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A Reliable and High Yielding Method for Isolation of Genomic DNA from *Ammi majus*

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Abstract

The developed protocol describes a cheaper, quicker and reliable method for the isolation of pure DNA from medicinal herbs, such as Ammi majus, which produces the secondary metabolites xanthotoxin and berganpectane having immense medicinal importance. Use of CTAB, liquid nitrogen and EDTA in different isolation protocols analyzed for A. majus, all were ended with polysaccharide and protein contamination with low purity of DNA ($A_{260/280}$ = 1.3 – 1.6), revealed a need for method modification for the inexpensive and rapid isolation of pure DNA. Developed reliable and competent protocol isolated enough pure DNA ($A_{260/280}$ = 1.81) without following time consuming lengthy steps and hazardous chemicals used in other protocols, which increase experimental costs, risk, and need expertise to perform. The explained protocol requires few chemicals and little time to obtain pure DNA having yield 688 µg/g of A. majus. A higher quantity of isolated DNA obtained from young fresh leaf samples than from either the callus or stem. A. majus is a pharmaceutically important medicinal herb, and the present protocol aids in the analysis and modification of its genes.

Keywords: Ammi majus, DNA Isolation, secondary metabolites, xanthotoxin, berganpectane.

Introduction

Modification of plant metabolic pathway for higher production of medically important secondary metabolite or byproduct requires basic changes in the plant at DNA level. Application of molecular technology would increase and facilitate production of these substances[1]. Studying about plants for their product forming pathway by using modern biotechnology methods, like PCR amplification, gene transformation, molecular mapping and marker identification, requires a native component of the plant, genomic DNA. Polyphenols as powerful oxidizing agents can reduce the yield and purity of extracting DNA[2]. Medicinal plants, including *A. majus* contain high levels of polysaccharides, polyphenols, several pigments, and other secondary metabolites, which makes DNA unusable for downstream work in molecular biology research[3]. Polysaccharides make DNA viscous, glue – like and non – amplifiable in PCR by inhibiting Taq polymerase enzyme activity and also interfere with accurate DNA digestion[2]. Because plants contain high amounts of many different substances, it is unlikely that just one nucleic acid isolation method suitable for all plants can ever exist[4].

Photo – reactive furocoumarins, psoralens, have been identified in medicinal plant *A. majus*[5-7]. *A. majus* (Bishop's weed) is an annual plant in the Apiaceae family, often cultivated for its attractive flowering stems, originates in the Nile River Valley and also a commonly used spice in India[8]. Psoralens are substances that react with ultraviolet (UV) light to cause darkening of the skin, and are currently used together with UV light therapy to treat skin disorders. The fruit of *A. majus* has been used in the mediterranean and bordering regions in the treatment of leucoderma, psoriasis and vitiligo[9,10]. An inexpensive and competent DNA isolation protocol is not reported from *A. majus* till today.

Most of DNA isolation protocols having lengthy steps using different hazardous reagents and required to remove interfering substances that often co – precipitate with the extracted DNA[11]. CTAB method and its modifications[12,13] were extensively used in different laboratories, but these methods are time consuming[14]. The method containing CTAB, a cationic surfactant which is a hazardous chemical may cause irritation to skin and respiratory system. Sodium dodecyl sulfate (SDS) has also been used as an alternative to CTAB[15]. RNase treatment also consumes time and money mostly. Most of the methods required unsafe liquid nitrogen[16] or freeze – drying (lyophilization)[14,17] for proper tissue grinding and these facilities are more expensive to many laboratories. High cost per sample is main problem with commercially available DNA isolation kits[18,19] make them an unattractive option otherwise DNA isolation from large number of samples could be a costly affair in concern with money, safety and time.

After trying the protocols described by Doyle and Doyle[12], Edward[20], and Kotchoni and Gachomo[11], they were failing repeatedly to obtain pure DNA from *A. majus*. The procedure described here is modified method of Kotchoni and Gachomo[11] with containing least chemicals with a rapid procedure to get extremely pure DNA and consequently ideal for a large number of samples.

Material and Methods

Plant Material: Leaf, stem and callus of *A. majus* were taken from in vivo tissue cultured plants and used immediately for DNA extraction without freezing in liquid nitrogen or storing at – 80°C. The protocols described by Doyle and Doyle[12] (Protocol 1), Edward[20] (Protocol 2), and unmodified Kotchoni and Gachomo[11] (Protocol 3) followed to get rapid and pure DNA. Modified Kotchoni and Gachomo[11], (Protocol 4) developed by present study has been described in this paper and also describes a selection of sample type for high quality and quantity of DNA from callus, stem and leaves of *A. majus*.

DNA Extraction Reagents and chemicals: Extraction buffer: 1% SDS, 0.5 M NaCl, isopropanol (Chilled), 70% (v/v) ethanol, chloroform: isoamyl alcohol (24:1).

DNA Extraction Protocol: Take 50 mg fresh plant tissue (leaf or callus or stem) in eppendorf tube. Add 400 µL extraction buffer and a pinch of silica gel. Crush by plastic pestle completely. Incubate 10 min at 60°C incorporates additional step to protocol 3. Spin at 13000 RPM for 1 min at room temperature (RT). Take all solution in new eppendorf tube <150 µL. Add a double amount of chloroform: isoamyl alcohol (24:1) 300 µL which is another additional step needs to remove polyphenols and polysaccharides. Mix gently by inversion (no vertexing) and spin at 13000 RPM for 1 min at RT. Take liquid layer in new eppendorf tube. Add a double amount of isopropanol (pre – chilled). Mix gently by inversion and spin at 13000 RPM for 1 min at RT. Discard isopropanol and air dry the pellet. Dissolve pellet in 40 µL distilled water and store at – 20°C.

Quantification and visualization of DNA: DNA quantified by measuring optical density (O.D.) at A_{260} and A_{280} with UV/Vis spectrophotometer SL160 (Elico Ltd.). The quality of DNA was analyzed by agarose gel electrophoresis. Samples were prepared by taking 10 µl of DNA and 1 µl of 10X bromophenol blue dye (0.25% bromophenol blue and 50% glycerol) on a glass slide. Samples were subjected to electrophoresis in 1X TAE buffer for 1 hour at 80V on 0.8% agarose gels and photographed under UV light.

Results and Discussion

DNA isolation from medicinal plants is affected by their secondary metabolites yielding polyphenols and protein contaminations. Three different protocols, CTAB based Protocol 1, EDTA based Protocol 2, and unmodified SDS based Kotchoni and Gachomo[11] (Protocol 3) followed for isolation of DNA from *A. majus*, were used hazardous chemicals and consume different time span to complete procedure. Obtained quality and quantity of DNA, use of chemicals and time consuming steps of these three protocols were compared with the new modified method (table 1). Standardized Protocol 4 capitulate good quality and quantity of genomic DNA, giving $A_{260/280}$ ratio 1.81 indicating pure DNA than other followed protocols. Gel electrophoresis results shows (figure 1) the quality of DNA isolated with and without contaminants from *A. majus* using conventional as well as developed protocols.

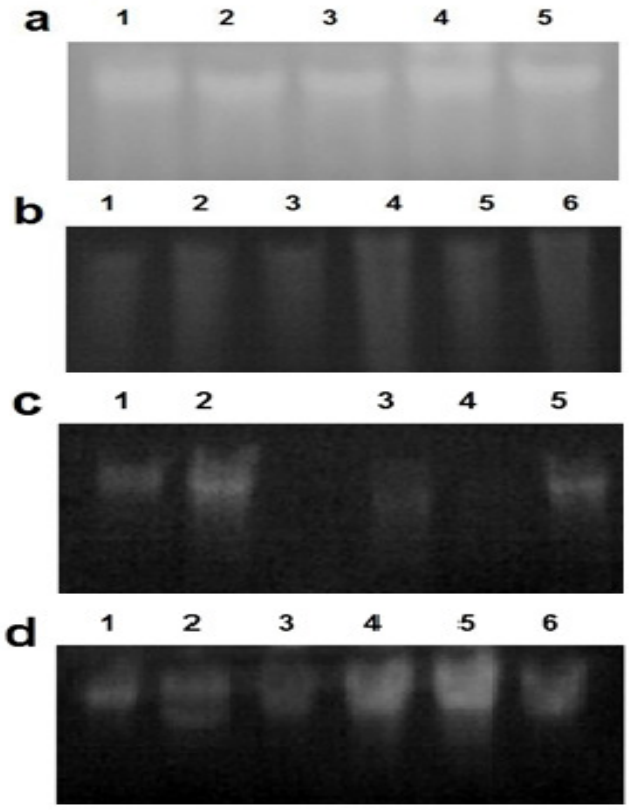

Figure-1

Analysis of purity of genomic DNA isolation of *A. majus* by different methods resolved on 0.8% agarose gel. (a) Protocol 1 – Use of CTAB and EDTA (Lane 1 to 5 leaf samples). (b) Protocol 2 – Use of EDTA and HCl (Lane 1 to 6 leaf samples). (c) Protocol 3 – Use of SDS and NaCl (Lane 3, 4 leaf samples) and Protocol 4 – Developed protocol (Lane 2, 5 leaf and Lane 1 callus samples). (d) Protocol 4 – Developed protocol (Lane 1 to 3 callus, 4, 5 leaf and 6 stem samples)

In Protocol 1, hazardous chemicals CTAB and EDTA were used and steps were more time consuming than others, making this protocol expensive. Also, isolated fraction was highly contaminated with proteins (figure 1a) yielding $A_{260/280}$ ratio 1.46 with yellow color shade. Protocol 2 were one of the famous and rapid method for DNA extraction, explains the use of EDTA and NaCl buffer, which was time and cost efficient, but protein contamination with $A_{260/280}$ ratio was 1.34 and observed no DNA band of *A. majus* (figure 1b). Without the use of EDTA and Tris – HCl, Protocol 3 was a successfully modified procedure described by Edwards[20] with more rapid way of DNA isolation, without handling any hazardous organic solvents, but failed to isolate pure DNA without proteins and polysaccharides contamination (figure1c). Comparative obtained data of different protocols presented in (table 1). In the present study, it was found that, Protocol 2 and Protocol 3 were fast, cost effective and less laborious but failed to isolate sufficient pure DNA from *A. majus* with $A_{260/280}$ ratio (1.3–1.6).

Table-1
Comparison of isolated DNA for purity, yield and isolation time using different protocols

	Required Chemicals	$A_{260/280}$	DNA Yield (µg/ml)	DNA yield (µg/g of plant material)	Required Time	Reference
Protocol 1	CTAB, HCl, EDTA, NaCl. Isopropanol, Chl:IAA, Ethanol.	1.46	18.4	368	55 min	12
Protocol 2	HCl, EDTA and NaCl	1.34	9.6	192	15 min	20
Protocol 3	SDS, NaCl, Isopropanol and Ethanol	1.59	22.4	448	10 min	11
Protocol 4	SDS, NaCl, Isopropanol, Chl:IAA and Ethanol	1.81	34.4	688	15 min	Modified Protocol

Modification in Protocol 3 shown best results to isolate pure and high quantity DNA which uses chloroform: isoamyl alcohol (24:1), chilled isopropanol and extra incubation time for buffer extraction steps (described in the DNA extraction protocol) differentiate the developed Protocol 4 from other protocols used here. These steps caused for fine – tuning of pure DNA isolation with A260/280 ratio 1.81 to its desired level yielding highest quantity of DNA per gram of sample shown in (figure 1d). Protocol 4 obtained DNA as transparent with no visible RNA contamination when electrophoresed on an agarose gel, gives sign of high purity. Isolated DNA can be directly used for PCR, RAPD or AFLP analysis. Present protocol analyzed of DNA isolation from callus, stem and leaf samples and the result shows young fresh leaf sample is ideal for isolation of genomic DNA of *A. majus* which gives higher quantity of DNA than from either the callus or stem shown in (table 2).

Table-2
Comparison of different tissue of *A. majus* for DNA yield

Plant material	$A_{260/280}$	DNA Yield µg /ml	DNA yield (µg/g of plant material)
Leaf	1.81	34.4	688
Callus	1.78	19.6	392
Stem	1.83	21.4	428

Conclusion

Above protocol is independent to use of liquid nitrogen, CTAB, HCl and EDTA with more advantages of its simplicity, rapidity and cost effectiveness. Addition of essential steps makes protocol reliable and yielding higher quantity of genomic DNA. An even inexperienced person could isolate pure DNA by following simple and safe steps described in given protocol. Use of general laboratory equipments and chemicals in present method gives further potential and scope for pure DNA isolation from other medicinal and herbal plants.

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