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BIOCHEMICAL STUDIES ON ABNORMAL CLONES OF THE OIL PALM *(Elaeis guineensis)*

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A preliminary study was conducted on the biochemistry of oil palm clones producing mantled fruits. The kernels, mesocarp, leaves and callus tissues derived from both normal palms and those with mantled fruits were analysed. The results showed that the mantled material seemed to have a higher level of reducing sugars, particularly in the kernels and the callus tissues. The difference in the reducing sugar content of leaf extracts from normal palms and those producing mantled fruits was not highly significant except in the P38 clone. The content of soluble protein and ethanol-soluble nitrogenous compounds appeared inconsistent between different batches of leaf samples though there was generally an increasing trend in material from palms producing mantled fruits. No differences were observed between the activities of polyphenol oxidases, esterases or peoxidases in the leaf extracts from normal palms and those with mantled fruits. Analyses of total amino acids in the mesocarp tissues of mantled fruit from clones PIO, P12, P15 and P38 showed that there was an increase in serine content but a decrease in. amino acids related to aspartate.

INTRODUCTION

Plant tissue culture techniques have the potential to be powerful tools for the

micropropagation and multiplication of the oil palm *Elaeis guineensis* Jacq. However, the application of this novel technology was disrupted when Corley et al. (1986) identified abnormal flower development in oil palms, propagated by tissue culture. The variability and heterogeneity that existed within a clone made it difficult to identify the cause of the abnormalities. Thus, some individual palms produced bunches with all the fruits mantled, while others of the same clone produced both normal and mantled fruits in a single bunch, and still others produced all. The abnormality ranges from mild to severe. Preliminary evidence for a genetic cause for the abnormalities was described by Rao and Donough (1990). It is likely that this problem arises at the molecular level affecting the metabolism of macromolecules such as proteins, lipids, carbohydrates and nucleic acids. A preliminary study was, therefore, conducted to investigate the biochemistry of the clones producing mantled fruits Tissues from normal and mantled fruits, and from the palms produced were sampled and analysed. The objectives of the study were to try to understand the mechanisms related to the mantled condition and to identify metabolic steps associated with the abnormality.

MATERIALS AND METHODS

Plant materials

Fronds and fruits were taken from palms producing all normal fruits and from other individuals of the same clone in which the fruits were mantled. (Mantled fruits were taken from bunches containing no normal fruits).The samples were obtained from Golden Hope's Oil Palm Research Station, Banting (clones 31A, 90A and 115E, and also D × P palms) and from PORIM's Teluk Intan Research Station (clones P10, P12, P15 and P38).

Preparation of plant materials

The mesocarp of oil palm fruits was separated from the kernels and the middle portions of the selected leaf samples (fron frond No. 17) were cut into smaller pieces. The samples were frozen until they were analysed. The embryo-derived callus tissues were cultured on MS (Murashige and Skoog, 1962) solid medium containing 3% sucrose, 1% casein hydrolysate, 0.1% glutamine and 2.5% activated charcoal. The plant hormone used was 100 mg/L 2.4-D. The cultures were maintained in the dark and subcultured at four-week intervals. Two-week old callus tissue was used in this study.

Analyses of total reducing sugars and ethanol-soluble nitrogenous components

The samples were homogenized in 10 ml of 80% cold ethanol. The extracts were centrifuged at 10 000 rpm for 30 min at 4°C. The supernatants from mesocarp, kernel and callus tissues were used for total reducing sugar analyses by the colorimetric method described by Somogyi (1945). The supernatant from the leaf extracts was analysed for ethanol-soluble nitrogen content using the ninhydrin method of Spies (1957).

Protein and isoenzyme studies

Leaf samples were ground in a a chilled mortar and pestle in twice their volume of the extraction buffer, contained 0.1 M TRIS-HCl (pH 8.0), 1.0 mM EDTA, 0.1% (v/v) β-mercaptoethanol and 2% (w/v) insoluble polyvinyl polypyrrolidone (PVP). The extract was squeezed through two layers of muslin, centrifuged at 10 000 rpm for 20 min at 6°C and the supernatant was used for protein determination and isoenzyme studies. Protein was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard. Disc gel electophoresis of the enzyme preparation on 7.5% polyacrylamide gel was carried out according to the method of Davis (1964). The isoenzymes, polyphenoloxidases, esterases and peroxidases, were detected by incubating the gels in their respective substrates.

Amino acid analyses

The mesocarp tissues were extracted and analysed for total amino acid content as described by Cohen et al. (1989). Samplings and the respective analyses were carried out three times.

RESULTS AND DISCUSSION

Generally, the levels of reducing sugars were increased in tissues from mantled fruits. The results were significant in the mesocarp tissues (Table I), the kernels ***(Table*** 2) and the callus tissues (Table 3). The reducing sugar content of tissues derived from mantled fruit of clone P15 was about double in the mesocarp, 12 times higher in the kernel and 50% higher in callus tissues, as compared with the level in material from a normal individual of the same clone. A larger increase was observed in the callus tissues of P38 clone. Because of severe abnormality no kernel was produced in mantled fruit of P38 clone. The sugar content in the leaves of normal and abnormal palms did not seem to differ significantly except in P38, where it was increased in the samples from clones with mantled fruits (Table *4*).

The results in ***Table*** 5 indicate that the concentration of ethanol-soluble nitrogen was consistently higher in the leaf extracts from cloned palms producing mantled fruit than in extracts from normal palms of the same clones (31A, 90A and 115E). A similar trend was observed for the soluble protein content, but, the results were inconsistent, depending on the batches of samples collected. (Four samplings were made for the study). Leaves from D x P palms with mantled fruit also had more ethanol-soluble nitrogen than those from normal D x P palms ***(Table 5).***

The accumulation of ethanol-soluble nitrogen and probably soluble protein in the leaves of palm producing mantled fruits appeared to be due to abnormal synthesis of proteins. Studies on the isoenzyme patterns from the leaf extracts showed that there were no variations in the activities of polyphenol oxidases, esterases or peroxidases, suggesting that these enzymes are not directly involved

TABLE 1. REDUCING SUGAR CONTENT IN THE MESOCARP OF MANTLED AND NORMAL OIL PALM FRUIT

Clone		Reducing sugars[a] (mg/g fresh weight)
P15	Mantled	25.28 ± 0.96
	Normal	10.56 ± 1.12
P38	Mantled	11.84 ± 0.16
	Normal	10.74 ± 0.74

[a]average of twenty replicates

TABLE 2. REDUCING SUGAR CONTENT IN THE KERNEL OF MANTLED AND NORMAL OIL PALM FRUIT

Clone		Reducing sugars[a] (mg/g fresh weight)
P15	Mantled	6.08 ± 2.24
	Normal	0.48 ± 0.16

[a]average of twenty replicates.

TABLE 3. REDUCING SUGAR CONTENT IN CALLUS TISSUES OF OIL PALM

Clone		Reducing sugars[a] (mg/g fresh weight)
P15	Mantled	21.81 ± 0.57
	Normal	14.81 ± 0.55
P38	Mantled	11.75 ± 3.25
	Normal	6.00 ± 0.25

[a]average of twenty replicates.

TABLE 4. REDUCING SUGAR CONTENT IN THE LEAVES OF OIL PALM

Clone		Reducing sugars[a] (mg/g fresh weight)
P10	Mantled	4.75 ± 0 50
	Normal	4.42 ± 0.30
P12	Mantled	1.33 ± 0.30
	Normal	1.25 ± 0.30
P15	Mantled	3.00 ± 0.30
	Normal	4.25 4 0.30
P38	Mantled	3.58 ± 0.15
	Normal	2.00 ± 0.25

[a]average of twenty replicates.

TABLE 5. SOLUBLE PROTEIN AND ETHANOL-SOLUBLE NITROGEN CONTENT OF THE LEAVES OF OIL PALMS

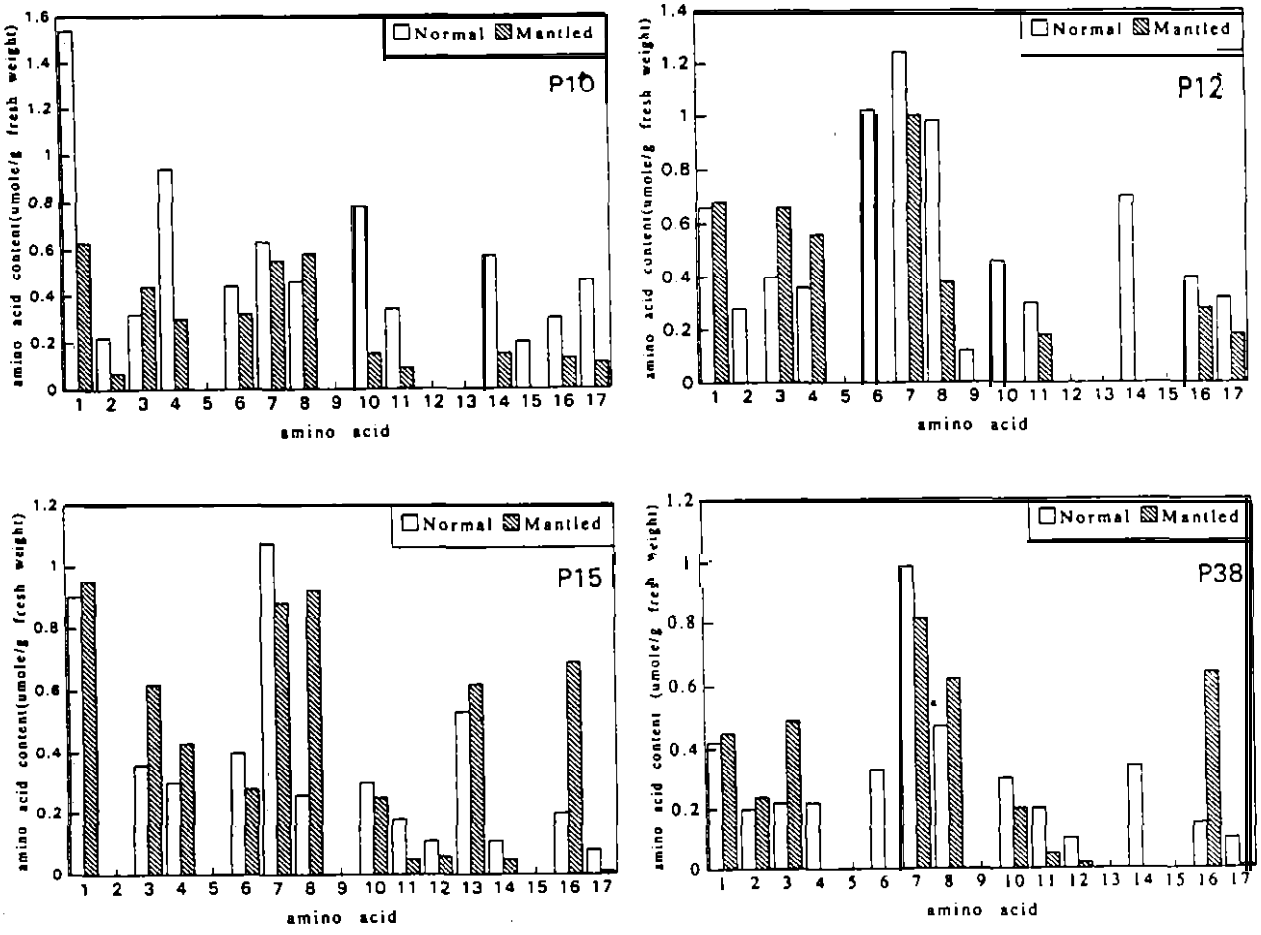
Clone	Soluble protein[a] (mg/g fresh weight)	Ethanol-soluble N[a] (mg/g fresh weight)
31A Mantled	33.8	14.5 ± 1.5
31A Normal	20.0	8.9 ± 1.1
90A Mantled	31.3	22.6 ± 3.6
90A Normal	25.0	9.6 ± 1.6
115E Mantled	35.0	24.6 ± 2.3
115E Normal	23.8	18.4 ± 0.9
Dura × Pisifera Mantled	19.8	22.4 ± 1.2
Dura × Pisifera Normal	17.1	17.6 ± 1.0

[a]average of twenty replicates.

in the metabolic activities associated with abnormalities. The activities of other isoenzymes need to be examined further, especially in fruit tissues, in order to elucidate the correlation between abnormalities and biochemical activities.

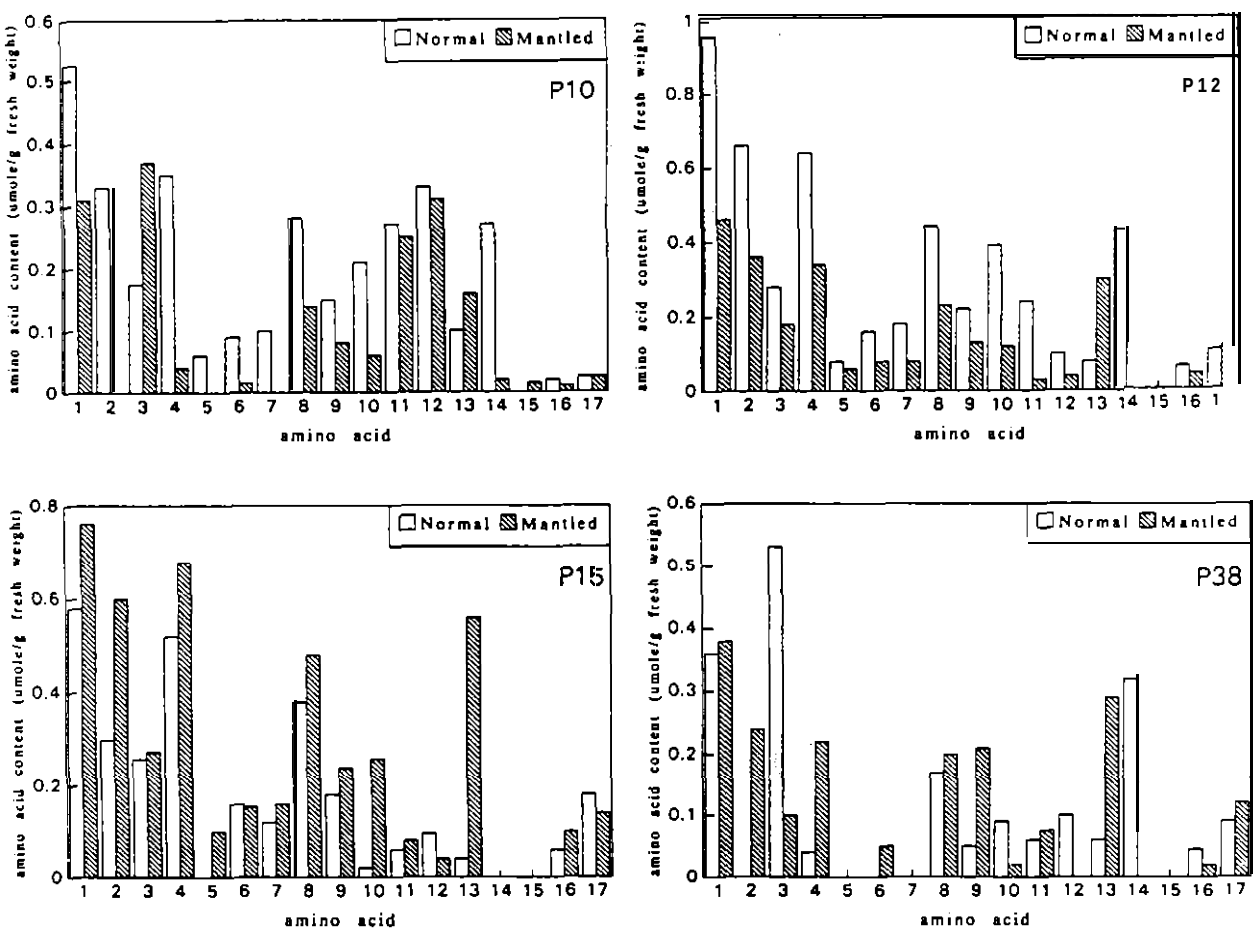
The total amino acid content of the mesocarp tissues and leaves from different oil palm clones produced by tissue culture are shown in *Figures 1* and 2. There were variations in the amino acid profiles of the four clones, P10, P12, P15 and P38. An increase in the level of serine was observed in the mesocarp tissues of mantled fruit from all four clones; however, the levels of lysine, threonine and valine were lowered. Arginine, tyrosine, leucine, isoleucine and methionine were either decreased or not detected. Histidine and proline were not detected in either the normal or the mantled material. In the acid hydrolysates of leaf samples from 'mantled' palms cysteine was increased while methionine, isoleucine and leucine were either reduced or not detected. The pattern of other amino acids appeared inconsistent.

Isoleucine and lysine belong to the same amino acid family – the aspartate family. Valine, leucine, threonine and methionine are also directly related to aspartate (Figure 3). The presence of an increased concentration of aspartate but a decrease in the amino acids related to it suggests that there is an inability to utilize aspartate or that there is a defect in the metabolic steps leading to the formation of these amino acids. Some amino acids were not easily detected, especially in material derived from clones P12 and P38. They are possibly present in small quantities. There was a variation in the age of the fruits and it is likely that some of the differences observed in the amino acid composition may be due to the differences in the physiological stage of the fruits. The increase in serine in mantled material could be due to an inability to utilize it as a metabolic precursor. Serine is known to be one of the precursors for cysteine synthesis; however, this pathway appeared to be unaffected. An increase in cysteine and a decrease in methionine in the leaf extracts were also observed. The amino acid profile of the ethanol-soluble fraction appeared inconsistent, suggesting that the free amino acids present were in a dynamic state and, therefore, do not reflect the mantled condition. The mesocarp tissues probably contained more stable proteins. The amino acid pattern of the leaf samples showed a similar inconsistent profile, again suggesting that observed abnormalities are closely related metabolically and genetically to the fruit tissues.



1 = asp	6 = arg	10 = tyr	14 = ileu
2 = glu	7 = thr	11 = val	15 = leu
3 = ser	8 = ala	12 = met	16 = phe
4 = gly	9 = pro	13 = cys	17 = lys
5 = his			

Figure 1. Total Amino Acid Content in the Mesocarp Tissues of Oil Palm Fruit. The Samples were Homogenized and Hydrolysed in 6N HCL.



1 = asp
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7 = thr
8 = ala
9 = pro
10 = tyr
11 = val
12 = met
13 = cys
14 = ileu
15 = leu
16 = phe
17 = lys

Figure 2. Total Amino Acid Content in the Leaf Tissues of Oil Palm. The Fresh Samples were Homogenized and Hydrolysed in 6N HCL.

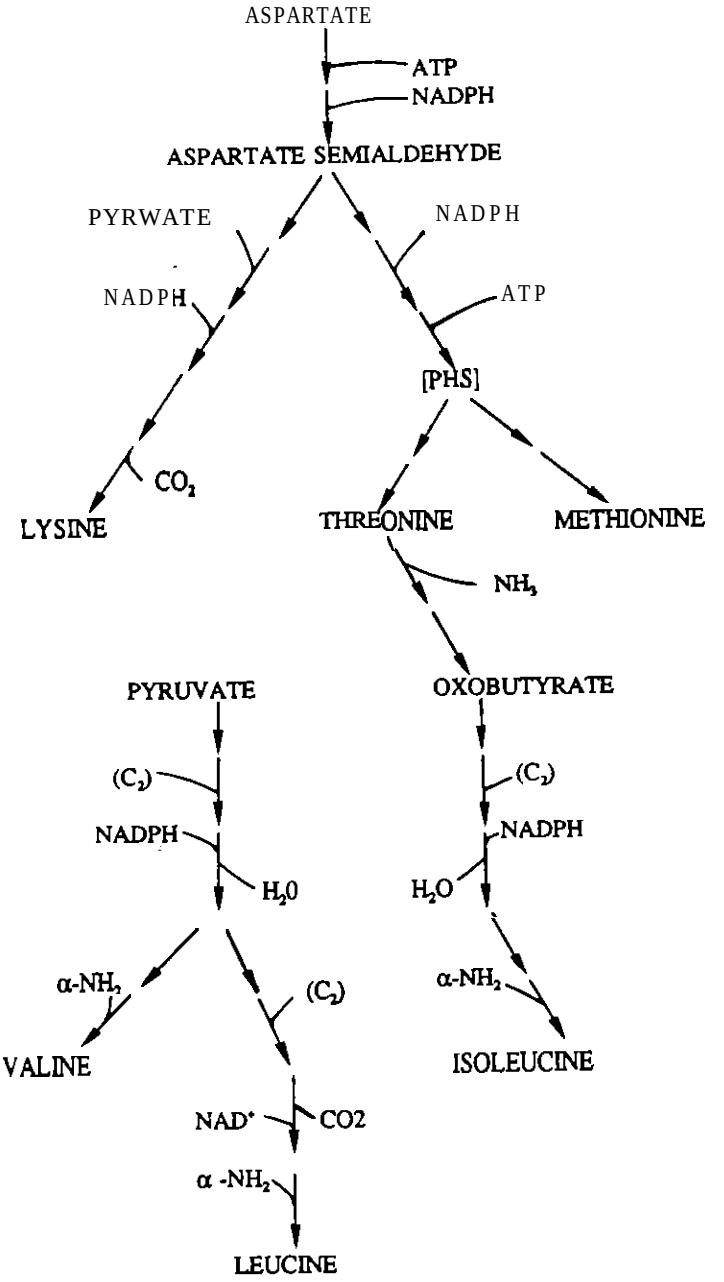

Figure 3. Relationships Among Members of the Aspartate Family and Branched Amino Acids (Bryan, 1980)

The absence or reduction of glutamate in both normal and 'mantled' samples showed that it is efficiently utilized and possibly served as a major amino-donor during transamination reactions.

We may conclude from our study that the production of mantled fruits in oil palm has resulted in extensive changes related to protein and sugar metabolism. Further studies need to be conducted on the biochemical changes that occur and on the accompanying abnormalities.

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