Journal of Plantation Crops, 2004, 32 (2): 13-15

Allozyme variation in populations of dwarf coconut cultivars

P. Geethalakshmi, V. Niral and V.A. Parthasarathy**

Central Plantation Crops Research Institute, Kasaragod-671 124, Kerala, India.

(Manuscript Received 2-7-2003; Revised : 18-8-2003; Accepted : 5-5-2004)

Abstract

Six dwarf coconut cultivars viz., COD, MYD, CGD, MOD, GDD and GBGD were studied for isozyme polymorphism using seven enzyme systems - *EST, PER, PPO, MDH, ACP, ADH* and *GOT*. Among the cultivars, higher enzyme polymorphism was observed in GDD and least in GBGD. Of the seven isozyme systems, PPO showed higher polymorphism followed by *EST, GOT, PER* and *MDH. ACP* and *ADH* did not show any intrapopulation variation.

Key words: Coconut, dwarfs, isozyme, PAGE, intra-population, polymorphism

Introduction

Coconut, (*Cocos nucifera* L.) exhibits a lot of variability within this monotypic genus and the palms are broadly classified into two groups – Talls and Dwarfs. At CPCRI, coconut germplasm is being characterized based on morphological characters. These traits are influenced to a greater extent by the environment in which they grow. Therefore, there is a need for developing fast, economical and reliable methods for assessing genetic diversity in coconut populations for use in breeding programmes. Isozymes are useful genetic markers as they are codominant with low level of environmental interaction (Gottlieb, 1981). Isozyme studies in coconut are quite limited. Meunier (1992) studied 17 enzyme systems in different tissues of the coconut palm and observed clear and consistent banding in pollen and haustorium tissues. On the other hand, Hengky and Hartana (1994) recorded stable enzyme activity in leaf tissue. Canto-Canche *et al.*, (1992) used inflorescence and leaf extracts to characterize Malayan Dwarfs and Atlantic Talls by protein/enzyme electrophoresis and detected possible biochemical markers using leaf extracts. Fernando and Gajanayake (1997) used leaf tissue for isozyme analysis to characterize coconut populations used for breeding purposes. In the present study, isozymes have been used for identification of genetic similarity and evaluation of heterogeneity. Standardization and optimization of enzyme extraction and staining protocols were undertaken and six dwarf ecotypes were screened for isozyme polymorphism.

Materials and Methods

Enzymes were extracted from leaf tissue of adult coconut palms in WCT and COD palms. Spindle leaf extract gave better and consistent enzyme activity and was hence used for further enzyme studies.

Enzymes were extracted with 0.1 M Tris-HCl buffer (pH 6.8) containing 0.1% β-mercaptoethanol 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 (Davis, 1964). Polyacrylamide gels of 7.5-10% were used and the gels were run for 4 hours under constant current. The gels were stained for isozymes using standard protocols (Table 1). Seven enzyme systems were studied – Peroxidase (*PER*), Esterase (*EST*), Acid phosphatase (*ACP*), Malate dehydrogenase (*MDH*), Polyphenol oxidase (*PPO*), Alcohol dehydrogenase (*ADH*) and Glutamate oxaloacetate transaminase (*GOT*).

**Corresponding author

Present Address: Director, Indian Institute of Spices Research, Marikunnu P.O.-673 012, Calicut, Kerala, India

Tel.: 0091-495-2731346; fax: 0091-495-2730294

E-mail address: parthasarathy@iisr.org

Table 1. Buffer composition and electrophoretic conditions used to resolve coconut isozymes

Enzymes resolved	Staining solutions	Running condition
Peroxidase (*PER*)	Benzidene in 0.2 M Sodium acetate buffer (pH 5.0)	I) Gel Buffer: i) Stacking gel
Esterase (*EST*)	Fast Blue RR & α-napthyl acid acetate in 0.2 M phosphate buffer (pH 6.5)	0.125M Tris-HCl (pH 6.7) ii) Separating gel:
Acid phosphatase (*ACP*)	Sodium α-napthyl phosphate & Fast Black K in 0.2 M Sodium acetate buffer (pH 5.0)	0.375M Tris-HCl (pH 8.8)II) Electrode buffer:0.025M Tris, 0.192M Glycine (pH 8.3)
Polyphenol oxidase (*PPO*)	DOPA in 0.05M Sodium phosphate buffer (pH 7.0)	
Glutamate oxaloacetate Transferase (*GOT*)	Fast Blue BB and pyridoxal 5 phosphate in 0.1 M Tris-HCl buffer (pH 8.5) containing Aspartic acid and α-ketoglutaric acid	
Alcohol dehydrogenase (*ADH*)	NAD in 0.5 M Tris-HCl buffer (pH 7.1)	
Malic dehydrogenase (*MDH*)	NAD in 0.1 M Tris-Malate buffer (pH 7.5)	

To assess the variability in dwarf coconuts, seven palms each in six different cultivars viz. Malayan Orange Dwarf (MOD), Malayan Yellow Dwarf (MYD), Chowghat Orange Dwarf (COD), Chowghat Green Dwarf (CGD), Ganga Bondam Green Dwarf (GBGD) and Gudanjali Dwarf (GDD) were assayed for different enzyme systems.

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 analyzed. 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.

Results and Discussion

Differences in isozyme banding pattern were observed both between and within ecotypes. There were no specific differences in banding pattern of different varieties.

Esterase: Seven *EST* bands were observed in dwarf cultivars (Table 2), of which four bands (band 1, 2, 5 and 6) were polymorphic. Allelic frequencies of *EST* isozymes were highest in GDD and GBGD (1.00) and least in MYD (0.71).

Table 2. Table of Mean : Allelic Frequencies for Allozymes in six dwarf coconut cultivars

Cultivar / Isozymes	COD	MYD	CGD	MOD	GDD	GBGD	Mean	Mean Rf Value
Esterases	0.96	0.71	0.82	0.86	1.00	1.00	0.89	0.38
Peroxidases	0.71	0.71	0.71	0.71	0.57	0.43	0.59	0.23
Polyphenol oxidases	0.37	0.31	0.31	0.25	0.48	0.30	0.34	0.53
Malate dehydrogenase	0.67	0.67	0.67	0.64	0.67	1.00	0.72	0.43
Alcohol dehyrogenase	1.00	1.00	1.00	1.00	0.50	0.50	0.83	0.44
Glutamate-Oxaloacetate transaminases	0.86	0.67	1.00	0.75	0.75	0.75	0.80	0.36

Peroxidase: Seven bands were observed and only four bands (band 1, 2, 4 and 6) were polymorphic (Table 2). Band number 1 and 2 (Rf value 0.12 and 0.15) were specific to GDD and observed only in some trees of this ecotype. Band number 6 was absent in GDD and GBGD while band 4 was absent in GBGD and present in very low frequency in GDD. Cultivars COD, MYD, CGD and MOD had similar peroxide profiles and highest allelic frequency (0.71) whereas allelic frequency was least in GBGD (0.43).

Polyphenol oxidase: Differences in the banding pattern were observed both between and within cultivars. Eighteen PPO bands were observed (Table 2); of them only band numbers 3 and 5 were monomorphic. Allelic frequency was highest in GDD (0.48) and least in MOD (0.25). Bands 1 and 2 appeared to be specific to GDD, band 10 to MYD, band 12 to MOD, band 15 to COD and band 18 to CGD and were observed only in some of the palms of these cultivars. In addition band 6 was seen only in CGD palms and a few palms of MOD.

Table 3. Polymorphic index of dwarfs using *EST, PER, PPO, MDH, ACP, ADH* and *GOT*

Cultivar/Enzyme	*EST*	*PER*	*PPO*	*MDH*	*ACP*	*ADH*	*GOT*	Mean
COD	0.029	0.000	0.068	0.000	0.000	0.000	0.061	0.023
MYD	0.000	0.000	0.077	0.000	0.000	0.000	0.055	0.019
CGD	0.064	0.000	0.023	0.000	0.000	0.000	0.000	0.012
MOD	0.070	0.000	0.065	0.020	0.000	0.000	0.000	0.022
GDD	0.000	0.087	0.084	0.000	0.000	0.000	0.000	0.024
GBGD	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.003
Average	0.027	0.015	0.057	0.003	0.000	0.000	0.019	0.017

Malate dehydrogenase: Polymorphic banding pattern was observed. Six bands were observed in GBGD. Band number 4 (Rf 0.50) and 6 (Rf 0.55) were specific to the cultivar GBGD (Table 2). Band number 1 (Rf 0.25) showed very low polymorphism and was absent in some samples of the ecotype MOD. Allelic frequency was highest in GBGD (1.00) and least in MOD (0.64).

Acid phosphatase: A single monomorphic band (Rf 0.12) was observed in all cultivars.

Alcohol dehydrogenase: Two bands were observed, with the faster band (Rf 0.47) being polymorphic (Table 2). This band was absent in cultivars GBGD and GDD and therefore, the allelic frequency was low in these cultivars.

Glutamate oxaloacetate transaminase: Four *GOT* bands were observed (Table 2) and two bands (band no. 1 and 2) were polymorphic. Allelic frequency was highest in CGD (1.00) and least in MYD (0.67).

Intra/Inter population polymorphism : Of the six dwarfs studied, GDD showed (Table 3) highest enzyme polymorphism (0.024) while GBGD showed least enzyme polymorphism (0.003). Within each enzyme system, differences in intra population variation were observed between cultivars except for *ACP* and *ADH*. For *EST*, among the dwarfs, MOD showed highest intra-population variation (0.070), while MYD, GDD and GBGD did not show any intra-population variation. For *PER*, only GDD showed intra-population variation (0.087). For *PPO*, GDD showed highest intra-population variation (0.084), while both CGD and GBGD had least intra-population variation (0.023). For *MDH*, only MOD showed intra-population variation (0.020). For *GOT*, COD showed higher intra-population variation (0.061), while CGD, MOD, GDD and GBGD did not show any intra-population variation.

A comparison of polymorphic indices of the dwarfs (Table 3), indicates that among seven enzyme systems, *PPO* showed highest intra-population polymorphism (0.057) followed by *EST* (0.027), *GOT* (0.019), *PER* (0.015) and *MDH* (0.003). *ACP* and *ADH* did not show any intra-population polymorphism. Fernando and Gajanayake (1997) studied six enzyme systems in coconut breeding populations and found that only *EST* and *PER* gave a polymorphic pattern. Studies on isozyme polymorphism in coconut by other workers (Meunier, 1992; Asmono *et al.*, 1993; Fernando and Gajanayake, 1997) indicated high polymorphism in Talls as compared to Dwarfs. Cardena *et al.* (1998) were able to distinguish four cultivars and two hybrids based on the leaf protein polymorphism. Canto-Canche *et al.* (1992) detected slight differences in protein/isozyme profile of Malayan dwarfs and Atlantic Talls. However, they were unable to distinguish between the three Malayan dwarfs (MYD, MGD and MOD). None of the earlier workers have attempted to study the available variability in dwarf coconut cultivars. The present study indicates existence of isozyme variability in dwarf coconut cultivars, though at a low level. It also reflects on the breeding behaviour of the Dwarf populations. Generally Dwarf cultivars are self-pollinated due to their pollination behaviour as there is overlapping of male and female phases within a spadix. The cultivar GDD showed highest polymorphism, while, GBGD showed least polymorphism, indicating that the population GBGD is more homogenous than GDD. Substantial variability appears to have persisted in GDD population possibly due to differential selection pressure. It was observed that though CGD and GBGD nuts are morphologically similar in colour they are genetically dissimilar as indicated by their polymorphic index. Thus, the isozyme studies in Dwarf coconut populations reveal that isozymes can be successfully used as markers for genetic studies.

References

Asmono, D., Hartana, A., Guhardja, E. and Yahya, S. 1993. Genetic diversity and similarity of 35 coconut populations based on isoenzyme banding pattern analysis. *Bulletin Pusat Penelitian Kelapa Saniet,* **1:** 39-54.

Canto-Canche, B., Quintal-Salazar, E. and Villanueva, M.A. 1992. Biochemical markers of variety in *Cocos nucifera* L. from Yucatan. *Turrialba.* **42:** 375-381.

Cardena, R., Oropeza, C. and Zizumbo, D. 1998. Leaf proteins as markers useful in the genetic improvement of coconut palms. *Euphytica.* **102:** 81-86.

Davis, B.J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121:** 404-427.

Fernando, W.M.V. and Gajanayake, G. 1997. Patterns of isozyme variation in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantations, Recherche, Developpement.* **4:** 256-261.

Gottlieb, L.D. 1981. Electrophoretic evidence and plant populations. In: *Progress in Phytochemistry* (eds). L. Reinhold, J.B.Harbone, T. Swain, Vol. 7, Pergamon Press, Oxford, pp.1-46.

Hengky, N. and Hartana, A. 1994. Isoenzymes in development and differentiation of coconut palms. *Journal Biologi Indonesia,* **1:** 35-39.

Meunier, J. 1992. Appendix. XV. Genetic diversity in coconut. In: *Coconut Genetic Resources*, Papers of an IBPGR Workshop, Cipanas, Indonesia, 8-11, Oct. 1991. *International Crop Network Series No. 8.* International Board for Plant Genetic Resources, Rome, pp.59-64.