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RESEARCH PAPER

Selection of Index Leaf of Oil Palm for Biochemical Analysis

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ABSTRACT

There was no report on an index leaf in oil palm (*Elaeis guineensis* Jacq.) for biochemical analysis, grown under irrigated condition. In oil palm growing countries, frond (leaf) no. 17 was used as an index leaf for different analysis including biochemical analysis. In India, oil palm is being grown as irrigated crop and hence, it is necessary to select an index leaf/ leaves, which can be sampled for analyzing different biochemical constituents as well as leaf nutrients. Some of the biochemical constituents (chlorophylls, carotenoids, total carbohydrates and soluble protein) and nitrogen content in different leaves of oil palm were analysed for the selection of index leaf.

It was observed that chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were highest in frond no. 25. Total carbohydrate and soluble protein were highest in frond no 20, where as nitrogen was found highest in frond no. 9. For all these parameters, several other fronds were also on par with the highest value.

For the analysis of pigments, mature leaves (beyond frond no. 6) were found suitable. More mature fronds (19^{th}-25^{th}) showed higher protein and carbohydrate content.

For the analysis of nitrogen and biochemical parameters (like pigments, soluble protein and total carbohydrate) from single sampling, 9^{th} frond was found to be most suitable, as it showed the value on par with highest values in all the cases. Hence, 9^{th} leaf could be suggested as an index leaf.

Key Words : Index leaf, frond, chlorophyll, carotenoids, protein, carbohydrate, nitrogen

INTRODUCTION

Oil palm is a newly introduced crop in India and for the first time in the world as an irrigated crop. Leaf analysis of oil palm is a routine exercise for the estimation the macro and micronutrient content to assess the available nutrient status and requirement of the palms.

Biochemical analysis of any crop is a part of basic research and it is applicable for oil palm too. Several biochemical parameters like estimation of protein, sugars, phenols, carotenoids, chlorophyll and other pigments, enzyme activity, isozyme analysis etc. involves the extraction from leaf samples. The content of the different bio-molecules differ with the age of the leaf sample as it happens in the case of different leaf nutrients. It is important to sample a leaf, which contains the maximum amount of the particular bio-molecule and also present in active form if required. Hence, it is very important to analyse all the leaves to determine the index leaf/ leaves for sampling.

Oil palm usually have more than 25 fronds (leaves), of which 17^{th} frond was used as index leaf for nutrient and biochemical analysis in various research laboratories (Kanapathy *et al*., 1974; Knecht *et al*., 1974; Foster and Goh, 1977; Foster and Chang, 1977; Marziah and Rohani, 1995). So far, the similar procedure was followed in our laboratories for nutrient, chlorophyll and protein analysis (Suresh *et al*. 2002, Mandal *et al*. 2001). Choong *et al.* (1996) extracted enzyme from first fully opened frond for isozyme analysis.

In India, oil palm is being grown as irrigated crop and it is necessary to find out index leaf (standard leaf) for nutrient as well as biochemical analysis. Preliminary study was reported by Suresh *et al*. (2002) on leaf nutrient analysis, but no report is available on biochemical analysis

of oil palm leaf under irrigated condition.

Keeping the above points in view, a study was taken up to determine the index leaf/leaves for some important and common biochemical parameters namely chlorophyll, carotenoids, soluble protein, total carbohydrate and also total nitrogen content.

MATERIALS AND METHODS

Leaf samples of oil palm from total six *tenera* palms, two each from three different sources were sampled from NRCOP, Pedavegi experimental fields during January 2003. The age of the palms were four to nine years (after planting).

All the twenty-five leaves/ fronds from each palm were sampled. Six Leaflets from the middle of each frond were collected. Mid-rib of each leaflet was removed and the middle portion of the leaflet was taken for analysis. chlorophyll 'a', chlorophyll 'b', carotenoids, total carbohydrates, soluble protein and nitrogen content of the leaves were estimated.

Chlorophyll was estimated by the method of Hiscox and Israelstam (1979) using DMSO. Finely cut leaf samples (25 mg) were put in 5 ml of DMSO (Dimethyl sulphoxide) in a test tube. The colour of the DMSO solution was measured at 645 nm and 663 nm after incubating the tubes at room temperature for overnight in dark. Chlorophyll 'a' and chlorophyll 'b' were estimated by using the following formulae: Chl'a' = (12.7 x O.D. at 663 nm - 2.69 x O.D. at 645 nm) x (V/1000 x W) and Chl'b' = (22.9 x O.D. at 645 nm - 4.68 x O.D. at 663 nm) x (V/1000 x W); where,
V= volume of DMSO (ml); W = weight of the leaf sample (mg). Same DMSO extract mentioned above was measured at 480 and 510 nm for the estimation of carotenoids. Carotenoids content in mg/ g Fr. Wt. was calculated by using the formula: Carotenoids = (7 x O.D. at 480 nm – 1.47 x O.D. at 510 nm) x (V/1000 x W).

Total carbohydrates were estimated by modified method of Hedge and Hofreiter, (1962). Leaf sample (100 mg) was hydrolysed with 5ml of 2.5N-HCl by keeping the tubes in a boiling water bath for three hours and cooled to room temperature. The hydrolysate was neutralised with solid sodium carbonate until the effervescence ceases and the volume was made up to 50ml with distilled water. An aliquot of 50?l was taken and made up to 1ml with distilled water. Anthrone reagent (2% anthrone in concentrated H_2SO_4) was added (4 ml) to each tube and heated for eight minutes in a boiling water bath, the tubes were cooled rapidly and the green colour was measured at 630nm. Carbohydrate content was calculated from the standard curve. Soluble protein was estimated by the method of Lowry *et al*., (1951).

Nitrogen content of the dried leaf samples was estimated by Micro-Kjeldhal method (Bremmer, 1965) using a Kjel-Plus unit.

The data were statically analysed by using statistical software MSTATC Version 2.1. Duncan's Multiple Range Test (DMRT) was performed to determine the significant difference.

RESULTS AND DISCUSSION

Results of biochemical and leaf nitrogen analysis are presented in Table 1. It was observed that chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were highest in leaflets from frond no. 25 (Chart 1 and 2). Highest chlorophyll value was on par with other leaves except frond no. 1,3,6,20 and 24. Similarly all leaflets from other frond except frond no 1 to 6, 20 and 24 were on par with highest carotenoid value.

The results indicated that the younger leaves has less carotenoids.

Total carbohydrates and soluble protein were highest in frond no. 20 (Table 1 & Chart 3). This was on par with leaflets from frond no: 22, 25, 21, 19, 24, 9, and 5 in both

Chart 1: Chlorophyll content in different Fronds of mature oil palm

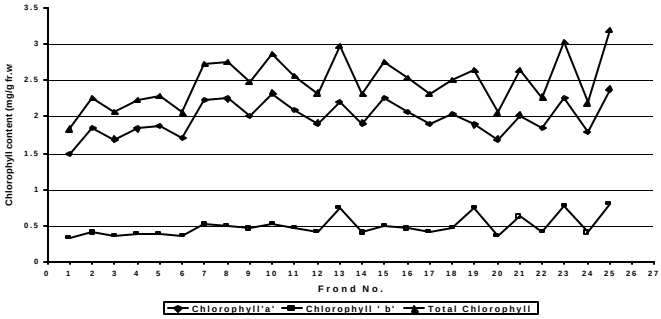
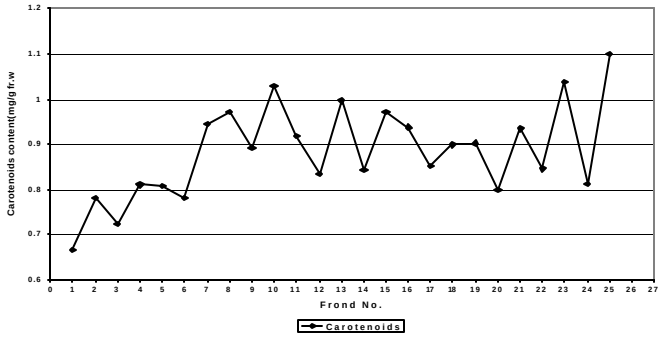


Chart 2 : Carotenoids content in different fronds of mature oil palm



Chat 3 : Total Carbohydrate content in different fronds of oil palm

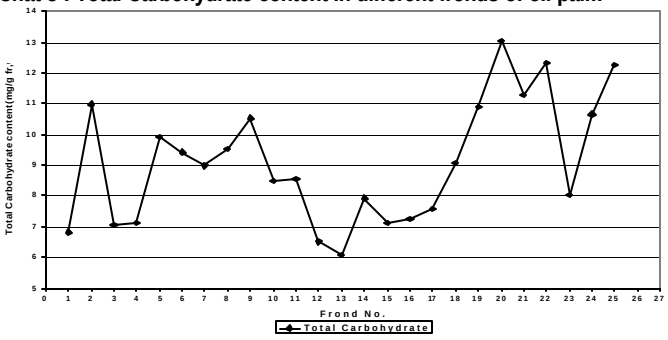


Table 1: Chlorophyll, carotenoids, CHO, soluble protein and nitrogen content in the different fronds of mature oil palm

Frond No.	Chl-a (mg-^{1}g Fr. Wt)	Chl-b (mg-^{1}g Fr. Wt)	Total Chl (mg-^{1}g Fr. Wt)	Carotenoids (mg-^{1}g Fr. Wt)	Total CHO (mg-^{1}g Fr. Wt)	Soluble Protein (mg-^{1}g Fr. Wt)	Nitrogen (%)
1	1.501	0.335	1.836	0.666	6.824	26.880	2.49
2	1.852	0.403	2.255	0.782	10.980	29.330	2.66
3	1.692	0.369	2.061	0.725	7.064	36.950	2.67
4	1.835	0.389	2.224	0.810	7.124	31.990	2.69
5	1.880	0.397	2.276	0.807	9.938	35.230	2.74
6	1.700	0.359	2.060	0.783	9.418	32.340	2.73
7	2.220	0.514	2.734	0.945	8.983	34.030	2.65
8	2.248	0.501	2.750	0.971	9.523	39.420	2.66
9	2.015	0.458	2.473	0.892	10.520	36.720	2.84
10	2.326	0.528	2.854	1.028	8.506	39.220	2.57
11	2.096	0.469	2.564	0.919	8.555	38.230	2.64
12	1.914	0.414	2.327	0.836	6.503	36.510	2.59
13	2.209	0.758	2.967	0.996	6.102	37.080	2.57
14	1.913	0.402	2.315	0.845	7.929	32.870	2.48
15	2.255	0.497	2.752	0.972	7.140	32.460	2.50
16	2.074	0.464	2.537	0.938	7.273	34.270	2.66
17	1.895	0.418	2.312	0.851	7.555	37.560	2.58
18	2.040	0.472	2.511	0.899	9.043	35.390	2.49
19	1.890	0.757	2.647	0.903	10.900	39.810	2.43
20	1.694	0.361	2.055	0.799	13.050	43.230	2.59
21	2.026	0.623	2.650	0.935	11.280	41.000	2.42
22	1.847	0.424	2.270	0.846	12.330	38.090	2.34
23	2.263	0.772	3.035	1.038	8.038	41.350	2.36
24	1.786	0.405	2.191	0.812	10.650	41.770	2.25
25	2.384	0.803	3.188	1.099	12.260	36.920	2.32
CD (at 5%)	0.504	0.370	0.792	0.229	2.884	6.821	0.20

the cases. Results indicated the higher level of carbohydrate in the leaves from 19th frond onwards (except 23).

Soluble protein content was found higher in leaflets from the middle fronds (8, 9, 10, 11,12 and 13.) and older fronds 17th leaf onwards (Table 1 & Chart 4).

Among the nutrients, nitrogen is most important and was analysed, moreover, total protein content can be derived by multiplying 6.25 to the nitrogen content. It was found highest in frond no. 9 (Table 1 & Chart 5). In fact, younger fronds (2 to 9) had the higher nitrogen level. Suresh *et al. (*2002) reported that 7th frond contains highest nitrogen in mature palms. In the present study, 7th frond ranked 9th as per nitrogen content but on par with highest nitrogen containing frond no: 9. Earlier reports from other oil palm growing countries indicated 17th frond as index leaf for nutrient analysis (Kanapathy *et al*., 1974; Knecht *et al*., 1974; Foster and Goh, 1977; Foster and Chang, 1977). However, our study on nitrogen was different, might be due to irrigated condition.

Chart 4 : Soluble Protein content in different fronds of oil palm

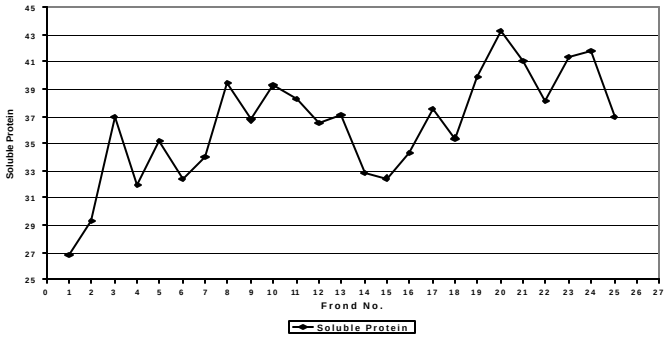
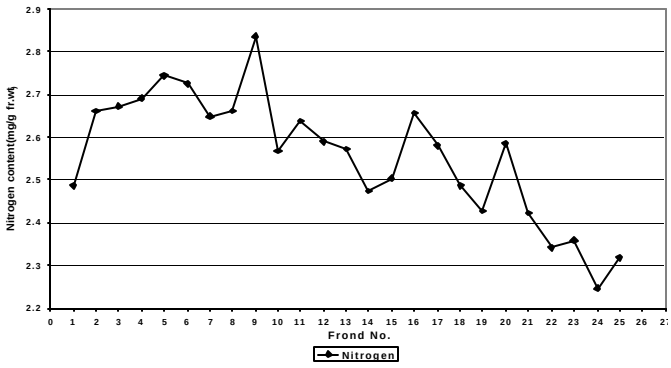


Table 2: High level of Biochemical constituents and nitrogen content in different fronds of mature oil palm

Frond no	Chl-a	Chl-b	Total -Chl	Carotenoids	Total CHO	Soluble Protein	Nitrogen
1	-	-	-	-	-	-	-
2	★	★	★	-	★	-	★
3	-	★	-	-	-	★	★
4	★	★	★	-	-	-	★
5	★	★	★	-	★	★	★
6	-	★	-	-	-	-	★
7	★	★	★	★	-	-	★
8	★	★	★	★	-	★	★
9	✪	✪	✪	✪	✪	✪	✪
10	★	★	★	★	-	★	-
11	★	★	★	★	-	★	★
12	★	★	★	★	-	★	-
13	★	★	★	★	-	★	-
14	★	★	★	★	-	-	-
15	★	★	★	★	-	-	-
16	★	★	★	★	-	-	★
17	★	★	★	★	-	★	-
18	★	★	★	★	-	★	-
19	★	★	★	★	★	★	-
20	-	★	-	-	★	★	-
21	★	★	★	★	★	★	-
22	★	★	★	★	★	★	-
23	★	★	★	★	-	★	-
24	★	★	-	-	★	★	-
25	★	★	★	★	★	★	-

★ Indicates no significant difference among the leaves.

Chart 5 : Nitrogen content in different fronds of oil palm



In case of biochemical analysis, Marziah and Rohani (1995) sampled 17th frond, however, first open frond was sampled by Choong *et al*.(1996) for isozyme analysis. In a similar study, Fernando and Ganjanayake (1997) first selected the best tissue for enzyme extraction and sampled unopened cabbage to the last opened leaf. Finally last unopened leaf was found suitable. Their experiment indicated that a suitable leaf /frond sampling is important for different biochemical parameters.

From the present experimental results, a table (Table 2) is prepared showing the fronds having highest level of biochemical constituents and leaf nitrogen. It indicates that any leaf from 7 to 25th frond can be sampled (except 20 and 13th frond) for pigments (Chlorophyll-a, Chlorophyll-b, total chlrophyll and Carotenoids)

The 20th frond showed highest soluble protein and total carbohydrate content, but it had lower level of pigments. For analysis of soluble protein and carbohydrate, any frond from 19 to 25th could be sampled (except 23rd frond in case of carbohydrates). For analysis of nitrogen, any young frond from 2 to 9th could be sampled.

However, for nitrogen and biochemical parameters (like pigments, soluble protein and total carbohydrate) 9th frond showed the value on par with highest value in all the cases. Hence, when single leaf sample is required to be analysed for nitrogen as well as biochemical parameters mentioned above, 9th leaf could be selected as an index leaf.

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REFERENCES

Bremmer, J.M. 1965. Methods of soil analysis.2.Chemical and microbiological methods. Americansociety of Agronomy, Madison, Wisconsin.

Choong, C.Y., Shah, F.H., Rajanaidu, N. and Zakri, A.H. 1996. Isozyme variation of zarean oil palm (*Elaeis guineensis* Jacq.) germplasm collection. *Elaeis*, **8**: 45-53.

Fernando W.M.U. and Gajanayake, G. 1997. Patterns of isozyme variation in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantations, recherché, developpement,* **July-Aug 1997:** 256-261.

Foster, H.L. and Chang, K.C. 1977. Seasonal fluctuations in oil palm leaf nutrient levels. *MARDI-Research-Bulletin*. **5 :** 74-90.

Foster, H.L. and Goh, H.S. 1977. Yield response of oil palm to fertilizers in West Malaysia. III. Leaf nutrient levels. *MARDI-Research-Bulletin.* **5 :** 56-73.

Hedge, J.E. and Hofreiter, B.T. 1962. In: *Carbohydrate Chemistry* **17** (Eds Whistler, RL. And Be Miller, J.N.) Academic Press New York.

Hiscox, J.D. and Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, **57**:1332-1334.

Kanapathy, K., Tan, S.H. and Cheah, T.E. 1974. Available micronutrients in some peninsular Malaysian soils as indicated by the oil palm. *Malaysian-Agricultural-Journal*. **49:** 357-364.

Knecht, J.C.X., Ramachandran, R. and Narayanan, R. 1974. Variability and other features of leaf K + Ca + Mg in oil palm leaf sampling. *Oleagineux*. **30:** 99-105.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. J. *Biol. Chem*.,**193:** 265.

Mandal P.K., Pillai, R.S.N. and Jayashree, E. 2001. Biochemical analysis of oil palm, In: Annual Report of National Research centre for Oil Palm 2000-2001. Peadvegi-534 450, Andhra Pradesh. pp. 31-38.

Marziah, M. and Rohani, O. 1995. Biochemical studies on abnormal clones of the oil palm (*Elaeis gunneensis*). *Elaeis*, **7:** 10-17.

Suresh, K., Reddy, V.M., Bhanushri, A. and Sivasankara Kumar, K.M. 2002.Ontogenic changes in the leaf nutrient contents of Oil Palm grown under irrigated conditions. *J. Plantaion Crops (supplementary Issue)* (In Press).