International Journal of Oil Palm, **6(2)** : 7-11, December 2009

RESEARCH PAPER

Esterase and Peroxidase Isozyme Profile along with its Activity in Oil Palm (*Elaeis guineensis Jacq.*)

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ABSTRACT

Oil palm is the highest edible oil yielding crop. The varieties of oil palm can only be identified by dissecting the fruits, and so far no marker has been identified. A comparative study on peroxidase and esterase isozyme pattern, and peroxidase enzyme activity was carried out in all the active leaves of a dura and pisifera variety of oil palm. Thirty two leaf samples were collected from last unopened leaf (0-leaf) to 31 leaf (31-leaf) from a mature oil palm plantation situated at DOPR, Pedavegi. Leaf-0 or Leaf-1 was found to be suitable for sampling for esterase isozyme analysis in both the varieties of oil palm. However, any leaf from 9 to 20 could be sampled for peroxidase isozyme analysis in case of both the varieties. Presence of Rf 0.44 bands in 7-9 leaf in the case of pisifera and in 21 -31 leaf in dura, also the presence of Rf 0.44 band in 28-31 leaf dura (absent) in pisifera could be used as markers. Older leaves, from 23 leaf onwards, which showed significantly higher amount of peroxidase activity in pisifera than that of dura, could be used as variety specific marker. No bands could be identified as a marker in case of esterase isozyme.

Key words : Oil palm, esterase, peroxidase, isozyme

INRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) has been originated from West Coast of Africa which is capable of yielding 4-6 T oil /ha/year. There are three fruit forms available in oil palm *viz.,* dura, pisifera and tenera, which are also called varieties. Only teneras are cultivated commercially due to its low shell thickness and high mesocarp as well as kernel content. Tenera is a hybrid between thick-shelled dura and shell less, female sterile pisifera (Hartley, 1988). These varieties can only be identified by dissecting the fruits, and so far no marker is identified, which can be associated with the shell thickness of the oil palm fruits. Molecular approaches are also attempted and similar efforts are also being continued at DOPR (Mayes *et al*., 1997; Mandal and Pillai, 2007). However, biochemical markers also can complement the study.

Oil palm produces two leaves per month and possess approximately 30-32 healthy leaves. Different leaves are at different growth stages. Hence the protein, enzymes and other metabolites may also be differing from one leaf to another. This academic study helps to know the pattern of the different biochemical parameters in leaves starting from last unopened leaf ('0' leaf) to the fully mature leaf in oil palm. This is more important since the condition of growth is entirely different in terms of irrigation as well as climatic condition in India, and so far, not many studies have been conducted on biochemical parameters of irrigated oil palm (Mandal, 2003). Moreover, studies of all the leaves in dura and palms from same environment and of same age group would generate information, which could be useful for identifying some biochemical markers associated with the fruit forms. Keeping this in mind a comparative study on peroxidase and esterase isozyme pattern, and peroxidase enzyme activity was taken up in all the active leaves of dura and pisifera variety of oil palm.

MATERIALS AND METHODS

The leaf samples were collected from healthy pisifera and dura palms from a mature oil palm plantation, from DOPR, Pedavegi experimental field. Three palms from each variety were sampled, and from each palm, 32 leaf samples were collected starting from last unopened leaf (0-leaf) to 31st leaf (31-leaf). Leaflets from the central portion of each leaf were taken for study, (excluding the midrib). All the sampled leaves were healthy and without damage.

While collecting sample, the leaflets were kept wet with filter paper and away from direct sun light. Peroxidase activity was estimated by method of Putter (1974). The activity is expressed as the change of O.D. ($\tilde{N}_t$) per minute. For protein and isozyme analysis, leaf samples were extracted in pre-chilled pestle and mortar with 1:1 (w/v) 50mM Tris-Cl, pH 7.6 buffer containing 5mM ß-mercaptoethanol and 5 mM EDTA and centrifuged at 15000 x g for 15 min at 4°C. An aliquot (100ml) of each sample containing 10% glycerol and 0.4% saturated bromophenol blue was used for PAGE in 7.5% gel to separate the isozymes. Gels were stained for peroxidase and esterase isozymes by following standard procedures using Fast Blue RR and Benzidine respectively.

RESULTS AND DISCUSSION

Zymogram was drawn for peroxidase and esterase isozymes patterns of 32 leaves of both pisifera and dura palms. In case of peroxidase, it was observed that all leaves of both dura (Fig. 1) and pisifera (Fig.2) palms contained one single specific dark intensity band of Rf = 0.190. Other two isozymes of Rf = 0.44 and 0.48 with low intensity were also present in most of the leaves in both the varieties, however, the band intensity was distinctly differently in some specific leaves between the two varieties. In case of dura, Rf 0.44 was present in all the leaves from 0-31. However, Rf 0.48 started appearing from 8th leaf onwards and continued till the 31st leaf in case of dura. Intensity of both Rf 0.44 and 0.48 bands was higher in the central leaves in dura. In case of pisifera, intensity of Rf 0.44 band was very light, and no Rf 0.48 band appeared till 6th leaf. Both the bands were prominant up to 19th leaf and intensity of the both bands gradually decreased. Nothing was visible after 20th leaf for Rf 0.48 band and after 27th leaf for Rf 0.44 band. Presence of Rf 0.48 bands in 21st-31st leaf and Rf 0.44 band in 28th -31st leaf in dura palms (absent in pisifera) could be used as markers for these two varieties. But, these markers have very less relevance since the palms could be identified easily once they bear fruit and identification at seedling stage is important. However, any leaf from 9th to 20th could be sampled for peroxidase isozyme analysis in case of both dura and pisifera varieties.

Esterase isozyme showed similar pattern in both dura (Fig. 3) and pisifera (Fig.4) palms. Two major esterase isozymes were observed (Rf = 0.48 & 0.60). Unopened leaf (0-leaf) and first leaf contained these two bands with higher intensity, however, Rf 0.48 band was thicker in dura than that of pisifera, where as Rf 0.60 band was thicker in case of pisifera than that of dura. From the second leaf onwards only one band (Rf = 0.60) was observed, intensity of which was decreased from 2nd leaf then increased from 6th to 8th leaf and again decreased gradually from 13 leaf onwards, finally no esterase isozyme band was observed from 18th leaf onwards. Hence, Leaf-0 or Leaf-1 was found to be suitable for sampling for esterase isozyme analysis in both the varieties of oil palm and could be used as markers.

Result of peroxidase activity revealed that pisifera has higher activity than that of corresponding dura leaf with a few exceptions (Table 1). However, in most of the leaves, the peroxidase activity value were on par in both the palms except leaf no. 5, 8, 13, 15, 16, 19, 23-27, 30 and 31, where pisifera leaves had significantly higher values than that of dura leaves. To use this result as a dura and pisifera specific marker, older leaves, from 23rd leaf onwards, could be used as an index leaf for both the varieties for assaying peroxidase activity.

There was no report on biochemical analysis of all the 32 leaves of oil palm (*Elaeis guineensis* Jacq.) for the selection of index leaf. Earlier 17th leaf was used as an index leaf for different nutrient analysis in different oil palm growing countries. Some of the biochemical constituents (chlorophylls, carotenoids, total carbohydrates and soluble protein) and nitrogen content in different leaves of oil palm were analyzed for the selection of index leaf and 9th leaf was found to be more suitable (Mandal *et al.,* 2003). The present study would be useful for sampling leaves for esterase and peroxidase isozymes, as the index leaf could be selected. This result, especially, peroxidase enzyme activity and isozyme patterns would be useful towards selection of marker for varietal identification.

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Table 1: Peroxidase activity in 0-31 leaves of dura and pisifera variety of oil palm

Leaf No.	POD Activity (t_t/min/g tissue)				
		Dura	Range	Pisifera	Range
0	OP	2.09	W	2.15	VW
1	OP	2.66	QRSTUVW	2.72	PQRSTUVW
2	OP	3.16	IJKLMNOPQRSTUVW	2.40	TUVW
3	OP	3.09	KLMNOPQRSTUVW	3.11	JKLMNOPQRSTUVW
4	OP	3.27	FGHIJKLMNOPQRSTUVW	3.69	DEFGHIJKLMNOPQR
5	S	2.29	UVW	3.52	DEFGHIJKLMNOPQRST
6	OP	3.79	DEFGHIJKLMNOPQ	3.99	BCDEFGHIJKLMNO
7	OP	4.39	ABCDEFG	5.52	A
8	S	2.57	RSTUVW	4.35	ABCDEFGH
9	OP	4.07	BCDEFGHIJKLMNO	4.59	ABCD
10	OP	4.31	BCDEFGHI	4.07	BCDEFGHIJKLMN
11	OP	3.34	EFGHIJKLMNOPQRSTUV	4.43	ABCDEF
12	OP	4.22	BCDEFGHIJKL	4.51	ABCDE
13	S	2.97	MNOPQRSTUVW	5.14	AB
14	OP	3.42	DEFGHIJKLMNOPQRSTU	4.09	BCDEFGHIJKLM
15	S	3.64	DEFGHIJKLMNOPQRS	5.05	ABC
16	S	3.47	DEFGHIJKLMNOPQRSTU	5.53	A
17	OP	3.89	CDEFGHIJKLMNOP	3.35	EFGHIJKLMNOPQRSTU
18	OP	3.76	DEFGHIJKLMNOPQR	3.07	KLMNOPQRSTUVW
19	S	2.66	QRSTUVW	4.03	BCDEFGHIJKLMNO
20	OP	3.12	IJKLMNOPQRSTUVW	3.43	DEFGHIJKLMNOPQRSTU
21	OP	3.21	GHIJKLMNOPQRSTUVW	3.19	HIJKLMNOPQRSTUVW
22	OP	3.05	LMNOPQRSTUVW	3.83	DEFGHIJKLMNOPQ
23	S	2.88	OPQRSTUVW	4.30	BCDEFGHIJ
24	S	3.02	MNOPQRSTUVW	5.04	ABC
25	S	2.89	NOPQRSTUVW	4.23	BCDEFGHIJKL
26	S	2.35	TUVW	3.81	DEFGHIJKLMNOPQ
27	S	3.17	HIJKLMNOPQRSTUVW	4.44	ABCDEF
28	OP	3.36	EFGHIJKLMNOPQRSTU	4.22	BCDEFGHIJKL
29	OP	3.44	DEFGHIJKLMNOPQRSTU	4.24	BCDEFGHIJK
30	S	2.58	RSTUVW	4.15	BCDEFGHIJKLM
31	S	2.49	STUVW	4.11	BCDEFGHIJKLM
CD at 5%		**1.193**			

Note: OP= On par; S = Significant

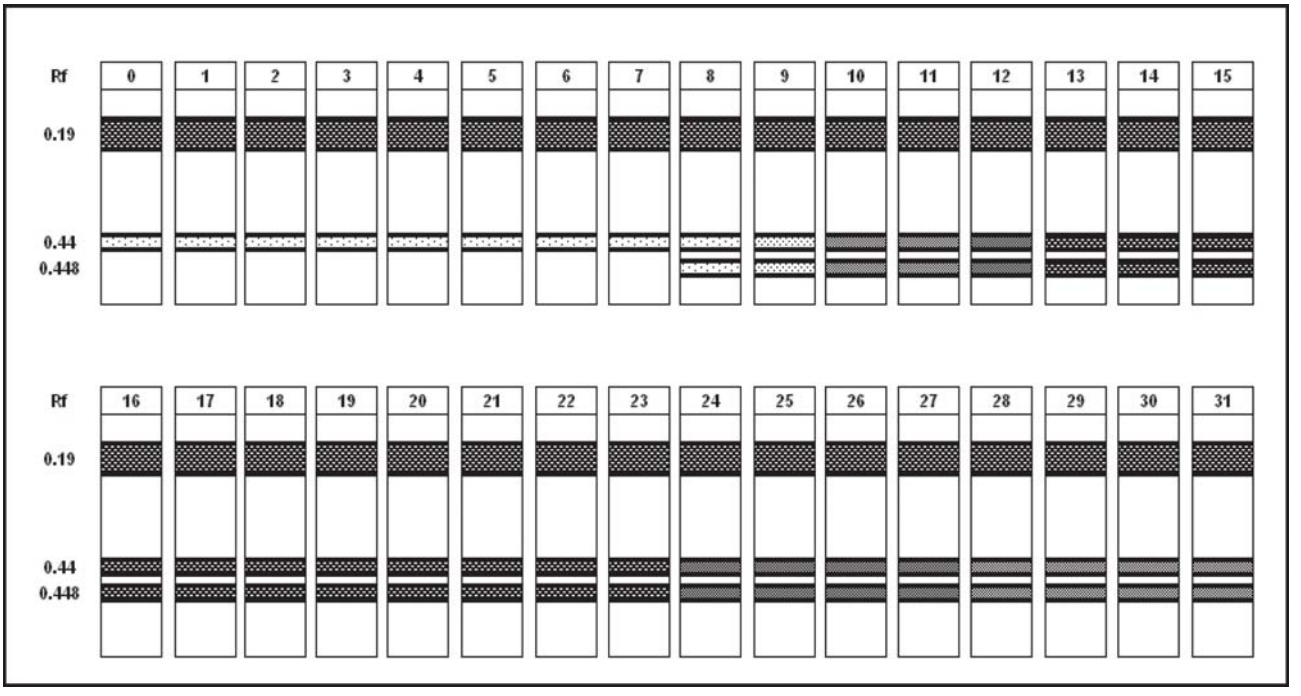

Fig. 1 : Peroxidase isozyme patterns of 0-31 leaves of dura variety of oil palm

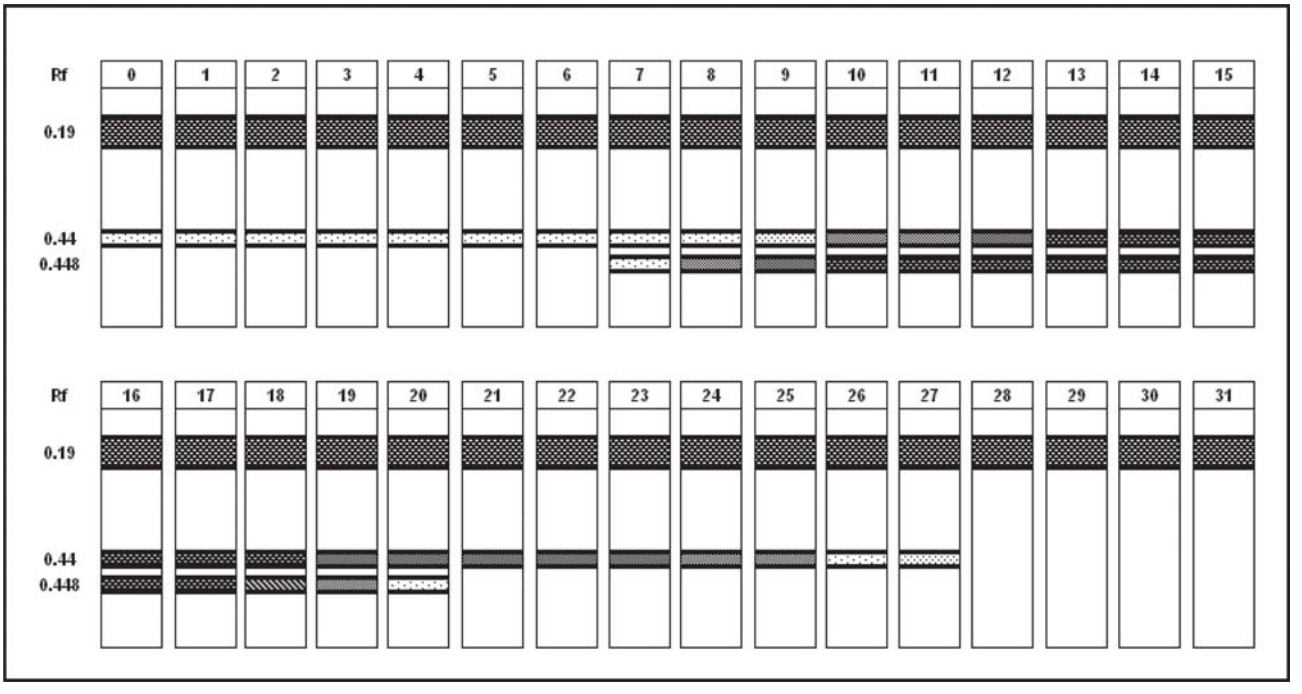

Fig. 2 : Peroxidase isozyme patterns of 0-31 leaves of pisifera variety of oil palm

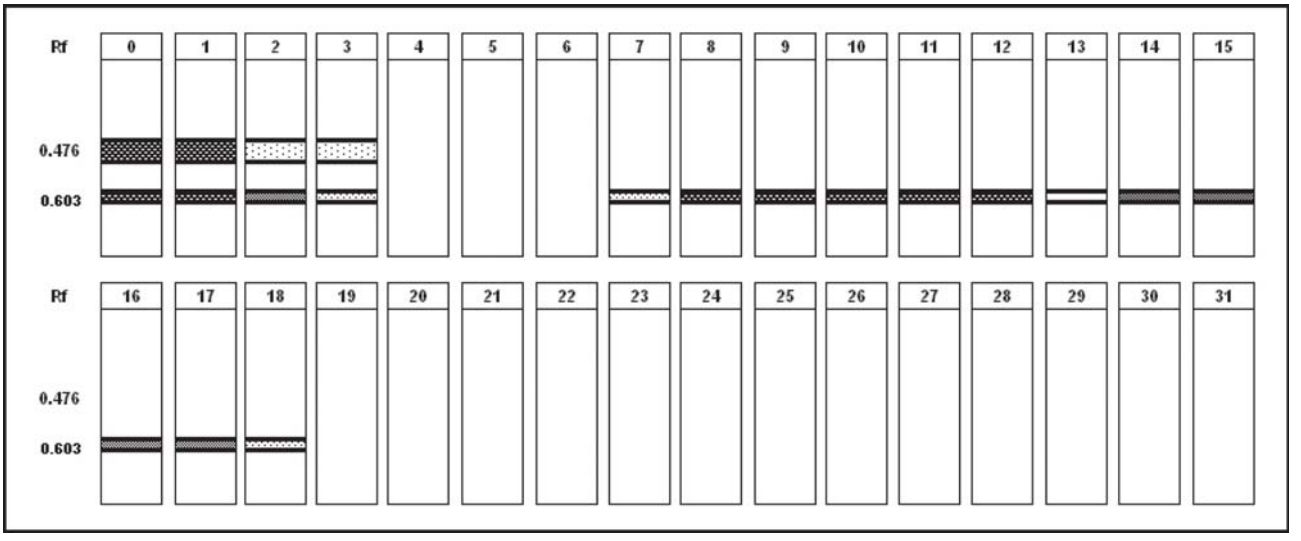

Fig. 3 : Esterase isozyme patterns of 0-31 leaves of dura variety of oil palm

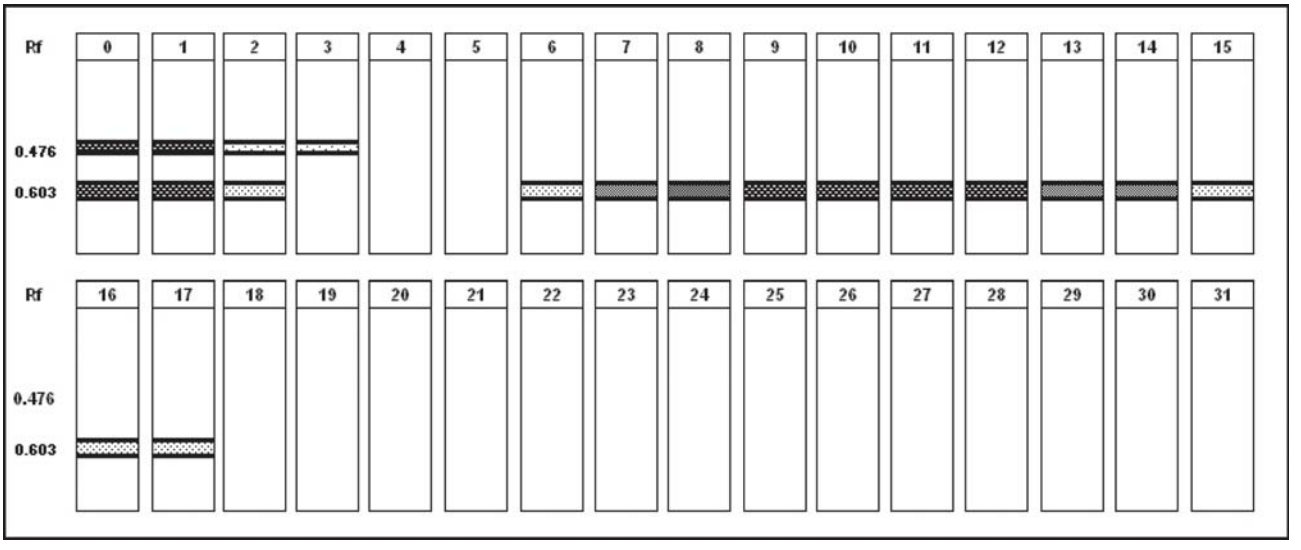

Fig. 4 : Esterase isozyme patterns of 0-31 leaves of pisifera variety of oil palm