

OIL PALM (*Elaeis guineensis*, JACQ.) ESTERASE GENETIC POLYMORPHISM REVEALED BY ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GEL

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ABSTRACT

Oil Palm pollen extracts of 97 palms from 31 open pollinated progenies from Bahia State, Brazil, were analyzed for fluorogenic esterases through polyacrylamide gel isoelectric focusing at two pH gradients. There were a large number of bands distributed over five activity regions. Regions A, B and C presented monomorphic patterns and isozymes of region D were difficult to analyze.

The observed profile for region E (esterases E1 and E2) suggests that the control of this esterase is determined by two alleles and that this enzyme has a monomeric structure. Allelic and genotypic frequencies are presented for this locus. A hypothesis for genetic control of the two isozyme variants detected is presented.

INTRODUCTION

Esterases (EC 3.1.1.1.) have been widely studied in plant tissue by means of several electrophoretic techniques and are very useful tools for genetic studies. Esterase zymograms obtained after starch or polyacrylamide gel electrophoresis are usually complex and difficult to analyze. Ghesquiére (1983) reported low reproducibility for complex patterns of Oil Palm pollen extracts after starch gel electrophoresis. We present a methodology that enables the detection of two Esterase isozyme variants at one locus, an important and reliable tool for genetic analyses of *Elaeis guineensis* materials.

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MATERIAL AND METHODS

Pollen samples (dried, sieved and vacuum stored) were obtained from 97 palms from 31 open-pollinated progenies of the Oil Palm Germplasm Bank at the Amazon Research Center for Agriculture and Cattle Raising of EMBRAPA. These progenies were originated from bunches collected in sub-spontaneous groves (material introduced and dispersed by African slaves during the colonial period), located near three small villages of the Bahia state coast, namely: Nazaré (39°01'W; 13°02'S); Valença (39°05'W; 13°22'S) and Taperoá (39°07'W; 13°34'S).

Solubilization of enzymes was obtained by homogenization of 100 mg of dry pollen in 1 ml of a 0.1 M Tris-HCl buffer pH 7.2 at 4°C. The material was then centrifuged at 20,000 x g at 4°C during 30 minutes and the supernatant recovered.

Polyacrylamide gel (190 x 11 x 0.7 mm) solutions consisted of 1.6 g sucrose; 8.8 ml deionized water; 2.8 ml 28% acrylamide (Merck); 2.8 ml 2% bisacrylamide (Serva); 720 µl Pharmalyte (Sigma no. PO658) pH 4.5-5.4 and 80 µl Pharmalyte (Sigma no. P1522) pH 3.0-10.0 (these were substituted by 800 µl of Pharmalyte, Sigma no. P1772, for gels of pH gradient 4.0-6.5); 27 µl TEMED (CANALCO), 800 µl ammonium persulfate 15 mg/ml (Merck). Acrylamide and bisacrylamide were recrystallized according to Loening (1967).

As electrolytes, 0.2 M histidine was used at the cathode and 0.04 M glutamic acid at the anode. Maximum settings of the power supply (LKB 2298 MACRODRIVE 5) were 1500 V, 30 mA and 10 W. After 30 minutes of a prerun, paper wicks (5 x 10 mm) were soaked with 20 µl of supernatant and applied to the gel on a strip 1.5 cm from the cathode. The wicks were removed after 20 minutes and an additional 2.5 hours of power was applied.

Detection of esterases was performed as described by Coates *et al.*, 1975, using 4-methyl-umbeliferil acetate as the enzyme substrate. Immediately after visualization, the esterase focusing points were marked and the gel surface briefly rinsed with distilled water. The pI for each isozyme was then estimated by measuring the pH of the gel at different points with a contact electrode.

RESULTS AND DISCUSSION

Description of the phenotypes

Gradient 1 (4.0-6.5 ampholytes)

Sample zymograms (Figure 1) reveal bands distributed over five regions tentatively denominated A, B, C, D and E. In all samples analyzed A, B and C presented one major band or high activity, with estimated pI's of 5.95, 5.60 and 5.35, respectively.

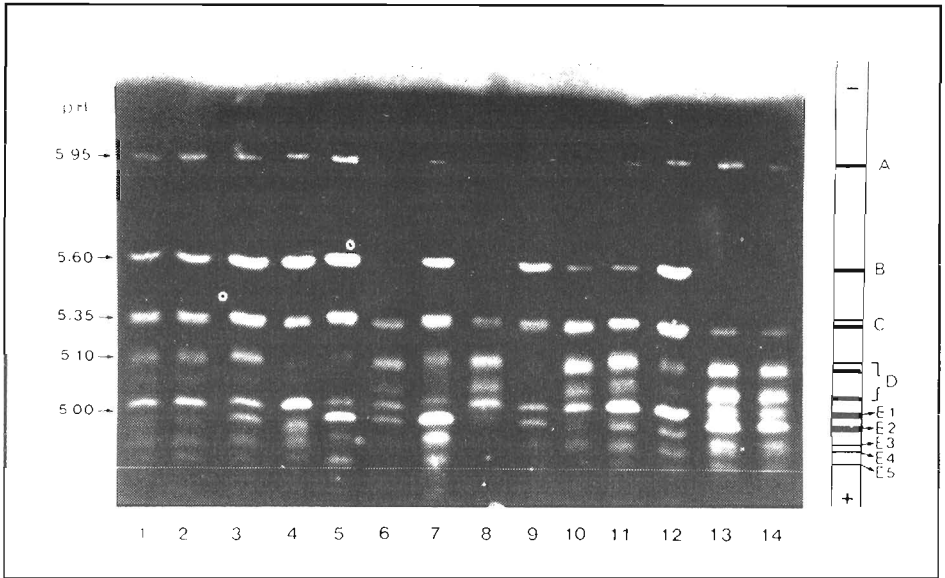


Figure 1 - Fluorogenic esterases phenotypes and pI's after PAGIF for pII gradient 1 (4.0-6.5 ampholytes). Lane 1 and 2 - sample A315718; 3 - A314002; 4 - A314508; 5 - A316110; 7 - A316007; 8 - A315005; 9 - A316018; 10 - A315514; 11 - A313410; 12 - A314913; 13 and 14 - A314604.

Region D displayed a pattern of low reproducibility when this pH gradient was used. One band of high intensity was detected at pH 5.10, often accompanied by others in a profile somewhat difficult to analyse. Five bands were detected at region E: E1 and E2 with a pI of approximately 5.0, forming three phenotypes denoted E1/E1, E2/E2. Three other more anodal bands (E3, E4 and E5) were detected in all samples studied.

Gradient 2 (90% of 4.5-5.4 and 10% of 3.0-10.0 ampholytes)

Sample zymograms (Figure 2) presented bands distributed over four regions denominated B, C, D and E. According to the previous description for gradient 1, regions B and C each presented one major band with pI of 5.60 and 5.35, respectively. Region D presented up to five bands, representing complex phenotypic patterns. The higher intensity band at this region had a pI of 5.15, as shown in Figure 2.

Region E presented a variation identical to that observed in gradient 1, with improved fractioning of the detected variants. Bands E1 and E2 had their pI's estimated at 5.02 and 4.98, respectively.

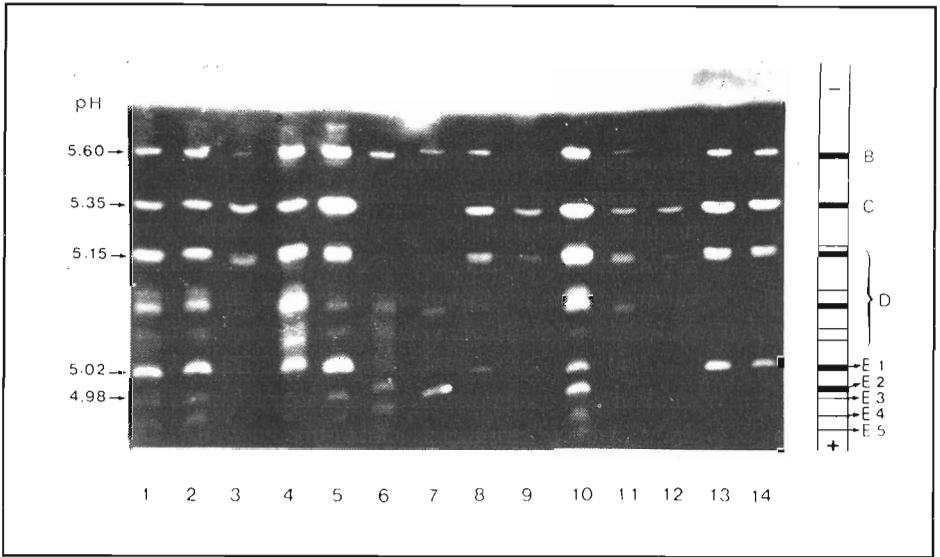


Figure 2 - Fluorogenic esterases phenotypes and pI's after PAGIF for gradient 2 (4.5-5.4 (90%) and 3-10 (10%) ampholyte mixture). Lanes 1 and 2 - sample A314110; 3 - A316018; 4 - A315005; 5 - A314508; 6 - A315705; 7 - A314604; 8 - A315718; 9 - A314002; 10 - A316110; 11 - A315515; 12 - A315908; 13 and 14 - A314913.

Interpretation of phenotypes

The high number of esterase isozymes detected in this work is in accordance with observations made for this enzymatic system in other species (Tanskey and Orton 1983). On the other hand, the better separation and higher resolution of the isozymes after PAGIF in expanded pH gradients allows the reliable distinction of some variants, overcoming difficulties observed when less sensitive techniques are employed.

Regions A, B and C, showed invariant isozymes for both gradients employed. Low reproducibility of phenotypes was observed at region D. This and the large number of isozymes variants detected at this region, did not allow an accurate interpretation of the profiles.

The analysis of region E demonstrated three phenotypes, composed of bands E1 and E2, according to the expected segregation of two codominant alleles (tentatively called *E1* and *E2*), coding for a monomeric enzyme (Harris, 1980). Accordingly, the allelic frequencies of *E1* and *E2* were estimated as 0.54 and 0.46, respectively, for the 97 plants analyzed. The genotypic frequencies were estimated as 0.32, 0.43 and 0.25 for *E1/E1*, *E1/E2* and *E2/E2*, respectively.

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RESUMO

Extratos de pólen de 97 palmeiras obtidos de 31 progênies de polinização aberta originárias do Estado da Bahia foram analisados para Esterase (E.C. 3.1.1.1) com substrato fluorogênico, após focalização isoeletrica em gel de poli-acrilamida em dois gradientes de pH (4.5-5.4 e 4.0-6.5).

Os resultados mostram um grande número de eletromorfos distribuídos em cinco regiões de atividade. Regiões A, B e C mostraram padrões eletroforéticos monomórficos enquanto as isoenzimas identificados na região D apresentam baixa reutilização dos perfis observados. O perfil da região E (esterases E1 e E2) sugere que esta enzima é monomérica e que as duas formas observadas são determinadas por dois alelos codominantes. Frequências alélicas e fenotípicas são apresentadas para este locus. Hipótese para o controle genético de duas variantes isoenzimáticas é apresentada.

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