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Intrapopulation allelomorphism in tall and dwarf populations of the coconut

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Six Dwarf and eight Tall types of coconut were used to study intrapopulation variation based on protein/isozyme polymorphism. Total soluble proteins and seven isozyme systems – peroxidase (*PRX,* EC 1.11.1.7), esterase (*EST,* EC 3.1), acid phosphatase (*ACP,* EC 3.1.3.2), malate dehydrogenase (*MDH,* EC 1.1.1.37), polyphenol oxidase (*PPO,* EC 1.14.18.), alcohol dehydrogenase (*ADH,* EC 1.1.1.1) and glutamate oxaloacetate transaminase (*GOT,* EC 2.6.1.1) were used for the study. Among Dwarfs, the highest enzyme polymorphism was observed in Gudanjali Dwarf, while Gangabondham Dwarf showed the lowest polymorphism. Of the eight Talls studied, Java Tall showed the highest isozyme polymorphism, while the lowest polymorphism was seen in SNRT. Overall, among seven isozyme systems, *PPO* showed the highest polymorphism, while *ACP* did not show any polymorphism. Differences in the allelic frequency were obtained even though there were no specific differences in the banding pattern of varieties.

Key words: Coconut, cultivars, isozyme, intrapopulation, polymorphism

Introduction

The coconut (*Cocos nucifera* L.), one of the most useful palms in the world, grows in more than 80 countries of the tropics. The palms are of two types – Talls and Dwarfs. Though represented by a monotypic species, because of its predominantly cross-pollinating nature, there are several cultivars or types, widely differing from each other in the morphological characters, particularly with respect to fruit. Morphological descriptors and agronomic traits (AKPAN 1994, SUGIMURA et al. 1997) are used to describe the coconut cultivars. Since most of the traits are polygenic, a clear-cut demarcation among cultivars is rather difficult to achieve. Advances in biochemistry, genetics and molecular biology have provided descriptors based on proteins including isoenzymes. In plants and animals, isozymes can be used to generate genetic fingerprints, since these are universal and codominant, with a low level of environmental interaction (GOTTLIEB 1981). Even though isozyme studies have been carried out in other species, the research carried out on the coconut is quite limited. The work on isozyme analysis in coconut began at IRHO in 1978 in order to understand the structure of genetic diversity. Isozyme analysis has been used for cultivar identifi-

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cation, progeny legitimacy and diversity analysis (PARTHASARATHY et al. 2004, GEETHALAKSHMI et al. 2004). Different researchers used different tissues of the palm. HENGKY and HARTANA (1994), ASMONO et al. (1993), CARDENA et al. (1998), FERNANDO and GAJANAYAKE (1997), JAYALEKSHMY (1999) used leaf tissues to study isozyme profiles, while BHATTACHARYA et al. (1993) used pollen tissue. CANTO-CANCHE et al. (1992) used different tissues such as leaf, endosperm and inflorescence. In the present study six Dwarf and eight Tall ecotypes were screened for the study of intrapopulation variation and heterogeneity.

Materials and methods

To assess the intrapopulation variation, seven palms each of six Dwarf cultivars, Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Chowghat Green Dwarf (CGD), Malayan Orange Dwarf (MOD), Gudanjali Dwarf (GDD), Ganga Bondam Dwarf (GBGD) and eight Tall cultivars, West Coast Tall (WCT), Java Tall (JVT), Laccadive Ordinary Tall (LCT), Philippines Ordinary Tall (PHOT), Andaman Ordinary Tall (ADOT), San Ramon Tall (SNRT), Kappadam Tall (KPDT) and Laccadive Micro Tall (LMT) were assayed for different isozyme systems. Protocols that gave better staining were used to study the seven different isozyme systems, *viz.* peroxidase (*PRX,* EC 1.11.1.7), esterase (*EST,* EC 3.1), acid phosphatase (*ACP,* EC 3.1.3.2), malate dehydrogenase (*MDH,* EC 1.1.1.37), polyphenol oxidase (*PPO,* EC 1.14.18.1), alcohol dehydrogenase (*ADH,* EC 1.1.1.1) and glutamate oxaloacetate transaminase (*GOT,* EC 2.6.1.1) and proteins as detailed by PARTHASARATHY et al (2004). The data were analysed as per the procedure outlined by GEETHALAKSHMI et al. (2004).

Individual bands, represented by their R_f value, were considered as alleles and used for the estimation of variability. The allelic frequency (band frequency) of different isozyme systems was scored as the ratio of the number of the samples analyzed. Intrapopulation variation was studied by computing the polymorphic index (PI), which was calculated by using the formula,

$$\text{Polymorphic Index, PI} = \Sigma Pi\ (1–Pi)/N$$

where, Pi = i^{th} allele (band) frequency and N = Number of bands.

Heterogeneity in the coconut germplasm was worked out based on the allelic frequency and polymorphic index obtained from isozyme analysis. Within population, genetic diversity for each accession was estimated by using the Shannon information index, which is defined as,

$$H = -\Sigma^{K}_{j=1}\ Pi\ \log_e Pi$$

where H denotes the diversity of isozyme markers in a population, k the number of isozyme markers and Pi denotes the frequency of the i^{th} isozyme marker in a given accession i=1 (ASHBURNER et al., 1997).

Results

The spindle leaf extracts from six Dwarfs (COD, MYD, CGD, MOD, GDD and GBGD) and eight Talls (WCT, JVT, LCT, PHOT, ADOT, SNRT, KPDT and LMT) were subjected to PAGE and stained for seven isozymes, namely, *EST, PRX*, *PPO, MDH, ACP,*

ADH and *GOT* and *PROT*. Differences in the allelic frequency were obtained even though there were no specific differences in the banding patterns of varieties.

Out of seven *EST* bands observed in Dwarf cultivars, four bands (band no. 1, 2, 5 and 6) were polymorphic. The allelic frequency in Dwarfs (Tab.1) for *EST* isozymes was the highest in GDD and GBGD (1.00) and the lowest in MYD (0.71). Five bands (bands no. 2, 6, 7, 8 and 9) were polymorphic among the nine *EST* bands observed in Tall cultivars. The allelic frequency was the highest in WCT and LCT (0.87) and the lowest (0.56) in SNRT

Differences in the peroxidase banding pattern were observed both among and within cultivars. Seven bands were observed in Dwarfs, while in Talls only six bands were observed. A band with R_f value 0.32 was present only in the Dwarf populations. In Dwarf cultivars only four of the seven bands (bands no. 1, 2, 4 and 6) were polymorphic. The allelic frequency (Tab.1) was the highest in COD, MYD, CGD and MOD (0.71) and the lowest in GBGD (0.43). In Tall cultivars, of the six bands, only band no. 5 was monomorphic. The allelic frequency (Tab.2) was the highest in ADOT (0.78) and the lowest in SNRT (0.50).

Differences in the PPO banding pattern were observed both among cultivars and within a single cultivar. Eighteen *PPO* bands were observed in Dwarf cultivars, and of them, only bands no. 3 and 5 were monomorphic. The allelic frequency was the highest in GDD (0.48) and the lowest in MOD (0.25) (Tab.1). All the 21 *PPO* bands observed in Tall cultivars were polymorphic. The allelic frequency (Tab.2) was the highest in PHOT (0.50) and the lowest in SNRT (0.24).

Of the six MDH bands observed in Dwarf cultivars, bands no.1, 4 and 6 were polymorphic. The allelic-frequency (Tab.1) was the highest in GBGD (1.00) and the lowest in MOD (0.64). In Tall cultivars only two bands (bands no.4 and 6) were polymorphic. The allelic frequency (Tab.2) was the highest in SNRT (1.00) and the lowest in KPDT (0.71).

In the case of ACP a monomorphic banding pattern with a single band (Rf value 0.12) was observed in all Talls and Dwarfs.

As regards ADH, in Dwarf cultivars, out of the two bands only band no.2 (Rf value 0.50) was polymorphic. Band no.2 was absent in GDD and GBGD. The allelic frequency (Tab.1) was the highest in COD, MYD, CGD and MOD (1.00) and the lowest in GDD and GBGD (0.50). Among the *ADH* bands observed in Tall cultivars, band no.3 (Rf value 0.50) was monomorphic. The allelic frequency (Tab.2) was the highest in WCT (0.75) and the lowest in LCT and SNRT (0.25).

Four *GOT* bands were observed in Dwarf cultivars, and of them, two bands (band no.1 and 2) were polymorphic. The allelic frequency (Tab.1) was the highest in CGD (1.00) and the lowest (0.67) in MYD. In Tall cultivars, among the six bands observed, all bands were polymorphic. Among them, the allelic frequency (Tab.2) was the highest in WCT, LCT and PHOT (0.67) and the lowest in SNRT (0.50). Bands no.4 and 5 were present only in JVT and PHOT.

The polymorphic banding pattern for PROT was observed. Thirty two clear *PROT* bands were observed in Dwarf cultivars, of which six bands (bands no. 5, 25, 27, 30, 31 and 32) were monomorphic. The allelic frequency (Tab.1) of *PROT* was the highest in GDD (0.83) and the lowest in CGD (0.56). Thirty three clear *PROT* bands were observed in Tall cultivars, of which four bands (band no. 26, 30, 32 and 34) were monomorphic. The allelic frequency (Tab.2) was the highest in KPDT (0.91) and the lowest in JVT (0.64).

Among the Dwarf cultivars, COD (0.75) showed the highest value of mean allelic frequency (Tab.1) while GBGD showed the lowest value (0.67). Among Dwarfs, the isozymes *EST* showed the highest value (0.89) and *PPO* showed the lowest value (0.34). WCT showed the highest mean allelic frequency (0.70) among Talls (Tab.2), while SNRT showed the lowest (0.53) value. Among Talls, *MDH* showed the highest mean allelic frequency (0.86), while *PPO* showed the lowest value (0.37).

Tab. 1. Mean allelic frequency of different isozymes for Dwarfs

Cultivar/Isozyme	*EST*	*PRX*	*PPO*	*MDH*	*ADH*	*GOT*	*PROT*
COD	0.96	0.71	0.37	0.67	1.00	0.86	0.70
MYD	0.71	0.71	0.31	0.67	1.00	0.67	0.66
CGD	0.82	0.71	0.31	0.67	1.00	1.00	0.56
MOD	0.86	0.71	0.25	0.64	1.00	0.75	0.59
GDD	1.00	0.57	0.48	0.67	0.50	0.75	0.83
GBGD	1.00	0.43	0.30	1.00	0.50	0.75	0.72
Mean	0.89	0.64	0.34	0.72	0.83	0.80	0.68

Tab. 2. Mean allelic frequency of different isozymes for Talls

Cultivar/Isozyme	*EST*	*PRX*	*PPO*	*MDH*	*ADH*	*GOT*	*PROT*
WCT	0.87	0.64	0.31	0.90	0.75	0.67	0.79
JVT	0.81	0.55	0.35	0.76	0.64	0.62	0.64
LCT	0.87	0.62	0.42	0.90	0.25	0.67	0.86
PHOT	0.84	0.71	0.50	0.76	0.43	0.67	0.78
ADOT	0.72	0.78	0.44	0.89	0.38	0.61	0.86
SNRT	0.56	0.50	0.24	1.00	0.25	0.50	0.67
KPDT	0.75	0.62	0.33	0.71	0.47	0.57	0.91
LMT	0.75	0.69	0.33	0.95	0.50	0.60	0.88
Mean	0.77	0.64	0.37	0.86	0.46	0.61	0.80

The polymorphic index helps in evaluating the degree of polymorphism in a population and aids in comparing them. Of the six Dwarfs studied, GDD showed (Tab.3) the highest enzyme polymorphism (0.024) while GBGD showed the lowest enzymepolymorphism (0.003). Within each enzyme system, differences in intra population variation were observed among cultivars except in *ACP* and *ADH*. For *EST*, among the Dwarfs, MOD showed the highest intrapopulation variation (0.070), while MYD, GDD and GBGD did not show any intrapopulation variation. For *PRX*, only GDD showed intrapopulation variation (0.087). GDD showed the highest intrapopulation variation (0.084) for *PPO*, while CGD and GBGD had the lowest intrapopulation variation (0.023). For *MDH*, only MOD showed intrapopulation variation (0.020), while the rest of the cultivars did not show any intrapopulation variation. COD showed the highest polymorphism (0.061), while CGD, MOD, GDD and GBGD did not show any polymorphism for *GOT*. A comparison of the polymorphic indices of the Dwarfs (Tab.3) indicated that among the seven enzyme systems studied, *PPO* showed the highest polymorphism (0.057) followed by *EST* (0.027), *GOT* (0.019), *PRX* (0.015) and *MDH* (0.003). *ACP* and *ADH* did not show any polymorphism.

Of the eight Talls studied (Tab.3), JVT showed the highest (0.078) and SNRT the lowest enzyme polymorphism (0.004). Within each enzyme system (except *ACP*), differences in intrapopulation variation were observed among cultivars. For *EST*, JVT showed the highest intrapopulation variation (0.050), while it was absent in SNRT. Similarly for *PRX*, JVT showed the highest intrapopulation variation (0.144), and SNRT none at all. For *PPO*, PHOT showed the highest (0.121) and for SNRT the lowest intrapopulation variation (0.030). For *MDH*, ADOT showed the highest intrapopulation variation (0.071), followed by WCT, JVT, LCT, PHOT (0.069) and KPDT, LMT (0.040) while it was absent in SNRT. For *ADH*, ADOT showed the highest intrapopulation variation (0.063) and KPDT the lowest (0. 030) with WCT, LCT, SNRT and LMT exhibiting zero values. In the case of *GOT,* JVT showed the highest intrapopulation variation (0.120), and ADOT the lowest (0.037), WCT, LCT and SNRT showing no intrapopulation variation.. A comparison of the polymorphic indices of the Talls (Tab.3) indicates that among seven enzyme systems studied, *PRX* showed the maximum polymorphism (0.092) followed by *PPO* (0.066), *MDH* (0.053), *GOT* (0.039), *ADH* (0.026) and *EST* (0.025). *ACP* did not show any polymorphism.

Among the Dwarf cultivars studied, MOD showed the highest intrapopulation polymorphism (0.111) and GDD showed the lowest polymorphism (0.028) for *PROT.* Among the Tall cultivars studied, the highest *PROT* polymorphism was seen in JVT (0.114), while it was the lowest in KPDT (0.018) (Tab.3).

Considering all Talls and Dwarfs, for intrapopulation variation, among the seven isozyme systems, *PPO* showed the highest polymorphism (0.062) followed by *PRX* (0.054), *GOT* (0.029), *MDH* (0.028), *EST* (0.026) and *ADH* (0.013), while *ACP* did not show any polymorphism (Tab.3). Among the Dwarfs, maximum bands were present in GDD and COD (63), while minimum bands were seen in MYD (53). Among the different

Tab. 3. The polymorphic index of Dwarf and Tall cultivars

Cultivar/Enzyme	*EST*	*PRX*	*PPO*	*MDH*	*ACP*	*ADH*	*GOT*	*PROT*
Dwarfs								
COD	0.029	0.000	0.068	0.000	0.000	0.000	0.061	0.098
MYD	0.000	0.000	0.077	0.000	0.000	0.000	0.055	0.039
CGD	0.064	0.000	0.023	0.000	0.000	0.000	0.000	0.053
MOD	0.070	0.000	0.065	0.020	0.000	0.000	0.000	0.111
GDD	0.000	0.087	0.084	0.000	0.000	0.000	0.000	0.028
GBGD	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.057
Talls								
WCT	0.013	0.115	0.058	0.069	0.000	0.000	0.000	0.036
JVT	0.050	0.144	0.099	0.069	0.000	0.061	0.120	0.078
LCT	0.013	0.122	0.083	0.069	0.000	0.000	0.000	0.114
PHOT	0.036	0.089	0.121	0.069	0.000	0.051	0.074	0.048
ADOT	0.040	0.120	0.052	0.071	0.000	0.063	0.037	0.071
SNRT	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.052
KPDT	0.023	0.089	0.035	0.040	0.000	0.030	0.041	0.036
LMT	0.023	0.060	0.047	0.040	0.000	0.000	0.041	0.018

Tall cultivars studied (Tab.4) maximum bands were seen in PHOT (74), while the minimum number was seen in SNRT (52).

Tab. 4. Number of bands in Dwarf and Tall cultivars for different enzyme systems. * – Figures in parenthesis, indicate number of polymorphic bands

Cultivar/Enzyme	*EST*	*PRX*	*PPO*	*MDH*	*ADH*	*GOT*	*ACP*	*PROT*	Total
Dwarfs									
COD	7	5	10	4	2	4	1	30	63
MYD	5	5	9	4	2	3	1	24	53
CGD	7	5	7	4	2	4	1	24	54
MOD	7	5	9	4	2	3	1	27	58
GDD	7	6	12	4	1	3	1	29	63
GBGD	7	3	6	6	1	3	1	27	54
Total bands*	7 (4)	7 (4)	18 (16)	6 (3)	2 (1)	4 (2)	1 (0)	32 (26)	77 (56)
Talls									
WCT	8	6	9	6	3	4	1	32	69
JVT	8	5	14	6	3	6	1	29	72
LCT	8	6	14	6	1	4	1	32	72
PHOT	8	5	15	6	2	6	1	31	74
ADOT	7	6	11	6	2	4	1	31	68
SNRT	5	3	7	6	1	3	1	26	52
KPDT	7	6	10	6	2	4	1	31	67
LMT	7	6	10	6	2	4	1	31	67
Total bands*	9 (5)	6 (5)	21 (21)	6 (2)	3 (2)	6 (6)	1 (0)	33 (29)	85 (70)

Based on the allelic frequency obtained from the isozyme analysis, heterogeneity in Talls and Dwarfs was calculated (Tab.5). Among the Dwarfs, GDD showed the highest heterogeneity (0.112), while GBGD showed the lowest heterogeneity (0.018). Among the different Talls, JVT was more heterogenous (0.159), while WCT showed the lowest heterogeneity (0.076). In a comparison of Talls and Dwarfs, Tall cultivars showed more heterogeneity (0.121) than Dwarfs (0.073).

Discussion

The coconut is a highly cross-pollinating species likely to contain a high proportion of genetic variation within populations, as in populations of another palm species, *Acrocomia aculeate* (LOPES et al. 1992). Talls particularly contain immense variation because of their allogamous nature, which was confirmed by isozyme studies (FERNANDO and GAJANAYAKE 1997) and by RAPD and AFLP analysis (ASHBURNER et al. 1997, PERERA et al. 1998, RATNAMBAL et al. 2001 and UPADHYAY et al. 2002). The total genetic variation of a species is likely to be distributed among populations as the impact of natural selection varies among populations due to genetic drift and environment (LAWRENCE and RAJANAIDU 1985). Therefore with germplasm conservation programmes, it is imperative to measure accurately the amount of genetic diversity and its distribution within and among populations. In the present study, all the populations showed many common alleles for the loci analysed in spite of their broad eco-geographic range of origins. Of the seven isozyme systems studied in the

Tab. 5. Heterogeneity in coconut cultivars

Cultivar	Heterogeneity Index
Dwarfs	
COD	0.090
MYD	0.093
CGD	0.048
MOD	0.075
GDD	0.112
GBGD	0.018
Mean	0.073
Talls	
SNRT	0.076
JVT	0.159
LCT	0.124
PHOT	0.148
KPDT	0.099
LMT	0.103
WCT	0.148
ADOT	0.109
Mean	0.121

Cultivar	Heterogeneity Index
Exogenous	
JVT	0.159
PHOT	0.148
SNRT	0.076
MYD	0.093
MOD	0.075
Mean	0.125
Indigenous	
COD	0.090
CGD	0.048
GDD	0.112
GBGD	0.018
ADOT	0.109
LMT	0.103
KPDT	0.099
WCT	0.148
LCT	0.124
Mean	0.087

present work, apart from protein, *ACP* did not show any polymorphism. The present study revealed that dehydrogenases are not ideal for studying the allelic polymorphism as reported by FERNANDO et al. (1993) and GEETHALAKSHMI et al. (2004). The study of enzymatic polymorphism provides useful information on genetic diversity and on its structuring among populations. Studies in Sri Lanka showed high intrapopulation variation in Sri Lankan Talls than in Dwarfs using microsatellite markers (PERERA et al., 2001) as revealed by the present work. This shows that in Talls, due to cross pollination, high genetic variation exists and in Dwarfs due to inbreeding nature low genetic diversity is present. The result was supported by the work of FERNANDO and GAJANAYAKE (1997) in which, the level of polymorphism was high in Tall cultivars compared to Dwarfs. As the polymorphic index studies showed, more heterogeneity was observed for Talls than Dwarfs indicating higher genetic diversity in Talls. Very few studies have been carried out in the coconut to draw definite conclusions on the genetics of isozymes. Even though isozyme genetics in the coconut is in its infancy, it will not be difficult to use it as a type of successful genetic marker in crop improvement programmes if more work is done in this area.

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