

Polymorphism of isoperoxidases in coconut cultivars

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ABSTRACT

Banding pattern of spindle leaf isoperoxidases were studied in six dwarf and eight tall coconut varieties using polyacrylamide gel electrophoresis. Differences in banding patterns were observed both within and between cultivars. Only Gudanjali Dwarf showed intrapopulation polymorphism among dwarfs while, Java Tall showed highest intrapopulation variation among tall.

Key words: Coconut, Isoperoxidase, polymorphism.

INTRODUCTION

Coconut (*Cocos nucifera* L.) an economically important palm belongs to the family Palmae. It is a monotypic genus with no known wild forms. Hence, the variability exists only within local types/populations. Characterization and evaluation of coconuts by morphological and agronomic traits (Akpan *et al.*, 1; Sugimura *et al.*, 11) do not provide an accurate measure of genetic diversity because many characters exhibit complex inheritance and are influenced by both environmental and genetic factors. Parthasarathy *et al.* (8) used isozyme to analyse the coconut cultivars. Since isozymes are codominant in nature and cause no deleterious changes in plant phenotypes through recessiveness or pleiotropy, it is the best marker for genetic studies. Although isozymes have a lot of advantages, such as inexpensive, non-destructive etc., little work has been done on polymorphic isozymes like peroxidase (PRX) in coconut (Cardena *et al.*, 3; Carpio, 4; Fernando and Gajanayake, 5; Jayalekshmy, 6). In the present study, different tall and dwarf coconut ecotypes were screened for the analysis of intrapopulation variation using PRX.

MATERIALS AND METHODS

Spindle leaves of adult palms gave good consistent pattern. So this tissue was used in the present studies. To study the variation, four to seven palms from six dwarf cultivars namely, Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Chowghat Green Dwarf (CGD), Malayan Orange Dwarf (MOD), Gudanjali Dwarf (GDD) and Gangabondam Dwarf (GBGD), and eight tall cultivars namely, West Coast Tall (WCT), Java Tall (JVT), Laccadive Ordinary Tall (LCT), Philippine Ordinary Tall (PHOT), Laccadive Micro Tall (LMT), Kappadam Tall (KPDT), Andaman Ordinary Tall (ADOT) and San Ramon Tall (SNRT) were selected.

Two hundred fifty milligrams of polyvinyl pyrrolidone (PVP) was added during the extraction to 1 g leaf tissue in order to prevent the damage caused by phenolics. Tris-HCl buffer (pH 6.8 with 10% glycerol and 0.1% β -mercaptoethanol) was used for the extraction. Extracts, after the centrifugation (10,000 rpm for 20 min. at 4°C) were used for electrophoresis using discontinuous buffer system (Ornstein, 7). Polyacrylamide gel (7.5%) was used and electrophoresis was carried out using constant current. The gels were stained using benzidine in 0.2 M sodium acetate buffer of pH 5.0 (Veech, 12). Variability was calculated by considering each band as an allele. Allelic frequency was calculated using the formula:

$$\text{Allelic frequency} = \frac{\text{No. of samples in which band was present}}{\text{Total no. of samples analyzed}}$$

Polymorphic index was computed (Rajanaidu *et al.*, 10) as follows:

$$\text{Polymorphic index, PI} = \sum (1 - P_i) / N$$

where, P_i = allele (band) frequency and N = number of bands.

RESULTS AND DISCUSSION

Peroxidase exhibited polymorphic banding pattern for tall and dwarfs. Differences in the banding pattern was observed both between and within cultivars. Seven bands were observed in dwarfs, while only six bands were observed in tall. Band with R_f value 0.32 was present only in the dwarf populations. In dwarfs, only four of the seven bands (band No. 1, 2, 4 and 6) were polymorphic. Allelic frequency was highest in COD, MYD, CGD and MOD (0.70), while it was least in GBGD (0.43). Only GDD showed polymorphism among dwarf cultivars studied (Table 1). In tall cultivars, only band No. 5 of the six bands were monomorphic. Allelic frequency was highest in ADOT (0.78) and least in SNRT (0.50). Among tall, JVT showed highest

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Table 1. Allelic frequency and polymorphic index (Pi) for *PRX* in dwarf and tall coconut genotypes.

| Cultivar/Band No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | P.I. |
|-------------------|------|------|------|------|------|------|------|-------|
| Rf value | 0.12 | 0.15 | 0.18 | 0.23 | 0.29 | 0.32 | 0.34 | |
| Dwarf | | | | | | | | |
| COD | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.000 |
| MYD | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.000 |
| CGD | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.000 |
| MOD | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.000 |
| GDD | 0.43 | 0.43 | 1.00 | 0.14 | 1.00 | 0.00 | 1.00 | 0.087 |
| GBGD | 0.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.000 |
| Mean | 0.07 | 0.07 | 1.00 | 0.69 | 1.00 | 0.66 | 1.00 | 0.015 |
| Tall | | | | | | | | |
| WCT | 0.86 | 0.71 | 0.86 | 0.43 | 1.00 | 0.00 | 0.00 | 0.115 |
| JVT | 0.71 | 0.29 | 0.57 | 0.71 | 1.00 | 0.00 | 0.00 | 0.144 |
| LCT | 0.86 | 0.43 | 0.57 | 0.86 | 1.00 | 0.00 | 0.00 | 0.122 |
| PHOT | 0.71 | 0.71 | 1.00 | 0.86 | 1.00 | 0.00 | 0.00 | 0.089 |
| ADOT | 0.50 | 0.50 | 1.00 | 0.67 | 1.00 | 0.00 | 1.00 | 0.120 |
| SNRT | 0.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.000 |
| KPDT | 0.29 | 0.29 | 1.00 | 0.14 | 1.00 | 0.00 | 1.00 | 0.089 |
| LMT | 1.00 | 0.14 | 0.14 | 0.86 | 1.00 | 0.00 | 1.00 | 0.060 |
| Mean | 0.62 | 0.38 | 0.77 | 0.57 | 1.00 | 0.00 | 0.50 | 0.092 |

intrapopulation variation. It was absent in SNRT (Table 1). Among dwarfs, GDD showed maximum numbers of bands (6) for *PRX* (Table 2), while GBGD showed minimum number of bands (3). Among tall, WCT, LCT, KPDT and LMT showed the maximum numbers of bands (6) and SNRT showed the least number (3), which is in tune with the polymorphism shown by the respective cultivars.

Among dwarfs, only GDD showed intra-population variation (0.087). JVT showed highest intra-population variation (0.144), while it was absent in SNRT among tall (Table 2). Among the cultivars, tall showed more polymorphism (0.092) than the dwarfs (0.015) due to the occurrence of more cross-pollination in them. Asmono *et al.* (2) found variation for *PRX* enzymes by starch gel electrophoresis. They found five bands in leaf tissue of coconut. Four cultivars and two hybrids were analysed by using *PRX* by Cardena *et al.* (3). Polymorphism was found for *PRX* by Fernando and Gajanayake (5).

Peroxidase thus provides useful information about genetic diversity and on its structuring between populations. Polymorphism among individuals of tall and dwarf were revealed by *PRX* with six different alleles. This confirms *PRX* as a better isozyme system for the genetic studies. Tall showed more polymorphism than dwarf. It was also seen in other enzyme systems of coconut. Besides these, the results shown by microsatellite markers (Perem *et al.*, 9)

Table 2. Number of bands present in different coconut dwarfs and tall for *PRX*.

| Cultivar/Isozyme | No. of bands |
|------------------|--------------|
| Dwarf | |
| COD | 5 |
| MYD | 5 |
| CGD | 5 |
| MOD | 5 |
| GDD | 6 |
| GBGD | 3 |
| Tall | |
| WCT | 6 |
| JVT | 5 |
| LCT | 6 |
| PHOT | 5 |
| ADOT | 6 |
| SNRT | 3 |
| KPDT | 6 |
| LMT | 6 |

showed the same result. This shows that in tall, due to cross pollination, high genetic variation exists and in dwarf due to inbreeding nature low genetic diversity is present. The result was supported by the work of Fernando and Gajanayake (5) in which the level of polymorphism was high in tall. One additional band

was seen in dwarfs, except in GDD and GBGD. This may be the representative band for dwarfness. It also shows that GDD and GBGD show special breeding behaviour than the rest of the dwarfs. Band No. 1 and 2 were present only in GDD among dwarfs, but these bands were present in all tall except SNRT. This result indicates that, due to cross pollination, GDD is showing more heterogeneity inclining towards tall. Morphologically also, GDD shows distinct fruit shape, confirming the above statement. In SNRT these bands were absent. It indicates that SNRT is shifting towards dwarfness due to the result of its inbreeding behaviour. Band No. 4 (Rf value, 0.23) was absent in GBGD among dwarfs and SNRT among tall indicating their heterogeneity from rest of the dwarfs and tall. All these observations indicate that *PRX* can be successfully used in the study of genetic variation and cultivar characterization.

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REFERENCES

1. Akpan, E.E.J. 1994. Evaluation of tall coconut (*Cocos nucifera* L.) genotypes within the Nigerian coconut germplasm bank. *Oleagineux*. **49**: 13-20.
2. Asmono, D., Hartana, A., Guhardja, E. and Yahya, S. 1993. Genetic diversity and similarity of 35 coconut populations based on isoenzyme banding pattern analysis. (Diversitas dan kemiripan genetic 35 populasi kelapa berdasarkan analisis pola pita isozirn). *Buletin Pusat Penelitian Kelapa Sawit*. **1**: 39-54.
3. Cardena, R., Oropeza, C. and Zizumbo, D. 1998. Leaf proteins as markers useful in the genetic improvement of coconut palms. *Euphytica* **102**: 81-86.
4. Carpio, C.B. 1982. Biochemical studies of *Cocos nucifera* L. Kalikasan. *Philippines J. Biol.* **11**: 319-38.
5. Fernando, W.M.U. and Gajanayake, G. 1997. Patterns of isozyme variations in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantations Res. Develop.* **4**: 256-61.
6. Jayalekshmy, V.G. 1999. Genetic finger printing in coconut cultivars. *Proc. Eleventh Kerala Science Congress*, Kasaragod, February-March 1999. pp. 200-4.
7. Ornstein, L. 1964. Disc electrophoresis I. Background and theory. *N.Y. Acad. Sci.* **121**: 321-49.
8. Parthasarathy, V.A., Geethalakshmi, P. and Niral, V. 2004. Analysis of coconut cultivars and hybrids using isozyme polymorphism. *Acta Bot. Croatia* **63**: 69-74.
9. Perera, L., Russell, J.R., Provan, J. and Powell, W. 2001. Levels and distribution of genetic diversity of coconut (*Cocos nucifera* L., var. *typica* form *typica*) from Sri Lanka assessed by microsatellite markers. *Euphytica* **122**: 381-89.
10. Rajanaidu, N., Sapurah, R., Rao, V., Zakri, A.H. and Embi, Md. Noor 1993. Isoenzyme variation in Nigerian oil palm (*Elaeis guineensis*) germplasm. *Proc. 1993 ISOPB International Symposium on Recent Developments in Oil Palm Tissue Culture and Biotechnology*. Kuala Lumpur, Malaysia, 24-25 September.
11. Sugimura, Y., Itano, M., Salud, C.D., Otsuji, K. and Yamaguchi, H. 1997. Biometric analysis on diversity of coconut palm: cultivar classification by botanical and agronomic traits. *Euphytica* **98**: 29-35.
12. Veech, J.A. 1969. Localization of peroxidase in infected tobacco susceptible and resistant to black shank. *Phytopath.* **59**: 566-71.

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