

# Estimation of Genetic Diversity in Jackfruit Employing PCR Based D-19 RAPD Fingerprinting

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## Abstract

Jackfruit (*Artocarpus heterophyllus Lam.*) belongs to the family Moraceae, grows in all evergreen forest zones of the world, which include hundreds of varieties thousands of cultivars diversification based on the jacalin (lectins) concentration in the fruits. Among DNA based molecular markers, Random Amplified Polymorphic DNAs (RAPDs) provide an excellent tool for studying genetic relationships. Investigation on DNA fingerprinting to estimate the genetic diversity in jackfruit using D19 primer (OPD19 – CTGGGGACTT) was used to estimate genetic diversity in five high yielding jackfruits accessions obtained from different location in GKV campus. Good quality of DNA was extracted from five different varieties of young tender leaves of *Artocarpus heterophyllus* by CTAB method with minor modification of high detergent concentration of 2 percent PVP and 3 percent CTAB. The quality of DNA was checked by Agarose Gel Electrophoresis. The isolated DNA was quantified using UV spectrophotometer at 260nm and 280nm. And Quantified DNA was subjected for PCR amplification. 67 unambiguous, readable and reproducible RAPD markers were produced using the selected primer (OPD19 – CTGGGGACTT). The number of bands obtained the average of 13.4 bands using single primer, with the sizes ranging from 250bp to 10000bp. Of the 67 bands, 18 (21.1%) were polymorphic and shared between five individuals, while 45 (52.9%) were monomorphic in five individuals. The present study showed low to moderate genetic diversity among the 5 jackfruit accessions.

**Key words:** Jackfruit, Genetic diversity, DNA, PCR and RAPD.

## Introduction

Jackfruit (*Artocarpus heterophyllus Lam.*) is generally regarded as an underutilized fruit. It grows in Bangladesh, India and in many part of Southeast Asia (Odoemelam, 2005). Jackfruit is native to India and grows wild in the rain forests of Western Ghats of India. Western Ghats is a rich source of biodiversity for a number of plant species specifically wide diversity of jackfruit trees. Jackfruit is also commonly grown in Burma, Malaysia and to a considerable extent in Brazil. Jackfruit is not easy to eat of hand owing to difficulty in separating fruit bulbs from rind (Jagadeesh *et al.*, 2007). The fruit has been reported to contain high levels of protein, starch, calcium and thiamine. In Bangladesh, it is commonly referred to as “poor man's food”. The nutritious seeds are boiled or roasted and eaten like chestnuts, added to flour for baking, or cooked in dishes. The tree is also known for its durable timber, which ages to an orange or reddish brown colour, with anti-termite properties. The leaves and fruit waste provide valuable fodder for cattle, pigs and goats. Many parts of the plant, including the bark, roots, leaves and fruit, have medicinal properties. Jackfruit wood chips yield a dye, which is used to give the famous orange-red colour to the robes of Buddhist priests (Simon *et al.*, 2007). Hot water extract of mature jack leaves is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus. An extract of jack leaves improves the glucose tolerance in normal human subjects and diabetic patients. Photochemical screening has revealed that the hot water extract contains flavonoids, leucoanthocyanins, anthocyanins and tannins as components (Chandrika *et al.*, 2006).

The diversity in specificity of lectins or Jacalin per seed, might have arisen due to various hidden factors such as multimeric, multichain, and partial glycosylation (Anagh *et al.*, 2004). Cultivar identification and estimates of genetic diversity using phenotypic markers have several limitations, especially in perennial crops. Molecular differences, using DNA and protein-based markers are more authentic and unaffected by environmental factors. Hence, characterization of genotypes at the genetic level, supplemented by phenotypic characters, provides the first step towards more efficient conservation, maintenance, and utilization of existing genetic diversity. Among DNA based molecular markers, Random Amplified Polymorphic DNAs (RAPDs) provide an excellent tool for studying genetic diversity and genetic relationships (Simon *et al.*, 2007). The present investigation aims at estimation of genetic diversity in jackfruit using D-19 RAPD marker for correlating their similarity and distance between five different jackfruit accessions employing DNA fingerprinting.

## Materials and Methods

The young green tender leaves of *Artocarpus heterophyllus* were obtained from Horticulture farm, University of Agricultural Science, The Division of Horticulture Science, GKV Campus, Bangalore, India. Five different varieties of *Artocarpus heterophyllus* (TCJ1, TCJ3, FH14, FH9, NC1) leaves were collected. The Selection of named varieties was based on morphological characters from Karnataka, India. The leaves were washed using distilled water, wiped with Sodium hypo chlorite initially and later by 70 percent ethanol to remove the dirt and

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microorganisms and air dried. The leaves were then sealed in airtight plastic bags, labeled and stored at 4°C for further use.

The total genomic DNA was isolated from five different varieties jack fruit genotypes using CTAB Method. 0.5g to 1g of leaf tissue was ground to a fine powder using mortar and pestle with the help of liquid nitrogen. 3ml of extraction buffer pre heated for about 5-10 minutes in hot water bath, i.e., at 65°C was added to the grounded leaves and 40mg of PVP was added. This slurry was transferred into two clean poly propylene (centrifuge tube) eppendorf tube. 15µl of β-Mercaptoethanol was added to each tubes and mixed thoroughly by inverted the tubes. The tubes were incubated at 65°C for 1 hour and cooled to room temperature. The content was centrifuged at 5000 rpm for 6 minutes. The aqueous phase was transferred to a fresh centrifuge tube and added 400µl of chloroform: isoamyl alcohol i.e., in ratio of 24:1 to the tube. The contents were mixed well for 25-30 times by inverting the tube gently and spin at 7000 rpm for 10-15 minutes at 4°C and again the supernatant was transferred to fresh tube. The procedure was repeated to get a clear form of supernatant. To the supernatant half the volume of 5M NaCl and one volume of ice cold propanol was added and incubated overnight at 4°C. The content was centrifuged at 10000 rpm for 20 minutes at 4°C. The supernatant was discarded and the pellet was washed with 70 percent ethanol and air dried. The pellet was dissolved in 200µl of TE Buffer. The quality of DNA was verified by 0.8 percent agarose gel electrophoresis. Extracted DNA was subjected to purification. 5µl of RNase (1µg/µl) was added to the DNA samples and incubated at 37°C for 1 hour. 5µl of Proteinase (1µg/µl) was also added to the DNA samples and incubated at 37°C for 1 hour. 200µl to 300µl of phenol was added and centrifuged at 7000 rpm for 10 to 15 minutes and transferred and supernatant was collected in fresh tubes. To the supernatant 200µl to 300µl of phenol: chloroform 1:1 was added and centrifuged at 7000 rpm for 10 to 15 minutes and the supernatant was transferred to fresh tubes. To the supernatant 200µl to 300µl of chloroform was added and then centrifuged at 7000 rpm for 10 to 15 minutes at 4°C and the supernatant was transferred to fresh tubes. Half of the volume of 5M NaCl and 1 volume of ice cold propanol was added to the supernatant and incubated overnight at 4°C. The tubes were centrifuged at 10000 rpm for 20 minutes and the supernatant was discarded. The pellet was washed with 70 percent ethanol. The pellet was dissolved in 200µl of TE Buffer was stored at 4°C. The DNA was checked in 0.8 percent agarose gel electrophoresis and quantified using UV spectrophotometer. 10µl of isolated DNA sample from each variety of Jackfruits were taken and made up to 2000µl or 2 ml with TBE / TAE buffer. The entire isolated DNA samples were quantified using UV spectrophotometer (JASCO) at 260nm and 280nm. The ratio OD 260/280 should be determined to assess the purity of the sample.

$$\text{DNA concentration}(\mu\text{g}/\mu\text{l}) = \text{OD at } 260 \text{ nm} \times 50 \text{ (dilution factor)} \times 50(\mu\text{g}/\text{ml})$$

1000

PCR was carried out in Applied Biosystems PCR Thermocycler. PCR Amplification was preceded according to the method of Simon et al (2007). The PCR reaction was carried out in a final volume of 25µl reaction mixture contained PCR stock DNA (5 µl), Cocktail (10 µl), 5 Pmoles Primers (2.5 µl), Sterile water (7.5 µl). The Mixture was over layered with a drop of mineral oil. This reaction mixture involved in PCR amplification conditions (hot start at 94°C for 4minutes, Denaturation at 94°C for 1minutes, Annealing at 35°C for 1minutes, Extension at 72°C for 2minutes, 45 cycles, Final Extension at 72°C for 10minutes, Holding at 4°C for 10minutes and end). Amplified fragments from RAPD primer were scored manually for their presence (1) absence (0) and a RAPD

matrix of the 5 accessions of jackfruit using D19 primer (OPD19 – CTGGGGACTT) was assembled for statistical analysis the sizes of the fragments were estimated using DNA 1 kb ladder. Co-electrophoresis with the PCR products was also done. A genetic dissimilarity matrix was developed using squared Euclidean distances which estimates all pairwise differences in the amplification products. A cluster analysis was based on Wards method using a minimum Variance Algorithm.

## Results and Discussion

Healthy, fresh 5 different varieties of young tender leaves were collected for the isolation of good quality of DNA. Extraction and Purification of DNA from fresh 5 different varieties of leaf tissues was done by modified CTAB method. Quality of DNA was done by Agrose gel electrophoresis. Gel was viewed under UV gel illuminator. DNA are viewed along with the RNA and Protein contamination (Plate 1) and without RNA and Protein contamination (Plate 2). The DNA quantity was determined by 260 & 280 nm ratio and the UV spectrophotometer readings showed that the presence of DNA from the PCR stock (Table 1). 67 unambiguous, readable and reproducible RAPD markers were produced using the selected primer (OPD19 – CTGGGGACTT). The number of bands

Sl.No.	Varieties	260nm	280nm	260/280nm
1	TCJ1	0.121	0.080	1.51
2	TCJ3	0.144	0.099	1.45
3	NC1	0.132	0.094	1.40
4	FH9	0.121	0.078	1.47
5	FH14	0.140	0.091	1.53

Table 1. Quantification DNA from 5 different jackfruit accessions.



Figure:1 The gel profile of Artocarpus heterophyllus DNA Extraction (before Rnase) by CTAB Method. Lane 1 - Non cracking 1 (NC 1) Lane 2 - Farm house 14 (FH 14) Lane 3 - Farm house 9 (FH 9) Lane 4 - Tissue Culture jack 1 (TCJ 1) Lane 5 - Tissue Culture jack 3 (TCJ 3)

obtained the average of 13.4 bands using single primer, with the sizes ranging from 250bp to 10000bp. Of the 67 bands, 18 bands (21.1%) were polymorphic and shared between in five individuals, while 45 bands (52.9%) were monomorphic in five individuals. A representative polymorphic gel profile using the primer (OPD19 – CTGGGGACTT) is shown in Plate 3. A pair-wise genetic dissimilarity matrix was calculated using Squared Euclidian Distances. The highest genetic dissimilarity more than 2% was between NC1, TCJ1, TCJ3 and FH9, FH14. The lowest genetic dissimilarity (1.4%) was between NC1 and TCJ1, TCJ3. TCJ1, TCJ3 and FH9, FH14 clustered together, indicating a close genetic relationship at a linkage distance of 1.0% in the dendrogram (Figure 1).

Jackfruit is the most widespread species of the genus. It forms forest associations with homesteads, tropical rain forests, dry evergreen forests, and the montane vegetation of mountain groups (Hossain and Nath, 1995). Jacalin, the major protein from the jackfruit (*Artocarpus heterophyllus*) seeds, is a tetrameric two-chain lectin combining a heavy

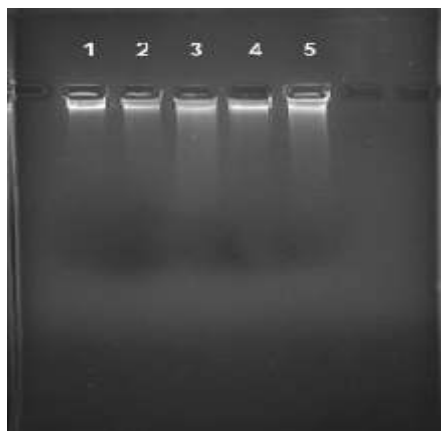


Figure: 2 The gel profile of *Artocarpus heterophyllus* DNA extraction (after Rnase) by CTAB method  
Lane 1 - Non cracking 1 (NC 1)  
Lane 2 - Farm house 14 (FH 14)  
Lane 3 - Farm house 9 (FH 9)  
Lane 4 - Tissue culture jack 1 (TCJ 1)  
Lane 5 - Tissue culture jack 3 (TCJ 3)

chain of 133 amino acid residues with a light chain of 20–21 amino acid residues (Kabir,1998). Healthy, fresh 5 different varieties of young tender leaves were collected for the isolation of good quality of DNA. Matured leaves were avoided because they are rich in phenol, secondary metabolites and polysaccharides. Extraction and Purification of DNA from fresh 5 different varieties of leaf tissues

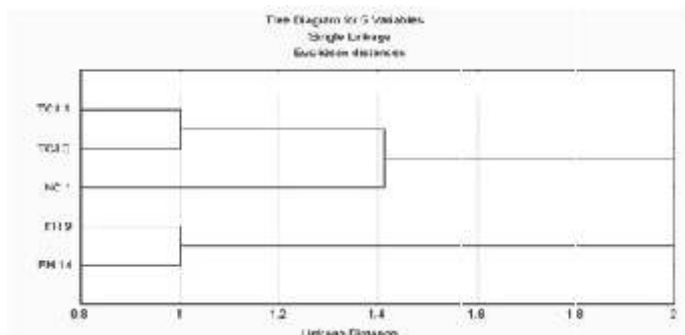


Figure 1. Dendrogram showing RAPD marker - based genetic relationships among 5 jackfruit accessions

was done by modified CTAB method. Similarly, Puchooa, (2004) also used the CTAB method of extraction without modification was used which gave DNA. However, it was contaminated with polysaccharides and phenolics as shown by the spectrophotometer readings.

The quality of DNA was confirmed by 0.8 percent Agarose Gel Electrophoresis. The DNA quantity was determined by 260 & 280 nm ratio and the UV spectrophotometer readings showed that the presence of DNA from the PCR stock. Similarly, Katarina *et al.*, (2006) confirmed the quality of DNA by 0.8 percent Agarose Gel Electrophoresis. The DNA quantity was determined by 260 & 280 nm ratio. PCR is the technique of choice for nucleic acid quantification. However, successful quantification depends crucially on the quality of the sample DNA analyzed. Whereas, Puchooa, (2004) reported the yield of DNA per gram of leaf tissue extracted using a UV-VIS Spectronic Genesys spectrophotometer at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. Quantified DNA was subjected to PCR amplification. 67 unambiguous, readable and reproducible RAPD markers were produced using the selected primer (OPD19 – CTGGGGACTT). The number of bands obtained the average of 13.4 bands using single primer, with the sizes ranging from 250bp to 10000bp. Of the 67 bands, 18 (21.1%) were polymorphic and shared between five individuals, while 45 (52.9%) were monomorphic in five individuals. A pair-wise genetic dissimilarity matrix was calculated using Squared Euclidian Distances. Whereas Fernandez, (2006) analysed genetic similarities between 8 cultivars of

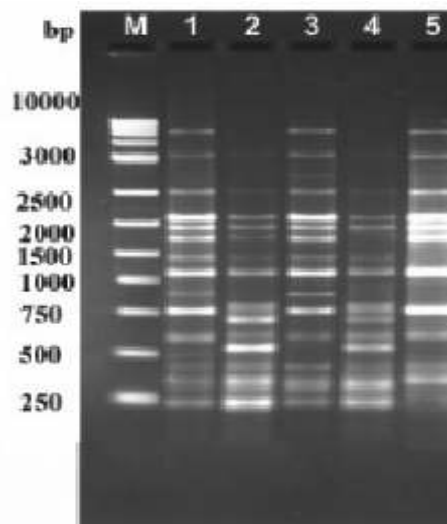


Figure: 3 RAPD - PCR Profiles of Jackfruit accessions, TCJ3, TCJ1, FH9, FH14 AND NC1 using RAPD-PCR primer OPD-19 (CTGGGGACTT), M, 400 bp standard DNA Marker.

M - Marker OPD - 19  
1- Tissue culture jack 1 (TCJ 1)  
2 - Farm house 9 (FH 9)  
3 - Tissue culture Jack 3 (TCJ 3)  
4 - Farm house 14 (FH 14)  
5 - Non cracking 1 (NC 1)

strawberry. Using 10 primers for their analysis, they obtained 116 bands, 79 (68%) of which were polymorphic and 37 (32%) were monomorphic. These results were used to calculate genetic distances and to prepare a dendrogram. The similarity between the analysed variants ranged from 62 to 89% indicating their close relation. The results obtained confirm the usefulness and applicability of the RAPD method in determining genetic similarity. Although these all genotypes represented

different geographical locations in India, the majority of them had morphologically similar attributes such as broad, thick, pubescent leaves and elongated fruits with sharp vines and dark green rind.

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