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Genetic variation studies on selected varieties of *Cocos nucifera* using RAPD molecular marker

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INTRODUCTION

Coconut palm (*Cocos nucifera*) is a member of the palm tree family (*Arecaceae*) and the only living species of the genus *Cocos*. The estimation of genetic diversity between different genotype is the first process in any plant breeding program. Characterization and evaluation of coconut population, based on morphological and agronomical traits is time consuming, labour intensive and subject to environmental effects. Therefore sensitive and reliable techniques for assessing genetic diversity and screening agronomic traits are necessary to establish effective collecting and gene banking strategies. PCR based techniques such as Random Amplified Polymorphic DNAs (RAPD) has proved its importance in genetic diversity studies. This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. RAPD assay has been used by several groups as efficient tools for identification of markers linked to agronomically important traits, which are introgressed during the development of near isogenic lines.

Jayalekshmy (1996) reported RAPD analysis to be effective method for developing molecular markers to differentiate the coconut genotypes. Ashburnerst et al., (1997) carried out genetic diversity study of 17 distinct South Pacific populations by means of RAPD technique using 14 primers. The study revealed that over 60 percent of the observed variability occurred within populations. Duran et al (1997) studied 48 East African Tall (EAT) coconut genotypes using 22 RAPD primers. A total of 238 amplicons were produced out of which (86%) were polymorphic and others were monomorphic. RAPD markers were used by Ratnambal et al., (2001) to characterize the coconut germplasm. Hundreds of primers were screened for their ability to detect polymorphism in coconut. Among this only 34 primers were polymorphic. The number of polymorphic bands per primer ranged from 1-16. Daher et al. (2002) assessed the genetic divergence among 19 coconut tree populations by RAPD. The markers used permitted the identification of each of the populations showing that they were genetically different.

Anuradha Upadhyay (2002) used RAPD markers to analyze genetic diversity and genetic relationship among coconut accessions DNA from 81 palms representing 20 accesssions, 15 Indian and 5 exotic was used to amplify with 8 highly polymorphic primers. To understand the genetic variation between 2 different coconut ecotypes, Parthasarathy *et al*. (2005) conducted studies on WCT (West Coast Tall) and AST (Assam Tall) under two different ecosystems. 6 individuals each of WCT and AST were used for the analysis. Very negligible polymorphism was observed (0.17) in both the varieties indicating that both the local varieties are genetically similar. It was observed that similar heterogeneity value for both the varieties indicating the absence of genetic variation. Manimekalai *et al*. (2006) determined the effectiveness of RAPD markers to identify polymorphism among 33 coconuts (*Cocos nucifera* L.) germplasm accessions. Rajesh *et al*. (2008) investigated RAPD markers for the tall/dwarf trait in coconut using a bulked DNA approach. Screening of tall and dwarf palm bulk DNA with 200 primers revealed a RAPD primer OPBA3 which was able to clearly differentiate both the tall and dwarf bulks. Sankaran *et al*. (2012) analysed molecular characterization of the 30 coconut accessions collected from Pacific Ocean Islands and Nicobar Islands by using RAPD markers. Out of the 45 primers screened, only 13 primers showed expression and all of them were used for polymorphism survey. Masumbuko *et al*., (2014) used RAPD markers to estimate levels of genetic diversity and structure among twelve East African Tall (EAT) coconut (*Cocos nucifera* L.) accessions that are maintained at Chambezi station. The main objective of this research work was to analyze the genetic variability among 5 different varieties of coconut using RAPD technique.

MATERIALS AND METHODS

SAMPLE COLLECTION

Five *Coconut* varieties selected from Coconut Developmental Centre, Palayad, Kannur, Kerala, India *Coconut* varieties such as ***TxD, WCT, Dwarf, NCD and Chenthengu*** were used for the study.

DNA ISOLATION

Total cellular genomic DNA was isolated and purified from young leaves by the CTAB extraction procedure (Doyle and Doyle 1990). Young leaves were ground to very fine powder in liquid nitrogen in a mortar and pestle. CTAB buffer was added and centrifuged at 10,000 g for 10 min. Following extraction with an equal volume of Phenol: chloroform: isoamylalcohol (25:24:1), the aqueous phase was transferred to new centrifuge tube where RNA was eliminated by treatment with RNase. The aqueous phase was re-extracted twice with chloroform and DNA was precipitated by the addition of 0.6 volumes of ice-cold isopropanol (100%).

AGAROSE GEL ELECTROPHORESIS

The quality of the isolated DNA was checked by agarose gel electrophoresis. Two µl of loading buffer was added to 8 µl of DNA and the samples were loaded to 0.8% agarose gel prepared in 0.5X TBE buffer. Electrophoresis was carried out at 70 Volts for nearly 2 hours until the bromophenol dye front has migrated to the bottom of the gel. Staining was carried out with 0.5 µg/ml of ethidium bromide. The gel was visualized using gel documentation system and was photographed under UV light.

PCR AMPLIFICATION

8 µl of template DNA, 4µl of RAPD primer, 25µl PCR master mix and 13µl nuclease free water were added into microfuge tube. The solution was mixed gently and amplification were carried out using the following reaction condition (**Table 1**)

Temperature	94°C	94°C	35°C	72°C	94°C	38°C	72°C	72°C
Time	5 min	45 s	1 min	1.5 min	45 s	1 min	1 min	10 min
Steps	Initial denaturation	Denaturation	Annealing	Extension	Denaturation	Annealing	Extension	Final extension
Cycles		8 cycles			35 cycles			

Table 1. PCR conditions for the RAPD profile of *Cocos nucifera*

RESULTS AND DISCUSSION

Genomic DNA from *Cocos nucifera* was isolated in good quality and quantity (**Fig. 1**). Five random primers RP6, RP7, RP8, RP9 and RP10 were selected for the RAPD analysis of five varieties amplified 104 different reproducible bands **(Fig. 2 to Fig. 6**).

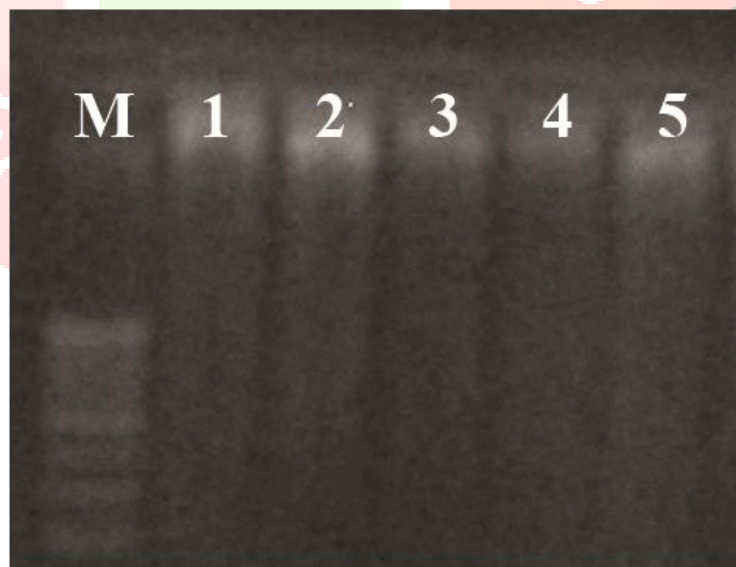

Figure 1. Genomic DNA isolated from *Cocos nucifera*

The number of bands per primer product ranged from 1-21. Average bands per primer are 17.3. The size of the amplified product ranged from 100 bp – 3 kb. Five primers reveal polymorphism between cultivars. Out of 104 scored the maximum number of bands found to be polymorphic. Maximum numbers of polymorphic bands (21) were obtained with the primer 9 and 10. The average number of polymorphic bands per primer is 20.8.

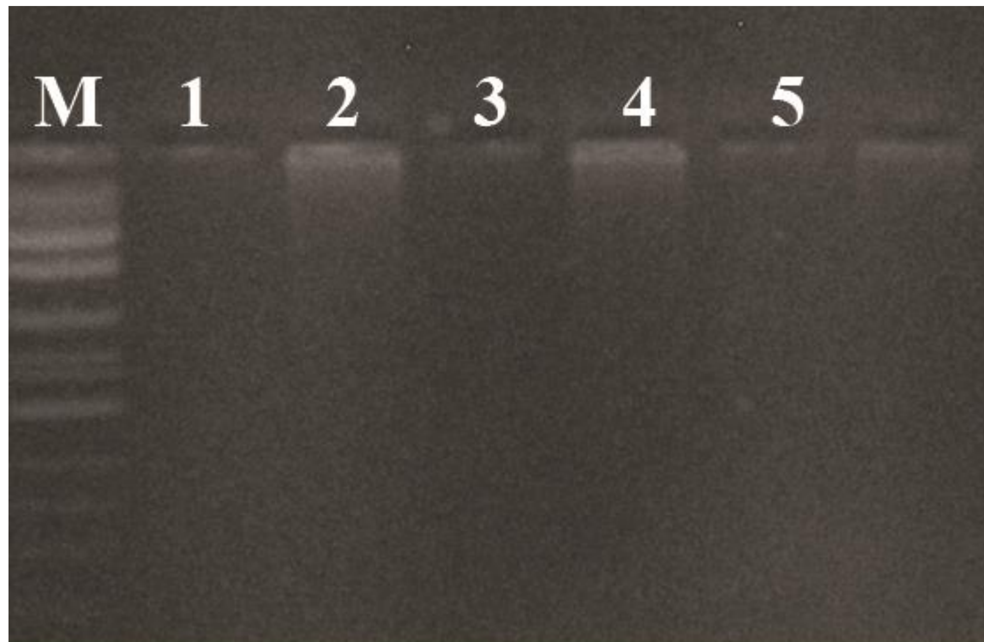

Figure 2. RAPD profile of *Cocos nucifera* using random primer 6. M = Mol wt marker, Lane 1 = Tall×Dwarf hybrid, Lane 2 = West Coast Tall (WCT), Lane 3 = Dwarf, Lane 4 = Naturally Crossed Dwarf, Lane 5 = Chenthengu

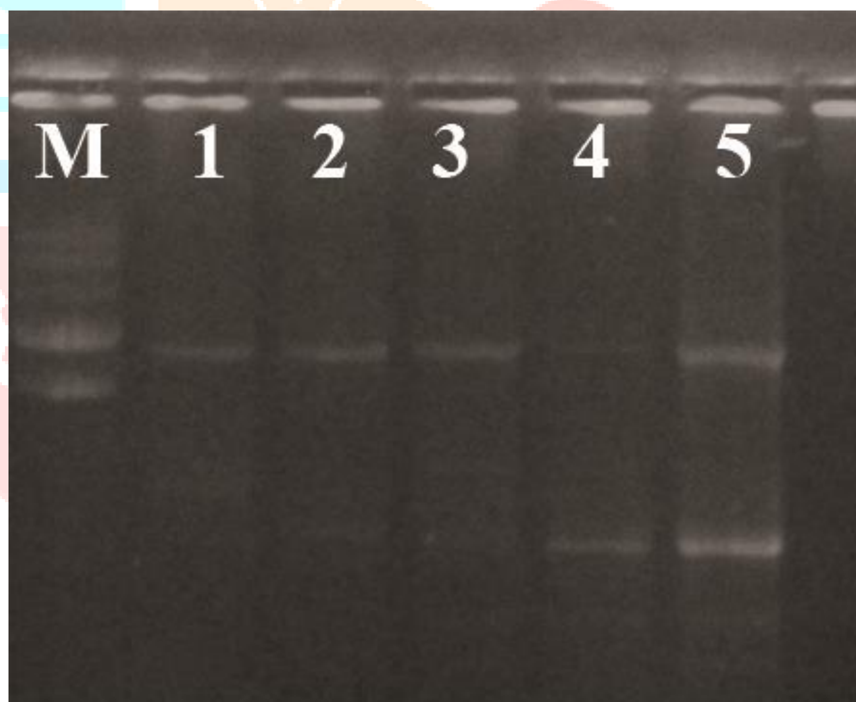

Figure 3. RAPD profile of *Cocos nucifera* using random primer 7. M = Mol wt marker, Lane 1 = Tall×Dwarf hybrid, Lane 2 = West Coast Tall (WCT), Lane 3 = Dwarf, Lane 4 = Naturally Crossed Dwarf, Lane 5 = Chenthengu

Figure 4. RAPD profile of *Cocos nucifera* using random primer 8. M = Mol wt marker, Lane 1 = Tall×Dwarf hybrid, Lane 2 = West Coast Tall (WCT), Lane 3 = Dwarf, Lane 4 = Naturally Crossed Dwarf, Lane 5 = Chenthengu

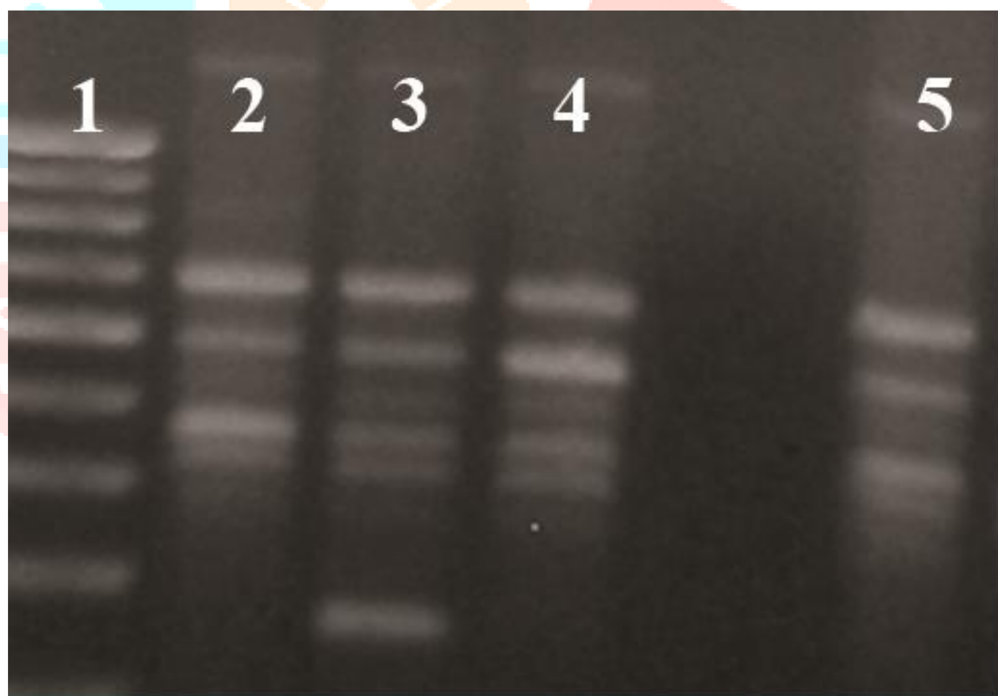

Figure 5. RAPD profile of *Cocos nucifera* using random primer 9. M = Mol wt marker, Lane 1 = Tall×Dwarf hybrid, Lane 2 = West Coast Tall (WCT), Lane 3 = Dwarf, Lane 4 = Naturally Crossed Dwarf, Lane 5 = Chenthengu

Figure 6. RAPD profile of *Cocos nucifera* using random primer 10. M = Mol wt marker, Lane 1 = Tall×Dwarf hybrid, Lane 2 = West Coast Tall (WCT), Lane 3 = Dwarf, Lane 4 = Naturally Crossed Dwarf, Lane 5 = Chenthengu

Characterization and quantification of genetic diversity have been major goal in evolutionary biology and plant breeding. Study of genetic polymorphism provide scientific basis for utilization of germplasm resources efficiently in crop improvements. The coconut palm (*Cocos nucifera.L*) is a major plantation crop in tropical areas.

Molecular markers are powerful techniques that reflect variation at the DNA sequence level. The molecular markers are present in unlimited numbers compared to phenotypic markers; hence they detect diversity at very high resolution. The molecular markers are helpful in crops for screening of germplasm for new sources of variation, identification of genotype making of single genes controlling commercially important trait, morphing quantitative trait loss, study of genetic introgression, population genetics and anatomic studies. Molecular markers in contrast to phenotypic markers are independent of environment and they reflect variations at the DNA sequence level. Germplasm knowledge is quite important in breeding program, since it is a source of variation that can be used for selection.

RAPD is useful for the characterization of DNA from plants and other organisms. This technique involves short oligo nucleotide primers that arbitrary selected to amplify a set of DNA fragments randomly distributed throughout the genome. The genetic resources of coconut palm in south pacific island was characterized using fruit morphological and molecular character (RAPD) was concluded that there was continuous variation in fruit morphology and characters throughout the region (Ashburner et.al, 1997).

CONCLUSION

RAPD is useful for the characterization of DNA from plants and other organisms. The current RAPD profile can be used to develop an effective molecular marker system for marker assisted selection and breeding for an effective crop improvement programme in *Coconut*. The RAPD bands show that Coconut plants are genetically heterogeneous as a high level of variability was observed. This high level of genetic variability may be due to the different geographical locations from which the isolates were obtained or it could indicate that the isolates may have originated from the same species with a wide genetic base or from closely related species

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