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ISOENZYME VARIATION OF ZAIREAN OIL PALM (*Elaeis guineensis* JACQ.) GERMPLASM COLLECTION

Keywords: Isoenzyme; polymorphism; polymorphic index; peroxidase; superoxide dismutase; glutamate dehydrogenase

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Genetic variation was studied in a Zairean oil palm germplasm collection by employing native polyacrylamide gel electrophoresis of isoenzymes. Three isoenzymes extracted from leaves, namely superoxide dismutase (SOD), peroxidase (PRX) and glutamate dehydrogenase (GDH) were investigated. Polymorphism was detected in SOD and PRX systems but there was no genetic variation in GDH system. Polymorphic indices obtained from SOD and PRX systems revealed that the degree of polymorphism increased from the family to the ecotype level. This study also showed that the degree of polymorphism is influenced climatically and geographically.

INTRODUCTION

In the last few decades, most studies on plant population genetics have focused on soya bean, rice, wheat and other important crops, and have contributed significantly towards progress in plant improvement through selection procedures. These studies have made extensive use of morphological traits in determining the genetic structure of populations. However, such traits are strongly linked with environmental factors, which has made these unreliable as indicators of the diversity in a population at the gene level (Marshall and Brown, 1975). Besides, this method is time consuming due to the length of the life cycles of some species. Molecular markers such as Restricted Fragment Length Polymorphism (RFLP) and Randomly Amplified Polymorphic DNA (RAPD), and also isoenzymes have become very popular and commonly used among plant breeders.

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The use of isoenzymes to determine genetic polymorphism developed in the 1970s and 1980s. Although more and more studies have utilized molecular methods, such as RFLP and RAPD (Huff *et al.*, 1993; Isabel *et al.*, 1993 and Marney *et al.*, 1994), isoenzyme technique is considered the most convenient method available for the detection of genetic variation which is close to the DNA level. The ease of handling and lower cost of screening have made it preferable to other methods (Brown, 1978). Isoenzyme studies in oil palm are still scarce and more effort is needed to determine the usefulness of this method for oil palm improvement. The earliest work on isoenzymes in oil palm was traced back to the work done by Ghesquiere (1984), who studied the genetic variability of oil palm, *Elaies guineensis*, from four different geographic origins from West Africa and South-East Asia, using electrophoretic techniques. Using pollen extracts of selfs and cross plants, he had revealed some linkages among the isoenzyme loci and a considerable variability in the populations. Later, the same method was applied to materials from the two other geographic areas of West Africa and similar results were found (Ghesquiere, 1985). A study of *Elaeis oleifera*, a wild species relative of oil palm in the Amazon Basin showed that the genetic diversity of this species is low (Ghesquiere *et al.*,1987).

Intensive studies of genetic variability using measurement of morphological traits (such as height, leaf area, trunk, diameter, *etc.* and yield (number of bunches and bunch weight) have been done on some palm populations such as Surinam *Elaeis oleifera* (Rao *et al.*, 1989) and Nigerian *Elaeis guineensis* (Rajanaidu *et al.*, 1989). The oil content has been shown to be higher in the *E. oleifera* than in the *E. guineensis* (Rajanaidu *et al.*, 1989).

More recently, the RAPD method has been used to determine the genetic variability of African oil palm germplasm from' Zaire, Tanzania and Cameroon (Shah *et al.*, 1994). The RFLP of the rDNA loci of the oil palm has been studied to determine the variation between oil palm populations (Shah *et al.*, 1993). However, in view of its simplicity and cost effectiveness, the isoenzyme method is still commonly used. The objective of the present study was therefore to determine whether isoenzymes can be used as efficient genetic markers to screen large samples and evaluate the germplasm collections of oil palm and subsequently to study the genetic stucture of the different populations.

MATERIALS AND METHODS

A total of 182 plants were sampled from the PORIM/UKM Research station, Bangi. The materials are part of the Zairean oil palm germplasm collection collected by PORIM in 1984 and planted in the station (Rajanaidu, 1986). The samples consist of 13 populations (collections from 13 different locations), in which each population has two families (different bunches from a plant). Seven individuals (progenies germinated from seeds of a single bunch) are available per family. The 13 populations were categorised into five different ecotypes according to their climatic and geographic locations for the purpose of population studies (*Table 1*).

The first fully opened frond was collected for enzyme extraction. Leaflets were cut from the frond and washed before grinding to a powder in liquid nitrogen in a blender. Samples in powder form must be kept in the freezer at –20°C immediately after the grinding. Enzymes were extracted by adapting the method of Ghesquiere *et al.* (1987). The powder sample was ground with the extraction buffer (1g sample/4.0ml extraction buffer) in a mortar at 4°C. The extraction buffer contained 0.1M calcium phosphate at pH7.0, 0.1M L-cysteine, 0.1M L-dithiothreitol and 7% of PVPP. The extraction mixture was then centrifuged at 7000g for 20 minutes at 4°C. The supernatant were immediately electrophoresed or stored at –20°C for later use.

The electrophoresis method was according to the method of Laemmli (1970) with some modifications. A discontinuous native-polyacrylamide gel system which consisted of 7% stacking gel and 10% of separating gel was

TABLE 1. GEOGRAPHIC DESCRIPTIONS OF THE FIVE DIFFERENT ECOTYPES

Ecotype	Population	Geographic descriptions
1	A (18) B (19) C (20) D (22)	Haut-Zaire District Central Zaire Equatorial climate Rainfall 1800-2200 mm Altitude 500 m
2	E (24) F (25) G (26) H (27) I (28)	Equateur District Western Zaire Equatorial climate Rainfall 1800-2200 mm Altitude 500 m Swampy area
3	J (29)	Central Zaire District Southwestern Zaire Humid tropical climate Rainfall 1000-1400 mm Altitude 500 m
4	K (30) L (31)	Kivu District Eastern Zaire Humid tropical climate Rainfall 1000-1400 mm Altitude 2000 m Mountain area
5	M (32)	Bandundu District Southwestern Zaire Humid tropical climate Rainfall 1400-1800 mm Altitude 500 m

used throughout. Vertical migration was performed using a constant voltage of 200V for about five hours at 4°C.

Isoenzyme bands could be detected by using enzyme-specific histochemical stains in which coupled reactions occurred producing the bands. The staining solution for superoxide dismutase (SOD) (EC 1.15.1.1) was adapted from Beauchamp and Fridovich (1971); peroxidase (EC 1.11.1.7)(PRX) was according to Wendel and Weeden, (1989); and glutamate dehydrogenase (EC 1.4.1.2) (GDH) by adapting the method of Scheid *et al.* (1980).

The relative mobility of the isoenzyme band was expressed as its Rf value, which is the distance migrated relative to the distance migrated by the bromophenol blue marker. The frequency of each band was calculated according to the three different levels, namely family, population, ecotype. The frequency values were then used to determine the degree of polymorphism for each level by applying the formula (Marshall and Allard, 1970), that is

$$PI = \sum_{I=1^{i}}^{N} P_i\ (1-P_i i)/N$$

P_i refers to the frequency for i-th band and N is the total of bands that is present in the isoenzyme system. The polymorphic index (PI) could be in the range from 0 to 0.25. The higher the PI value the higher the degree of polymorphism.

RESULTS AND DISCUSSIONS

Although the enzymatic activity in leaves actually is much lower than that in the pollen in *Elaeis guineensis* (Ghesquiere *et al.*, 1987), leaves are still preferable in enzymatic polymorphism studies because of ease of extraction, stability of enzymatic activity and convenient harvesting of leaves compared to pollens. One problem encountered in the oil palm leaf samples is the presence of phenolic compounds. These can be rapidly oxidized to form quinones to inhibit plant enzyme significantly. Oxidation can be prevented by adding reducing agents such as L-dithiothreitol during the extraction (Anderson, 1968).

In this study, a total of 46 bands had been observed in three isoenzyme systems (*Figure 1*). There were 21 bands (Rf = 0.12, 0.14, 0.20, 0.23, 0.32, 0.35, 0.36, 0.37, 0.38, 0.40, 0.41, 0.43, 0.44, 0.47, 0.49, 0.60, 0.63, 0.70, 0.73, 0.75) for SOD, 13 bands for PRX (Rf = 0.09, 0.11, 0.16, 0.19, 0.21, 0.25, 0.26, 0.29, 0.31, 0.34, 0.41, 0.43, and 0.45) and 2 bands (Rf = 0.04, 0.06) for GDH. According to Marshall and Allard (1970) polymorphic bands are the bands with a frequency less than 95 % and monomorphic bands are those with a frequency of more than 95%. By these criteria there were 17 polymorphic bands (Rf = 0.14, 0.20, 0.23, 0.32, 0.35, 0.36, 0.37, 0.38, 0.40, 0.41, 0.43, 0.44, 0.47, 0.49, 0.60, 0.63,) for SOD, and 12 polymorphic bands for PRX (Rf=0.09, 0.11,0.19, 0.21, 0.25, 0.26, 0.29, 0.31, 0.34, 0.41, 0.43, and 0.45). Altogether seven monomorphic bands were observed, four for SOD (Rf = 0.12, 0.70, 0.73, 0.75), one for PRX (Rf = 0.16) and two for GDH (Rf = 0.64 and 0.06).

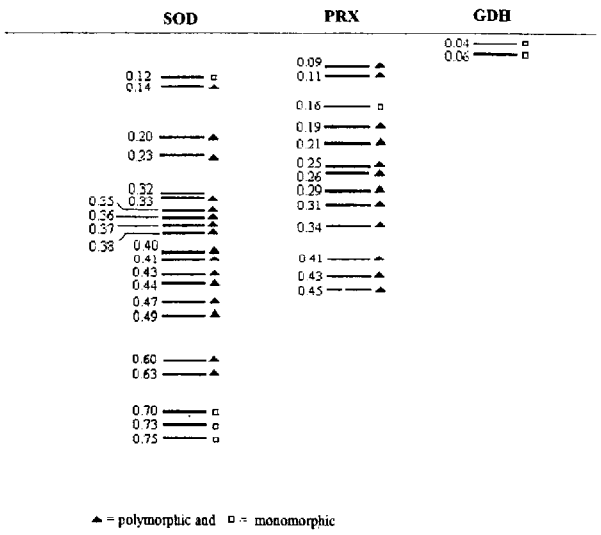

Figure 1. Bands for SOX, PRX and GDH in samples from the Zairean oil palm germplasm collection, with Rf values.

This study shows that SOD and PRX have complicated genetic control systems which result in the large number of bands on the electrophoresis gel. On the contrary, GDH has a simpler genetic control system because only two bands appeared on the gel. Although there is no previous report on SOD, PRX and GDH in oil palm, studies on other species have elucidated the basis of the genetic system of the three isoenzymes (Wendel and Weeden, 1989).

GDH

The electrophoretic data collected in the study showed no genetic variation in GDH because both the bands had a frequency of 100%, that is, the two bands were observed in all samples in the study. This implied that the GDH gene might be adapted and came to the gene fixation level. Nelson *et al.* (1977), by using starch gel electrophoresis, also demonstrated no genetic variation of GDH in 200 Caucasians, there being only a single band. On the electrophoretic gel, GDH have small Rf values of 0.04 and 0.06, which implies that the isoenzymes have high molecular weights as shown by Cammerts and Jacobs (1983) for the case of *Arabidopsis thaliana*. In this situation the two GDH isoenzymes could be coded by two different loci which could only give rise to two bands on the gel. The two GDH isoenzymes were most probably subjected to subcellular compartmentalization and contributed by a mitochondrial gene and a chromosomal gene separately, so that the subunits of gene product from different locations are prevented from adhering together to form a functional protein (Wendel and Weeden, 1989).

PRX and SOD

There were difficulties in determining the total loci coding for SOD and PRX owing to the condition of heteromultimerity, null alleles, modifier genes and the subcellular compartmentalization (Weeden and Wendel, 1989). Brewbaker *et al.* (1985) revealed nine different loci that coded for PRX in maize. Van Den Berg and Wijsman (1981) postulated that production of peroxidase in petunia involved several structural and regulatory genes, and post-transcriptional modification possibly contributing to the large number of peroxidase isoenzymes. Another possibility for the complexity of PRX zymogram is the difficulty in distinguishing peroxidase from polyphenoloxidase which has a similar redox reaction to that of the peroxidase (Cress *et al.* ,1991). Brubaker *et al.*, (1985) found that PRX has a dimer quaternary structure, whereas Garcia *et al.*(1982) reported that it is a monomeric isoenzyme.

SOD isoenzymes were found to be dimers or tetramers and can be found in the cytosol, chloroplast and mitochondria (Baum and Schandalios, 1981). Like PRX, SOD showed the optional quartenary structure and compartmentalization. Undoubtedly, the compartmentalization and complex quaternary structure of the isoenzymes has led to the observation of 21 different migrating bands in SOD.

Polymorphic Index

Genetic population structure can be studied without knowing the genotypes of the plants, in particular by using isoenzyme techniques and applying polymorphic indices (Marshall and Allard, 1970). In the computation of the polymorphic indices, each different migrating band is considered a variant. Each variant is scored quantitatively, presence of a band being designated with 1 and absence with 0. The frequency of the variants in a particular isoenzyme is then calculated and applied in the PI formula as stated above in Materials and Methods. However, in this study only PRX and SOD were used in the polymorphic index computation because of the monomorphic bands of GDH (no genetic variation) already mentioned.

Polymorphism at ecotype, population and family levels

For the convenience of the population genetic studies five different ecotypes have been established according to their geographic and climatic locations and designated

numerically (*Table 1*). Overall, the PI is much higher for PRX than for SOD. The average polymorphic index at the ecotype level ranged from 0.1301 to 0.0572 with Ecotype 1 showing the highest PI value and Ecotype 4 the lowest PI value (*Table 2*).

The high PI values of ecotypes 1, 2 and 3 are probably attributable to the location of the ecotypes. Ecotypes 1 and 2 are located in areas which are favourable for the growth of the palm with an annual rainfall of about 2000 mm, without marked dry seasons and at altitudes of about 300 m (Hartley, 1967). These ecotypes are abundant in palm groves where there is genetic variation. Furthermore, they originated in the Congo Basin along the Zaire River where human activity is high. Intensive human activity is thought to be one of the factors for increasing the degree of polymorphism because human interference in the native habitat can increase genetic variation by introgression (Harlan, 1975). In the oil palm native habitat, the actual physical spread of the plant by seed may be by animals and by water. However, the main agency since historic times in Africa is thought to have been humans carrying bunches from palm groves to homestead (Hartley, 1967). Ecotype 3 has a total precipitation of 1400-1800 mm and a marked dry season, conditions quite different from those of Ecotypes 1 and 2. However, it showed a PI value of 0.1166 which is higher than that of Ecotype 2. One of the reasons for this high degree of polymorphism could be human activity, as Ecotype 3 is highly populated. However, since only a single population of this ecotype was made available, the evaluation may be inaccurate. A larger sample size is being examined to test the validity of the results.

In contrast, Ecotypes 4 and 5 have low PI values of 0.0572 and 0.0871 respectively (*Table 2*). The reasons for their low degree of polymorphism are mainly due to geographic and climatic factors. Ecotypes 4 and 5 are located at the boundaries of the palm belt (Hartley, 1967). It is thought that the diversity of a species decreases outwards from its centre of diversity. Ecotype 5, originated from the southern part of Zaire where there is a dry season of 2 to 4 months, with total precipitation of 1400-1800mm and a high incidence of disease. These adverse conditions have made the ecotype very unusual (Hartley, 1967) and have possibly reduced the genetic variation of the palm dramatically because of adaptation for survival.

Ecotype 4 showed the lowest PI value of 0.0572 (*Table 2*). As mentioned previously, it is found at the boundaries of the palm belt, and also at a higher altitude where the climate is quite unfavourable for the growth of oil palm. This may have also caused the plant to struggle for adaptation and consequently lose some of its genetic variation. The area is also too high and isolated for extensive human activity, and this is thought to be another reason for the very low degree of polymorphism. Little is known about the relationship of altitude to bunch production in Africa, although production is said to be low in the high-altitude areas of Cameroon (Hartley, 1967). Although these highland palms do not give good yields, they may have other desirable characteristics which could be exploited for planting of oil palm at higher altitudes. It would be of interest if they were planted at similar altitudes in Malaysia to study their economic traits.

In the total of 13 populations in this study, Population K in Ecotype 4 showed the lowest PI value of 0.0350 while Population A in Ecotype 1 showed the highest PI value of 0.1256. Populations within Ecotype 1 have PI values ranging between 0.0872 and 0.1256, while Ecotype 2 has values from 0.0750 to 0.1041 and Ecotype 4 with values from 0.0350 to 0.0373 (*Table 2*).

At the family level, Family K1 has the lowest PI value and Family F2 the highest, at 0.0146 and 0.0768 respectively. The results showed that PI values increase from the family level to the ecotype level. Each ecotype has its own geographic and climatic specificities which can drive selection in different directions and diversify the populations, thus increasing their genetic variation.

Chi-square tests

Chi-square tests have been carried out to determine the significant differences at various

TABLE 2. POLYMORPHIC INDICES AT FAMILY, POPULATION AND ECOTYPE LEVELS OF THE ZAIREAN OIL PALM GERMPLASM COLLECTION

Code	Family			Population			Ecotype		
	SOD	PRX	Mean	SOD	PRX	Mean	SOD	PRX	Mean
A1	0.0525	0.0117	0.0321	0.0960	0.1551	0.1256			
A2	0.0350	0.0797	0.0574						
B1	0.0097	0.0525	0.0311	0.0615	0.1593	0.1104			
B2	0.0428	0.0991	0.0710				0.0895	0.1706	0.1301
C1	0.0505	0.0564	0.0535	0.0722	0.1452	0.1087			
C2	0.0661	0.0525	0.0593						
D1	0.0058	0.0466	0.0262	0.0785	0.0958	0.0872			
D2	0.0700	0.0408	0.0554						
E1	0.0505	0.0389	0.0447	0.0833	0.0907	0.0870			
E2	0.0525	0.0486	0.0506						
F1	0.0350	0.0330	0.0340	0.0782	0.1299	0.1041			
F2	0.0700	0.0386	0.0768						
G1	0.0447	0.0253	0.0350	0.0658	0.0585	0.0622	0.0979	0.1133	0.1056
G2	0.0272	0.0214	0.0243						
H1	0.0233	0.0525	0.0379	0.0668	0.0832	0.0750			
H2	0.0466	0.0408	0.0437						
I1	0.0486	0.0350	0.0418	0.0882	0.0867	0.0875			
I2	0.082	0.0408	0.0445						
J2	0.0984	0.0564	0.0774	0.0965	0.1366	0.1166	0.0965	0.1366	0.1166
J3	0.0408	0.0466	0.0437						
K1	0.0233	0.0058	0.0146	0.0326	0.0737	0.0350			
K2	0.0257	0.0117	0.0187				0.0590	0.0544	0.0567
L1	0.0058	0.0292	0.0175	0.0095	0.0651	0.0373			
L2	0.0166	0.0369	0.0268						
M2	0.0175	0.0350	0.0263	0.0483	0.1079	0.0781	0.0483	0.1079	0.0871
M3	0.0564	0.0311	0.0438						
Mean	0.0406	0.0428	0.0417	0.0675	0.1036	0.0856	0.0782	0.1166	0.0974

levels (*Table 3*). The results showed that most of the families within a population showed no significant differences. However there were significant differences between families within Populations B, D, F, J and M for SOD, and in Populations A, B and F for PRX. When the total (average of PRX and SOD) is considered, families within the Populations B, D, F and J showed significant differences. It can be concluded that differences within the families of A and M are small. All the families with significant differences between them come from ecotypes 1, 2 and 3 which have high polymorphic indices. Chi-square tests also showed significant differences between populations and between ecotypes. It can be inferred from the chi-square tests that although there were some differences at the family level, they did not affect much the total genetics variation. The genetics variation is contributed mainly by population and ecotype levels. This implies that collection of oil palm germplasm can be done effectively by increasing its samples in population and ecotype levels while reducing the number at family level.

By utilising polymorphic indices in isoenzyme studies, some light has been shed on the genetic population structure of PORIM's Zairean oil palm germplasm collection. The results are compatible with those of an earlier study using RAPD to estimate the genetic diversity of Ecotypes 1, 2 and 3 (Shah *et al.*, 1994). However, there is a slight discrepancy with regards to the ecotype with least diversity.

TABLE 3. CHI-SQUARE TESTS FOR FAMILY, POPULATION AND ECOTYPE LEVELS OF ZAIREAN OIL PALM GERMPLASM COLLECTION

Family in population	df	SOD		PRX	
		X^2	Probability	X^2	Probability
A	1	0.7	> 0.05	20.24	< 0.05
B	1	8.35	< 0.05	5.73	< 0.05
C	1	0.83	> 0.05	0.06	> 0.05
D	1	21.75	< 0.05	0.15	> 0.05
E	1	0.004	> 0.05	0.43	> 0.05
F	1	4.67	< 0.05	8.78	< 0.05
G	1	1.70	> 0.05	0.13	> 0.05
H	1	2.13	> 0.05	0.59	> 0.05
I	1	0.001	> 0.05	0.18	> 0.05
J	1	9.53	< 0.05	0.37	> 0.05
K	1	0.05	> 0.05	2.78	> 0.05
L	1	2.08	> 0.05	0.36	> 0.05
M	1	8.19	< 0.05	0.09	> 0.05
Populations	12	44.91	< 0.05	69.98	< 0.05
Ecotypes	4	10.80	< 0.05	24.90	< 0.05

More enzyme studies will be conducted to investigate this.

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ERRATUM

Vol 7(1) page 2 line 40
Should read as **0.1 g. L^{-1}**