ISOZYME ANALYSIS

OF PLANTLETS FROM SEVEN CLONES OF OIL PALM (*Elaeis guineensis* Jacq.)

Keywords: Starch gel electrophoresis, isozymes, *Elaeis guineensis*, oil palm, clones, plantlets, glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leusine aminopeptidase (LAP), phosphohexose isomerase (PHI), shikimate dehydrogenase(SHDH).

CHEE SENG OOI*; KHENG CHOO WOOI† and AM PAI CHONG*

*Starch gel electrophoresis of shoot extracts from plantlets of seven clones of oil palm (*Elaeis guineensis *Jacq.) was carried out and 12 enzyme systems were examined. Polymorphisms were detected for glutamate-oxaloacetate-transaminase (GOT), isocitrate dehydrogenase (IDH), leucine amino-peptidase (LAP), phosphohexose isomerase (PHI) and shikimate dehydrogenase (SHDH). Within clones, no differences could be detected. Using GOT, IDH, and SHDH as genetic markers, five of the clones could be differentiated, with the remaining two clones showing no isozyme differences between them.*

INTRODUCTION

Somatic embryogenesis using root or leaf tissues has made possible the cloning of elite or high-yielding oil palms, and clones have been planted out to assess their performance (Corley *et al.*, 1982). With the increase in the number of clones developed for further testing, there is also a greater need to identify every clone accurately. Protein differences and in particular, enzyme polymorphisms, have been used to study the genetic variability present in horticultural and plantation crop plants, and where applicable, to differentiate cultivars or material of different origins (Bringhurst *et al.*, 1981; Chevalier, 1988; Ghesquiére, 1985; Parfitt and Arulsekar, 1989; Stegemann and Hussein, 1987). Electrophoresis of pollen samples from oil palms has shown the presence of polymorphic enzymes (Ghesquiére, 1984). These enzymes could be used as biochemical markers for genotyping selected elite palms and the clones derived from them.

The recent report of floral abnormalities among clones planted in the field (Corley *et al.*, 1986) is not encouraging for the future of clonal propagation for oil palms as these are perennial trees with long economic life spans, and considerable costs could be incurred before the abnormalities were expressed and detected. The conditions of *in vitro* culture involving a

* Department of Genetics and Cellular Biology, University of Malaya, 59100 Kuala Lumpur, Malaysia.

† Tissue Culture Laboratory, Ebor Research, Sime Darby Plantations, P O Box 147, 41910 Klang, Selangor, Malaysia. (Address for requests for offprints)

callus phase induced by exposure to unnaturally high levels of growth-promoters, and the lengthy period of time required before somatic embryogenesis is finally achieved, have been recognized as probable causes for somaclonal variation in regenerated plants (Larkin and Scowcroft, 1981; Dulieu and Barbier, 1982; Austin and Cassels, 1983). Such somaclonal variation has been demonstrated in many plants and the oil palm is unlikely to be an exception, as such variation would be more likely to occur when clonal propagation is carried out through somatic embryogenesis than when it is achieved by proliferation of adventitious buds, which is just another form of vegetative propagation under sterile conditions.

In this preliminary study, extracts of oil palm plantlets from different clones were analyzed electrophoretically in order to look for intraclonal and interclonal variation. Intraclonal variation would indicate the presence of somaclonal variation at the structural enzyme level while interclonal variation could be useful for clone identification.

MATERIALS AND METHODS

Samples of plantlets (4-8cm tall) from seven different oil palm clones (*Table 1*) were obtained from Sime Darby Plantations, Malaysia. Extraction of soluble enzymes from each plantlet was done by grinding the shoot, including the youngest leaf, with a half volume of 0.005M dithiothreitol at a low temperature. Extracts of pollen, which have high enzymic activity, were included as controls to assess the effectiveness of the extraction and staining procedures for the shoot samples. The extracts were absorbed on to filter paper wicks (9mm × 6mm) that were then inserted into slots cut in the 10% starch gel (Sigma) prepared for horizontal slab electrophoresis. The electrophoresis was carried out at 4°C with the gel sandwiched between cooling plates and a constant voltage of 10 volts per cm applied across the gel (20.0cm × 13.5cm × 0.6cm) for four hours.

Two buffer systems were used, Tris-citrate (TC) pH 6.5 and Tris-EDTA-Magnesium chloride-Maleic anhydride (TEMM) pH 7.4. The TC bridge buffer was prepared by adjusting a 0.25M Tris solution to pH 6.5 with crystalline citric acid. A 1:10 dilution of this buffer was used for gel preparation. To prepare the TEMM bridge buffer, a solution containing 0.1M Tris, 0.1M Maleic anhydride, 0.01M EDTA and 0.01M Magnesium chloride was adjusted to pH 7.4 with 10N Sodium hydroxide. The buffer was diluted 10 times for gel preparation.

After electrophoresis, the gels were sliced and stained for the required enzymes using the stain recipes compiled by Vallejos (1983) with slight modifications. The agar overlay method was used for applying the staining mixtures on the freshly cut gel surfaces. When clear banding patterns had appeared, staining activity was stopped by fixing the gels with 4% acetic acid to prevent overstaining, which can reduce resolution. Overstaining also increases the appearance of secondary bands which could confuse the identification of the genotype. This is particularly true for the isozymes which show strong staining activity and often produce secondary bands after storage.

The TC gels were stained for isocitrate dehydrogenase (IDH, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), glutamate-oxaloacetate-transaminase (GOT, E.C.2.6.1.1), catalase (CAT, E.C.1.11.1.6), leucine aminopeptidase (LAP, E.C.3.4.11.1) and esterases (EST, E.C.3.1.1.2, using both the fluorescent as well as the diazonium method).

The enzymes stained on TEMM gels were phosphoglucomutase (PGM, E.C.2.7.5.1), phosphohexose isomerase (PHI, E.C.5.3.1.9), phosphogluconate dehydrogenase (PGD, E.C.1.1.1.44), malic enzyme (ME, E.C.1.1.1.40), shikimate dehydrogenase (SHDH, E.C.1.1.1.25), and peroxidase (PER, E.C.1.11.1.7). When buffer systems containing citric acid are used, IDH isozymes often appear during the staining for other NADP-requiring enzymes. So, such buffer systems should be avoided for these enzymes.

RESULTS

Good repeatable zymograms were obtained for all the enzyme systems examined. Within clones no differences were observed for these enzymes, but between clones polymorphisms were found for IDH, GOT, LAP, PHI and SHDH. Isozyme patterns observed for the polymorphic systems are shown in *Figure 1* and zymograms for IDH, PHI, SHDH and GOT in

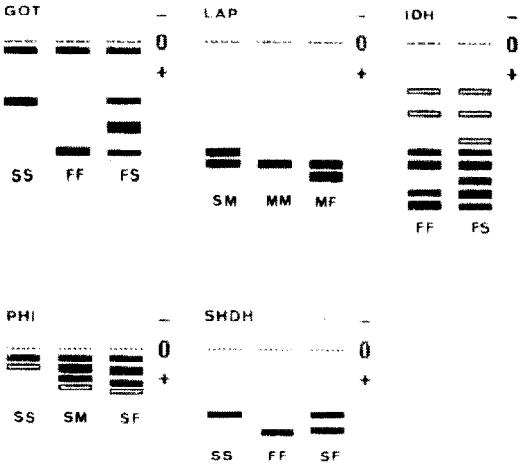

Figure 1. Diagrammatic representation of isozyme patterns observed for five polymorphic enzyme systems in the seven oil palm clones listed in Table 1.

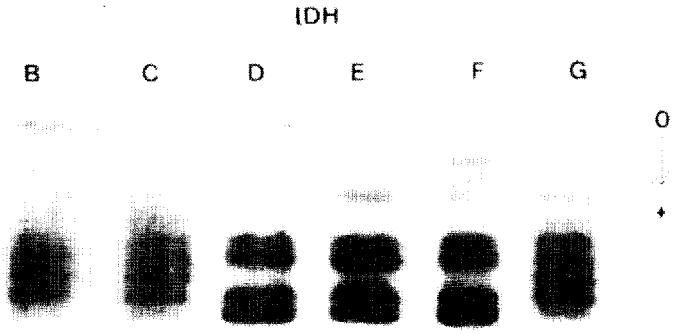

Figure 2. Electrophoretic patterns for isocitrate dehydrogenase in oil palm clones B to G. The identification of the clones is given in Table 1.

Figures 2, 3 and *4*. The putative genotypes of the seven clones for each system are given in *Table 1*.

Pollen extracts were generally observed to show stronger staining activity than shoot extracts for the enzyme systems studied. Secondary bands appeared for some enzymes (usually those with strong staining activity, *e.g.* MDH and PHI) when tissues had been stored frozen for sometime. The zymograms for IDH in fresh shoot extracts (*Figure 2*) showed the presence of two loci, the more anodal of which was polymorphic with two alleles. With prolonged staining, additional bands and zones of activity usually seen in pollen samples also appeared.

The PHI zymograms (*Figure 3*) showed the presence of one polymorphic locus with three alleles and a zone of activity close to the origin. The banding patterns for heterozygotes indicated that the enzyme is dimeric. The bands for PHI appeared very rapidly. Secondary bands and other zones with poor resolution also appeared if the staining process was not stopped. The appearance of these secondary bands could complicate genotype identification in the case of this enzyme.

TABLE 1. PUTATIVE GENOTYPES OF THE SEVEN OIL PALM CLONES SAMPLED

Sample	Clone	Number of plantlets	Enzyme locus				
			GOT	IDH	PHI	SHDH	LAP
A	2274	33	SS	FF	SS	SS	SM
B	2277	27	SS	SF	SS	SS	MM
C	2278	28	SF	SF	SF	SS	MM
D	2280	30	SF	FF	SS	SS	SM
E	2282	26	SF	FF	SS	SF	MM
F	2296	32	SF	FF	SS	SF	MM
G	2299	30	FF	SF	SM	FF	MF

Genotypes are named after the relative mobilities of their alleles: S – slow, M – medium and F – fast.

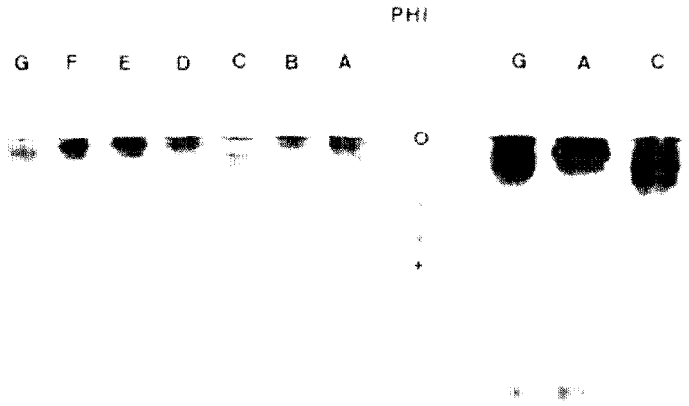

Figure 3. Electrophoretic patterns for phosphohexose isomerase in oil palm clones A to G. The patterns on the right for clones G, A and C are from a separate run and show the three observed patterns in greater detail. The identification of the clones is given in Table 1.

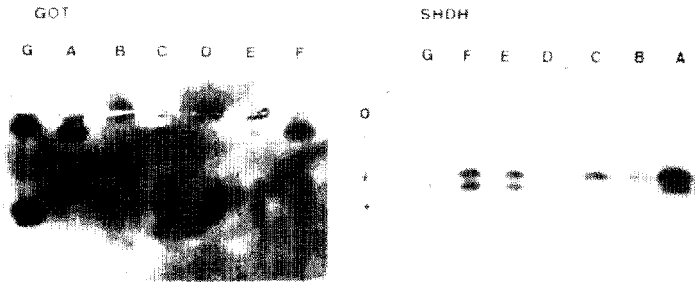

Figure 4. Electrophoretic patterns for glutamate oxaloacetate dehydrogenase and shikimate dehydrogenase in oil palm clones A to G. The identification of the clones is given in Table 1.

Isozymes for GOT showed strong staining activity in pollen and four zones of activity were observed. In shoot extracts, only two zones were consistently stained (*Figure 4*). Polymorphism was observed in the more anodal zone with two alleles detected at this locus.

A single zone of activity was observed for SHDH (*Figure 4*) and LAP. Both are monomeric as indicated by the two-banded heterozygotes observed. Two alleles were observed to be present for SHDH and three for LAP.

The enzyme systems that appeared to be monomorphic in the seven clones were EST, PER, CAT, ME, MDH, PGD, and PGM. Esterases showed different zones of activity when different substrates were used. With umbelliferyl acetate as the substrate, two anodic zones of strong activity were seen, one with high mobility and another close to the origin. When alpha-napthyl acetate was used, resolution was clearest for a cathodic zone near the origin. The anodic zone near the origin was not observed.

High PER enzyme activity was obtained in shoot extracts but not in pollen extracts. Four zones of activity were observed and all the clones showed similar banding patterns. Both

CAT and ME enzymes showed up in the zymograms as single-banded patterns close to the origin.

The MDH zymograms for fresh shoot extracts showed the presence of one strong band that appeared rapidly, but with longer staining resolution became poorer as weaker secondary bands and other zones of activity also appeared. Similar banding patterns were observed for pollen extracts but the bands appeared much more rapidly and stained more darkly.

The PGD enzyme, when stained on TEMM gels, appeared monomorphic with one zone showing a single band, but when TC gels were used, IDH isozymes also appeared in the same zone as PGD, which could then falsely appear to be polymorphic.

The PGM pattern observed in shoot extracts consisted of a single band but in pollen extracts this was also accompanied by a faster-moving secondary band. Pollen extracts also showed a slower zone of activity which was not seen in extracts of fresh shoot tissues.

The polymorphic enzyme systems, when used as markers for the seven clones, show that only two clones (2282 and 2296) are similar in genotype (*Table 1*). The five other clones could be distinguished from one another using GOT, IDH and SHDH as markers. Clone 2299 is different from the others by having unique alleles for LAP and PHI.

DISCUSSION

The polymorphic systems detected in this study have also been reported in earlier work carried out using pollen samples (Ghesquiére, 1984; 1985) from palms of seven geographical origins in Africa. These African palms showed a great deal of genetic variability in their isozymes. The variability obtained in this study is rather small as the materials used represent only a small selected sample; in addition, Malaysian oil palms are known to be derived from a small genetic base. An extensive isozyme survey of the planting materials in Malaysia is needed to determine the actual extent of isozymic variability present. Such a survey would also complement the use of genetic markers for clone identification: more polymorphic systems need to be uncovered to increase the effectiveness of clone identification, especially when the number of clones to be differentiated within a group is increased.

In the present sample of seven clones, five can be differentiated within the group. Inclusion of other additional markers might help to differentiate the remaining two.

The constancy of genotype within clones in the present study is not conclusive evidence for the absence of somaclonal variation at the enzyme level as the sample sizes are rather small. Sample sizes need to be increased, and plantlets regenerated from different lines and subcultures need to be examined. Morphological off-types have also been observed among regenerated plantlets and if such abnormalities have a detectable biochemical basis or relationship, isozyme analyses could be extremely useful for early elimination of potentially undesirable culture lines.

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