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Rapid and Efficient Procedure for Isolation of High Yielding DNA from Castor (*Ricinus communis L.*)

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Abstract **-** The ability to perform high-throughput seed DNA extractions is highly desirable and essential for plant breeding and other molecular screening techniques. DNA extraction methods for PCR-quality DNA from calluses and plants are not time efficient, since they require that the tissues be ground in liquid nitrogen, followed by precipitation of the DNA pellet in ethanol, washing and drying the pellet, etc. Secondly, it gets more difficult to isolate the DNA from seed containing oil. The need for a rapid and simple procedure is urgent, especially when hundreds of samples need to be analyzed. Hence, for this purpose, a simple and efficient method of isolating high-quality genomic DNA for PCR amplification and enzyme digestion from seed is developed.

Index Terms **–** Castor, DNA extraction, DNA purification, Genomic DNA, PCR amplification.

I. INTRODUCTION

The castor bean plant (*Ricinus communis* L.) is a member of the family Euphorbiaceae. In spite of its common name, the castor plant is not a true bean (i.e. leguminous plants belonging to the family, Fabaceae). The Euphorbiaceae family occurs mainly in the tropics, expresses mixed relationship with several other groups, and includes plants producing the most important economic products such as Para rubber (*Hevea brasiliensis*), Castor oil (*Ricinus communis L.*), Tung oil (*Aleurites fordii*) and Cassava meal (*Manihot esculenta)*. According to Hutchinson (1964) Castor and Jatrophas are commercially important non-edible oilseed crops in the Euphorbiaceae, very large genera with spurges and croton forming. The seeds of *R. communis* consist of 40-60% by weight of one of the world's most useful natural plant oils (Shamns, 2005). The seed of castor bean contains more than 48% oil and its oil is rich (80 to 90%) in an unusual hydroxyl fatty acid and ricinoleic acid (Jeong and Park, 2009). Castor oil is widely used for its lubricating properties and for medicinal purposes. In industry, castor oil is used for the manufacturing of soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceuticals and perfumes (Morris, 1996-2003; Franz and Jaax, 1997; Duke, 1998). Castor oil contains ricinoleic acid, which has been effective at inhibiting the growth of many viruses, bacteria, yeasts, and molds, which lends credence to the medicinal use of castor oil as a topical treatment for a variety of skin infections and ailments (Williams, 1995). Castor oil is also used as a laxative, as an emetic, and to induce labor in close to full-term pregnant women (Duke, 1998).

Castor is a plant that is commercially very important to the world. Castor is produced in about 30 countries lying in the tropical belt of the world (Milani *et al.,* 2009). The world production of castor seed hovers around at an average of 12.5 lakh tons and of castor oil is 5.5 lakh tons. The major producer countries of castor are India, China, Brazil, Paraguay, Ethiopia, Philippines, Russia, Thailand etc. The top most country in the list is India with around 65% of the share in production followed by China with 23% and Brazil with 7% of share. The world production levels observed a sharp rise in the year 2001 which took the level to 17.5 lakh tons but it again fell down due to crop failure (Hegde *et al.,* 2003). Due to high production and utilization of such crop, it gets essential to differentiate the varieties for benefit of mankind. In order to achieve that, the basic requirement is DNA isolation which becomes difficult due to its oil content. Hence, the below mentioned method is described to isolate the genomic DNA which can be further utilized for molecular based studies.

II. MATERIALS AND METHOD

Seed germination:

The seeds of castor were germinated for isolation of the genomic DNA as the castor seed contains approximate 48% of oil. Due to such higher oil content, isolation of genomic DNA from it becomes much difficult procedure so there is a need to germinate seed before isolation procedure. The seed is allowed to germinate *in vitro* for 5-7 days and the apical region of the hypocotyls is utilized for the purpose (Fig-1).

Figure 1: *In vitro* seed germinated of castor seeds.

Genomic DNA extaction:

Isolation of DNA from seed sample is very tedious as seed was hard and dry. There are very few protocols available for isolating DNA from seed (Anonymous, 2006). According to Doyle and Doyle (1990) generally for molecular studies, leaf and other soft tissues are being used for DNA extraction, therefore it requires costly column. In this study, DNA from the seed was isolated by the method of Patel and Jasrai (2010), which requires very less time and is cost effective.

Seed material:

Germinated Castor seeds were taken for DNA isolation. The apex region of the sprout of the germinated castor seed was taken and crushed with mortal and pestle in presence of extraction buffer. This extract was transferred to the 1.5 ml micro centrifuge tube.

Chemicals required:

- NaCl (AR, Ranbaxy Fine Chemicals Ltd. India)
- Hexadecyltrimethyl-Ammonium Bromide (CTAB) (AR, Ranbaxy Fine Chemicals Ltd. India)
- Tris HCl (AR, Ranbaxy Fine Chemicals Ltd. India)
- Na_2- EDTA (AR, Ranbaxy Fine Chemicals Ltd. India)
- Proteinase K (Banglore Genie, Banglore, India)
- RNase A (Banglore Genie, Banglore, India)
- β-mercaptoethanol (AR, Ranbaxy Fine Chemicals Ltd. India)
- Phenol (Banglore Genie, Banglore, India)
- Chloroform (AR, Ranbaxy Fine Chemicals Ltd. India)
- Iso-amyl alcohol (AR, Ranbaxy Fine Chemicals Ltd. India)
- Iso-propyl Alcohol (AR, Ranbaxy Fine Chemicals Ltd. India)
- Sodium Acetate (AR, Ranbaxy Fine Chemicals Ltd. India)
- Poly vinyl pyrrolidone (Loba cheme)
- Ethidium bromide (Sigma)

Chemical preparation:

1) Extraction Buffer: (1M Tris, pH 8.0, 5M NaCl, 0.5M EDTA)
   For 1 liter extraction buffer take 100 ml 1M Tris (pH8.0), 280 ml 5M NaCl, 40 ml 0.5M EDTA, 20g CTAB in an appropriate beaker. Stir it and heat up to 60-65˚C till CTAB dissolve completely .Adjust pH with HCl to pH 8.0, Adjust volume to 1 liter with de-ionized H_2O. Store it at room temperature up to 1 year. (NOTE: Add 1% w/v polyvinyl pyrrolidone (PVP) to the extraction buffer)

2) Proteinase-K: (20 mg/ml)
   Dissolve 200 mg Proteinase-K in 10 ml de-ionized H_2O. Store it at -20°C up to 2 years.

3) RNase-A: (100 mg/ml)
   Dissolve 100 mg RNase-A in 1 ml de-ionized H_2O. Store at -20°C up to 2 years.

4) 1X TE buffer: (1M Tris, pH 8.0, 0.5M sodium EDTA)
   Mix 10 ml of 1M Tris, pH 8.0 with 2 ml of 0.5M sodium EDTA and bring the final volume up to 1 liter with de-ionized H_2O.

5) Phenol:Chloroform:Iso-amyl alcohol (24:24:1):
   For 100 ml phenol:chloroform:iso-amyl alcohol (24:24:1), combine 49 ml phenol, 49 ml chloroform and 2 ml iso-amyl alcohol. Mix thoroughly and store it at room temperature under the fume hood for up to 6 months.

Isolation of genomic DNA:

DNA was extracted from 0.3-0.5g of germinated seed. 500 µl of extraction buffer was added to the mortal and was crushed. Then the extract was transferred to 1.5 ml micro-centrifuge tube. 25 µl of β-mercaptoethanol was also added to the above mixture. After that it was kept in the water bath for 1 hour at 55- 60°C. (The contents were mixed by inverting the micro-centrifuge tubes at an interval of 15 min.) The micro-centrifuge tubes were removed after 1 hour, and to that 500 µl of phenol:chloroform: iso-amyl alcohol in the ratio of 24:24:1 was added, so as to precipitate the cell debris and protein present in the crude extract. The micro-centrifuge tubes were mixed gently by inverting for 5 minutes and were kept for centrifugation at 9800 rpm for 13 minutes.

After centrifugation, 250 µl of the upper phase was pipetted out, and transferred to new micro-centrifuge tube. To this, 25 µl of 3M sodium acetate and 360 µl of iso-propyl alcohol/ethanol were added. The mixture was swirled gently and carefully. In this step, the DNA gets precipitated and appears like a white coil in the solution that is stored at -20°C for 30-40 min.

These tubes were centrifuged at 12,000 rpm for 12 minutes at low temperature. Then the supernatant was discarded by inverting the tubes. A translucent pellet appears on the wall of the tube that is pure DNA. The tubes were kept upside down on tissue paper to dry the sample for 2-3 hour. Removing the residual iso-propyl alcohol was critical especially if the DNA is to be used directly for PCR. The dried samples were stable at RT overnight. If air drying is used, the drying time advised is minimum of 1-h, but an overnight drying at RT was done.

TE buffer in the volume of 200 µl was added to the DNA pellet and incubated for another 10 min at 50°C. The DNA was solubilized by tapping gently and stored at -20°C until further use. Samples in TE can be stored at 4 or -20ºC.

Purification of DNA:

The dissolved DNA being crude requires further purification. 1 µl of proteinase-K was added to dissolve the protein constituent and 1 µl RNase-A was added for getting rid of RNA from the DNA extract. This was kept in the water bath for 1 hour at 55- 60°C. (The contents were mixed by inverting the micro-centrifuge tubes at an interval of 15min.)

The micro-centrifuge tubes were removed after 1 hour, and to that 200 µl of chloroform:iso-amyl alcohol in the ratio of 24:1 was added, so as to precipitate the cell debris and protein present in the crude DNA. The micro-centrifuge tubes were mixed gently by inverting for 5 minutes and were kept for centrifugation at 9800 rpm for 13 minute. .

After centrifugation, 150 µl of the upper phase was pipetted out, and was transferred to new micro-centrifuge tube. To this, 25 µl of 3M sodium acetate and 360 µl of iso-propyl alcohol/ethanol were added. The mixture was swirled gently and carefully. In this step, the DNA gets precipitated and appears like a white coil in the solution, then it was stored at -20°C for 30-40 min. These tubes were centrifuge at 12,000 rpm for 12 minutes at -20°C. The supernatant was discarded by inverting the tubes. A translucent pellet appears on the wall of the tube, is the pure DNA. The tubes were kept upside down on tissue paper for 2-3 hour to dry and to remove the residual iso-propyl alcohol. If air drying is used, the drying time advised is minimum of 1-h. but an overnight drying at RT was done and was far better than air drying. 200 µl of TE buffer was added to the DNA pellet and incubated for another 10 min at 50ºC. The DNA was solubilized by tapping gently and was stored at -20°C until further use. Samples in TE can also be stored for more period of time at -20ºC.

III. RESULTS AND DISCUSSION

Genomic DNA isolation and quantification:

In plants, genomic DNA extraction was carried out by CTAB protocol. CTAB is a cationic detergent that is compatible with the high salt concentrations often used to dissociate DNA from chromosomal proteins, and can also be used to selectively precipitate nucleic acids. CTAB-isolated plant DNA is now routinely used.

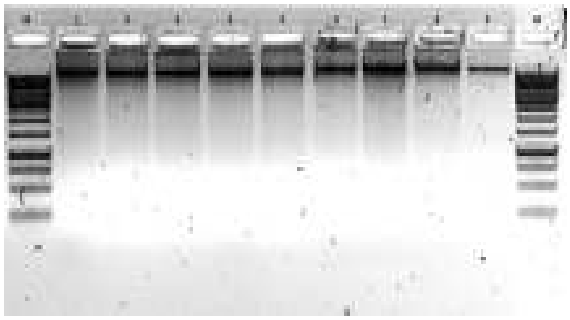

Figure 2: Ethidium bromide stained Genomic DNA of *Ricinus communis* (Castor) electrophoresed in 0.8% agarose gel. M- indicates the1Kb DNA ladder.

The quality of total DNA isolated was checked by using agarose gel electrophoresis, in which 2 µl of 6X loading dye (Fermentas, USA) to 10 µl of isolated DNA samples. 10 µl of this was loaded in a well of 1.2% agarose gel (Merck, India) containing 10mg/ml of ethidium bromide (EtBr). 1Kb DNA ladder (Fermentas, USA) was used as a marker. The intact DNA form a thick single band of good quality of DNA. The quality and quantity of DNA was further checked with the help of UV Spectrophotometer (at wavelengths 260 and 280 nm). Quality of extracted DNA was also confirmed by the agarose gel electrophoresis. The bands (Fig. 2) shows that the genomic DNA isolated from 9 different cultivars of castor plant was pure and lacks the other impurities like RNA and proteins. M (Fig. 2) indicates the DNA ladder which was used to measure the size of genomic DNA obtained. The data reported confirm that the extraction method is capable of isolating DNA from any kind of seed material and a suitable quality and quantity for subsequent PCR based detection applications is obtained.

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