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Interpopulation variation in coconut

P. Geethalakshmi, V. Niral and V.A. Parthasarathy*
Central Plantation Crops Research Institute, Kasaragod 671 124, Kerala

ABSTRACT

Eleven isozyme systems *viz.*, EST, PRX, PPO, MDH, ACP, ADH, GOT, a-AMY, PHOS, G-6PDH, SOD and PROT were used to study the interpopulation variation among 30 different coconut cultivars and hybrids. The rare alleles found in these populations may have an important role as genetic markers in coconut breeding programme. Genotypes AGT and SLT showed highest mean allelic frequency, while KTOD showed the least value. Among the isozymes and proteins studied, G-6PDH and ACP showed highest mean allelic frequency and ADH showed the least value. Out of the 11 isozyme systems, highest polymorphism was shown by PPO followed by PRX, ADH, MDH, SOD. EST, a-AMY, GOT, PHOS, GSPDH and ACP, respectively.

Key words: Coconut, isozyme, polymorphism, protein.

INTRODUCTION

Coconut (*Cocos nucifera* L.), is a monotypic genus with no known wild forms. Hence, variability exists only within local types/populations. It can be divided into two groups — Talls and Dwarfs. The practical identification of cultivars/hybrids is very important for the growers as well as for the research workers. The characterization and evaluation of coconut populations have relied mostly on nursery characters (Rao *et al.*, 17), morphological and agronomic traits (Akpan, 1; Sugimura *et al.*, 18). These procedures do not provide an accurate measure of genetic diversity because many characters exhibit complex inheritance and are influenced by both environmental and genetic factors. Isozyme studies overcome these difficulties. Recently, various biochemical and molecular techniques are being employed to study the origin, domestication, dissemination and diversity in coconut (Geethalakshmi *et al.*, 8-10; Parthasarathy *et al.*,15; Perera *et al.*, 16). Isozymes as genetic markers, have been proven to be reliable, consistent and essentially unaffected by environmental conditions (Bailey, 3). An additional advantage of using isozymes over other biochemical markers in that enzymes are nearly direct gene products and not the products of a series of biosynthetic reactions such as those leading to the production of pigments, oils and various other classes of compounds. Furthermore, characterizing germplasm on the basis of isozymes would help to eliminate duplications of collections. Hengky and Hartana (12) assayed *EST*, PRX and GOT to find the best organ/developmental stage in coconut, while, Asmono *et al.* (2) recommended coconut palm leaves for isozyme analysis. Cardena *et al.* (5) used PRX, END and Coomassie Blue stained proteins to study the isozyme profiles of different talls, dwarfs and hybrids. For breeding purpose, Fernando and Gajanayake (7) used leaf tissue for isozyme analysis to characterize coconut populations. Very few studies have been made till date to use isozymes in the study of interpopulation variation. The present experiments were undertaken to study the variability in coconut germplasm using isozyme polymorphism.

MATERIALS AND METHODS

The experimental material consisted of spindle leaves of adult palms of talls, dwarfs and hybrids of above ten years of age maintained in Central Plantation Crops Research Institute (CPCRI), Kasaragod. Polyacrylamide gel electrophoresis for isozymes was carried out according to Ornstein (15) and described earlier by Geethalakshmi *et al.* (9, 10). Three palms each from 19 talls-Fiji Tall (FJT), Ayiramkachi Tall (AYRT), Strait Settlement Green Tall (SSGT), Strait Settlement Apricot Tall (SSAT), Cochin China Tall (CCNT), Federated Malay States Tall (FMST), Andaman Giant Tall (AGT), Java Giant Tall (JVGT), Gonthembili Tall (GTBT), Seychelles Tall (SEYT), Ceylon Tall (SLT), New Guinea Tall (NGAT), Nadora Tall (NDRT), Zanzibar Tall (ZAT), Standard Kudat Tall (STDK), Ganga Pani Tall (GPNT), Benaulim Tall (BENT), Calangute Tall (CALT) and Spicata Tall (SPIC), seven dwarf cultivars-Malayan Green dwarf (MGD), King Coconut (RTB04), Kulasekharam Orange Dwarf (KOD), Kulasekharam Green Dwarf (KGD), Cameroon Red Dwarf (CRD), Kulasekharam Yellow Dwarf (KYD) and Kenthali Dwarf (KTOD) and four hybrids -

*Corresponding author's present address: Director, Indian Institute of Spices Research, Calicut 673 012; E-mail: parthasarathy@iisr.org

Chowghat Orange Dwarf x Chowghat Orange Dwarf (COD x COD), West Coast Tall x West Coast Tall (WCT x WCT), West Coast Tall x Chowghat Orange Dwarf (WCT x COD) and Chowghat Orange Dwarf x West Coast Tall (COD x WCT) were assayed for different enzyme systems. The tissue sampling and analysis was described earlier (Parthasarathy *et al.,* 15).

RESULTS AND DISCUSSION

Nine bands were observed for *esterase,* out of which three bands (band Nos. 6, 7 and 8) were polymorphic. The culirvars and hybrids showed an allelic frequency of 0.89 or 0.78 (Table 1). Out of the six bands observed for peroxidase, only two bands (band Nos. 5 and 6) were monomorphic. Polymorphic banding pattern was observed. In CCNT, FMST, AGT, JVGT, SLT, BENT, SPIC, CRD, WCT x COD and COD x WCT allelic frequency was 1.00. The least allelic frequency observed was 0.50 (Table 1). Band No. 1 and 2 were absent in FJT, NGAT, STDK, MGD, RTB04, KOD, KGD, KYD and KTOD, while band No. 2 and 3 were absent in AYRT, GTBT, SEYT, NDRT, CALT and WCT x WCT. Band No. 4 was absent in SSGT, SSAT, GTBT, ZAT, MGD, RTB04, KOD, KGD, KYD, KTOD and COD x COD.

High polymorphism was observed for polyphenol oxidase. Out of the 16 bands observed for PPO, only band No. 5 and 6 were monomorphic. Highest allelic frequency was shown by AGT (0.94) and least by MGD (0.25) (Table 1). Band Nos. 1 and 2 were absent in

Table 1. Mean allelic frequency of different isozymes and protein for coconut cultivars and hybrids.

Cultivar/isozyme	EST	PRX	PPO	MDH	ADH	GOT	a-AMY	PHOS	G-6PDH	SOD	ACP	PROT	Mean
FJT	0.89	0.67	0.44	1.00	0.25	0.75	1.00	0.78	1.00	0.80	1.00	0.48	0.76
AYRT	0.78	0.67	0.50	1.00	0.50	1.00	1.00	0.89	1.00	0.90	0.00	0.64	0.74
SSGT	0.89	0.83	0.75	0.67	0.25	0.75	1.00	0.89	1.00	0.70	1.00	0.55	0.77
SSAT	0.89	0.83	0.44	0.50	0.25	0.75	1.00	0.78	1.00	0.70	1.00	0.82	0.75
CCNT	0.89	1.00	0.44	1.00	0.50	1.00	0.75	0.67	1.00	0.80	1.00	0.67	0.81
FMST	0.89	1.00	0.56	1.00	0.25	1.00	0.75	0.67	1.00	0.80	1.00	0.61	0.79
AGT	0.89	1.00	0.94	1.00	0.50	1.00	0.75	0.67	1.00	0.80	1.00	0.70	0.85
JVGT	0.78	1.00	0.5	100	0.75	1.00	0.75	0.67	1.00	0.80	1.00	0.82	0.84
GTBT	0.78	0.50	0.38	1.00	0.75	0.75	0.75	0.67	1.00	0.80	1.00	0.88	0.77
SEYT	0.78	0.67	0.44	1.00	0.75	1.00	0.75	0.67	1.00	0.90	1.00	0.85	0.82
SLT	0.78	1.00	0.56	1.00	0.75	1.00	1.00	0.78	1.00	0.80	1.00	0.55	0.85
NGAT	0.89	0.67	0.63	1.00	0.50	1.00	0.75	0.67	1.00	0.80	1.00	0.81	0.82
NDRT	0.78	0.67	0.44	1.00	0.25	1.00	1.00	0.89	1.00	0.80	1.00	0.73	0.80
ZAT	0.78	0.83	0.56	0.67	0.50	0.75	0.75	0.67	1.00	0.70	1.00	0.73	0.75
STDK	0.78	0.67	0.56	0.67	0.25	1.00	1.00	0.89	1.00	0.70	1.00	0.82	0.78
GPNT	0.78	0.67	0.50	1.00	0.25	1.00	1.00	0.89	1.00	0.70	1.00	0.82	0.78
BENT	0.78	1.00	0.56	1.00	0.25	1.00	0.75	0.78	1.00	0.90	1.00	0.91	0.83
CALT	0.78	0.67	0.44	1.00	0.25	1.00	1.00	0.89	1.00	0.80	1.00	0.88	0.81
SPIC	0.89	1.00	0.50	0.67	0.50	1.00	1.00	0.78	1.00	0.70	1.00	0.73	0.81
MGD	0.89	0.50	0.25	0.50	0.25	1.00	1.00	0.89	1.00	0.60	1.00	0.82	0.74
RTB04	0.89	0.50	0.56	1.00	0.50	0.75	0.75	0.67	1.00	0.70	1.00	0.88	0.77
KOD	0.78	0.50	0.50	1.00	0.50	1.00	0.75	0.67	1.00	0.90	1.00	0.79	0.78
KGD	0.89	0.50	0.44	1.00	0.50	1.00	0.75	0.67	0.33	0.80	1.00	0.70	0.72
CRD	0.78	1.00	0.69	1.00	0.50	0.75	0.75	0.67	1.00	0.80	1.00	0.70	0.80
KYD	0.89	0.50	0.31	0.67	0.00	0.75	0.75	0.67	1.00	0.50	1.00	0.88	0.64
KTOD	0.89	0.50	0.5O	0.00	0.00	1.00	0.75	0.67	1.00	0.50	1.00	0.88	0.64
COD x COD	0.89	0.83	0.63	0.83	0.25	1.00	0.75	0.67	1.00	0.80	1.00	0.88	0.79
WCT x WCT	0.89	0.67	0.50	1.00	0.50	0.75	1.00	0.78	1.00	0.90	1.00	0.88	0.82
WCT x COD	0.89	1.00	0.50	1.00	0.50	1.00	0.75	0.67	1.00	0.90	1.00	0.85	0.84
COD x WCT	0.89	1.00	0.69	0.67	0.25	1.00	0.75	0.67	1.00	0.90	1.00	0.85	0.84
Mean	0.84	0.76	0.52	0.87	0.40	0.93	0.86	0.75	0.97	0.79	0.97	0.77	

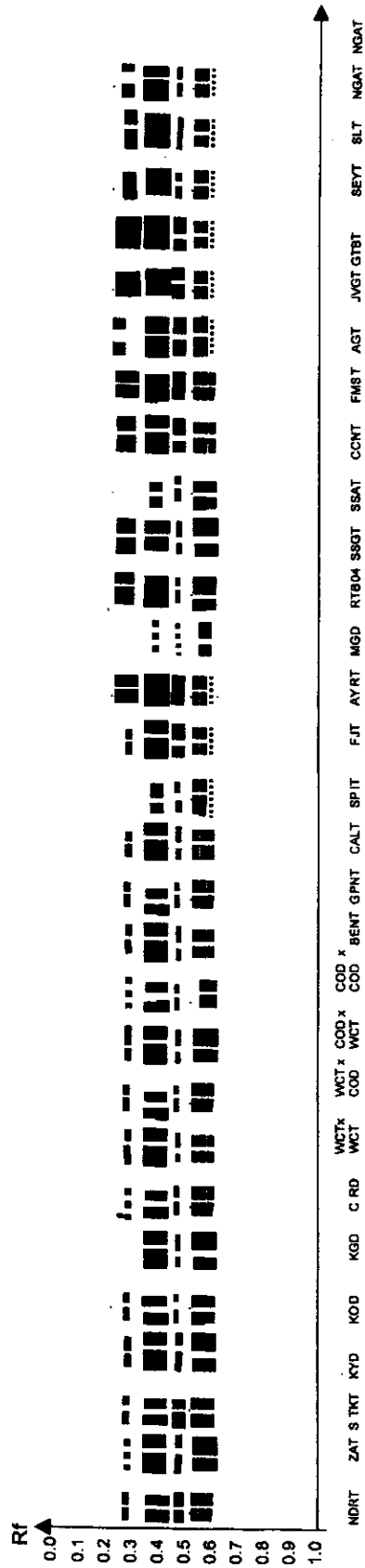

Fig. 1. Zymogram of malate dehydrogenase.

Fig. 2. Zymogram of alcohol dehydrogenase.

Fig. 3. Zymogram of glutamate oxaloacetate transaminase.

FJT, NGAT, STDK, CALT, MGD, RTB04, KGD and KYD. Band No. 7 was present only in few cultivars like AYRT, SSGT, FMST, AGT, NGAT, RTB04 and COD x WCT. Band No. 8 was present only in STDK, CRD, COD x COD and COD x WCT. Band No. 9 was absent in SSAT, CCNT, NGAT and MGD. Band No. 13 was present in SSGT, AGT and NGAT while band No. 19 was present in SSGT, SSAT, AGT, NGAT and RTB04. Band No. 16 was present only in AGT. Acid phosphatase showed limited polymorphism . Only one band (Rf value, 0.12) was present in all cultivars and hybrids except in AYRT, where null allele was seen.

Polymorphism was observed in super oxide dismutase. Four bands were found to be monomorpbic (band No. 6,8,9 and 10) out of 10 bands for SOD. Highest allelic frequency was 0.90 in AYRT, SEYT, WCT, BENT, KOD, KYD, WCT x COD and COD x WCT and the least was 0.50 in KTOD (Table 1). Band No. 1 was absent in MGD and KTOD. Band No. 2 was absent only in KTOD. Band No. 4 was present only in SSAT, SSGT, NDRT, ZAT, CALT, SPIC, MGD, RTB04 and KTOD. Band No. 7 was present only in AYRT, SEYT, NDRT, BENT, CALT, KOD, KYD, COD x WCT, WCT x COD and WCT x WCT.

Six bands were observed for *MDH* and all were polymorphic (Fig. 1). Majority of the cultivars and hybrids showed an allelic frequency of 1.00 and least was shown by SSAT, MGD and KGD (0.50). Null allele was seen in KTOD (Table 1). Band No. 1 was absent in SSAT, SPIC, MGD, KGD and KTOD while band No. 2 and 3 were absent in KTOD. Band No. 5 was absent in MGD, KTOD and COD x COD.

Table 2. Number of bands present in different cultivars and hybrids for different isozymes and protein.

Cultivar/isozyme	EST	PRX	PPO	WDH	ADH	GOT	a-AMY	PHOS	G-6PDH	SOD	ACP	PROT	Total
FJT	8	4	7	6	1	3	4	7	3	8	1	16	88
AYRT	7	4	8	6	2	4	4	8	3	9	0	21	76
SSGT	8	5	12	4	2	3	4	8	3	7	1	18	75
SSAT	8	5	7	3	1	3	4	7	3	7	1	27	76
CCNT	8	6	7	6	2	4	3	6	3	8	1	22	76
FMST	8	6	9	6	1	4	3	6	3	8	1	20	75
AGT	8	6	15	6	2	4	3	6	3	8	1	23	85
JVGT	7	6	8	6	3	4	3	6	3	8	1	27	82
GTBT	7	3	6	6	3	3	3	6	3	8	1	29	78
SEYT	7	4	7	6	3	4	3	6	3	9	1	28	81
SLT	7	6	9	6	3	4	4	7	3	8	1	18	76
NGAT	8	4	10	6	2	4	3	6	3	8	1	30	85
NDRT	8	4	10	6	2	4	3	6	3	8	1	24	77
ZAT	7	5	9	4	2	3	3	6	3	7	1	24	74
STDK	7	4	9	4	1	4	4	8	3	8	1	27	80
GPNT	7	3	8	6	1	4	3	7	3	9	1	30	86
BENT	7	6	9	6	1	4	4	8	3	8	1	29	82
CALT	7	4	7	6	1	4	4	8	3	8	1	29	78
SPIC	8	6	8	5	2	4	4	7	3	7	1	24	79
MGD	8	3	4	3	1	4	4	8	3	6	1	27	72
RTB04	8	3	9	4	2	3	3	6	3	7	1	29	78
KOD	7	3	8	4	2	4	3	6	3	9	1	26	76
KGD	8	3	7	3	2	4	3	6	1	8	1	23	69
CRD	7	6	11	6	2	3	3	6	3	8	1	23	79
KYD	8	3	5	4	0	3	4	7	1	9	1	21	66
KTOD	8	3	8	0	0	4	3	6	3	5	1	29	70
COD X COD	8	5	10	4	1	4	3	6	3	8	1	29	82
WCT X WCT	8	4	8	6	2	3	4	7	3	9	1	29	84
WCT X COD	8	5	8	6	2	4	3	6	3	9	1	28	83
COD X WCT	8	6	11	4	1	4	3	6	3	9	1	26	82

Four bands were observed for (ADH) and all bands were polymorphic (Fig. 2). Highest allelic frequency was 0.75 in JVGT, GTBT, SEYT and SLT, while no *ADH* activity due to presence of null allele was observed in KYD and KTOD (Table 1). Band No. 1 was present in AYRT, JVGT, GTBT, SEYT, SLT while band No. 2 was present in JVGT, GTBT, SEYT and SLT. Band No. 3 was absent only in KYD and KTOD.

Out of the four bands observed for Glutamate oxaloacetate tramaminase (GOT), only band No. 2 was polymorphic (Fig. 3). This band was absent in cultivars like FJT, SSGT, SSAT, GTBT, ZAT, RTB04, CRD, KYD and WCT x WCT. Majority of the cultivars showed an allelic frequency of 1.00, while the rest of the cultivars showed 0.75 (Table 1).

Four bands were observed for α-Amylase (Fig. 4) of which only one band (band No. 3) was polymorphic. This band was present in FJT, AYRT, SSGT, SSAT, SLT, NDRT, STDK, CALT, SPIC, MGD, KYD and WCT x WCT. Allelic frequency for some of the cultivars was 1.00 and for the rest it was 0.75 (Table 1).

Three bands (band Nos. 4, 6 and 8) were polymorphic in Phosphorylase (Fig. 5), out of nine bands observed for PHOS. Allelic frequencies observed were 0.89, 0.78 and 0.67 (Table 1). Band No. 4 had low allelic frequency (0.03) as it was present only in SSGT. Another band (band No. 6) also had low allelic frequency (0.23), which was seen in AYRT, NDRT, STDK, GPNT, BENT, CALT and MGD.

Polymorphism was observed in Glucose 6-phosphate dehydrogenase. (G-6PDH) showed three bands, of which two bands (band Nos. 1 and 2) were polymorphic (Fig. 6). Except for KGD and KYD (0.33), where band Nos. 1 and 2 were absent, rest of the cultivars and hybrids showed an allelic frequency of 1.00 (Table 1).

Polymorphic banding pattern for protein 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 mesomorphic (Fig. 2 E and F). Allelic frequency was highest in NGAT, where band No. 9,10 and 29 were absent and BENT (0.91), where band No. 8, 12 and 15 were absent and least in FJT (0.48), where band No. 1, 3, 6, 9, 11, 16, 17, 20, 21, 22, 26, 28, 30 and 31, 32 and 33 were present. Band No. 8 had low allelic frequency, which was present in JVGT, NGAT, KTOD and RTB04. AGT and SLT showed highest value of mean allelic frequency (0.85) for isozymes and proteins among the different cultivars and hybrids studied (Table 1), while KTOD (0.64) showed least value. Among the isozymes and proteins studied, G-6PDH and ACP showed the highest value (0.97) and ADH showed the least value (0.40). Among the cultivars and hybrids studied, maximum bands were present in BENT (86), while minimum bands (66) were seen in KYD (Table 2).

Table 3. Isozyme polymorphism in coconut cultivars.

Isozyme	Polymorphic Index
EST	0.082
PRX	0.145
PPO	0.151
MDH	0.130
ACP	0.030
ADH	0.140
GOT	0.053
a-AMY	0.060
PHOS	0.050
G-6PDH	0.043
SOD	0.095
Mean	0.089

Out of the 11 isozyme systems studied (Table 3), highest enzyme polymorphism was shown by PPO (0.151) followed by PRX (0.145), ADH (0.140), MDH (0.130), SOD (0.095), EST (0.082), a-AMY (0.060), GOT (0.053), PHOS (0.050), G-6PDH (0.043) and ACP (0.030).

High level of polymorphisms for isozyme phenotypes was observed among the coconut cultivars studied, as shown by Carpio (6). Significant differences in allelic frequencies were seen between populations and genetic discrimination between populations based on the allelic frequencies was carried out by principal component analysis. The PC1 and PC2 scores based on the principal component analysis of isozyme data of 26 accessions were plotted on the X and Y axes. The resulting graph showed most of the talls and dwarfs were scattered indicating the presence of genetic diversity among them. No geographical affinity was observed. Phenotypically and geographically diverse tall ecotypes like GPNT, BENT, JVGT, CALT, NDRT, GTBT, AYRT, SEYT and SLT grouped together indicating greater similarity between these cultivars (Fig. 5). The two spikeless ecotypes (Spicata and Standard Kudat) studied were observed to be divergent from each other. Among the dwarf ecotypes, two dwarfs, CRD and KGD appeared to be divergent from the rest of the dwarfs and closer to certain Tall ecotypes. The dwarf ecotypes such as KGD and CRD were scattered with Tails such as SPIC, ZAT and CCNT were distantly divergent from the rest of the dwarfs. Among talls, SSGT, STDK, SPIC, ZAT were found scattered from the rest of the talls indicating the genetic diversity as confirmed by Fernando and Gajanayake (7). In another group, NGAT, AGT, SSAT, SSGT and STDK were seen with most dwarfs, although STDK and SSGT were scattered apart. Canto-Canche *et al.* (4) also reported a wide range of variation in allelic frequencies for

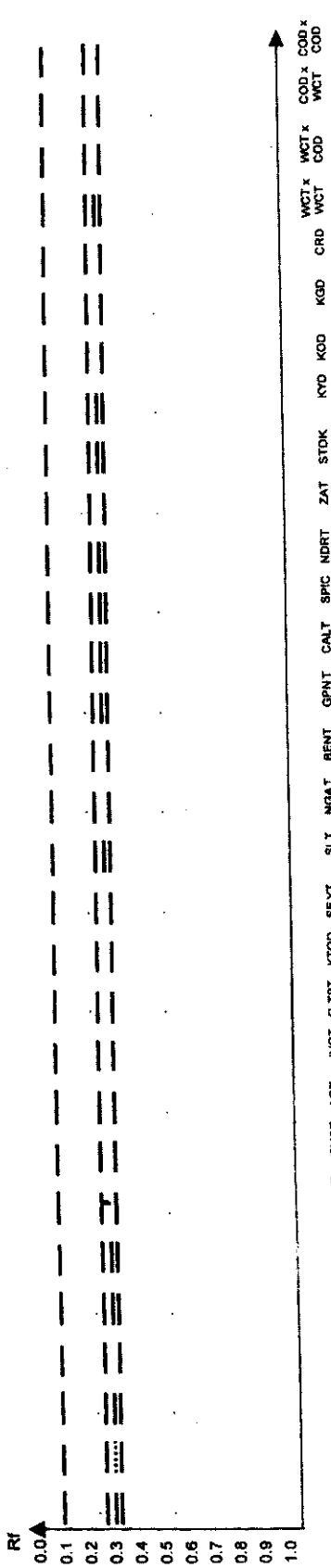

Fig. 4. Zymogram of α-amylase.

Fig. 5. Zymogram of phosphorylase.

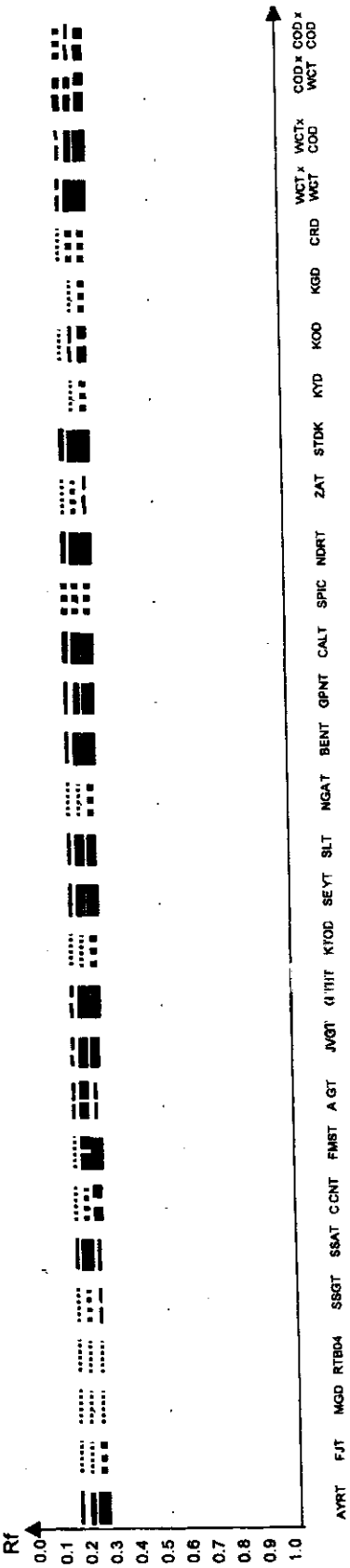

Fig. 6. Zymogram of glucose 6-phosphate dehydrogenase.

different isozymes for different cultivars. They reported the efficacy of the method to identify cultivars irrespective of their origin and concluded that the method should be of great value to identify cultivars in germplasm collections. Coconut cultivar identification will enable us to verify the homogeneity of *in vitro* shoots and to distinguish between the different cultivars. We should find and eliminate any variant produced, as well as the processes generating them without waiting for the first fructification. The present studies indicated tall varieties diverging slightly in the expression of some proteins. Total storage protein profiles did not show any specific variation between the different cultivars with respect to the major polypeptides. But minor polypeptides showed variation and are in tune with an early report on coconut (Jayalekshmy, 12). The present study indicates that isozymes like EST, PRX, PPO and SOD can be successfully used for the genetic diversity analysis and characterization of coconut populations.

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