

Diversity of esterase and peroxidase isozymes in a segregating population of coconut

C.G.N. Namboothiri, V. Niral and V.A. Parthasarathy*

Division of Crop Improvement, Central Plantation Crops Research Institute, Kasaragod 671 124

ABSTRACT

Esterase and peroxidase isozyme profiles in the F_2 populations of D X T coconut hybrids were compared with that of the parents and F_1 hybrids. The WCT parent showed greater number of bands than the dwarf COD parent for both enzyme systems. The F_1 s did not totally resemble either of the parents, indicating their hybrid nature. The F_2 palms showed differential segregation and among the 90 F_2 palms, five F_2 palms showed banding pattern similar to that of tall WCT parent (5.6%), while six F_2 palms were similar to the dwarf COD parent (6.6%). The rest of the palms showed a different pattern of banding than the parents, with 10% of the F_1 progenies of two F_1 families, showing similarities in the banding pattern to their hybrid parents (F_1 HB 96 and F_1 HB 136). The differential banding pattern is an indication of the segregation and recombination resulting in variability in the F_2 population. This study highlights the importance of isozyme analysis for hybridity testing and studying the segregation pattern of the F_2 population.

Key words: Isoenzyme, clustering, F_1 hybrid, F_2 population.

INTRODUCTION

The heterosis of the F_1 generation in coconut (*Cocos nucifera* L.) is well studied. However, the research on the behaviour of F_2 generation of the hybrids has not received much attention and there are only few reports on studies in the F_2 generation in coconut. The segregation for vegetative, reproductive and yield characters of the palms is of great importance with regard to selection for utilization in further breeding. The study of the segregation pattern in the F_2 population of D x T hybrid crosses will provide the information about the consequences of selfing on the characters of economic importance and determine the possibility of developing lines combining the favourable characters of dwarf and tall varieties in order to produce a viable alternative to the hybrids. The study of enzyme polymorphism provides useful information about the genetic diversity. Plant morphological characters have been the universally undisputed descriptors for genotype characterization. But these descriptors, besides being limited in number, are time consuming and less reliable owing to their interaction with environment in which the variety is grown. Therefore, isozyme and molecular markers are better alternatives for genotype characterization. Niral (8) has enumerated on the utilization of biochemical markers for studying genetic diversity in plantation crops. Geethalakshmi *et al.* (3, 4, 5, 7), Niral *et al.* (9) and Parthasarathy *et al.* (10) studied coconut genetic diversity at the protein

and isozyme levels using different tall, dwarfs and hybrids in coconut and elucidated the loci governing them and also reported the level of heterozygosity. The present study was undertaken to study the isozyme profile in the F_2 populations and confirm the process of segregation of genotypes in the F_2 generation of D x T coconut.

MATERIALS AND METHODS

The study was carried out at Central Plantation Crops Research Institute, Kasaragod to assess the extent of segregation by studying isozyme banding pattern in the F_2 populations of Chowghat Orange Dwarf x West Coast Tall (COD x WCT) coconut hybrid, in the name of Chandrasankara during 1985. Ninety F_2 palms of three F_1 families of COD x WCT (HB 96, HB 111 and HB 136), planted in a randomized block design with three replications during 1992 were used for the study. For comparison, the WCT and COD palms and the available parental F_1 palms (HB 96 and HB 136) were used for the study.

The spindle leaf was used for extraction of enzymes as per the standardized procedure and used for PAGE. The gels were stained for detection of peroxidase (PER) and Esterase (EST) isozyme banding profiles as per standard protocols detailed by Parthasarathy *et al.* (10). The Rf value was calculated for the individual bands. The information on presence and absence of band was converted into binary data, with 0 for absence and 1 for presence of a band. The binary data was used for obtaining similarity matrix based on Jaccard coefficient (Rohlf, 12). The similarity

*Corresponding author's address: Director, IISR, Marikkunnu, P.O., Calicut;
E-mail: parthasarathy@yahoo.com ; parthasarathy@spices.res.in

matrix was used for cluster analysis and a dendrogram was drawn based on unweighed pair group arithmetic average (UPGMA) method using NTSYS software.

RESULTS AND DISCUSSION

Polymorphic banding pattern was observed for peroxidase and esterase in the individuals of the F_2 populations and differences in the banding pattern within the families were observed. Four bands were observed for peroxidases (Fig. 1), of which band 1 (0.049), band 2 (0.066) and band 3 (0.10) were

polymorphic and the fourth band with Rf value 0.148 was present in all the palms. In COD, two bands with Rf values 0.066 and 0.148 were observed while in WCT three bands with Rf values 0.049, 0.10 and 0.148 were observed. In the case of F_1 HB 96, only two bands were observed with Rf values 0.049 and 0.148. Three bands with Rf values 0.049, 0.1 and 0.148 were observed in F_1 HB 136. The F_2 individuals showed a minimum of four and maximum of six bands for esterases (Fig. 2). The first, third, fifth and sixth bands with Rf values 0.30, 0.37, 0.43 and 0.47, respectively,

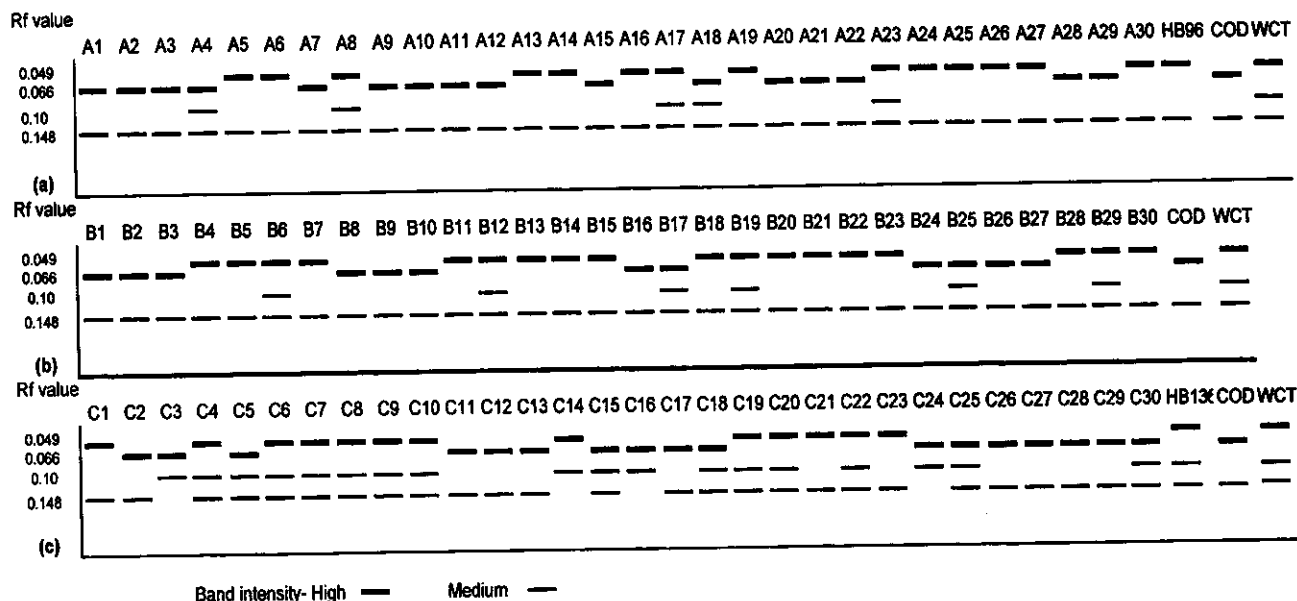


Fig. 1. Zymogram of peroxidase profiles in F_2 population of: (a) HB 96, (b) HB 111 and (c) HB 136.

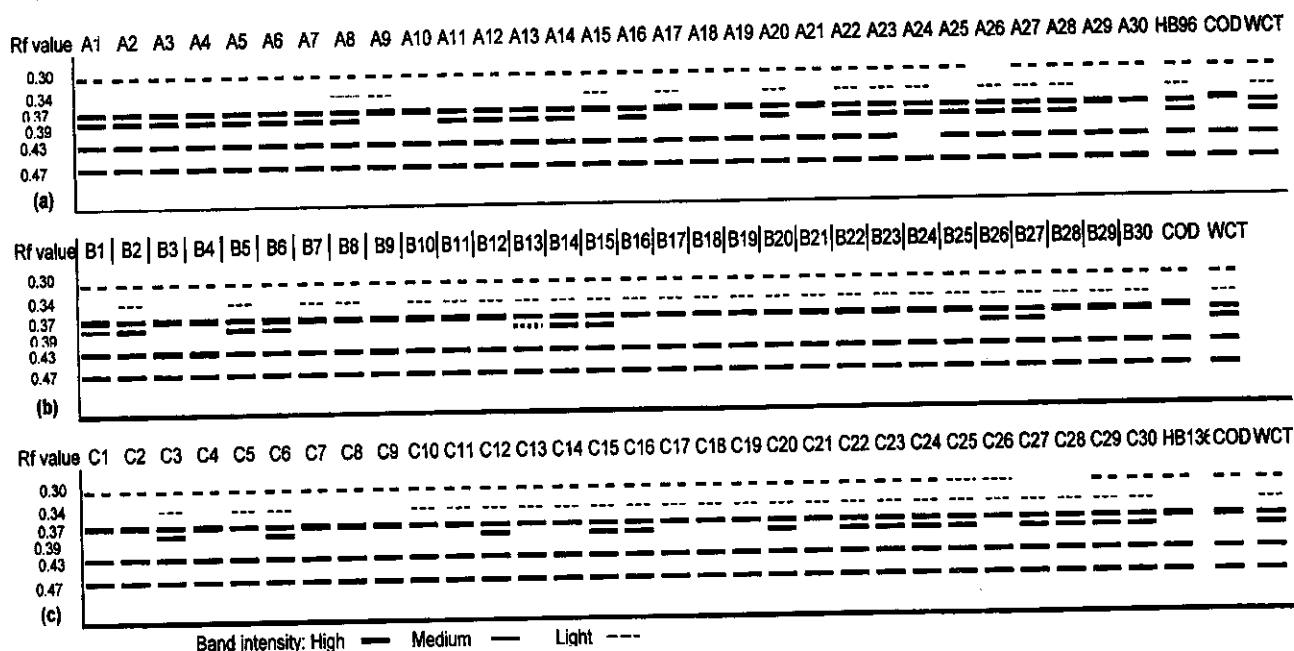


Fig. 2. Zymogram of esterase profiles in F_2 population of: (a) HB 96, (b) HB 111 and (c) HB 136.

were present in all the F_1 progenies. The second and fourth bands with Rf values 0.34 and 0.43 were polymorphic. In COD, only four bands with Rf values 0.3, 0.37, 0.43 and 0.47 were observed while in WCT six bands with Rf values 0.30, 0.34, 0.37, 0.39, 0.43 and 0.47 were observed. In the case of F_1 HB 96, six bands were observed and in the case of F_1 HB 136 four bands with Rf values 0.30, 0.37, 0.43 and 0.47 were observed.

In coconut, the tall palms are predominantly out breeding and the dwarf palms are predominantly in breeding (Child, 1). The tall coconut is the commercially grown type and each individual is a unique heterozygous genotype than the dwarfs. The chances of self-pollination in the dwarf brings about homozygosity in dwarfs. The parent WCT is highly cross pollinating when compared to the dwarf COD parent (Ratnambal *et al.*, 11). In the present study, on scoring the banding patterns, it was observed that WCT showed the maximum number of bands for peroxidase and esterase while the dwarf COD parent showed least number of bands for both enzymes. Fernando and Gajanayake (2), Geethalakshmi *et al.* (6), and Niral *et al.* (9) reported high levels of polymorphism in tall cultivars as compared to dwarfs. Parthasarathy *et al.* (10) reported that the F_1 hybrids clustered closer to the female parent. In the present study, though the banding pattern of the F_1 hybrids was not exactly intermediate between the parents, still heterozygosity was observed with respect to the two enzyme systems studied. The F_1 's did not totally resemble either of the parents, indicating their hybrid nature. The F_1 hybrids HB 96 and HB 136, showed differences between each other in the banding pattern for both enzymes. The F_1 HB 96 showed similarity in banding pattern with COD for peroxidase and with WCT for esterase, while the F_1 hybrid HB 136 showed similarity with WCT for peroxidase and with COD parent for esterase. Therefore, isozyme markers can be used as a marker for testing hybridity at the seedling stage in the nursery for identification of hybrid seedlings. This result also indicates the heterozygosity of the parents for the different enzyme loci and the need for stringent selection of parental palms for obtaining more uniform F_1 populations.

Cluster analysis based on the similarity matrix generated from the EST and PER banding data produced a dendrogram with two major groups, one towards the WCT side and the other towards the COD side (Fig. 3). Within each major group, 10 clusters were formed. The two F_1 hybrids were towards the WCT side. The distribution of the F_2 progenies was almost equal in both the major groups. The first major group contained 14 palms of HB 96, 19 palms of HB 111 and 13 palms of HB 136. The second major group

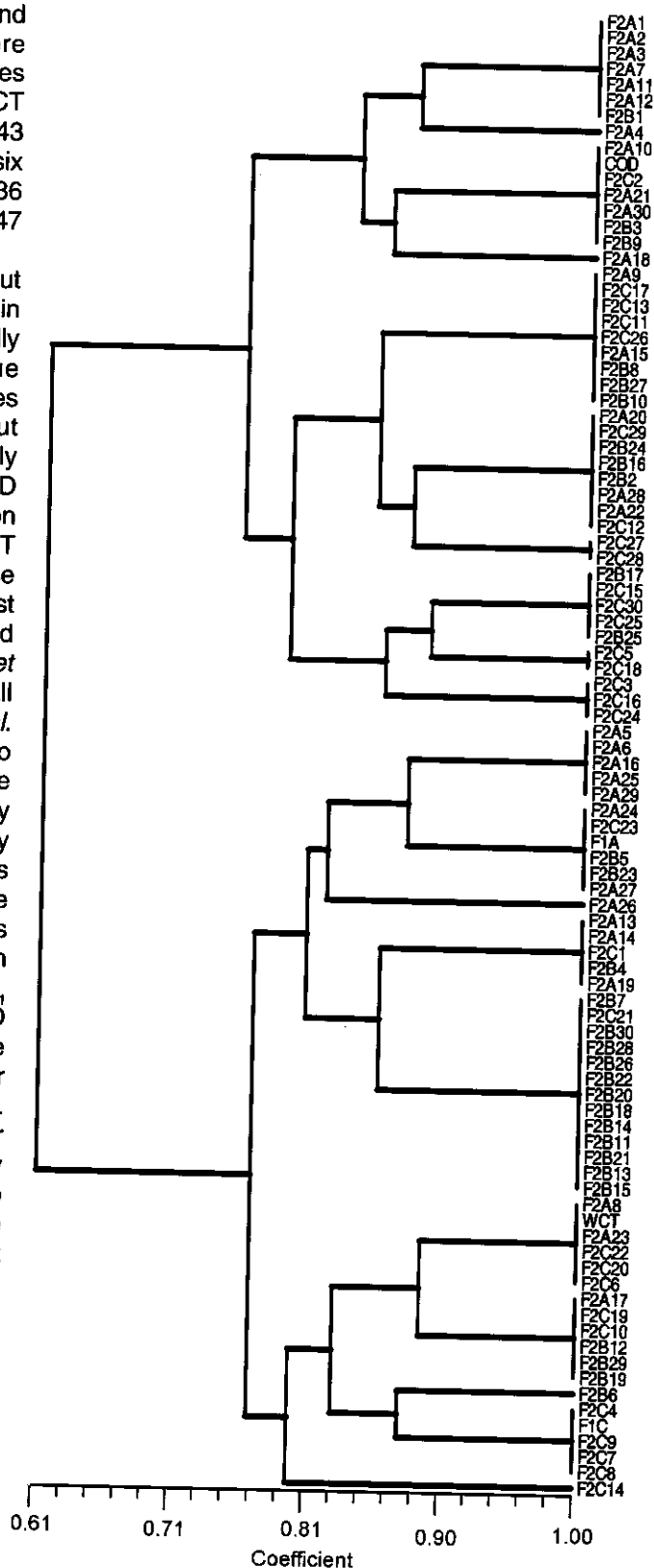


Fig. 3. Dendrogram showing genetic relationship in the F_2 populations of coconut.

included 16 palms of HB 96, 11 palms of HB 111 and 17 palms of HB 136. Five palms formed single accession clusters and two of them were towards the COD side and three of them were towards WCT side. Further, five F_1 progenies were typically similar in banding pattern to that of tall WCT parent (5.6%), while six F_1 progenies were similar to dwarf COD parent (6.6%) showing a clear 1:1 distribution of F_1 progenies towards the parents on the basis of isozyme study. Out of 60 F_1 progenies of two F_1 families (F_1 HB 96 and F_1 HB 136), six F_1 progenies (10%) in the F_2 generation showed similarities in the banding pattern to their hybrid parents (F_1 HB 96 and F_1 HB 136). Differential segregation was seen in the rest of the F_1 progenies as evidenced by the different pattern of banding as shown in the zymogram. This differential banding pattern is an indication of the segregation and recombination resulting in the variability of the F_2 population. This further indicates that F_2 palms can't be utilized for commercial seed production, as the resulting progenies will be too variable.

The F_1 hybrids clustered in the WCT group. This clustering of the F_1 hybrids towards the male WCT parent that was observed in this study is in contrast to the report of Parthasarathy *et al.* (10). These contradictory results may be due to the differential genetic makeup of the parents used for crossing. The study of peroxidase and esterase isozymes profiles in the F_2 populations of coconut has indicated the occurrence of differential segregation and recombination, and also the utility of isozyme markers in testing hybridity.

REFERENCES

1. Child, R. 1974. *Coconuts*, 2nd edn., Longman Group Ltd., London. 335 p.
2. Fernando, W.M.U. and Gajanayake, G. 1997. Patterns of isozyme variations in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantations Recherche Develop.* 4: 256-61.
3. Geethalakshmi, P., Nirai, V. and Parthasarathy, V.A. 2000. Allozyme variation in populations of dwarf coconut cultivars. In: *Abstracts National Seminar on Recent Advances in Plant Biology-an Interdisciplinary Approach to Unravel Plant Functions* (Eds., Rajagopal, V., Naresh Kumar, S., Anuradha Upadhyay and Nirai, V.). 3-5 February 2000, C.P.C.R.I., Kasaragod. 10 p.
4. Geethalakshmi, P., Nirai, V. and Parthasarathy, V.A. 2004. Allozyme variation in population of dwarf coconut cultivars. *J. Plantn. Crops*, 32: 13-15.
5. Geethalakshmi, P., Nirai, V. and Parthasarathy, V.A. 2005a. Characterization of coconut germplasm based on protein polymorphism. *Indian J. Hort.* 62: 118-21.
6. Geethalakshmi, P., Parthasarathy, V.A. and Nirai, V. 2005b. Genetic Diversity among coconut (*Cocos nucifera* L.) genotypes using isozymes. *Asian J. Plant Sci.* 4: 678-83.
7. Geethalakshmi, P., Nirai, V. and Parthasarathy, V. A. 2006a. Interpopulation variation in coconut. *Indian J. Hort.* 63: 439-45.
8. Nirai, V. 1999. Biochemical markers in characterizing genetic diversity. In: *Improvement of Plantation Crops* (Eds., Ratnambal, M.J., Kumaran, P.M., Muraleedharan, K., Nirai, V. and Arunachalam, V.), C.P.C.R.I., Kasaragod. pp.164-70.
9. Nirai, V., Geethalakshmi, P. and Parthasarathy, V.A. 2007. Intra-population allelomorphisms in tall and dwarf population of coconut. *Acta Bot. Croatica* 66: 35-42.
10. Parthasarathy, V.A., Geethalakshmi, P. and Nirai, V. 2004. Cluster analysis of coconut cultivars and hybrids using isozymes. *Acta Botanica Croatica* 63: 69-74.
11. Ratnambal, M.J., Nair, M.K., Muralidharan, K., Kumaran, P.M., Rao, E.V.V.B. and Pillai, R.V. 1995. *Coconut Descriptors* Part.1. Central Plantation Crops Research Institute, Kasaragod.
12. Rohlf, J.F. 1993. Numerical Taxonomy System (NTSYS). Exeter Software. Version 1.70. Applied Biostatistics Inc. New York.

Received: February, 2007; Revised: February, 2008;
Accepted : March, 2008)