

OPTIMISED METHODOLOGY FOR HIGH QUALITY DNA ISOLATION FROM LEAVES AND SEEDS OF FENNEL (*FOENICULUM VULGARE*)

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Abstract: In this study, an efficient, simple and rapid protocol is described for high quality DNA isolation from leaves and seeds of fennel (*Foeniculum vulgare*). The protocol gives highly reproducible results and can be carried out easily. Young leaves and seeds of fennel were kept at -80° C for 20 min to freeze the tissues and make the grinding easy without any tissue damage. This protocol eliminates the use of liquid nitrogen. The protocol is inspired by the CTAB method and Sambrook principles.

Keywords: Seed spices, DNA, Fennel, Seeds, Leaves

INTRODUCTION

Fennel (*Foeniculum vulgare*), is a highly aromatic and flavourful herb with culinary and medicinal uses from the family Apiaceae. It has a long history of herbal uses and widely cultivated in India, Pakistan, Suria and Egypt, for its edible strongly flavoured leaves and seeds. This crop is the very rich source of antioxidants and used in many medicines to cure diseases (Oktay, 2003, Bruyas-Bertholon V, 2012 and Lucinewton S, 2005). Saravanaperumal and Terza (2012) also studied and recommended the Polyphenolics free DNA isolation from mature and young leaves of fennel. Fennel is the very potential seed spice and plays a significant role in Indian economy and yet very limited information is available about genome of this crop. Now a day's use of advanced biotechnological tools is becoming a very important part of breeding programmes. Conventional breeding processes are very tedious and time consuming but molecular marker assisted breeding is time saving process. Molecular markers are the very essential for advance breeding programmes but unfortunately presently very few molecular markers are available for seed spices. It's a great need to do some molecular studies of these crops in this aspect we have isolated DNA using an effective and rapid method following principles of Sambrook (1989).

The DNA extraction process involves separation of DNA from naturally occurring plant cell constituents such as polysaccharide and polyphenolic compounds (Porebski *et al.*, 1997) followed by removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites from the aqueous solution containing the DNA and then precipitation and purification of DNA. DNA extraction and purification by CTAB method for various plants were standardized by Krizman *et al.*,(2006).

MATERIAL METHOD

Plant material

Fennel dry seeds and fresh leaf tissue were used for DNA isolation. Plant materials (seeds) were obtained from the seed bank of NRCSS (AF-12). The seeds were placed on a moist filter paper in a Petri dish, 10 seeds/plate at 25°C. After germination seeds were transplanted in pots for growth, young leaves from plants and dry seeds were taken for DNA isolation.

Solutions and reagents

Extraction Buffer, Chloroform, Isopropanol (pre-chilled), Isoamyl Alcohol, Ethanol, Tris-EDTA, RNase A, 70% ethanol, Absolute ethanol, Double distilled water, Concentrated HCl and NaOH pellets

Equipments

High speed centrifuge, Agarose gel electrophoresis equipment, Power supply, Vortex mixer, Refrigerator (-80° C), Mortar and pestle, Balance, Gloves, Forceps, Centrifuge tubes, Centrifuge, Micropipettes and tips and Water bath

Protocol (method)

- 100mg fennel seeds and approx 100mg young fennel leaves were kept at -80° C for 20 minutes; mortar and pestle were also kept in freezer for 30 minutes. Freezed seeds and leaves were grinded separately immediately in freezed mortar and pestle and transferred in 50ml tubes.
- 22.5ml extraction buffer was added and mixed well (cetyl trimethyl ammonium bromide (CTAB) which disrupts the membranes, β mercaptoethanol which helps in denaturing proteins and EDTA which chelates the magnesium ions). The samples were incubated at 65° C for one hour.
- 22.5ml chloroform: isoamyl alcohol (24:1) solution was added and mixed well for five minutes. Samples were centrifuged at 5000rpm for 10minutes. (To denature the contaminants

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which accumulate in the organic phase and the nucleic acids preserved in the aqueous phase). Supernatant was removed and was transferred to fresh labelled 50ml tube. Step was repeated twice for contamination.

- Samples were centrifuged for 10 minutes at 6000rpm and transferred to a new tube. 2volume of ice cold isopropanol was added and kept in freeze for 30 minutes (nucleic acid precipitation). Samples were centrifuged at 6000rpm for 10minutes. Solution was pipette off, taking care not to lose the DNA pellet at the bottom of tubes.
- 12.5ml of 70% ethanol was added. Samples were centrifuged for 10 minutes at 6000rpm. Supernatant was removed and DNA pellet was air dried till small of ethanol lasts and diluted with 2.5ml TE and stored at 4°C for future use.

RESULT AND DISCUSSION

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as “pure” for DNA (Leninger, 1975). If the ratio is appreciably lower, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm but our samples are showing no contamination by protein or polysaccharides (table 1). The 260/230 Ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid

are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm. The isolated DNA was measured by using the Nano-Drop spectrophotometer where the measurement at OD 260/280 was ranged 1.81 for seed and 1.80 for leaf tissue where as at OD 260/230 purity (nm) was 2.05 for seed and 2.10 for leaf. Total yield for seed DNA was 1130.1ng/μl and for leaf DNA yield was 1271.1ng/μl (table 1). DNA concentrations were confirmed using agarose gel electrophoresis. Ten microliters of purified DNA from the proposed procedure was run on a 1% (w/v) agarose gel containing 0.1 μg/mL of ethidium bromide. DNA was visualized using the Gel Doc System gel was showing very good results (Fig.1). The quantity of DNA was much higher in fennel leaf tissues in comparison with the fennel dry seeds.

In order to facilitate the efficiency and reliability of the DNA extraction method and the quality of the extracted DNA. The purified DNA was incubated with RNaseA (10mg/ml) at 37°C and precipitated following phenol: chloroform extraction to remove the RNase. The resulted DNA was amplified using RAPD primers and PCR product was run on 1.5% agarose gel (Fig. 2). A good PCR product indicates the good quality of DNA.

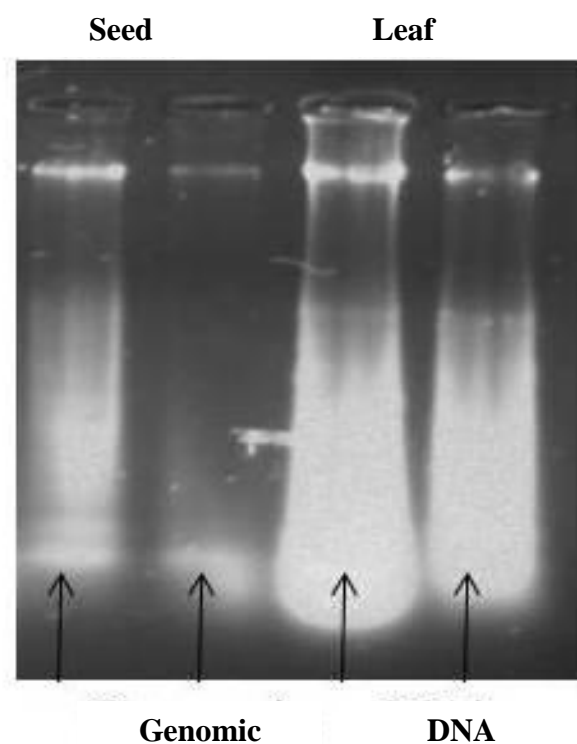


Fig.1 A gel image showing genomic DNA of Fennel Seed and Leaf (AF-12)

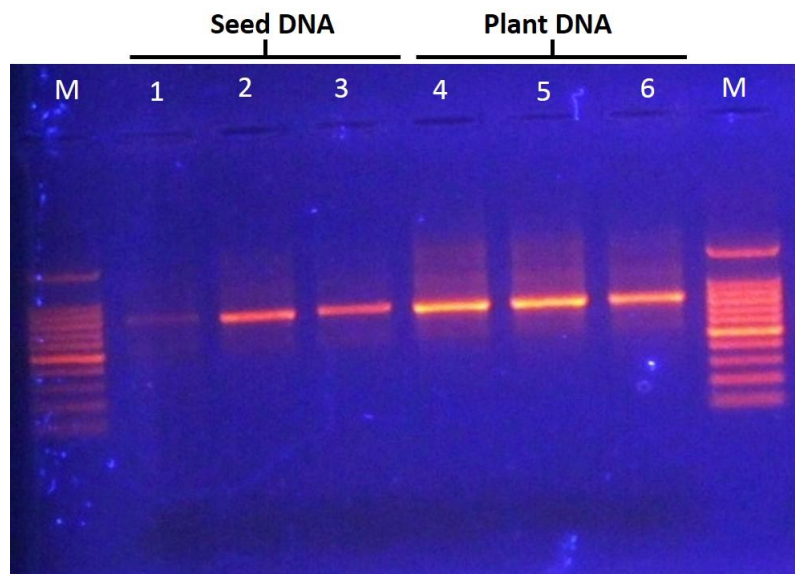


Fig.2 PCR result showing good amplification with fennel seed DNA (1-3) and leaf tissue DNA (4-6) with RAPD primer OPD-04

Table 1. The ratios of OD A_{260}/A_{280} and OD $A_{260}/230$ of Genomic DNA

Crop/Accession	DNA		
	Purity (nm)		Yield
	$A_{260}/280$	$A_{260}/230$	Con.(ng/ μ l)
Fennel-1 (seed)	1.81	2.05	1130.1
Fennel-2 (leaf)	1.80	2.10	1271.1

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