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Characterization of coconut germplasm based on protein polymorphism

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ABSTRACT

Characterization of different coconut cultivars and hybrids was done by analyzing the banding profiles of native proteins using 10% poly acrylamide gel electrophoresis. Intrapopulation variation was studied in six Dwarfs, eight Talls, two hybrids and their parents. Among Dwarfs, MOD showed highest intrapopulation variation and GDD showed least polymorphism. Among Talls, JVT showed highest and KPDT showed least intrapopulation variation. While among the hybrids and their parents, WCT showed highest and LCT and GBGD showed least variation. Interpopulation variation was studied in seven Dwarfs, 19 Talls and four hybrids. Allelic frequency was highest in NGAT and BENT, while least in FJT.

Key words: Electrophoresis, protein bands, coconut, polymorphism.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a monotypic species with pantropic distribution. It is economically a very important palm. There are two types of coconut palms namely, Talls and Dwarfs. Traditionally, morphological characters are used for evaluation and classification of germplasm. However, germplasm characterization based on morphological traits is time consuming and needs to be complemented with molecular and protein profiling. In coconut, very limited work has been done on proteins and isozymes to characterize coconut palms (Parthasarathy *et al.*, 7; Cardena *et al.*, 2; Jayalekshmy, 5; Carpio, 3). The present study was undertaken to study the protein profiles of different coconut cultivars.

MATERIALS AND METHODS

Protein analysis was carried out using spindle leaf extracts from different cultivars. Eight Tall and six Dwarf genotypes were used for intra-population variation and thirty different cultivars and hybrids were used for the study of inter-population variation. Four to seven palms from six Dwarf and eight Tall genotypes were studied as given below:

a. ***Dwarfs:*** Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Chowghat Green Dwarf (CGD), Malayan Orange Dwarf (MOD), Gudanjali Dwarf (GDD) and Ganga Bondam Dwarf (GBGD).
b. ***Talls:*** West Coast Tall (WCT), Java Tall (JVT), Laccadive Ordinary Tall (LCT), Phillippines Ordinary Tall (PHOT), Andaman Ordinary Tall (ADOT), San Ramon Tall (SNRT), Kappadam Tall (KPDT) and Laccadive Micro Tall (LMT).
c. ***Hybrids and their parents:*** Laccadive Ordinary Tall (LCT), Ganga Bondam Tall (GBGD), West Coast Tall (WCT), Strait Settlement Green Tall (SSGT), Laccadive Ordinary Tall x Ganga Bondam Dwarf (LCT x GBGD) and West Coast Tall x Strait Settlement Green Tall (WCT x SSGT).

Inter-population variation: Seven Dwarfs, 19 Talls and four hybrids were used for the analysis.

a. ***Dwarfs:*** Kenthali Dwarf (KTOD), King Coconut (RTB04), Malayan Green Dwarf (MGD), Kulasekharam Yellow Dwarf (KYD), Kulasekharam Orange Dwarf (KOD), Kulasekharam Green Dwarf (KGD) and Cameroon Red Dwarf (CRD).
b. ***Talls:*** Strait Settlement Green Tall (SSGT), Strait Settlement Apricot Tall (SSAT), Fiji Tall (FJT), Ayiramkachi Tall (AYRT), Cochin China Tall (CCNT), Federated Malay States Tall (FMST), Andaman Giant Tall (AGT), Java Giant Tall (JVGT), Gonthembili Tall (GTBT), Seychelles Tall (SCT), Ceylon Tall (SLT), New Guinea Tall (NGAT), Benaulim Tall (BENT), Ganga Pani Tall (GPNT), Calangute Tall (CALT), Spicata Tall (SPIT), Nadora Tall (NDRT), Zanzibar Tall (ZAT) and Standard Kudat Tall (STKT).
c. ***Hybrids:*** West Coast Tall x West Coast Tall (WCT x WCT), Chowghat Orange Dwarf x Chowghat Orange Dwarf (COD x COD), West Coast Tall x Chowghat Orange Dwarf (WCT x COD) and Chowghat Orange Dwarf x West Coast Tall (COD x WCT).

Extraction was done with 0.1M Tris-HCl buffer (pH 6.8) containing Poly Vinyl Polypyrrolidone (PVP), glycerol and b-Mercaptoethanol in cold. Clear extracts after centrifugation (for 20 min. and 10,000 rpm) were used for electrophoresis (at constant current, 25 mA per gel) in cold (4°C). Discontinuous polyacrylamide gel electrophoresis was used in the experiment. The

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gel was incubated in 12.5% TCA solution at room temperature for one day and then stained using 3% Brilliant Blue R solution for protein (PROT) bands. The gels were then destained using 3% NaCl solution. After destaining the gels were fixed in 7% acetic acid and finally stored in distilled water.

By noting the number of samples in which a particular band was present, allelic frequency was calculated. Polymorphic Index was calculated (Choong *et al.*, 4) based on the allelic frequency as Polymorphic Index (PI) = aPi (1-Pi)/N, where, Pi = ith allele (band) frequency and N = number of bands.

RESULTS AND DISCUSSION

Polymorphic banding pattern was observed and thirty two clear protein (PROT) bands were observed in Dwarf cultivars (Table 1), of which, six bands (band No. 5, 25, 27, 30, 31 and 32) were monomorphic. Allelic frequency of *PROT* was highest in GDD (0.83) and least in CGD (0.56). Thirty three clear *PROT* bands were observed in Tall cultivars (Table 2), of which four bands (band No. 26, 30, 32 and 34) were monomorphic. Allelic frequency was highest in KPDT (0.91) and least in JVT (0.64). Thirty two clear *PROT* bands were observed in hybrids and their parents (Table 3), of which 16 bands were monomorphic. Highest allelic frequency was observed in LCT x GBGD and WCT x SSGT (0.92) and least in WCT (0.81). Band with Rf value 0.17 was absent in Dwarfs, while band with Rf value 0.53 was absent in hybrids and their parents. Both the above bands were present in Tall cultivars.

Among the Dwarf cultivars studied, MOD showed highest intrapopulation polymorphism (0.111) and GDD showed least polymorphism (0.028) for *PROT*. Upadhyay *et al.* (8) also observed high polymorphism for MOD using RAPD markers. Among the Tall cultivars studied, highest PROT polymorphism was seen in JVT (0.114), while it was least in KPDT (0.018). Among

Table 1. Intra-population allelic frequency for PROT in Dwarfs.

Cultivar/ Band No.	Rf value	COD	MYD	CGD	MOD	GDD	GBGD	Mean
1	0.03	0.71	0.00	1.00	1.00	1.00	1.00	0.79
2	0.05	1.00	1.00	1.00	1.00	1.00	0.71	0.95
3	0.06	1.00	1.00	0.14	0.57	1.00	1.00	0.79
4	0.08	0.43	0.57	0.00	0.29	0.57	0.00	0.31
6	0.14	1.00	0.57	1.00	0.43	1.00	0.29	0.72
7	0.16	0.29	0.86	0.86	0.57	0.71	0.57	0.64
8	0.21	1.00	1.00	1.00	0.57	1.00	1.00	0.93
9	0.22	0.71	0.00	1.00	0.71	1.00	1.00	0.74
10	0.23	0.71	0.00	1.00	0.71	1.00	0.43	0.64
11	0.24	0.43	1.00	0.00	1.00	1.00	0.43	0.64
12	0.27	0.43	1.00	0.00	1.00	0.14	0.00	0.43
13	0.28	0.43	0.00	0.14	000	1.00	1.00	0.43
14	0.29	0.86	0.14	0.29	0.57	1.00	1.00	0.64
15	0.31	0.86	1.00	1.00	1.00	0.29	0.29	0.74
16	0.33	0.14	0.00	0.57	0.43	1.00	1.00	0.52
17	0.35	0.86	0.86	1.00	0.71	1.00	0.00	0.74
18	0.37	0.71	0.71	0.14	0.43	1.00	1.00	0.67
19	0.40	1.00	1.00	0.14	0.29	0.86	1.00	0.72
20	0.46	1.00	1.00	0.00	0.43	1.00	1.00	0.74
21	0.51	0.71	1.00	0.00	0.43	1.00	0.86	0.67
22	0.52	0.14	0.00	0.00	0.14	0.00	0.00	0.05
23	0.53	0.00	0.00	0.29	0.00	0.00	0.00	0.05
24	0.58	0.29	0.00	0.00	0.00	0.00	1.00	0.22
26	0.64	0.86	1.00	0.57	0.00	1.00	0.86	0.72
28	0.68	0.00	0.29	0.00	0.00	1.00	0.86	0.36
29	0.70	1.00	1.00	0.71	0.71	1.00	0.86	0.88
Mean		0.70	0.66	0.56	0.59	0.83	0.72	

Band Nos. 5, 25, 27, 30, 31 and 31 are monomorphic with mean allelic frequency of 1.0.

hybrids and their parents, WCT showed highest polymorphism (0.047) and LCT and GBGD showed least polymorphism (0.008). Interestingly, cultivars showing highest polymorphism at interpopulation level was revealing low intra-population variation. Polymorphic banding pattern for PROT was observed with a total of 33 bands of which, nine bands (band No. 6, 21, 22, 26, 28, 30, 31, 32 and 33) were monomorphic. Allelic frequency was highest in NGAT and BENT (0.91) and least in FJT (0.48).

Coconut is a highly cross-pollinating species containing a high proportion of genetic variation within populations (Upadhyay *et al.*, 8; Parthasarathy *et al.*, 7) as in populations of another palm species *Acrocomia aculeata* (Lopes *et al.*, 6). The total genetic variation of a species, therefore is likely to be distributed among populations as the impact of natural selection, varies among population due to genetic drift and environment (Choong *et al.*, 4). Therefore, with germplasm conservation programmes, it is imperative to accurately measure the amount of genetic diversity and its distribution within and between populations. Protein profiles revealed high allelic frequencies within GDD among Dwarfs and very low allelic frequencies was recorded in case of JVT among the Talls. Protein showed least intrapopulation variation for GDD, while MOD showed highest intrapopulation variation. This needs further investigation because in earlier studies GDD was found to be intermediate and it clustered with Talls, while MOD was a typical Dwarf (Parthasarathy

Table 2. Intrapopulation allelic frequency for PROT in Talls.

Cultivar/ Band No.	Rf value	WCT	JVT	LCT	PHOT	ADOT	SNRT	KPDT	LMT	Mean
1	0.03	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	0.96
2	0.05	1.00	0.57	1.00	1.00	1.00	1.00	1.00	1.00	0.95
3	0.06	0.57	0.29	0.71	1.00	1.00	1.00	1.00	1.00	0.82
4	0.08	1.00	0.71	0.86	0.71	0.83	0.25	1.00	1.00	0.80
5	0.09	0.29	0.57	1.00	0.29	1.00	1.00	1.00	1.00	0.77
6	0.14	1.00	0.86	0.14	0.71	1.00	1.00	1.00	1.00	0.84
7	0.16	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	0.96
8	0.17	0.43	0.29	0.71	0.00	0.00	0.00	0.00	0.00	0.18
9	0.21	1.00	0.86	1.00	1.00	0.67	0.75	1.00	1.00	0.91
10	0.22	0.71	0.71	0.71	0.29	1.00	1.00	1.00	1.00	0.80
11	0.23	0.71	0.71	1.00	1.00	1.00	1.00	1.00	1.00	0.93
12	0.24	0.86	0.57	1.00	1.00	0.67	0.00	0.00	0.00	0.51
13	0.27	0.71	0.29	1.00	1.00	1.00	0.50	0.86	1.00	0.80
14	0.28	0.71	0.29	0.14	0.57	1.00	1.00	1.00	1.00	0.71
15	0.29	1.00	1.00	0.86	0.57	1.00	1.00	1.00	0.43	0.86
16	0.31	1.00	0.71	0.43	0.43	0.50	0.00	1.00	1.00	0.63
17	0.33	0.57	0.71	0.86	0.14	0.67	0.25	0.57	1.00	0.60
18	0.35	1.00	0.71	1.00	0.71	1.00	1.00	1.00	1.00	0.93
19	0.37	1.00	0.71	1.00	0.71	1.00	1.00	1.00	0.71	0.89
20	0.40	1.00	1.00	1.00	1.00	0.50	1.00	1.00	1.00	0.94
21	0.46	1.00	1.00	1.00	1.00	1.00	1.00	0.86	0.71	0.95
22	0.51	1.00	1.00	1.00	1.00	0.83	0.00	1.00	1.00	0.85
23	0.52	0.14	0.00	1.00	1.00	1.00	0.25	1.00	1.00	0.67
24	0.53	0.14	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.39
25	0.58	0.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00	0.45
27	0.64	1.00	1.00	0.86	0.57	0.83	0.25	1.00	1.00	0.76
28	0.67	0.71	0.14	1.00	1.00	1.00	1.00	1.00	1.00	0.86
29	0.68	0.43	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80
31	0.80	1.00	1.00	1.00	1.00	0.83	0.00	0.86	1.00	0.84
Mean		0.79	0.64	0.86	0.78	0.86	0.67	0.91	0.88	

Band Nos. 26, 30, 32 and 33 are monomorphic with mean allelic frequency of 1.0.

Table 3. Intra-population allelic frequency for PROT in hybrids and their parents.

Cultivar/ Band No.	Rf value	LCT	LCT x GBGD	GBGD	WCT	WCT x SSGT	SSGT	Mean
3	0.06	1.00	1.00	1.00	0.50	1.00	1.00	0.92
4	0.08	1.00	1.00	1.00	0.50	1.00	1.00	0.92
8	0.17	0.00	0.33	0.00	1.00	0.50	1.00	0.47
12	0.24	0.50	0.66	0.00	0.00	0.75	1.00	0.49
13	0.27	1.00	1.00	1.00	0.50	1.00	1.00	0.92
14	0.28	0.00	0.66	0.00	1.00	1.00	1.00	0.61
15	0.29	0.00	0.33	1.00	1.00	0.25	0.00	0.43
17	0.33	1.00	1.00	0.00	0.50	1.00	1.00	0.75
18	0.35	1.00	1.00	0.50	1.00	1.00	1.00	0.92
20	0.40	1.00	1.00	1.00	1.00	1.00	0.50	0.92
21	0.46	1.00	0.66	1.00	0.50	1.00	1.00	0.86
22	0.51	1.00	1.00	1.00	0.50	1.00	1.00	0.58
23	0.52	1.00	1.00	1.00	0.00	1.00	1.00	0.83
24	0.58	1.00	1.00	1.00	0.00	1.00	1.00	0.83
25	0.60	1.00	0.66	1.00	1.00	1.00	1.00	0.94
26	0.64	1.00	1.00	1.00	1.00	1.00	0.50	0.92
Mean		0.89	0.92	0.86	0.81	0.92	0.91	

Band Nos. 1, 2, 5, 6, 7, 9, 10, 11, 16, 19, 27, 28, 29, 30, 31 and 32 are monomorphic with mean allelic frequency 1.0.

et al., 7; Upadhyay *et al.,* 8). This might be due to the high degree of homozygosity detected in certain loci by proteins. But, among Talls, JVT showed highest intrapopulation variation for proteins. The present studies indicated Tall varieties diverging slightly in the expression of some proteins. Similar observation has been made by Canto-Canche (1). Total leaf protein profile did not show any specific variation between the different cultivars with respect to the major polypeptides. However, minor polypeptides showed variation and is in tune with an earlier report (Jayalekshmy, 5) on coconuts. Protein profiles can therefore be used as one of the components for the characterization of coconut germplasm.

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