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Article *in* Indian Journal of Horticulture · January 2005

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Indian J. Hort. 62(4), December 2005 : 315-318

Molecular diversity in coconut eco-types of coastal and inland riverine ecosystems

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ABSTRACT

To understand the variation between coconuts growing in in the two different ecosystems, molecular studies was conducted on two ecotypes, WCT and AST under two ecosystems, *viz.* coastal and inland respectively. These two varieties showed very little difference between them shelf and the slight differences noted may be due to the climatic difference. It also shows that the Assam Tall has adapted the cold tolerance. The West Coast Tall and Assam Tall are both Tall group cultivars separated geographically, a distance of more than 3000 km but the two varieties did not show much difference as evidenced by the isozyme patterns. On the other hand, DNA fingerprinting using molecular markers detect variation at the DNA level overcoming most of the limitations of morphological and biochemical markers. This study on two distinct eco-geographical zones, yielded useful information on the diversity of coconuts. The Assam Tall types are more uniform genetically and this is the first molecular study, which shows that the cultivar is related to West Coast Tall. The application of molecular tools was found useful in bio-geographical studies. It was found that Assam Tall and West Coast Tall though genetically similar differ only in their adaptation.

Keywords: Isozymes, RAPD analysis, genetic relationship, coconut.

INTRODUCTION

India leads in coconut production and productivity in the world. It is considered to be a crop of coastal region even though it is grown in inlands like Assam. Assam has a typical inland eco-system where coconut grows in an area of 19,600 ha and producing 126.9 million nuts (6,442 (nuts/ha), while Kerala with its coastal ecosystem has an area of 10.1 lakh ha. producing 6,672 million nuts (6,188 nuts/ha). This indicates that coconut productivity in Assam is as high as in Kerala and coconut can successfully be grown in the subtropical, high humid and highly acidic soils of Assam as commercially as in Kerala. Climatologically and geographically, Assam is a humid subtropical region with an annual average rainfall of 300 cm with sub-tropical rainforest ecosystem. Kerala is a humid tropical region with an annual average rainfall of 305 cm. The ecosystem of Kerala is mixed with both tropical rainforest as well as coastal landscape. Considering this distinct diversity, the present investigations were undertaken to study the molecular diversity of the coconut ecotypes grown in the two different ecosystems.

Isozymes are useful genetic markers as they are codominant with low level of environmental interaction. Isozymes were successfully used in coconut to detect variation among various cultivars (Parthasarathy *et al.,* 7). Hence, isozyme studies were conducted for the two local varieties (WCT and AST) of coconut to understand whether their origin was from the same source or not. On the other hand, DNA fingerprinting using molecular markers detect variation at the DNA level overcoming most of the limitations of morphological and biochemical markers. As demonstrated by their use in various plant species, molecular markers are best suited for estimation of genetic diversity and varietal identification (Anuradha *et al.,* 2). Another important factor associated with DNA markers is that they are not affected by the developmental or environmental stage of the crop. In the present study, isozymes and Random Amplified Polymorphic DNA (RAPD) analysis were used for identification of genetic similarity and evolution of heterogeneity in coconut.

MATERIALS AND METHODS

Standardized protocols (Geethalakshmi, 3) of enzyme extraction and staining were used for the two local varieties of Assam (AST) and Kerala (WCT), respectively. For DNA fingerprinting, PCR-based RAPD approaches were used. Surveys were conducted in Kamrup district of Assam and Kasaragod district of Kerala as described earlier (Utpala *et al.,* 11). Leaflets from first open frond were collected from various ecotypes. Enzymes were extracted from leaf tissue of

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adult coconut in WCT and AST palms. Spindle leaf extract gave better and consistent enzyme activity and was hence used for further enzyme studies. To assess the variability, fifteen palms each in the two cultivars *viz.,* AST and WCT were assayed for two different enzyme systems namely peroxidase and esterase. Enzymes were extracted with 0.1 M Tris-HCI buffer (pH 6.8) containing 0.1% p-mercaptoethenol and 10% glycerol in cold. The clear extracts after centrifugation at 10,000 rpm at 4°C were used for electrophoresis using discontinuous buffer system. Polyacrylamide gels of 7.5-10% (w/v) were used and were run for 4 h under constant current. The gels were stained for isozymes using standard protocols of the two standard enzyme systems, i.e. peroxidase (PER) and esterase (EST). For estimation of variability, individual bands were considered as allelic variants and represented by their Rf values. The allelic frequency for the different enzyme systems was scored as the ratio of the number of samples in which the allele was present to the total number of samples analysed. The polymorphic index (PI) was then computed to study intra- and inter-population variation using the formula.

$$PI = \Sigma P_i (1-P_i)/N$$

where P_i = $_i$th allele (band) frequency and N = Number of bands.

Leaflets of first open frond of 15 AST and 15 WCT assassins were taken for RAPD analysis. The DNA was extracted from 1g fresh leaf. DNA extraction protocol suggested by Upadhaya *et al.* (9) was followed. Six primers identified as highly polymorphic from previous work (Upadhaya *et al.,* 10) were used for PCR amplification of the 30 DNA samples. Amplified products resolved by electrophoresis in a 1.2% agarose gel, 1 x TAB (Tris: acetate: EDTA) at 60V for 4 h. The gel was stained with ethidium bromide and viewed under UV light. Each band was considered as a RAPD marker and was identified by its molecular weight. The genetic diversity or heterogeneity was calculated according to the following formula,

$$H \text{ (Heterogeneity)} = 1- \frac{\Sigma x^2}{N}$$

$$\text{Where, } X = \frac{\text{No. of occurence of a particular band}}{\text{Total number of samples}}$$

N = Total number of bands in all the primers used.

The presence or absence of a DNA band was entered into a binary matrix as discrete variables (1 for the presence and 0 for the absence of a homologous band). Pair-wise genetic distance was calculated based on Nei and Li coefficient (Nei and Li, 6). Using computer package RAPD distance (Armstrong *et al.,* 1).

RESULTS AND DISCUSSION

Data in Table 1 and Fig. 1 show the isozyme band numbers and Rf values of the two varieties and the two strains. It is seen that in the case of esterase, WCT shows 11 bands and Assam Tall shows 8 bands, whereas in case of peroxidase, the number of bands is lesser in both the varieties. Similarly, the polymorphic index is also higher in esterase than peroxidase.

Assam Tall showed low polymorphic index (0.048 in esterase and 0.025 in peroxidase) than WCT (0.058 in esterase and 0.32 in peroxidase) indicating that the variation within the population in the case of Assam Tall is less than the intra-population variation of WCT. It is evident that in Kamrup district, the coconut population is more homozygous possibly because of human selection or they arose from single source.

Table 1. Isozyme band numbers and Rf values of the two coconut varieties.

Band No.	Allelic frequency value of esterase		Allelic frequency value of peroxidase	
	WCT	AST	WCT	AST
1	0.93	1.0	0.8	0.06
2	5.93	1.0	0.4	1.00
3	0.91	0.9	0.6	0.13
4	0.93	1.0	0.3	1.00
5	1.0	1.0	1.0	1.00
6	1.0	0.2	1.0	1.00
7	1.0	0.9	1.0	
8	1.0	1.0		
9	0.6	-		
10	0.8	-		
11	1.0	-		
Total No. of bands	11.0	8.0	6	7
Polymorphic index	0.058	0.05	0.32	0.025

The inter-cluster distance and similarities between and within the cultivars were examined with the help of dendogram and similarity matrix (Fig. 2). The West Coast Tall and Assam Tall are both Tall group cultivars separated geographically, i.e. a distance of more than 3,000 km in their place of cultivation. Assam has a cool winter compared to warm winters of Kerala. In spite of such a difference in the eco-climate, the two varieties did not show much difference as evidenced by the isozyme patterns.

The difference between Assam Tall and West Coast Tall was only marginal. However, in the cluster and dendrogam analyses (Fig. 2), it was found that all

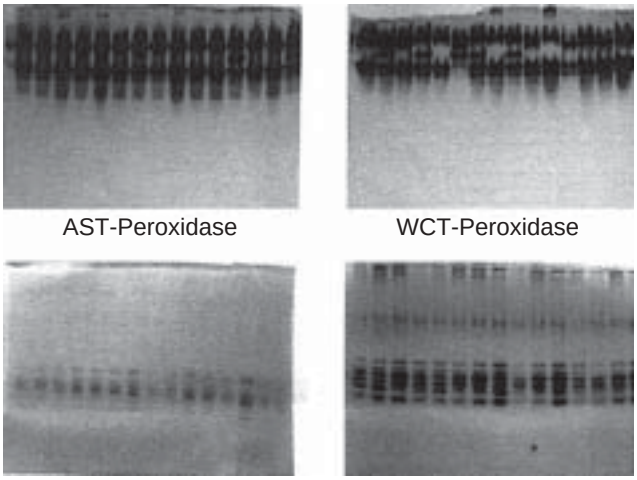

Fig. 1. Intra population variation- Banding pattern of WCT and AST.

the fifteen West Coast Tall ecotypes clustered together while all the Assam Tall clustered together indicating similar origin for the both ecotypes. However, WCT 12 showed more isozyme variation than other ecotypes. Variations within Assam Tall were less compared to the variation within West Coast Tall.

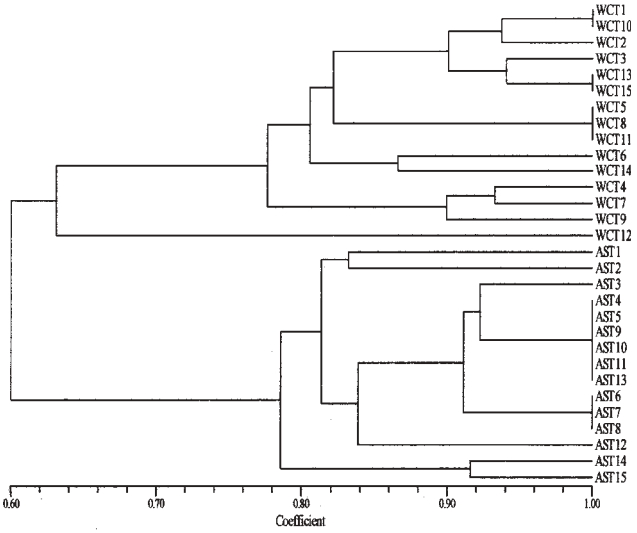

Fig. 2. Dendrogram based on Jaccard's similarity level showing the genetic relationship between the varieties.

In order to further study the polymorphism at DNA level, 6 ecotypes each of WCT and AST were tested using RAPD markers. The polymorphism was found to be very negligible (0.17) in both the varieties indicating that both the local types are similar genetically (Table 2 & Fig. 3).

Table 2. The primer sequence and number of polymorphic bands.

S. No.	Name of the primer	Primer sequence	Total No. of bands	No. of polymorphic bands
1.	OPC-2	GTGAGGCGTC	12	4
2.	OPC-5	GATGACCGCC	10	Nil
3.	OPC-9	CTCACCGTCC	12	5
4.	OPE-2	GGTGCGGGAA	15	2
5.	OPB-5	TGCGCCCTTC	11	1
6.	OPB-6	TGCTCTGCCC	10	Nil

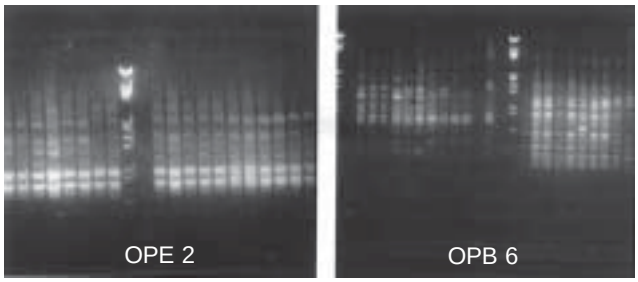

Fig. 3. RAPD profile of WCT and AST coconut cultivars.

It was noticed that heterogeneity for WCT was 0.5 and for AST it was 0.52, indicating negligible variation between the two ecotypes.

The Assam Tall types were found more uniform genetically and there were three distinct types, AST1, AST3 and the remaining in one group (Fig. 4). This study of two distinct eco-geographical zones indicated

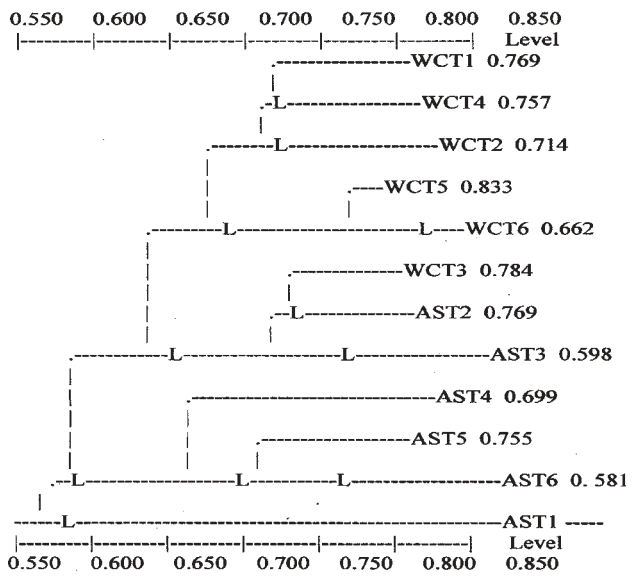

Fig. 4. RAPD dendrogram showing intra and between similarities of the two varieties WCT and AST.

very useful information on the diversity of coconut. This needs further investigation.

A similar attempt was made by Lebrun *et al.* (4) using similar molecular techniques to study the spread and domestication of coconut palm.

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(Received : March, 2005; Revised : July, 2005; Accepted : September, 2005)