

Determination of quality and quantity of DNA template using modified CTAB protocol

Abstract

DNA extraction protocol on *Jatropha* plant needs to be modifying in order to obtain good template DNA for PCR amplification. Total DNA was extracted using modified CTAB extraction protocol. This modify protocol provided enough quantities and quality of DNA for PCR amplification that produced ~530bp PCR product. It indicated that Dry leaves samples has high DNA quantity than the fresh samples, but they are almost at the same quality which confirmed by Spectrophotometer.

Keywords: DNA, genome, *jatropha*, mosaic viruses, medicinal plant, CTAB

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Introduction

Jatropha (*Jatropha curcas*) is one of the 170 known species that belongs to the family *Euphorbiaceae*.¹ *Jatropha* is a small shrub tree which has pale-green leaves and produces latex when the plant is cut.¹ *Jatropha* plant derives its name from the Greek word iatros (doctor) and trophe (food) which indicates the plant's medicinal value in ancient times.¹ The species name 'curcas' comes from the name physic nut found in Malabar, India.¹ *Jatropha* plant grows to a height of up about 3–5 meters, but under favourable agronomic conditions it grows up to 8–10 meters.²

Jatropha curcas can be found in tropical and subtropical regions and it can also grow in drought conditions although *jatropha* yields better under irrigated cultivation.² For many years scientists attempted to identify the origin of *Jatropha* plant but the sources are still controversial.¹ Martin et al.,³ reported that *jatropha* originated from Ceara state of Brazil, and Dehgan et al.,⁴ supported this by reporting the Mexico and Central America are the likely to be origin of the Physic nut (*Jatropha*).¹ Today *jatropha* is found worldwide, especially in developing countries (Centre for *Jatropha* Promotion 2007). In the last decade the cultivation of *jatropha* increased dramatically because of its importance as its seeds serve as raw material for biofuel synthesis.² When compared to other crops that are used in biofuel productions *jatropha* produces the maximum yield; for example from corn 200 barrels of biofuel can be used per square mile area per year in USA, rice can produce 1000 barrels and *Jatropha* produces up to 2000 barrels of oil per square mile in a year.

However *jatropha* is also useful as a source of organic manure, medicinal plant, lubricant and used in soap making. *J. curcas* was also found in Nigeria especially in the rural areas as hedge plants to demarcate farm boundaries and to protect crops against invasive wild and domestic animals. Only recently with the development of biofuel industry *jatropha* is being used as a source of income in Nigeria.⁵

Today *Jatropha* is cultivated intensively in many developed and developing countries which have lead to increased number of pests and diseases being affecting the plant including begomoviruses which cause *Jatropha* mosaic disease (JMD). JMD was first reported from Puerto Rico on *Jatropha gossypifolia* and it was also present in Jamaica, Mexico, Mali, Cuba and America.⁶ Furthermore JMD was also reported in 2004 from India, and is associated with *Jatropha* mosaic Indian virus (JMIV), which is transmitted, by *Bemisia tabaci*.^{7,8} Based on sequencing of the partial genome of JMIV, it confirmed that it's closely related to cassava mosaic viruses infecting cassava in India.⁸ However, the precise taxonomic position of the virus (JMIV) could not be confirmed because of the limited data available, which requires an immediate investigation consider the global importance of the crop.⁷

Methodology

Protocol for DNA extraction from the infected leaves samples

Total DNA was extracted from the infected *Jatropha* leaves sample, using CTAB method according to Lodhi et al.,⁹ which modified by Dr Maruthi of the NRI University of Greenwich.

Step 1: Leaves samples of *Jatropha* plant were collected out of freezer at –80°C; about 0.1g (100mg) of the disease plant was placed into a thick gauged plastic bag.

Step 2: The plant tissues were grounded with the used of roller and mixed with 10 volumes (1ml) of the CTAB extraction buffer.

Step 3: About 750µl of each sample was poured in to 1.5ml eppendorf tube, the samples were then heated at 60°C for 30min.

Step 4: The samples were mixed with an equal volume of (750µl) of phenol:chloroform:isoamylalcohol (1:24:1) and mixed gently to form an emulsion, then centrifuged at 13000 rpm for about 10min.

Step 5: The top aqueous phase was transferred in to 1.5ml eppendorf tubes with the used of wide bore pipette tips (1ml tips).

Step 6: The DNA samples were precipitated by adding 0.6 volume (300µl) of cold (−20°C) isopropanol and incubated at −20°C for at least 1hr. The samples solution was be kept at −20°C weeks.

Step 7: The samples were collected from freeze (−20°C), and centrifuged at 13000 rpm at 4°C for about 10min and the supernatant was discarded.

Step 8: The pellet was washed in 0.5ml 70% ethanol by vortexing and then centrifuged for about 5min at 13000 rpm.

Step 9: The ethanol was removed and the pellet was vacuum to dry for 5min. The dried pellet was suspended in 100µl 1 x TE buffer and stored at −20°C.

Results and discussion

Total DNA was extracted from the *Jatropha* Plant sample which shows mosaic symptoms using CTAB method⁹ which modified at Natural Resources Instituted University of Greenwich UK. This modified CTAB method provides enough quantity and quality of DNAs, for PCR amplification which confirm by Spectrophotometer at absorbance mean A260/A280.

DNA qualities of the fresh and dry samples (Tables 1&2).

Table 1 The above result of DNA purity indicates that the samples are of good quantity. Normally a good DNA template quantity should be above 200 ug/ml. In the above result only sample 2 and 6 have less DNA quantity but they are of good quantity to produce PCR amplicons. However the Spectrophotometer result above indicate that Dry leaves samples were of high DNA quantity with the average mean 492.1 ug/ml, than the fresh samples with average mean of 257.6ug/ml

JMIV	Fresh ug/ml	Dry ug/ml
Sample 1	312.6	420.1
Sample 2	172.9	236.3
Sample 3	384	851.8
Sample 4	160.9	460.2

Table 2 The above result of DNA purity indicates that the samples are of good quality. Normally a good DNA template quality has reading in between ~1.8 absorbance ratios A260/280. The absorbance ratios were between 1.85 to 2.7 shows that DNA samples are of good purity

JMIV	Fresh	Dry
Sample 1	2.07	2.05
Sample 2	1.9	1.85
Sample 3	2.05	1.89
Sample 4	1.86	2

However there were no any significant differences on the above result, which indicated that the samples are free from proteins, phenol or other contaminants that usually absorb higher at A280. Generally the modified protocol produces enough quality and quantity of DNA that produces enough Template DNA of ~530bp PCR product.

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None.

Conflicts of interest

The author declares there are no conflicts of interest.

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