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CYTOLOGICAL ANALYSIS OF *Elaeis guineensis* (*tenera*) CHROMOSOMES

Keywords: *Elaeis guineensis (tenera)*; karyotype and cytogenetics

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Cytological analyses were performed on chromosomes of the oil palm Elaeis guineensis *of the tenera fruit phenotype using metaphase chromosome spreads. Root tips were obtained from three- to four-week old seedlings and pretreated with 2mM 8-hydroxyquinoline for 5–6 hours at 18°C to increase the metaphase index. Using a protoplast technique, chromosome spreads free of cell wall and cytoplasmic debris were obtained. Cytological analysis on six metaphase spreads showed that oil palm contains 2n = 32 chromosomes and paired t-tests performed showed no significant difference between homologues for chromosome pairs Nos. 1–5, 7, 8, 10 and 12–16, whereas chromosome pairs Nos. 6, 9 and 11 showed significant differences between members. Significant differences may be caused by varying degrees of chromosome condensation between spreads and slide preparations, and also errors incurred during measurement. Based on length, chromosome pair No. 1 was assigned to group I, Nos. 2–9 were assigned to group II and Nos. 10–16 to group III. Group I consists of the longest chromosome (10.83% of total chromosome length), group II of medium length chromosomes (6.21%–8.42% of total chromosome length) and group III of medium short chromosomes (3.17%–5.37% of total chromosome length).*

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INTRODUCTION

Elaeis guineensis belongs to the family *Palmae*, which contains over 225 genera and 2600 species (Purseglove, 1975).However, the chromosome numbers of only 35 genera and 75 species including *Elaeis guineensis* are known (Imam, 1982). Technical difficulties in studying the cytogenetics of the *Palmae* family have impeded progress in this field. Research on the cytogenetics of *Palmae* has been done by Sharma and Sarkar (1956), Sato (1949), Darlington *et al.* (1945) and Ventakusubban (1945). *Elaeis guineensis* contains 2n = 32 chromosomes (Poerk 1943; Sato 1949; Darlington *et al.*, 1966 and Tan 1976). Sato (1949) suggested that oil palm had two complementary groups of chromosomes, so that 2n = 8A + 24C, and that they consisted of 4 pairs of long chromosomes and 12 pairs of short chromosomes which had either submedian or subterminal constrictions. However, Sharma and Sarkar (1956) suggested that the complementary groups of chromosomes consisted of 3 long pairs, 4 medium length pairs and 9 pairs of short chromosomes. The chromosomes are small (1.15–2.97 microns). Jones *et al.* (1982) estimated the amount of DNA in the diploid nucleus of oil palm as 2 pg and by calculation, the genome size as 1.8×10^9 base pairs. There appears not to be much information on the cytogenetics of oil palm. This is possibly due to the small size of the chromosomes and the difficulty in spreading them (Imam, 1982). The objective of the present research was to characterize the karyotype of oil palm chromosomes.

MATERIALS AND METHODS

Obtaining metaphase spreads

Metaphase spreads were obtained by using a protoplast technique modified from the methods of Ambros *et al.* (1986) and Schwarzacher *et al.* (1989). Root tips from 3 to 4-week old germinated seedlings were pretreated with 2 mM 8-hydroxyquinoline for 5–6 hours; 6 or 7 root tips were then rinsed several times with citric-citrate buffer, pH 4.6, and an approximately 1 mm length was cut from each root tip meristem. This material was incubated in an enzyme solution containing 2% cellulase and 20% pectinase in citric-citrate buffer at 37°C for 1 hour. The softened tissues were collected by centrifuging for 3 min at 800 g. The pellet was resuspended in citric-citrate buffer, and the suspension was centrifuged again. This washing was repeated twice more. The pellet was then resuspended in fixative (absolute ethanol: glacial acetic acid, 3:1). It was again collected by centrifugation and finally resuspended in 40 µl aceto orcein for 5–10 minutes; 40 µl 45% acetic acid was then added. Then 10–20 µl of suspension was placed on a glass slide and covered with a coverslip. A few layers of Whatman filter paper were placed over the coverslip and the tissue was squashed using firm thumb pressure. The edges of the coverslips were sealed with nail polish to prevent drying. Slides were screened under phase contrast using a Carl Zeiss Axioplan microscope.

Selecting metaphase spreads

Metaphase spreads were considered good if they clearly showed 32 well-spaced chromosomes. Such spreads were then photographed at 1000x magnification using black and white Kodak Technical Pan film. *Figures 1a–1f* show selected metaphase spreads. Photographs were developed on Kodabromide Grade 2 photography paper. These photographs were further enlarged. The length of each chromosome was measured three times and averaged (in mm), then converted to actual size (in µm) (*Table 1*). Chromosome pictures were then cut out and paired according to morphology, banding pattern and nearest absolute length. They were then pasted on pieces of paper to produce karyograms. *Figures 2a–2f* show karyograms of metaphase spreads numbered one to six. The lengths of chromosome arms were not measured because centromeric locations were not clear. Statistical analysis was therefore based on morphology and absolute lengths of chromosomes.

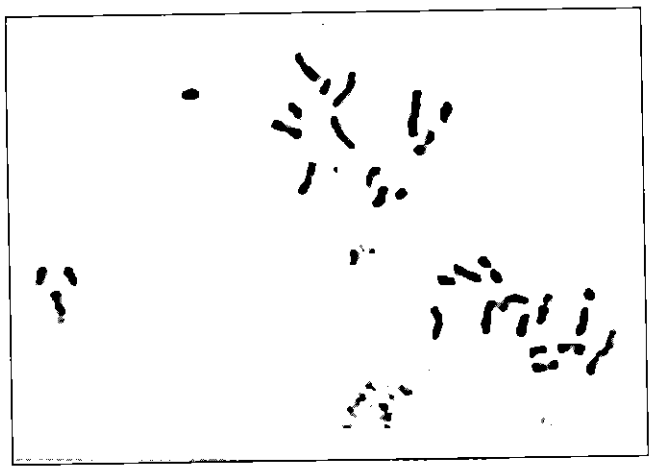

Figure 1a. First metaphase spread.

Figure 1b. Second metaphase spread.

Figure 1c. Third metaphase spread.

Figure 1d. Fourth metaphase spread.

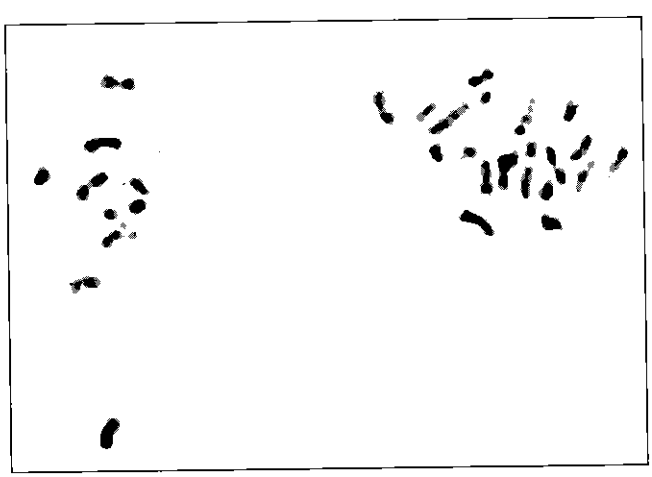

Figure 1e. Fifth metaphase spread.

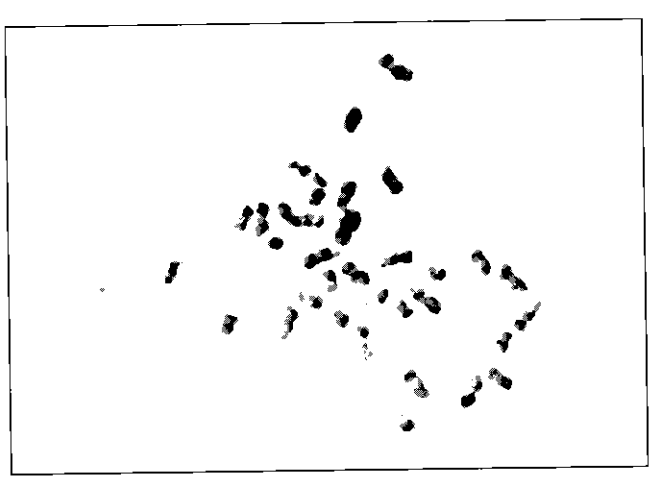

Figure 1f. Sixth metaphase spread.

TABLE 1. ABSOLUTE LENGTH (L) OF EACH CHROMOSOME FOR SIX METAPHASE SPREADS

Chromosome Number	Absolute length (μm)																	
	Spread 1			Spread 2			Spread 3			Spread 4			Spread 5			Spread 6		
	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
1a	3.40	3.80	4.00	3.42	3.61	3.99	3.08	3.30	3.60	3.00	3.40	3.80	3.36	3.36	3.26	3.99	4.10	4.20
1b	3.40	3.40	3.40	3.80	3.80	3.80	3.08	3.30	3.74	3.40	3.20	3.40	2.94	2.84	2.73	3.57	3.68	3.78
2a	3.00	3.00	2.80	2.85	2.66	2.85	2.64	2.64	2.40	2.40	2.40	2.40	2.63	2.73	2.73	3.15	3.15	2.94
2b	3.00	3.00	2.80	2.85	2.85	2.85	2.42	2.64	2.42	2.40	2.40	2.20	2.73	2.73	2.42	2.94	2.94	2.73
3a	2.60	2.60	3.00	2.47	2.66	2.47	2.64	2.64	2.64	2.40	2.30	2.40	2.84	2.56	2.21	2.42	2.48	2.63
3b	3.00	2.60	2.60	2.47	2.66	2.47	2.42	2.42	2.42	2.40	2.20	2.20	2.84	2.52	2.10	2.21	2.42	2.69
4a	2.40	2.40	2.40	2.40	2.47	2.47	2.66	2.64	2.64	2.64	2.40	2.20	2.42	2.31	2.31	2.31	2.52	2.73
4b	2.40	2.40	2.40	2.47	2.47	2.47	2.42	2.42	2.42	2.00	2.20	2.40	2.63	2.63	2.10	2.21	2.31	2.31
5a	2.40	2.40	2.40	2.47	2.47	2.47	2.20	2.53	2.64	2.20	2.20	2.00	2.10	2.31	2.52	2.10	2.52	2.63
5b	2.40	2.40	2.40	2.47	2.47	2.47	2.20	2.42	2.64	2.00	2.00	2.00	2.42	2.52	2.31	2.31	2.31	2.52
6a	2.20	2.40	2.20	2.47	2.47	2.47	2.42	2.53	2.42	2.00	2.00	2.40	2.21	2.31	2.42	2.52	2.63	2.52
6b	2.00	2.00	2.00	2.47	2.47	2.47	2.42	2.42	2.42	2.00	2.00	2.00	2.52	2.42	2.10	2.21	2.31	2.21
7a	2.20	2.20	2.00	2.47	2.47	2.47	2.20	2.20	2.20	2.00	2.00	2.00	2.10	2.21	2.10	2.31	2.52	2.63
7b	2.00	2.20	2.00	2.47	2.47	2.47	2.42	2.42	2.42	2.00	2.00	2.00	2.21	2.31	2.10	2.63	2.31	2.31
8a	2.20	2.20	2.00	2.28	2.28	2.28	2.20	1.98	1.98	2.00	2.00	2.00	2.10	2.10	2.10	2.63	2.31	2.31
8b	2.00	2.20	2.00	2.28	2.47	2.47	2.20	2.20	2.20	2.00	2.00	2.00	2.21	2.21	2.21	2.63	2.63	2.31
9a	2.00	2.10	2.00	2.09	2.28	2.09	2.20	2.20	2.20	1.80	2.00	2.00	2.10	1.89	1.79	2.02	2.21	2.42
9b	1.80	2.00	2.00	1.90	1.90	2.09	1.76	2.20	2.20	1.80	1.80	1.80	1.58	1.79	1.79	2.10	2.21	2.10
10a	1.60	1.60	1.60	1.71	1.71	1.71	1.76	1.76	1.76	1.70	1.60	1.40	1.58	1.68	1.68	2.08	2.10	1.89
10b	1.60	1.80	1.80	1.71	1 90	1.90	1.76	1.76	1.76	1.70	1.80	1.80	1.58	1.58	1.58	1.89	1.89	1.68
11a	2.00	1.40	1.80	1.71	1.52	1.71	1.76	1.76	1.76	1.50	1.60	1.60	1.68	1.58	1.58	1.89	1.79	1.79
11b	1.60	1.60	1.60	1.52	1.52	1.52	1.32	1.54	1.54	1.40	1.40	1.40	1.16	1.68	1.89	1.79	1.79	1.89
12a	1.60	1.60	1.60	1.62	1.62	1.62	1.76	1.76	1.76	1.76	1.20	1.40	1.60	1.26	1.26	1.79	1.68	1.68
12b	1.60	1.60	1.60	1.52	1.43	1.43	1.54	1.32	1.32	1.60	1.40	1.20	1.26	1.26	1.26	1.79	1.89	1.79
13a	1.40	1.40	1.40	1.52	1.52	1.52	1.54	1.54	1.54	1.20	1.40	1.60	1.26	1.26	1.26	1.58	1.58	1.58
13b	1.40	1.40	1.40	1.52	1.62	1.76	1.54	1.54	1.40	1.40	1.40	1.20	1.26	1.26	1.26	1.58	1.58	1.58
14a	1.40	1.40	1.40	1.52	1.52	1.52	1.54	1 54	1.54	1.30	1.20	1.00	1.26	1.26	1.26	1.47	1.47	1.26
14b	1.40	1.40	1.40	1.52	1.52	1.52	1.54	1.54	1.54	1.00	1 00	1.00	1.26	1.26	1.26	1.26	1.37	1.47
15a	1.40	1.40	1.40	1.33	1.33	1.33	1.54	1.54	1.54	1.20	1.20	1.20	1.26	1.26	1.26	1.26	1.47	1.47
15b	1.40	1.40	1.40	1.33	1.33	1.33	1.54	1.54	1.54	1.00	1.00	1.00	1.26	1.26	1.26	1.26	1.26	1.05
16a	1.00	1.00	1.00	1.14	1.14	1.14	1.10	0.88	0.88	0.80	0.90	1.00	1.05	1.05	1.05	1.05	1.05	1.05
16b	1.00	1.00	1.00	1.14	1.14	1.14	1.10	1.10	1.10	0.80	1.00	0.80	1.05	1.05	1.05	1.05	1.05	1.05

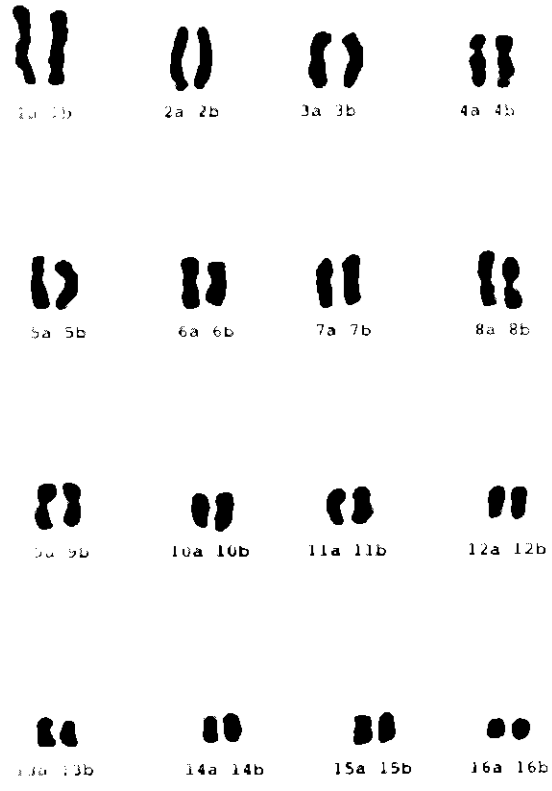

Figure 2a. Karyogram of first metaphase spread.

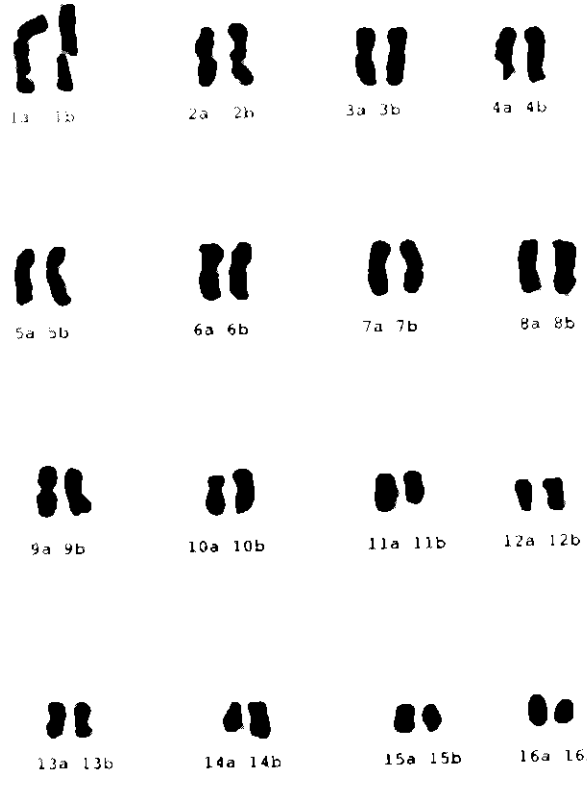

Figure 2b. Karyogram of second metaphase spread.

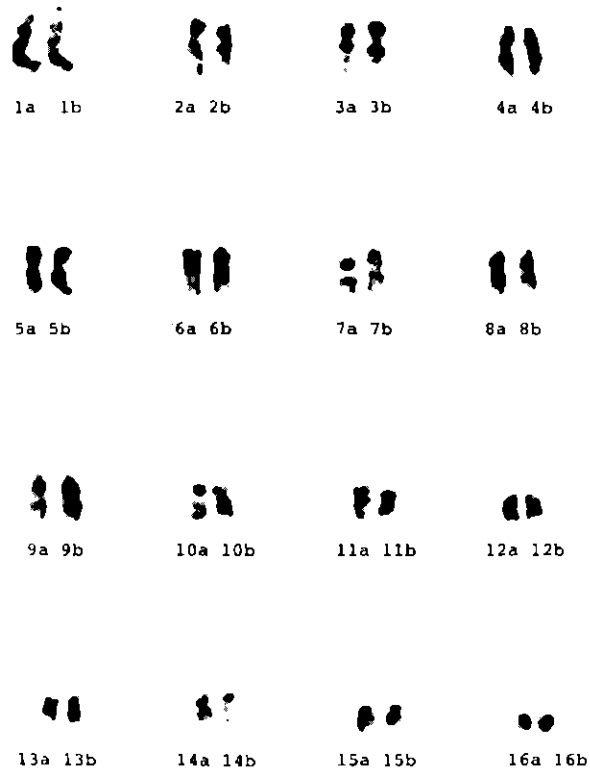

Figure 2c. Karyogram of third metaphase spread.

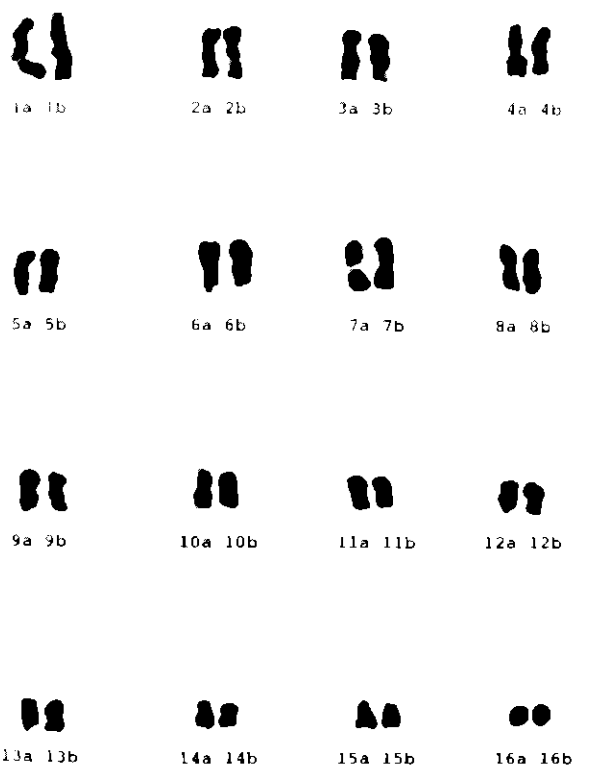

Figure 2d. Karyogram of fourth metaphase spread.

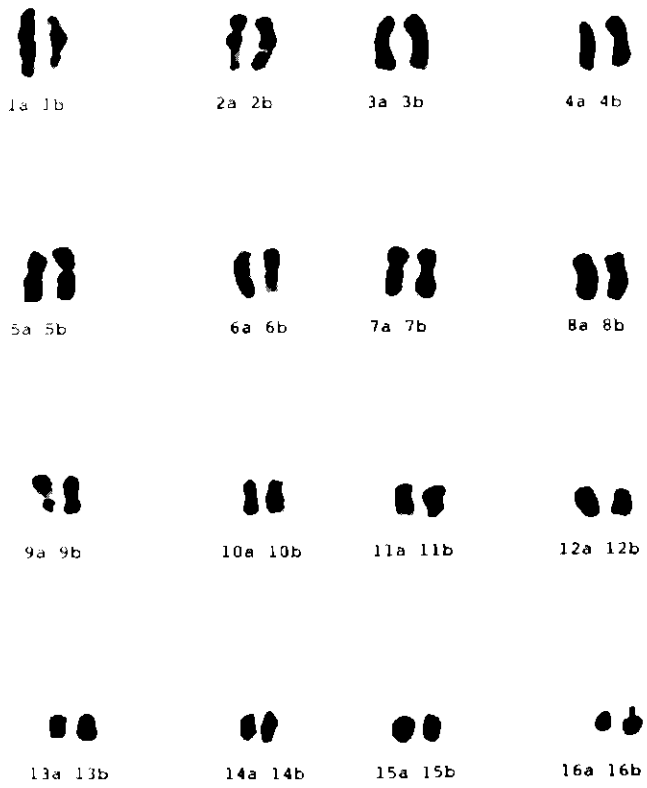

Figure 2e. Karyogram of fifth metaphase spread.

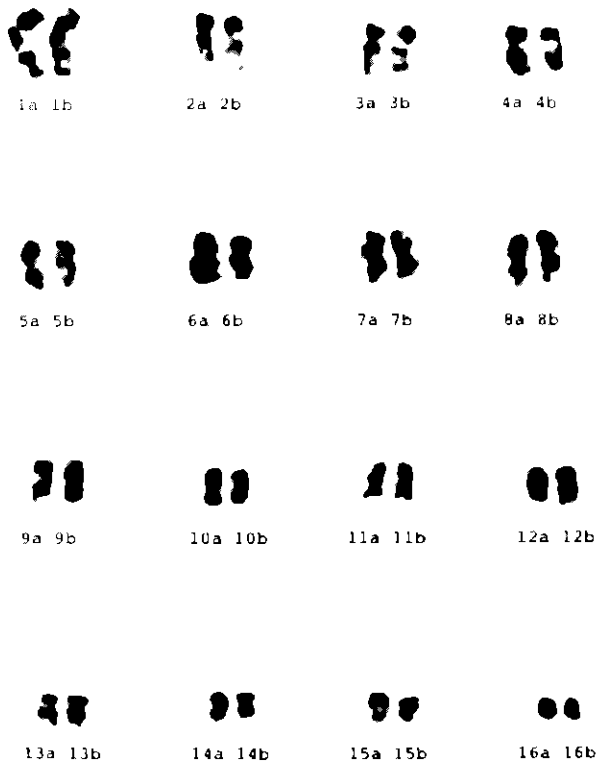

Figure 2f. Karyogram of sixth metaphase spread.

RESULTS

Cytological analysis of six metaphase spreads confirmed that oil palm has a complement of 2n = 32 chromosomes. Paired t-tests were done to ascertain whether pairings of homologues were accurate. *Table 2* shows calculated t values and whether H_o was accepted or rejected. Acceptance of H_o indicates that pairings of homologues have no significant differences while rejection of H_o indicates significant differences. Paired t-tests showed no significant difference between homologues for chromosome pairs Nos. 1–5, 7, 8, 10 and 12–16, whereas chromosome pairs Nos. 6, 9 and 11 showed significant differences between members.

Mean relative lengths (in %) for each chromosome pair are shown in *Table 3*. The average length of each chromosome was obtained from three measurements. This method was used to obtain the mean length of homologous chromosomes for each metaphase spread. Total mean lengths of chromosome pairs Nos. 1–16 were obtained and percentage relative lengths of individual chromosome pairs were calculated.

TABLE 2. ACCEPTANCE OR REJECTION OF PAIRED T-TESTS ON CHROMOSOME PAIRS

Chromosome Pair Number	t_{value}	$t_{critical}$	H_o accepted/ rejected
1	1.629	1.697	Accepted
2	1.000	1.697	Accepted
3	1.130	1.697	Accepted
4	1.470	1.697	Accepted
5	0.190	1.697	Accepted
6	1.856	1.697	Rejected
7	0.376	1.697	Accepted
8	1.100	1.697	Accepted
9	2.530	1.697	Accepted
10	0.640	1.697	Accepted
11	2.250	1.697	Rejected
12	1.050	1.697	Accepted
13	0.450	1.697	Accepted
14	0.526	1.697	Accepted
15	0.940	1.697	Accepted
16	0.570	1.697	Accepted

Oil palm chromosomes can be divided into three groups on the basis of length. Chromosome pair No. 1 is assigned to group I, Nos. 2–9 are assigned to group II, and Nos. 10–16 to group III. Group I consists of the

TABLE 3. MEAN PERCENTAGE OF CHROMOSOME LENGTH

Chromosome Number	Mean chromosome length (μm) for metaphase spread						Mean chromosome length (μm) for metaphase spread						Mean % of chromosome length
	A	B	C	D	E	F	A	B	C	D	E	F	
1	3.57	3.74	3.35	3.37	3.08	3.89	10.99	11.04	10.14	11.49	2.00	11.29	10.83
2	2.93	2.82	2.57	2.37	2.66	2.98	9.02	8.33	7.78	8.08	8.64	8.65	8.42
3	2.73	2.53	2.53	2.32	2.51	2.48	2.41	7.47	7.66	7.91	8.15	7.19	7.80
4	2.40	2.50	2.53	2.24	2.40	2.40	7.39	7.38	7.66	7.64	7.79	6.96	7.74
5	2.40	2.47	2.44	2.07	2.36	2.40	7.39	7.29	7.38	7.06	7.66	7.96	7.29
6	2.13	2.47	2.44	2.07	2.33	2.4	6.56	7.29	7.39	7.06	7.57	6.96	7.14
7	2.10	2.47	2.31	2.00	2.17	2.45	6.47	7.29	6.99	6.82	7.05	7.11	6.96
8	2.10	2.34	2.13	2.00	2.16	2.47	6.47	6.91	6.45	6.82	7.01	7.17	6.81
9	1.98	2.06	2.13	1.87	1.83	2.18	6.10	6.08	6.45	6.38	5.94	6.32	6.21
10	1.67	1.77	1.76	1.67	1.61	1.92	5.14	5.23	5.33	5.70	5.23	5.57	5.37
11	1.67	1.58	1.58	1.48	1.59	1.82	5.14	4.67	4.78	5.05	5.16	5.28	5.01
12	1.60	1.54	1.58	1.40	1.26	1.77	4.93	4.55	4.78	4.77	4.09	4.58	4.53
13	1.40	1.58	1.58	1.40	1.26	1.58	4.31	4.67	4.78	4.77	4.09	4.58	4.53
14	1.40	1.52	1.54	1.08	1.26	1.38	4.31	4.49	4.66	3.68	4.09	4.00	4.21
15	4.40	4.33	4.54	1.10	1.26	1.30	4.31	3.93	4.66	3.75	4.09	3.77	4.09
16	1.00	1.14	1.03	0.88	1.05	1.05	3.08	3.37	3.12	3.00	3.41	3.05	3.17
Total Mean length of chromosomes Nos 1–16	32.48	33.86	33.04	29.32	30.79	34.47	100%	100%	100%	100%	100%	100%	100%

longest chromosome (10.83% of total chromosome length), group II of medium length chromosomes (6.21%–8.42% of total chromosome length) and group III of medium short chromosomes (3.17% –5.37% of total chromosome length).

DISCUSSION

There have been varying reports on oil palm chromosomes. Imam (1982) studied mitosis in *Elaeis guineensis* of the Deli *dura* fruit type and reported the chromosome number as 2n = 32. Karyotype analysis showed three groups of chromosomes designated as A, B and C (A = 3, B = 5 and C = 8). For the *tenera* fruit type, Low (1990) found that pachytene chromosomes had n = 16, therefore 2n = 32. Of 16 chromosomes, 8 were submedian, 3 median and 5 subterminal, while chromosomes No. 4 and No. 7 were found to carry the nucleolar organizer regions (NOR). In the present study, two pairs of chromosomes were also found to carry these regions. One pair belonged to group II (medium length chromosomes) and another pair to group III (short chromosomes).

Wong (1992) reported *tenera* chromosome pairs as submetacentric except for pairs No. 14 and No. 15, which were reported to be subterminals. Pang (1993) found chromosome No. 8 to be metacentric and the other chromosomes submetacentric. The differences in observations are due to the difficulty of determining the location of centromeres. The cytological analysis we have carried out used absolute length as the parameter because of the difficulty mentioned. Therefore, observations by Wong (1992) and Pang (1993) could not be verified.

In summary, cytological analysis on six metaphase spreads showed that the oil palm complement is 2n = 32 chromosomes. Paired t-tests performed showed no significant differences for chromosomes pairs Nos. 1–5, 7, 8, 10 and 12–16, whereas chromosome pairs Nos. 6, 9 and 11 showed significant differences between members. Such differences may be caused by varying degrees of chromosome condensation between spreads and slide preparations and also by errors incurred during measurement. On the basis of length, oil palm chromosomes can be divided into three groups. Pair No. 1 is assigned to group I, pairs Nos. 2–9 are assigned to group II and Nos. 10–16 to group III. Group I consists of the longest chromosome (10.83% of total chromosome length), group II consists of chromosomes of medium length (6.21%–8.42% of total chromosome length) and group III of medium short chromosomes (3.17%–5.37% of total chromosome length).

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