD primer	2 µl
6.	Genomic DNA	1 µl
7.	Taq DNA Polymerase	1.2 µl
Total Volume		20 µl

Table 2. Thermocycling conditions optimized for RAPD Reaction.

Steps	Temp	Time	Cycles
1.	94°C	4 minutes	1
2.	94°C	1minute	40
3.	32°C	1minute	
4.	72°C	2 minutes	
5.	72°C	10 minutes	1
6.	4°C	Infinite	Continue

Table 3. Pre mix used for SSR reaction.

S.No	REAGENT	QUANTITY
1.	H_2O	11 µl
2.	dNTPs	1 µl
3.	$MgCl_2$	1.5 µl
4.	10xTaq Buffer	2.5 µl
5.	Forward primer	1 µl
6.	Reverse primer	1 µl
7.	Reverse primer	1 µl
8.	Taq DNA Polymerase	1 µl
Total Volume		20 µl

Table 4. Thermocycling conditions optimized for SSR Reaction.

STEPS	TEMP	TIME	CYCLES
1.	94°C	5 minutes	1
2.	94°C	1minute	35
3.	30°C	1minute	
4.	72°C	2 minutes	
5.	72°C	10 minutes	1
6.	4°C	Infinite	Continue

Discussion

Castor seed consists of high quantity of oil. “Recin oil” is extracted from its fruit which is used as lubricant in the industries,also as laxative, purgative and in treatment of terminal cancer in folk medicine (Correa, 1984; Feijao, 1963). High amount of phosphorus is present in seeds, 90% in phytic form. The major constituent of castor oil is ricinoleic acid,

with a small amount of dihydroxystearic acid, oleic acid, linoleic acid and stearic acid. (Anonymous 1948-1976). Most chemical constituents of medicinal plants interfere in DNA isolation (Puchooa and Venkatasamy 2005) and isolated DNA that contains impurities, is not suitable for PCR amplification and restriction digestion Lodhi *et al.*, (1994). Due to maximum concentration of oil in caster bean seeds, DNA is not extracted easily by using the protocol of Doyle & Doyle (1990). In order to overcome these issues Purohit *et al.*,2012 method was developed and successfully isolated the genomic DNA, we tested the same protocol, but in Pakistani *Ricinus* germplasm it failed to amplify the DNA, therefore we made certain changes for the purpose and the failure of the protocol might be the remaining of oil continents in the sample, which might be responsible for problem in isolation of genomic DNA. It was found that Purohit *et al.* (2012) protocol was best for isolating DNA from young seedlings but was unable to isolate DNA from the seeds of the castor. For this purpose the DNA from the seeds of castor was isolated by the modified protocol of Purohit *et al.* (2012).

Fig. 1. Morphological feature of different *Ricinus communis* L. Lines.

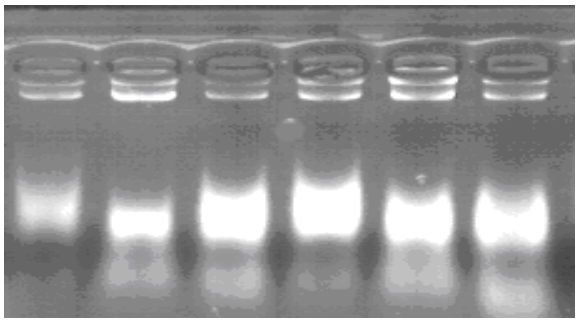

Fig. 2. DNA extracted from seeds of caster before RNSase treatment.

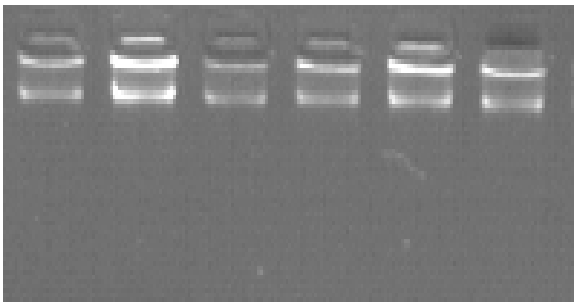

Fig. 3. DNA extracted from seeds of Caster after RNSase treatment.

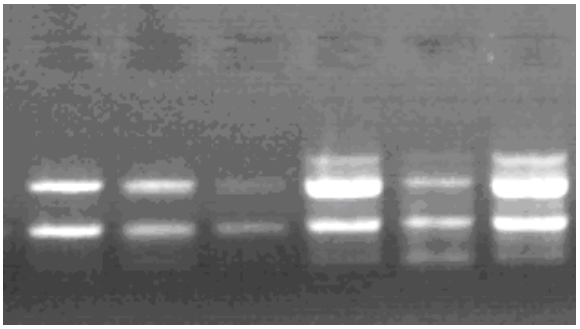

Fig. 4. Show the PCR amplification of DNA with RAPD primers.

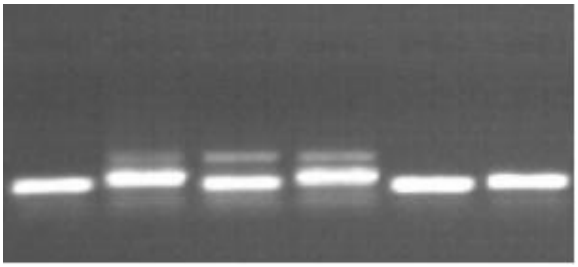

Fig. 5. Show the PCR amplification of DNA with SSR primers.

Inplant molecular biology extraction of high quality of DNA from plant material is an important for genome characterization(Barzegari *et al.*, 2010; Li *et al.*, 2010; Smyth *et al.*, 2010). The newly developed protocol is user friendly and extremely helpful as fresh leaves may not be available in all seasons. Hence this protocol was optimized to isolate the genomic DNA from the seeds castor bean.

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