B-12:Patterns of isozyme variations in *Cocos nucifera*

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At present the evaluation and characterization of coconut germplasm is carried out only on the basis of morphological and physiological traits. The tall form of coconut which is the commercially grown type is predominantly outbreeding and each individual is a unique heterozygous genotype which justifies the importance of evaluating at the genotypic level. Isozymes are accepted as the best descriptors of genetic variability since they are codominant and also exhibit very low levels of environmental interactions. Therefore isozyme studies would help to elucidate the genetical structure of coconut populations which would assist in adjusting the sampling strategies and would aid in screening valuable genotypes for breeding purposes.

Young actively growing leaf tissues obtained from the most recently opened fronds of palms and haustorium tissues were used for the extraction of enzymes. The extraction buffer consisted of 0.05M Tris (pH 7.5) with 50 μl 2- Mercaptoethanol /50 ml. The samples were crushed under chilled conditions in proportions of 1.5 g of finely cut tissues with 2.5 ml of extraction buffer and 40 mg of Polyvinylpolypyrillidone (PVPP). A clear supernatant was obtained and was absorbed onto 3 mm wide paper wicks

which were then placed on a 12.5% horizontal starch gel. Electrophoresis was carried out at a constant voltage of 200 V until the front had migrated about 9 to 10 cm from the origin. The gel was prepared with 32 g of hydrolysed potato starch (Sigma) and 225 ml of Tris citrate (0.05M, pH 8.3) and 25 ml of Lithium borate
(0.02M; pH 8.3).

The staining procedures were carried out according to Wendal and Weeden (1989) for the isozyme systems Alcohol dehydrogenase (ADH) Esterase (EST) and Peroxidase (PER).

Leaf tissues showed the maximum polymorphism and the banding pattern was clear than the haustorium tissues for all 3 enzyme systems used. Considering the zones of activity for EST isozymes, it could be surmised that 2 different loci are expressed for leaf and haustorium tissues. The outer covering of the haustorium had a higher concentration of enzymes than the inside part with a darker set of bands appearing after electrophoresis for esterase isozyme. The stage of maturity of the leaf tissues was identified to be very important in obtaining a clear resolution in the banding patterns. Slightly matured leaf tissues which contain a certain amount of chlorophyll gave best results following electrophoresis.

Individuals of the Tall form showed polymorphism for the esterase enzyme system with a maximum of 5 different alleles. Individuals with homozygous banding pattern (single bands) were low in frequency within the Tall form of coconut but were more frequently found in the dwarf type. However common bands appear for both dwarf and Tall forms at this enzyme system with a differentiation possible only among the individuals. A difference in the frequency of alleles was observed among different coconut populations.

Polymorphism among individuals of the Tall form was revealed for the peroxidase system with 2 different alleles. One allele was found to be fixed in most individuals whilst only a few showed the other allele.

No polymorphism was observed among the individuals for the dehydrogenase enzyme system.

A method for extraction of leaf isozymes in coconut was established along with modifications in the appropriate buffer systems for best resolution of the banding patterns. Among the 3 isozymes EST, ADH and PER, EST showed

the maximum polymorphism among individuals with 5 different alleles. The difference in the allele frequency among various populations indicates the possibility of estimating the genetic diversity existing in the germplasm and also the possible linkage of certain allelic forms to important quantitative characters.

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