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Fast and Efficient DNA Extraction Method for a *Plumeria Acuminata* with Medicinal Potential

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Abstract

Many herbs are used from ancient to cure diseases. Plumeria species is of *Apocynaceae* family, *Plumeria accuminata* belonging to this, is also considered as ornamental plant. The plant is full of medicinal uses as it cures infections, digestive diseases, bronchitis, bleeding piles, dysentery, blood disorder, blennorrhagia, leprocy, rheumatism. The biological Activities are anti-inflammatory, wound healing activity, anti-pyretic action, anti-tumor potential and antioxidant properties, antifertility, antimicrobial, hepatoprotectant activities.

A simple, rapid and efficient method developed for the extraction of genomic DNA from *Plumeria accuminata* leaves. The protocol was based on a modified cetyl trimethylammonium bromide (CTAB) extraction method, allowed the rapid DNA extraction from little amounts of leaf material. This protocol gives good quality DNA, yield and a shorter duration of extraction would be ideal for genotyping and further studies.

Keywords: CTAB; DNA; Genotyping; Medicinal uses; *Plumeria acuminata.*

1. Introduction

Plumeria acuminata is a medicinal plant. *Plumeria* is a genus of about 155 genera having 2000 species, which are distributed primarily in the tropical and subtropical region. About eight species are known in India and of which *Plumeria acuminata* and *Plumeria rubra* are commonly grown. They are commonly known as "Temple tree" or "Champa" in India. According to location many common names also exist such as "Kalachuchi" in the Phillipines, Champa in laos, "Kembang kamboja" in Indonesia and Dead man's fingers in Australia. The plant material is widely used as purgative, remedy for pain, fever, diarrhoea and cure for itch. The milky juice is employed for the treatment of inflammation. The excessive doses of the latex are poisonous, and the root is a violent cathartic. The essential oil from the flowers possesses antifungal activity. In Unani practice, the medicinal herb is used to treat tumours and rheumatic pains. In the traditional medicine system, different parts of the plant have been useful in the treatment of a variety of diseases such as decoction of bark is used as purgative and febrifuge, the latex is used for itching with coconut oil, the flower is used as contraceptive etc. (Ashraf MD Farooque, 2012).

Plumeria acuminata is 3 to 7 m high, stem smooth, small tree and have shinning succulent with abundant white latex easily breaks. This plant is widely distributed all over the world among tropical region especially in southern part of India. In the traditional medicine system, different parts of the plant have been useful in the treatment of a variety of diseases such as decoction of bark is used as purgative and febrifuge, the latex is used for itching with coconut oil and the flower is used as contraceptive etc. This plant is also used as ornamental purpose. In this research article, various established facts related to the plant *Plumeria acuminata* have been compiled so that it may be a source of potential drugs in the days to come. This plant is a native to Mexico. It is reported to have been introduced from the Philippines and has become naturalised in India.

The search for a more efficient means of extracting DNA of both higher quality and yield has led to the development of a variety of protocols, however the fundamentals of DNA extraction remains the same. The crude DNA extraction procedure is still being adopted to allow multiple end uses. DNA extraction from plant tissue can vary depending on the material used.

The application of DNA technology in agricultural extraction have successfully been applied to many research has progressed rapidly over the last twenty plant species protocols mainly characterization as well as determination of population concern the ingredients (and the pH) of the extraction diversity in many plant species. The application of buffer, for example EDTA is generally included in DNA. This powerful tool in some plant species has however isolation buffers and storage solutions, since this compound has been constrained by lack of efficient nucleic acids compound chelates bivalent cations and thereby inhibits isolation techniques.

All molecular analysis to be successful, depend on obtaining DNA samples of quality. Therefore, the procedures are needed for efficient collection and storage of plant material, as well as isolation of the DNA molecule. The plants serve as bioreactors for natural products which produce large amounts of secondary metabolites and other substances of medicinal and industrial importance. Thus, while working with plant it is common to encounter problems arising from the presence of essential oils, polysaccharides, polyphenols and other secondary metabolites in the lysate and the DNA preparations. The polysaccharide is hindered by use of high amount of NaCl. The secondary compounds may interfere DNA isolation protocols. In our research, there are difficulties from the stage of cell lysis to DNA separation in the supernatant and subsequent reactions when following the procedures described by Murray and Thompson (1980), and Dellaporta et al. (1983) Major problems encountered were low DNA yield. Also, the procedure suffered from the precipitation of DNA and CTAB left in the supernatant during incubation of the mixture in cold temperatures. This protocol involves isopropanol precipitation of DNA initially at room temperature. Further, the polyphenols were removed from *Plumeria acuminata* by addition of high concentration of PVP. Phenolic content was removed using polyvinylpyrrolidone (PVP).

A CTAB-based mini-prep method for DNA extraction from *Gossypium* species and cultivars has been established that requires only one to three folded or nearly unfolded leaves collected in a 1.5-mL tube for tissue homogenization with an electrical drill which was proven more efficient (Jinfa Zhang et.al 2000). In another study, nucleic acid content of different parts of *Vinca rosea* were studied by using spectrophotometric analysis. Spectrophotometry has several advantages representing non-destructive technique and allows the sample recovery for further analysis or manipulation (Himesh et al. 2011). 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 (M. kazemi et.al 2012). The protocol included polyvinylpyrrolidone (PVP) to bind phenolic compounds, *β*-mercaptoethanol to inhibit the oxidation of polyphenols, and a high concentration of NaCl (2.5 M) to increase the solubility of polysaccharides, thus reducing their co-precipitation with DNA. (Sandra Lo Piccolo et.al 2012)

Extraction of quality DNA from other members of Meliaceae like *Azadirachta indica* and *Melia azedarach* was also carried out. In downstream applications, the extracted DNA was used for PCR amplification by using ISSR and SSR markers (Swati Rawat et.al 2016).

Modified DNA extraction and isolation of pure genomic DNA samples from medicinal plants were carried out and further downstream processes like PCR amplification were studied (Pallavi Sahare et. al 2012) Isolation of DNA using Triton-X-100 based extraction method with PVPP treatment efficiently removes metabolites and yield with high quality DNA from rice coleoptiles by Jhala et. al. The isolated DNA and RNA proved to be suitable for PCR and RT-PCR amplification, respectively (Jhala Vibhuti M et. al 2015).

Genomic DNAs were extracted from 12 soil samples of different crop roots. Above 10 μg of genomic DNA with approximately 20-kb fragment length was isolated from 1 g of soil sample. The absorption curves of the full wavelengths of the DNA extracted were consistent with that of pure nucleic acid. DNAs from different dilutions of pollutants were applicable for restriction enzyme digestion analysis, and the lambda DNA in 100-fold diluted soil samples had the same restriction enzyme digestion results as done in ddH2O (Jinbin Wang et.al 2013).

In another 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. (R. Balamurali Krishna et.al 2012)

The isolation of plant DNA is very challenging as the objective of our work was to isolate the DNA from *Plumeria acuminata* plant that has high amount of secondary substances. It is very challenging task as the extraction requires harsh procedure to obtain DNA extract. One of the methods of obtaining DNA is through CTAB based protocol that work very well, as it is not expensive and give good quality DNA. The proper bands appear in agarose gel electrophoresis and fine pictures appear in gel documentation. It effectively removes the polysaccharides and polyphenols. The kit based methods are more sophisticated and expensive.

2. Methodology

Overall methodology involved the DNA extraction method and obtaining purified DNA sample from leaves and stem of *Plumeria acuminata* and its electrophoresis.

2.1 DNA Extraction Protocol

Plant sample can be prepared by cutting the leaves with sterile scissor, then grinding tissue in mortar and pestle after wiping it with 70% ethanol. For each 100 mg, homogenized tissue, 500 µl of CTAB extraction buffer was used and mixed thoroughly in vortex shaker. Then transferred homogenate to microfuge tubes and incubated at 60ºC in Water Bath for 30 minutes. After the incubation period centrifuged the homogenate for 5 minutes at 12000 rpm. Supernatant transferred to a new tube. Chloroform: isoamyl alcohol (24:1) 250µl added and vortex for 5 minutes and centrifuged at 13000 rpm for 1 minute. Aqueous phase transferred to a new tube. Then 50µl of ammonium acetate was added with 500µl of ice cold absolute alcohol. Again, vortex these tubes. Tube incubated at -20ºCfor 30 minute. Then, centrifuged at 13000 rpm for 1 minute, at room temperature. Supernatant was discarded, pellet was taken and added with 70% ethanol(chilled). Vortexed well. Again, pellet was taken, and air dried it to remove alcohol but without completely drying. Lastly TE buffer or nuclease free water was added, mixed well and stored in -20ºC.

2.2 Electrophoresis of Extracted DNA

Agarose gel was prepared and mixed it with TBE/TAE buffer. Addition of ethidium bromide was done after boiling agarose and incubated in room temperature. Bromophenol blue dye added as a tracking dye and mixed on parafilm with 10µl of DNA sample. 15µl of sample loaded in the wells. Run the gel to 300 minutes at 100V.Expose the gel to UV light and photograph.

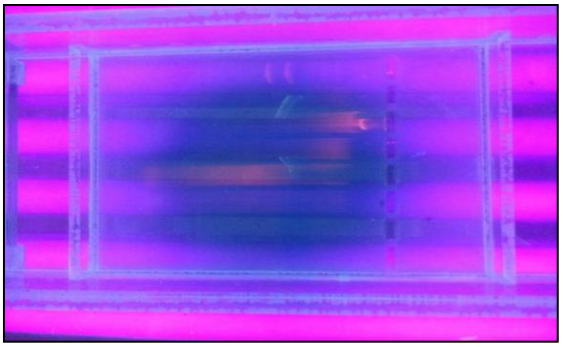

Figure 1: DNA bands of Stem and Root sample in Trans illuminator

3. Observation and Result

The success of any molecular study involves, firstly, the optimization of methods for collection and storage of plant material and DNA extraction. The extraction of high-quality genomic DNA is a primary and critical step in molecular biology. However, a low-cost and time-efficient extraction protocol is often hindered by two major challenges now. The first challenge is the reduction of secondary chemical reactions (including oxidation) in the initial crude tissue extract, which otherwise could lead to loss of DNA yield; another is the lack of a unique extraction protocol suitable for all organisms because of the differences in compounds between them (Kotchoni and Gachomo, 2009). Previously, genomic DNA purification methods included phenol-chloroform-based approaches with a popular method described by Sambrook et al. (1989), and methods based on grinding tissue in CTAB.

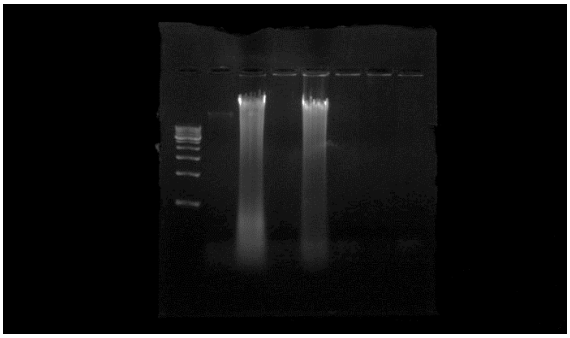

Figure 2: DNA bands of Stem and Root sample in Gel Doc

4. Conclusion

DNA isolation is a crucial technique which encompasses many genetic applications and analysis like techniques using molecular markers. All living things have DNA; it can easily be seen with the naked eye b when collected from thousands of cells. This work has proposed simple method to extract DNA from *Plumeria acuminata* and why each step is necessary due to the complex organization of DNA in cells.

Obtaining good quality DNA samples is the first step to succeed in such analysis and it depends on effective procedures for harvesting and preserving the plant material, and for DNA extraction. The use of fresh material is ideal for DNA isolation, in studies that involve the harvesting of wild plants this is not always possible, since in most cases, populations are distant from the research laboratory. The gel doc system provides automated gel imaging essential for quality images of gel. The procedures presented are simple, efficient and inexpensive, and can potentially be applied. Among the main advantages observed the use of inexpensive materials and ease of transport and storage of samples. Experience shows that the use of fresh material is ideal for holding the DNA isolation. However, the studies it is difficult to collect wild plants all the times freshly, because in most cases the plants are far from the research laboratory area.

The *Plumeria* plant is a source of many pharmacologically and medicinally important chemicals such as plumeride, isoplumeride, fluvoplumericin, irriod glycoside and other various minor secondary metabolites. Today's scenario is focused towards the application of non-toxic plant products. Thus, development of modern drugs from *Plumeria* species should be emphasized. Clinical trials should be conducted to support its therapeutic use. It is also important to recognize that its extracts may be effective not only isolation, but may have a modulating effect when given in combination with others.

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