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Genetic diversity in yellow dwarf populations of coconut assessed using RAPD markers

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ABSTRACT

RAPD markers were used to analyze genetic diversity and genetic relationship among three Yellow Dwarf coconut accessions. DNA from palms of Malayan Yellow Dwarf (MYD), Kulashekaram Yellow Dwarf (KYD) and Andaman Yellow Dwarf (AYD) were amplified with 16 highly polymorphic RAPD primers. Mean heterozygosity was higher in KYD (0.157) and MYD (0.153) and less in AYD (0.039). Similarity matrix based on Jaccards coefficient indicated a close association of the two indigenous yellow dwarfs (AYD and KYD) with the exotic Yellow Dwarf (MYD). Cluster analysis produced a dendrogram of two major clusters. One palm (KYD 16) formed a distinct single accession cluster while rest of the palms grouped into the second cluster which had 10 sub-clusters. The AYD palms formed a distinct sub-cluster within the second group indicating its distinctness and homogeneity. The MYD and KYD palms were scattered in different sub-clusters indicating presence of diversity within the accessions. The 3-D principal coordinate analysis also indicated a distinct grouping of the AYD palms but not the MYD and KYD palms. AMOVA indicated presence of greater diversity within the accessions than between the accessions. Therefore, it is likely that AYD and KYD evolved from a common progenitor, the MYD.

Key words: Cluster analysis, principal coordinate analysis, yellow dwarfs, heterozygosity.

INTRODUCTION

Coconut palm (*Cocos nucifera* L.), a member of the family Arecaceae, is a perenial oilseed plant found throughout the humid tropics. Owing to its widespread distribution, the exact centre of origin of this palm is still a matter of debate. However, the variability of local coconut types is reported to be the highest in South-East Asia. In India too, wide variability is observed in size and forms of coconut. Characterization and evaluation of coconut populations based on morphological and agronomic traits is protracted and laborious and provides a simplified picture of diversity. Molecular markers have the potential to increase efficiency of coconut genetic improvement, specifically in the areas of germplasm management, genotype identification and marker-assisted selection. Various molecular technologies have been applied for coconut diversity analysis *viz.,* isoenzymes (Geethalakshmi *et al.*, 4; Niral *et al.*, 8), ISTR (Duran *et al.*, 3; Rohde *et al.*, 13), RAPD (Ashburner *et al.*, 1; Upadhyay *et al.*, 15), RFLP (Lebrun *et al.*, 5), SSR (Devakumar *et al.*, 2; Merrow *et al.*, 7; Perera *et al.*, 10, 11), AFLP (Perera *et al.*, 9) and ISSR (Manimekalai and Nagarajan, 6). In the present study, an attempt was made to study the variability at the molecular level in three accessions of yellow dwarfs *viz.,* Malayan Yellow Dwarf, Kulasekharam Yellow Dwarf and Andaman Yellow Dwarf, being maintained at CPCRI. Malayan Yellow Dwarf (MYD) is a dwarf cultivar introduced from Malaysia. The leaf petioles, inflorescences and nuts of this cultivar are yellow in colour. It is widely used as a parent for production of high yielding coconut hybrids and is more homogeneous than the other Malayan Dwarfs namely, the Malayan Orange Dwarf and the Malayan Green Dwarf. Kulashekaram Yellow Dwarf (KYD) is synonymous to the MYD in gross morphology. However, they differ to a certain extent with regard to their breeding behaviour. This accession was introduced into the gene bank from Kulashekaram in Tamil Nadu. Andaman Yellow Dwarf (AYD) also resembles the MYD in gross morphology. It was introduced into the gene bank from the Andaman and Nicobar Islands. There are no reports available on the comparative evaluation of yellow dwarfs of coconut found in the country. A few workers from Sri Lanka and CIRAD, France have reported the molecular diversity and interrelationship of the yellow dwarfs, *viz.* the Indonesian Yellow Dwarf (Nias Yellow Dwarf), African Yellow Dwarf (Ghana Yellow Dwarf) and the Sri Lankan Yellow Dwarf (Lebrun *et al.*, 5; Perera *et al.*, 9, 11)

MATERIALS AND METHODS

Twenty-eight palms comprising three yellow dwarf coconut accessions, *viz.* Malayan Yellow Dwarf palms, Kulashekaram Yellow Dwarf palms and Andaman Yellow Dwarf palms conserved *ex situ* at CPCRI, Kasaragod were used for the present study. DNA was extracted from the spindle leaves of palms using the protocol of Upadhyay *et al.* (16). The DNA samples

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were used for random amplified polymorphic DNA (RAPD) analysis. Eighty primers of OP AH, OP AF, OP A, OP C and OP M series were used for screening, out of which 16 polymorphic primers were used for the study. PCR was carried out in a total reaction volume of 15 µl containing 20 µg/µl of sample DNA, 10X assay buffer (2 mM $MgCl_2$), 10 pmoles/µl of each primer, 10mM of each dNTPs and 5 units of *Taq* DNA polymerase. Amplification was performed in Eppendorf master cycler gradient with one cycle of initial separation at 94°C for 5 min followed by 39 cycles of 1 min. at 94°C, 1 min. at 42°C, 1.5 min. at 72°C. The last cycle was followed by an additional 7 min at 72°C. After PCR amplification, 2µl of dye 0.25% bromophenol blue and 40% (w/v) sucrose in water was added and the amplicons were resolved in 1.2% agarose gel. The RAPD banding profiles were scored in the form of a matrix with '1' and '0', indicating the presence and absence of bands, respectively. The total number of bands for each accession, the number of private bands, number of less common bands less than 25 and 50 percent, and heterozygosity was calculated using the software, GENALEX. Similarity matrix based on Jaccard's coefficient was used for cluster analysis and dendrogram was constructed by the unweighted pair-group method (UPGMA) for arithmetic mean using the NTSYS-PC software, version 2.0 (Rohlf, 14). Principal coordinate analysis (PCA) was done to obtain both 2-D and 3-D images, in order to visualize the difference between the individuals. A hierarchical analysis of molecular variance for partitioning variation among and within coconut accessions was done using GENALEX software with significance setting permutation value of 999.

RESULTS AND DISCUSSION

A total of 165 markers were produced across the 28 palms studied. The total number of bands per primer ranged from six (OP AF5, OP M8, OP AH15) to 15 (OP AF20). The RAPD profile generated by the primer OP M14 is given in Fig.1. The percentage of polymorphism ranged from 10 (OP M7) to 100 (OP AH13). An average of 10.31 bands per primer were observed, out of which an average of 5.06 bands were polymorphic. Rodriguez *et al.* (12) and Upadhyay *et al.* (15), based on RAPD analysis in coconut, reported polymorphic bands ranging from 1-3 and 1-16, respectively. The binary banding pattern obtained in the three accessions studied is tabulated (Table 1). There were no bands with a frequency of less than 5 percent. KYD and MYD produced a few private bands. Mean heterozygosity was greater in KYD and MYD as compared to AYD. Mean percentage of polymorphic loci was 28.69 percent, and a higher percentage of polymorphic loci were observed in KYD and MYD when compared to AYD (Table 2). In the earlier reports, the percentage of polymorphism in dwarf populations was reported to be around 20 percent (Upadhyay *et al.*, 15). Earlier studies on diversity in coconut populations (Devakumar *et al.*, 2; Meerow *et al.*, 7; Perera *et al.*, 11; Upadhyay *et al.*, 15) indicated higher diversity in talls and lower diversity in dwarfs. This is mainly related to the breeding behaviour.

Table 1. Binary band patterns by populations.

Population	KYD	AYD	MYD
No. of bands	161	144	158
No. of bands with frequency >= 5%	161	144	158
No. of private bands	6	0	4
No. of less common bands (<=25%)	0	0	0
No. of less common bands (<=50%)	0	0	0
Mean heterozygosity	0.157	0.039	0.153
SE of mean heterozygosity	0.016	0.010	0.017

Table 2. Percentage of polymorphic loci.

Population	Polymorphic loci (%)
KYD	40.61
AYD	9.09
MYD	36.36
Mean	28.69

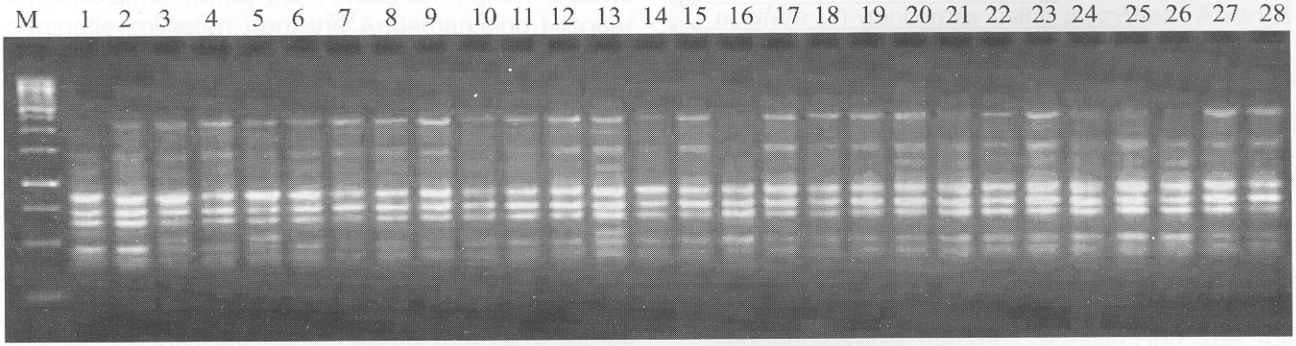

Fig. 1. RAPD marker profile using primer OP M 14 (M: 1 Kb ladder, Lanes 1-16: KYD, 17-19: AYD, 20-28: MYD).

Talls are generally allogamous while dwarfs are autogamous.

Hierarchical analysis of molecular variance based on RAPD data indicated a high within population variation and low between population variation (Table 3). The presence of significant within population variation might be due to the occurrence of a certain amount of outbreeding in these populations, which has resulted in greater percentage of variation being observed within the accessions. The absence of high inter-population variation could also be due to these accessions sharing a common genome, implying greater homology between the three yellow dwarf accessions, with local adaptation in the different regions wherever they have become established.

Similarity matrix based on Jaccard's coefficient indicated high similarity coefficient between many of the palms. Accessions wise also high similarity coefficients were observed (Table 4). Cluster analysis based on Jaccard's similarity matrix produced a dendrogram with two major clusters (Fig. 2). One palm, KYD16, formed a distinct single accession cluster while the rest of the palms grouped into the second cluster, which had 10 sub-clusters. The three Andaman Yellow Dwarfs formed a separate cluster within this second cluster. The MYD and KYD palms were grouped into many clusters. Perera *et al.* (9) reported distinct grouping of the indigenous dwarfs from Sri Lanka *viz.* Sri Lankan Green, Yellow and Brown Dwarfs. In a subsequent study, Perera *et al.* (11) observed that few palms of the Nias Yellow Dwarf scattered into different clusters and grouped along with other Indonesian Brown or Orange Dwarfs. These results indicate differences in the genetic makeup of different dwarf populations available in the different coconut growing countries. The 2-D principal coordinate analysis plot did not show any specific grouping but 3-D principle coordinate analysis plot showed a distinct separation of the three Andaman Yellow Dwarfs (Fig. 3,4). A few of the KYD palms (KYD 10, KYD 5, KYD 14, KYD 12, KYD 15, KYD 3, KYD 8, KYD 4, KYD 6, KYD 7 and KYD 9) formed a distinct group but the rest of the Kulashekaram Yellow Dwarfs were intermixed with MYD palms in the principal coordinate plot. Two of the MYD palms were distinctly different from the rest of the Yellow Dwarfs. The distinctness of some of the

Table 4. Pair-wise population matrix of Nei's genetic identity.

Genotype	KYD	AYD	AYD
KYD	1.00		
AYD	0.96	1.00	
AYD	0.97	0.95	1.00

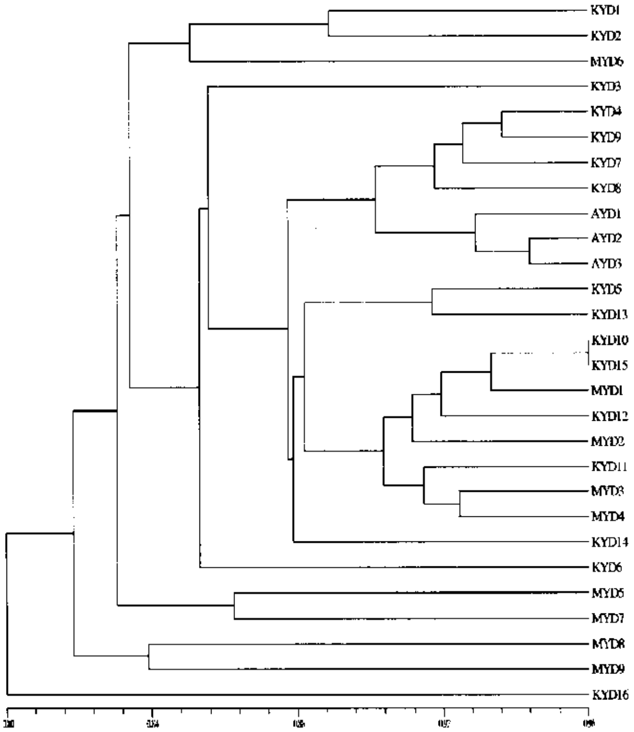

Fig. 2. UPGMA dendrogram of yellow dwarf palms based on Jaccard's coefficient.

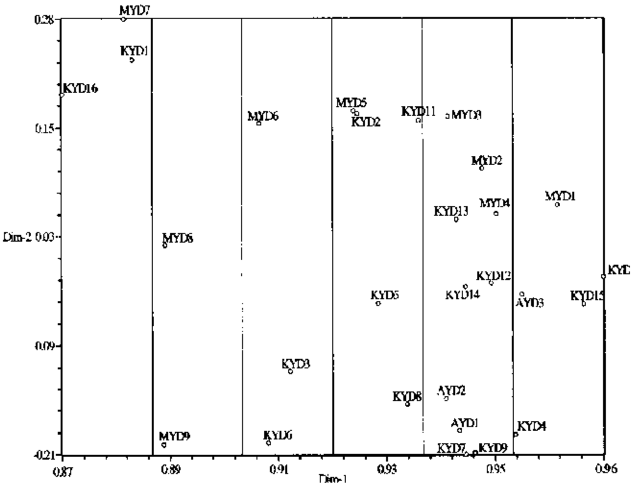

Fig. 3. 2-D Principal coordinate analysis plot of yellow dwarf palms.

Table 3. ANOVA based on RAPD data.

Source	df	SS	MS	Est. Var.	%	Stat	Value	Prob
Among Pops.	2	37.473	18.737	1.081	10			
Within Pops.	25	257.063	10.283	10.283	90	PhiPT	0.09512	0.010

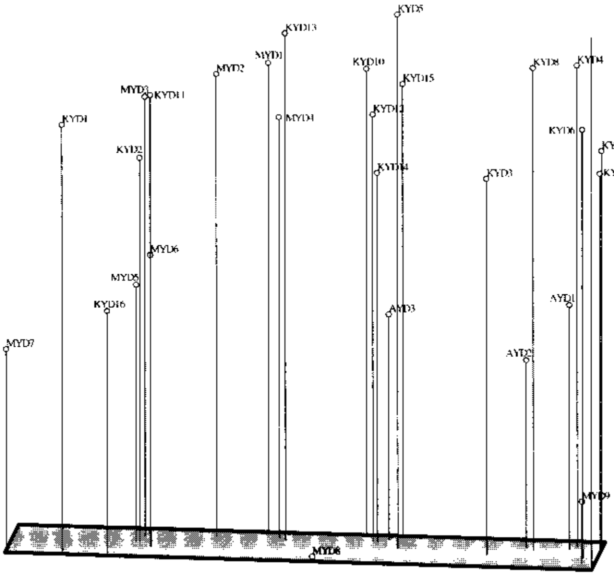

Fig. 4. 3-D Principal coordinate analysis plot of yellow dwarf palms.

palms within an accession further indicates that diversity is present within the population.

Based on the available evidence, it appears that the MYD and KYD populations share a higher degree of similarity at the molecular level. It is possible that the Kulashekaram Yellow Dwarfs may have originated from the Malayan Yellow Dwarfs. This is also supported by historical reports, which indicate that MYD was introduced from Sri Lanka to Kulashekaram in Tamil Nadu during the early part of the 20th century. Therefore, it is likely that Kulashekaram Yellow Dwarf has evolved from these MYD introductions. As a result of outbreeding and gene flow between the introduced MYD palms and the local coconut populations and adaptation to the local environment a certain amount of differentiation may have occurred leading to some distinctiveness between the MYD and KYD populations. However, they continue to share considerable similarity at the molecular level as evident by high similarity coefficient and the clustering pattern. On the other hand, the Andaman Yellow Dwarfs originally collected from the Andaman and Nicobar Islands also share considerable similarity with both KYD and MYD. However, it shows a very limited intrapopulation variation indicating a greater degree of homogeneity in the population. This may also be because of the lesser number of palms sampled in this accession. It is likely that AYD may also have originated from the MYD, as it is possible that seed nuts of MYD may have reached the Andaman and Nicobar Islands owing to the historic maritime contacts between the islands. It is possible that the sailors may have carried a few seeds of the MYD, which may have established in the islands giving rise to the present population of Andaman Yellow Dwarfs.

These results, indicating a close association of the two indigenous Yellow Dwarfs (AYD and KYD) with the exotic Yellow Dwarf (MYD) are also consistent with the earlier reports by a number of workers (Lebrun *et al.*, 5; Meerow *et al.*, 7; Perera *et al.*, 11) who postulated that the dwarfs originated in South-East Asia and from there were introduced to the different coconut growing countries. Lebrun *et al.* (5) observed that the Ghana Yellow Dwarf (GYD) from Africa was similar to MYD from Malaysia on the basis of RFLP analysis and based on the historical data they concluded that GYD was introduced to Africa from South-East Asia. Perera *et al.* (11) observed that Indonesian Yellow Dwarf (Nias Yellow Dwarf) and African Yellow Dwarf (GYD) clustered together and were closely related to Sri Lankan Yellow Dwarfs, indicating a common origin for these Dwarf populations. Therefore, based on historical facts and the presence of considerable similarity at the molecular level, it is postulated that Kulashekaram Yellow Dwarf and Andaman Yellow Dwarf evolved from a common progenitor, the Malayan Yellow Dwarf.

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