13. F. III

FINAL REPORT

1. Institute Code No: Gen IV (231)

2. I. C. A. R. Code No: P1-70/1-ICI-F 30/0311

3. Name and Address of Research Institute/Centre:

Central Plantation Crops Research Institute
Kasaragod 670 124 Kerala

4. Project Title: CYTOLOGICAL INVESTIGATIONS IN DIFFERENT VARIETIES OF *COCOS NUCIFERA* AND RELATED SPECIES

5. Name and Designation of Project Leader

1. Smt PK Thankamma Pillai (RA) 1970-71
2. Mr AA Mohammed Syed (RA) 1972-75
3. Mr K Vellaichamy (RA) 1975
4. Ms PM Zubaida (SRA) 1976
5. Mr. G Vijaya Kumar (S1) 1976-

6. Name (s) and Designation(s) of Project Associates including Project Leader and work to be done:

Sl. No.	Name and Designation			Time spent	work done
1.	PK Thankamma Pillai	RA	1970-71	3 months/yr	Pachytene analysis of CDG & meiosis in self and OP Pro
2.	AA Mohammed Syed	RA	1972-75		Meiosis in Dwarf cultivars
3.	K Vellaichamy	RA	1975		
4.	PM Zubaida	SRA	1976-77		
5.	MC Nambiar	Geneticist	1971		Meiosis in Dwarf cultivars
6.	KUK Nampoothiri	"	1972-73		Pollen sterility studies
7.	MK Nair	"	1974-75		
8.	G Vijaya Kumar	S1	1976-79	3 months/yr	Chromosome banding
9.	EVV Bhaskara Rao	S1	1978-79	2 months/yr	Pachytene analysis in WCT

7. Location of Research Project with complete address (Division/Section/Sub-Centre)

Division of Genetics
Central Plantation Crops Research Institute
Kasaragod 670 124
Kerala

8. Date of start

1970

9. Date of termination

December 1979

10. (a) Objectives (Not more than 150 words)

1. To study the cytological features and their applied significance in different varieties and forms of *Cocos nucifera*

2. Assess the genetic variation in different forms cytologically for the economic utilisation of available genetic material

(b) Practical Utility including background information (Not more than 150 words)

(as given in the project proposal form)

A thorough knowledge of the cytological behaviour of different species of the genus *Cocos* and different varieties and forms of *Cocos nucifera* is not only a taxonomic and phylogenetic significance but it would also provide explanations for such aspects like pollen sterility, lethality of gametes etc., and assist in directing the breeding programme in proper lines. For better understanding of the of the cytological behaviour, Karyomorphological studies as well as meiotic studies at different stages of microsporogenesis are essential.

13. Approximate expenditure incurred in the Project: (Give reasons for variation, if any, from original estimated cost)

ca. Rs.1,25,000/ (which includes the cost of Fluoresecent Microscope, Chemicals and proportionate salaries of the scientist)

14. Publications and material (one copy each to be supplied with this proforma)

a) Research papers Three

b) Popular articles

c) Reports

d) Seminars and workshops (Relevant to the Project) in which the Scientists have participated:

Smt. PK Thankamma Pillai and G Vijaya Kumar attended International Symposium of Coconut Research and Development, December 1976 Kasaragod and presented a paper entitled 'Cytology of Coconut'

e) Material developed such as new varieties of crops or breeds of farm animals, implements, products, etc.)

nil

15. Details (Nos. etc.) of Field/Laboratory Note books and final material and their location.

Project Log Book is available in Division of Genetics, Central Plantation Crops Research Institute, Kasaragod 670 124, Kerala.

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Cytology of Coconut

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Abstract

The chromosome number in coconut is $2n=32$. Karyomorphological studies show sli.ht differences between Tall and Dwarf varieties. Meiosis was generally regular in Tall cultivars and in hybrids between Tall and Dwarf, while several irregularities have been reported in the Dwarf and semi-tall cultivars. Pollen fertility is very high in Talls and hybrids as compared to that in Dwarfs and semi-talls. Meiotic irregularities are commonly met within the 'spicata' palms and these are believed to have originated from Talls.

Free nuclear division has been reported in coconut milk. C-mitosis leading to high ploidy level and several chromosome abnormalities occur in developing endosperm. In Philippines 'makapuno' nuts, the endosperm development is very abnormal. An inverse relationship between ploidy level and oil content has been also observed by some authors.

The two existing views on the origin of Dwarfs from Talls, namely, mutant origin or by continuous inbreeding, are presented. Another possibility that both Talls and Dwarfs might have had a common origin in a single ancestral form has been proposed.

Introduction

Cytological studies on coconut are few, though the palm is of great economic importance and has been used by man from time immemorial. The first report appeared in 1929 when Santos reported the gametic number of a Philippine coconut variety to be 16. Subsequently, reports on chromosome number have been published by several workers (Janaki Ammal, 1945; Venkatasubban, 1945; Sharma and Sarkar, 1956; Ninan, Pillai, and Joseph, 1960; Abraham, Mathew, and Ninan, 1961). Their studies and those that followed have confirmed the somatic number of coconut to be $2n=32$ (Nambiar and Swaminathan, 1960; Swaminathan and Nambiar, 1961; CCRS Annual Report, 1962-63; Raveendranath and Ninan, 1973). Nambiar and Upadhyaya (1961) outlined a pretreatment staining procedure—fixing—for the study of coconut root cells.

The review is divided into three parts, karyomorphology, meiotic

TABLE 2. Meiotic behaviour of some varieties and hybrids

Cultivar/ hybrid	Chiasma frequency		Percentage of irregularities at				Percentage of pollen sterility (non-stainability)
	per cell	per bivalent	Diakinesis and Metaphase	Anaphase I & II	Telophase I & II	Sporad stage	
WC Tall	28.9	1.8	6.4	1.9	0.3	0.7	3.6
Laccadive Ordinary	27.6	1.7	7.6	1.8	1.7	0.9	3.5
Andaman Giant	28.5	1.8	4.9	2.1	2.5	1.0	3.0
CDG	26.8	1.7	26.1	8.5	6.6	9.4	32.2
CDO	27.1	1.7	19.6	7.9	5.1	6.6	21.9
T×D hydrid	28.5	1.8	6.8	1.8	1.3	0.8	2.9
D×T hybrid	27.6	1.7	10.3	2.8	2.6	2.5	7.8

studies, and endosperm cytology, along with a concluding section on cytological evidences on the origin of Dwarf coconuts.

Karyomorphology

Nambiar and Swaminathan (1960) found that the majority of chromosomes in Tall palms had sub-median centromers and that they differed considerably in length. In the West Coast Tall (WCT) variety, two pairs of chromosomes were much longer and three pairs relatively short. They observed two satellited chromosome pairs, the satellites occurring on the long arm of chromosome VI and short arm of chromosome IX. Sharma and Sarkar (1956) also observed two pairs of SAT-chromosomes in the coconut variety studied by them. Raveendranath and Ninan (1963) compared the karyotypes of WCT, spicata, Chowghat Dwarf Green (CDG), and Chowghat Dwarf Orange (CDO) and found that the main distinguishing feature between the karyotypes of Tall and Dwarf forms was the presence of the secondary construction in Talls, on the long arm of chromosome VI, while in Dwarfs, on the long arm of chromosome III. But, this stated difference does not seem to be consistent. In the material studied in our Institute, chromosomes VI and XII were found to be satellited (Ann. Rep. 1962-63; 1973). The total length of the chromosome complement was higher in CDG than in WCT. Table 1 gives the comparative features of the WCT and CDG karyotypes.

TABLE 1. Relative length and index of the chromosomes of WCT and DG varieties*

Chr. no.	WC Tall		Dwarf Green	
	Relative length (in microns)	S.A./L.A. (index)	Relative length (in microns)	S.A./L.A. (index)
I	9.11	0.55	8.92	0.60
II	8.36	0.50	8.38	0.58
III	7.46	0.59	8.06	0.60
IV	7.04	0.59	7.28	0.58
V	6.91	0.61	7.04	0.65
VI	6.91	0.71[1]	6.80	0.72[3]
VII	6.80	0.74	6.58	0.65
VIII	6.49	6.68	6.43	0.71
IX	6.34	0.66[2]	5.80	0.74
X	6.01	0.81	5.80	0.80
XI	5.91	0.88	5.58	0.79
XII	5.40	0.89	5.37	0.75[4]
XIII	5.18	0.88	5.03	0.74
XIV	4.77	0.77	4.53	0.76
XV	4,32	0.72	4.23	0.80
XVI	3.76	0.89	3.97	0.83

*WCT data from Nambiar and Swaminathan (1960); DG data from Thankamma Pillai and Vijayakumar (unpublished).

[1]Satellite on the long arm.

[2]Satellite on the short arm.

[3]Satellite on the short arm.

[4]Satellite on the long arm.

Meiotic Studies

Nambiar and Swaminathan (1960) and Nambiar, Thankamma Pillai, and Vijayakumar (1970) observed significant differences in the meiotic behaviour between different cultivars of Tall and Dwarf forms, and between open pollinated and inbred populations. WCT and Laccadive Ordinary, another Tall cultivar, showed normal meiosis. Nambiar and Swaminathan (1960) observed many meiotic abnormalities in the cultivars Apricot and Dwarf Red.

Pachytene analyses conducted by the two senior authors on CDG showed that it had 7 subterminal, 7 submedian, and 2 median chromosomes. The short arm of chromosome VI and long arm of chromosome XII have nuclear organizer regions (Ann. Rep. CPCRI, 1973). This observation differs from that of Nambiar and Swaminathan (1960) on Talls and Raveendranath and Ninan on Dwarfs (1973). Thankamma Pillai and Vijayakumar (unpublished data) studied the meiosis of nine cultivars and hybrids (Table 2) and found that percentage of abnormalities was highest in Dwarf Green and Dwarf Orange. In D×T and T×D hybrids, chromosome abnormalities and sterility were very slow. The higher chromosome abnormalities and sterility in Dwarfs may be due to the higher degree of inbreeding in them. Nambiar et al. (1970) have also studied the cytology of open pollinated and inbred lines (also Ann. Rep. CPCRI, 1971).

Spicata are Tall palms differing from ordinary Talls in inflorescence character and breeding behaviour. They produce unbranched inflorescences and show a high degree of suppression of male flowers. Their cytology has been studied by Ninan and his coworkers (Ninan et al., 1960; Ninan and Satyabalan, 1963; Ninan and Nambiar, 1974). The meiosis was irregular with inversions, translocations, and many other abnormalities. Like Talls, spicata palms are predominantly outbreeders. They are believed to have arisen from Talls through mutation. However, Child (1974) considered spicata as a distinct variety because of the existence of a Dwarf form also.

In addition to the above studies on karyomorphology and meiosis, there have also been reports on the cytology of an abnormal palm (Thankamma Pillai and Vijayakumar, 1972), on palms affected with root (wilt) disease (Nambiar and Prasannakumari, 1964) and also on a bulbiferous palm (Raveendranath, Nair, and Ninan, 1975), Parthenogenesis was also known to occur (Venkataraman, 1928). The only report on haploidy was by Whitehead and Chapman (1962) who isolated a haploidy seedling from 31 twin seedlings raised by them. Ninan and Raveendranath (1965) has observed a haploid embryo in a WCT palm.

Endosperm Cytology

As early as in 1927 Quisumbing and Juliano studied the development of the ovule and the embryosac in coconut. The development of the endosperm has been described in detail by Cutter and coworkers (Cutter, Wilson, and Dube, 1952 a, b; Cutter and Freeman, 1854; Cutter, Wilson, and Freeman, 1955). They made interesting observations on the occurrence of free nuclei in the liquid syncytial endosperm commonly known as coconut milk. Free nuclear divisions in the coconut water have also been reported by Dutt (1953) and Abraham and Thomas (1962). But biochemical analysis of coconut water by Mondal, Mandal, and Biswas (1970a, b) did not support the above view on the presence of free nuclei.

Abraham and coworkers (Abraham and Mathew, 1963; Abraham, Ninan, and Gopinath, 1966) noted that in about 6 month old nuts, the nuclei of the developing endosperm tissue varied considerably in size. They found that the tissue adjacent to the endothelium was normally triploid ($3x=48$), less frequently hexaploid ($6x=96$), and still less frequently dodecaploid ($12x=192$). They proposed that higher ploidy levels arose by c-mitosis. They also recorded an inverse relationship between ploidy levels of endosperm and oil content. In the Tall variety, the outer, middle, and inner parts differed in the oil content, the percentage being 75.7, 54.1 and 41.1, respectively. They analysed Laccadive Tall, S.S. Apricot, CDG, CDO, and Philippine makapuno nuts and found that the last one had the lowest oil content and also the highest ploidy level (Abraham, 1963; Abraham et al., 1965).

The Philippine makapuno coconuts are peculiar in having loose jelly like endosperm filling the entire cavity. They do not germinate and this is ascribed to an incompatibility reaction between a normal embryo and abnormal endosperm (Abraham et al., 1965). These authors reported high ploidy levels ($48x$ and above) in the buttery endosperm which according to them arose through amitosis and nuclear fusion. Cruz and Ramariz (1968) found two types of cells in this endosperm, normal cells and micro-cells. They thought that the latter originated from normal cells through budding. Chromosome counts ranged from 32 to 96 ($2x$ to $6x$) with 48 as the most frequent number.

Recently, de Guzman and colleagues have succeeded in culturing the embryo from these nuts on artificial medium and have reported a high recovery of makapuno character (de Guzman, 1969; Balaga and de Guzman, 1970; de Guzman and del Rosario, 1974; de Guzman, Rafols, and del Rosario, *this Symposium*).

Cytological Evidence on the Origin of Dwarf Coconuts

Two hypotheses have been advanced to explain the origin of Dwarfs. The first one considers Dwarfs to have evolved by a recessive mutation from Talls (Handover, 1919; Anonymous, 1921; Jack and Sands, 1922; Pancho, 1960). According to the second hypothesis, the Dwarfs and semi-Talls occurring in nature are the products of several generations of inbreeding of Tall palms (Nambiar and Swaminathan, 1960; Swaminathan and Nambiar, 1961).

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Comparative studies on the meiosis of Talls and Dwarfs (Sharma and Sarkar, 1956; Nambiar and Swaminathan, 1960; Ninan et al., 1961; Abraham et al., 1961; Ann. Rep. CPCRI, 1972, 1973) reveal that meiosis in the former was more normal. It was therefore assumed that the Talls were the ancestral types, and that the Dwarfs, semi-Talls, and spicata were the derived ones. In support of this, it has been pointed out that the Philippine Dwarf palm Tambulilid is known to be of recent mutant origin (Pancho, 1960).

One criticism that can be levelled against the hypothesis on the origin of Dwarfs from Talls by a process of inbreeding is that in many Pacific Islands, natural inbreeding isolated populations of coconut have been existing for thousands of years. If continued inbreeding leads to Dwarfs, we would expect predominantly Dwarf populations in these areas. But this is not the case (Whitehead, 1966). There are also no cytological morphological, or genetical evidences to prove such a hypothesis, Moreover, some Dwarfs, such as the Fijian Dwarf, are highly heterogeneous and generally outbreeders. These conditions should not happen if the origin of Dwarfs has been through continuous inbreeding. It is therefore possible that both Talls and Dwarfs had a common origin from a single ancestral form and they underwent a certain degree of divergence during the course of evolution. The Dwarfs have got definite adaptive value in its early seed bearing habit, and greater seed production. In the course of domestication and co-existence much natural crossings might have taken place leading to the establishment of intermediate populations and the present day cultivated Dwarfs. Experimental verification of any of these hypotheses is not easy in a species like coconut, and much more basic studies are required before we can arrive at an acceptable conclusion on the origin of Dwarfs.

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CYTOLOGY OF AN ABNORMAL COCONUT PALM

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(Revised Mss. recd. 17–v–72; Accepted: 24–v–72)

A COCONUT palm (*Cocos nucifera* L.) showing defective fruit set was subjected to cytological analysis. This paper reports the deviations from normal cytological behaviour of this palm during meiosis.

MATERIALS AND METHODS

An abnormal plam in the progeny of a self-pollinated New Guinea palm at the Central Plantation Crops Research Institute, Kasaragod, was used for this study. The palm is fourteen years old, but no fruit set was recorded in this palm. Feulgen squashes of the root tip chromosomes pretreated with 0·002 M 8–hydroxy quinoline was used for the study of somatic chromosomes. For the study of meiosis, flower buds from the third inflorescence above the one that has opened were fixed in Carnoy's fixative (6 : 3 : 1) containing a trace of iron acetate. Analyses were carried out from temporary acetocarmine preparations. Pollen sterility estimates were based on acetocarmine stainiability.

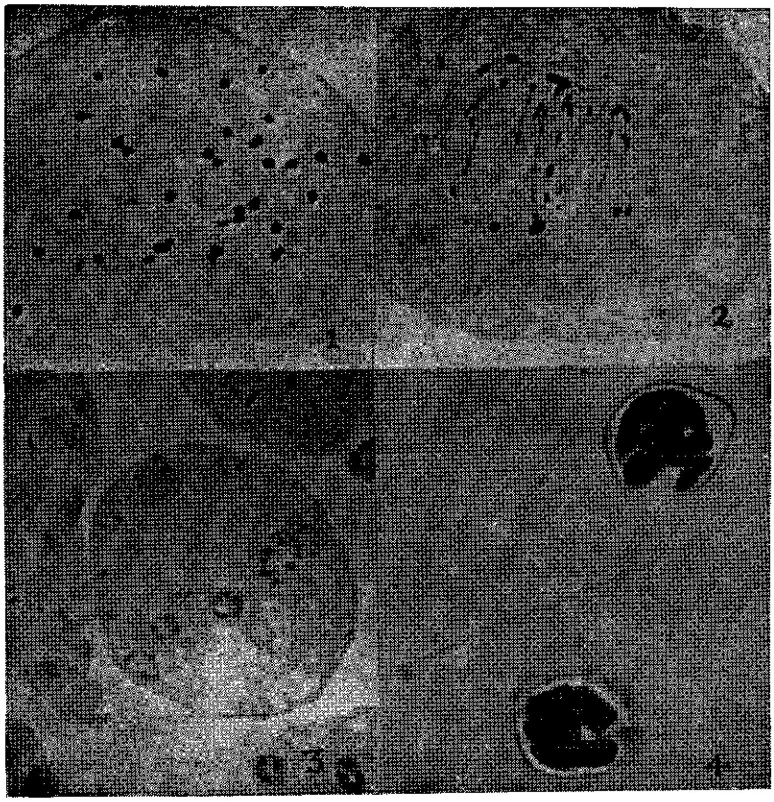
RESULTS AND DISCUSSION

The chromosome complement of *Cocos nucifera* is n=16 as reported by Santos (1929) and subsequently confirmed by several workers. Root tip squashes of the palm investigated showed 32 chromosomes. Thus, the somatic cells showed a normal chromosome complement.

The pollen mother cells underwent both the meiotic divisions; however, aberrations were recorded at all stages and in nearly all the pollen mother cells examined.

Deviations from normal pairing was observed at pachytene. At diakinesis and metaphase I, about 65% of the cells showed 32 univalents (Fig. 1) and 28 per cent exhibited 3 or 4 bivalents with the rest as univalents. Of the total number of cells examined, 5% were having 32 bivalents. About 2% of cells showed stickiness of chromosomes at metaphase I.

These abnormalities in the early stages of division were reflected in subsequent stages also. At anaphase I, 71·7% of the cells showed sticky bridges (Fig. 2), whereas inversion bridge was noticed in only one cell. At anaphase I and II, 23·4% of the cells contained laggards and 4·7% exhibited irregular spindle formation. During telophase II, almost all the cells showed more than four daughter nuclei (Fig. 3). Up to 25 daughter nuclei were observed in telophase II. At the end of meiosis, 28·2% of the pollen mother cells contained more than eight spores (Fig. 4). Octads (23·7%), heptads (18·9%), hexads (15·8%) and pentads (9·5%) were of common occurrence. About 3·4%



A note on the cytology of an abnormal coconut palm.

FIG. 1. A cell with 32 univalents at metaphase I. × 2000.

FIG. 2. Multiple bridge at anaphase I × 2000.

FIG. 3. Telophase II showing more than four daughter nuclei. × 760.

FIG. 4. Sporads with more than four spores. × 760.

of the cells showed monads also. Tetrads were observed (0·4%), but all the spores in one tetrad were not uniform in size. All the pollen grains were sterile.

One of the interesting facts noted here is that the plam under study is the selfed progeny of a selfed plant of New Guinea variety. Another progeny of the same mother palm also showed complete meiotic irregularities as well as complete pollen sterility (unpublished data). The mother palm itself, which is in the generation S_1, showed a high degree of meiotic irregularity (unpublished data). These irregularities in this naturally cross-fertilized species can be ascribed to inbreeding depression. Meiotic irregularities accompanying inbreeding in a normally cross-pollinated species have also been reported in many plant species such as rye (Lamm, 1936; Muntzing and Akdik, 1948), *Dactylis glomerata* (Myers and Hill, 1943) and coconut (Nambiar and Swaminathan, 1960).

Summary

Studies on the course of microsporogenesis in the progeny of a self-pollinated New Guinea palm revealed highly aberrant meiosis. The sterility in this palm has been attributed to inbreeding.

Acknowledgements

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CYTOLOGICAL BEHAVIOUR OF FIRST INBRED GENERATION OF COCONUT

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CYTOLOGICAL and breeding investigations in coconut (*Cocos nucifera* L.) are few as compared with other crop plant species for obvious reasons. Santos (1929) was the first to report the gametic number n=16 in coconut. This number has been subsequently confirmed by several workers (Sharma and Sarkar, 1956; Nambiar and Swaminathan, 1960; Ninan, Pillai and Joseph, 1960). A comparative study of meiosis in dwarf and tall varieties (Nambiar and Swaminathan, 1960; Swaminathan and Nambiar, 1961) showed that while meiosis is usually normal in the tall variety, the dwarf palms are generally heterozygous for chromosomal rearrangements like translocation and inversion. From the sum total of observations on cytological and breeding behaviour and morphology, Swaminathan and Nambiar (1961) have suggested that the dwarf palms occurring in nature may be the product of many generations of inbreeding in different varieties of coconut. Results of similar work reported in inbred rye by Lamm (1936), Prakken and Muntzing (1942), Muntzing and Akdik (1948), Rees (1955) and Heneen (1961) have clearly indicated that the behaviour of the chromosome is less efficient in inbred rye plant than in the open pollinated population. In coconut, information on the cytological consequences of inbreeding is very limited, though on vegetative and yield characters Harland (1957) expressed the view that, the ill iffects of inbreeding may not be universal in coconut. A comparative study of inbreds with their parental types and open pollinated sister plants (Ninan, Pankajakshan, Satyabalan and Gopinath, 1961) also show that different varieties of coconut respond to selfing in quite different ways and are affected to varying extents as far as vegetative and yield characters are concerned. The object of the present investigation was to study the cytological consequences of inbreeding in a few geographically distinct varieties of coconut.

MATERIALS AND METHODS

The inbred and open-pollinated progenies of ten exotic varieties of tall palms and five types of two geographically distinct varieties of dwarf palms available at the Central Coconut Research Station, Kasaragod, formed the material for this study. The inbred and outbred progenies of Andaman Giant,

TABLE 1

Sporad abnormalities and pollen sterility

Variety	Monads	Dyads	Tetrads	Pentads	Hexads	Heptads	Octads	Normal sporads %	Pollen sterility %
Andaman giant O.P.	—	—	400	3	1	—	—	99·00	2·97
Andaman giant S.P.	3	2	447	13	10	2	4	93·00	12·55
Cochin china O.P.	5	4	455	15	4	—	2	93·80	12·05
Cochin china S.P.	3	4	450	12	2	—	—	95·70	11·20
Laccadive O.P.	4	—	710	2	—	—	—	99·10	3·46
Laccadive S.P.	1	1	832	3	2	—	—	99·00	4·16
New guinea O.P.	—	—	302	13	10	3	—	92·00	12·00
New guinea S.P.	2	2	374	7	2	2	2	95·70	8·15
Philippines O.P.	—	—	220	3	—	—	—	98·64	5·56
Philippines S.P.	4	6	400	10	3	—	3	94·40	11·35
Dwarf red	18	10	1390	36	20	6	8	93·42	21·89
Dwarf green	21	14	1260	39	9	3	4	93·34	31·18
Dwarf yellow	5	2	200	4	1	—	2	93·00	26·90
Malayan dwarf green	4	20	540	25	5	3	28	86·40	40·11
Malayan dwarf yellow	13	6	655	13	22	4	11	90·35	25·23

TABLE 2

Chromosome association at different stages of meiosis

Variety	No. of trees examined	Total No. of cells examined	Diakinesis and Metaphase: Chiasma frequency per cell	Chiasma frequency per bivalent	Abnorm.	Cell with: Univalents	Quadrivalents	Stickiness	Chromosome mosaic	Anaphase I and II: No abnor.	Cells with: Laggards	Sticky bridge	Inversion bridge	Irregular spindle mechanism	More or less than 4 groups at A II	Telophase I and II: No. abnor.	Cells with: Micronuclei	More or less than 4 groups at T II
Andaman giant O.P.	1	773	28·53	1·78	202	1	—	9	—	275	6	—	—	—	—	273	7	—
Andaman giant S.P.	1	654	27·75	1·73	152	3	—	25	—	196	16	—	1	—	2	237	25	—
Cochin china O.P.	1	1049	28·24	1·76	434	9	—	69	1	198	15	—	—	—	1	312	14	—
Cochin china S.P.	1	528	28·00	1·75	235	4	—	32	1	105	6	—	—	—	2	140	10	—
Laccadive O.P.	2	1372	27·59	1·72	420	4	—	31	—	492	9	—	—	—	—	405	7	—
Laccadive S.P.	5	2872	27·52	1·72	973	9	—	64	—	870	14	1	—	—	—	883	18	—
New guinea O.P.	1	416	28·60	1·79	20	2	—	5	—	168	13	—	—	—	—	198	11	—
New guinea S.P.	1	712	28·40	1·77	140	8	—	20	—	251	15	—	—	—	—	276	18	—
Philippines O.P.	2	1656	28·30	1·77	527	8	—	53	—	545	11	—	—	—	—	503	9	—
Philippines S.P.	2	1889	27·61	1·72	607	21	6	94	7	592	35		1	3	2	484	33	1
Dwarf red	4	2915	27·13	1·69	840	56	9	204	5	670	40	11	3	4	—	1018	55	—
Dwarf green	4	2385	26·74	1·67	758	42	7	260	4	506	38	12	1	3	1	696	58	—
Dwarf yellow	1	654	26·60	1·66	205	11	—	92	—	135	7	2	1	1	—	190	10	—
Malayan dwarf green	1	882	27·17	1·69	270	15	4	55	4	255	24	3	1	3	3	210	27	8
Malayan dwarf yellow	1	657	26·28	1·64	185	11	1	37	3	175	15	3	—	1	—	210	14	2

Cochin China, Philippines, New Guinea and Laccadive and also the dwarf red, dwarf green, dwarf yellow and Malayan dwarf varieties were screened to study their cytological behaviour at different stages of meiosis.

Male flowers in the third inflorescence above the one that has opened were fixed in Carnoy's fluid (6:3:1) containing a trace of iron acetate. The anthers were smeared in aceto-carmine. The pollen sterility was determined by the aceto-carmine staining technique.

Results

The cytological observations are summarised in Tables 1 and 2.

LACCADIVE VARIETIES

Meiosis was comparatively regular in all the five inbred and two open pollinated progenies. There were 16 bivalents at diakinesis and metaphase I (Fig. 1). On an average 65% of the bivalents of a cell had chiasmata in both the arms and 35% were of the rod type. Among the ring bivalents 8% had three chiasmata each and among the rod bvialents 0·5% had two chiasmata on one of the arms.

Precocious separation of bivalents and stickiness of chromosomes at metaphase I were observed in a very low percentage of the cells in both inbred and open-pollinated progenies. A low percentage of lagging chromosomes at anaphase and micronuclei at telophase were also observed.

PHILIPPINES VARIETIES

The mean number of chiasmata per bivalent in the inbred progenies of Philippine varieties was comparatively lower than that in the open-pollinated ones. In the open-pollinated progeny of Philippines Laguna, chiasma frequency was 1·76 per bivalent and in the inbred 1·73. In Philippines Kalambahim, the chiasma frequencies in outbred and inbred progenies were 1·77 and 1·72 respectively.

Various types of abnormalities were observed in the inbred progenies of this variety. Out of 607 cells screened, six cells had one quadrivalent and 14 bivalents at metaphase I (Fig. 2) suggesting heterozygosity for reciprocal interchange. Precocious separation of bivalents (Fig. 3) and stickiness of chromosomes at metaphase I were comparatively higher in the inbred palms than in their open pollinated sister plants. Occurrence of microsporocytes with varying chromosome numbers (Fig. 4) were observed in 1·2% of the cells studied at metaphase I suggesting premeiotic irregularities.

At anaphase I, besides 3 cells with sticky bridges, a dicentric bridge and an acentric fragment were observed in one cell, indicating heterozygosity for an inversion in the inbred progenies. Lagging chromosomes at anaphase I and II and micronuclei at telophase were comparatively higher in the inbred plants. In the inbred progeny of Philippines Laguna a cell at anaphsae II with 8 groups

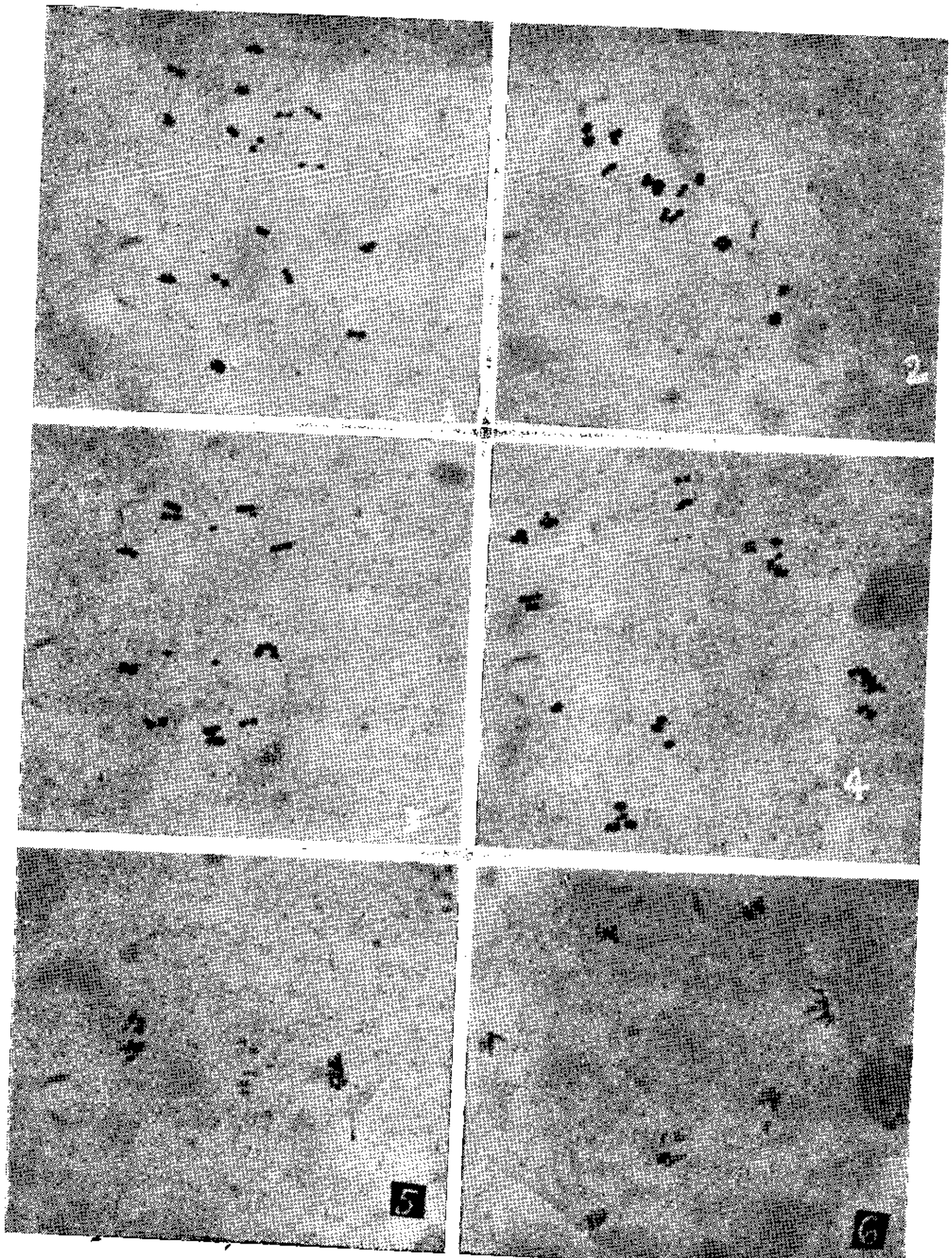

Fig. 1. Metaphase I in the Laccadive variety showing 16 bivalents.
Fig. 2. Metaphase I in inbred progeny of Philippines variety with 12 bivalents and 2 quadrivalents.
Fig. 3. Metaphase I in inbred progeny of Philippines variety showing 14 bivalents and 4 univalents.
Fig. 4. Pollen mother cell with 32 bivalents in inbred progeny of Philippines variety.
Fig. 5. Anaphase I in O. P. progeny of Cochin China showing lagging chromosomes.
Fig. 6. Anaphase II in Malayan dwarf green with 8 groups of chromosomes.

of chromosomes and in that of Philippines Kalambahim, cells with 3 groups at anaphase II were noted. Abnormal spindle mechanism and irregular distribution of chromosomes were also observed in the inbred progenies. Besides normal tetrads, sporads with 1, 5, 6 and 8 spores were also observed in these progenies. Pollen fertility was comparatively higher in the outbred giving 94·44% of well-stained pollen compared to 88·65% in the inbred progenies.

ANDAMAN GIANT VARIETY

Chiasma frequency in the outbred progeny of Andaman Giant was found to be comparatively higher than that of their inbred sister plants and was found to be 1·78 and 1·73 per bivalent respectively. In this variety also meiotic irregularities were slightly higher in the inbred progeny. These included the occurrence of stickiness at metaphase I, formation of lagging chromosomes at anaphase and micronuclei at telophase. Pollen sterility was found to be 12·55% in the selfed progeny compared to a low 2·97% in the open-pollinated progeny.

NEW GUINEA VARIETY

Both inbred and open-pollinated progeny of New Guinea variety now studied show higher frequency of chromosome aberrations. Chiasma frequency was found to be 1·79 in the outbred and 1·77 in the inbred progeny. Sticky association of chromosomes at metaphase I, laggards at anaphase and micronuclei at telophase were observed in both outbred and inbred progenies with a comparatively higher percentage in the former one. The inbred progeny gave an average of 91% of well-stained pollen, while the open-pollinated gave only 88% of well-stained pollen.

COCHIN CHINA

The inbred and open-pollinated progenies of Cochin China showed a comparatively higher frequency of chromosome aberrations. The chiasma frequency of the open pollinated palms was found to be 1·76 per bivalent and that of the inbred ones 1·75. On an average, 68·22% of the bivalents of a cell had chiasmata in both the arms and 31·78% were of rod type. Among the ring bivalents 10·95% had three chiasmata each. Precocious separation of bivalents and stickiness of chromosomes at metaphase I were observed in both the inbred and open pollinated progenies.

A comparatively higher frequency of laggards at anaphase was observed in the open-pollinated progeny compared to their sister palms. Upto eight lagging chromosomes at anaphase I (Fig. 5) were noticed. Micronuclei were observed in the outbred as well as in the inbred palms. Monads, dyads, pentads, hexads and octads were noted in both the inbred and outbred progenies. Pollen sterility was found to be 12·05% in the open pollinated palm and 11·20% in the selfed one.

DWARF PALMS

In all the dwarf palms the chiasma frequency was found to be comparatively lower. Several cells with meiotic irregularities were observed in the dwarf red, dwarf green, dwarf yellow and Malayan dwarf varieties. In the dwarf red variety, a cell with three nucleoli was observed. Quadrivalent association of chromosomes at metaphase I suggesting heterozygosity for reciprocal interchange were noted in all the types. Precocious separation of bivalents, and sticky association of chromosomes at metaphase I and cells with varying number of chromosomes at metaphase I were observed in several cells of these types.

Besides cells with dicentric bridge and acentric fragment, suggesting heterozygosity for an inversion, several cells with laggards, sticky bridges and abnormal spindle mechanisms and irregular distribution of chromosomes were observed at anaphase I and II in all these types. In the Malayan dwarf green variety few cells with 8 groups of chromosomes at anaphase II (Fig. 6) and 8 daughter nuclei at telophase II were noted. Several cells with micronuclei at telophase I and II and sporads with 1, 2, 5, 6, 7 and 8 spores were observed in all the types. Pollen sterility was found to be about 40 per cent in the Malayan dwarf green and 20–30 per cent in all the other forms.

Discussion

A reduction in chiasma frequency was observed in the inbred progenies of Philippines Laguna and Kalambahim and Andaman Giant and also in the dwarf varieties. Lamm (1936) has reported a decrease in the number of chiasmata in inbred rye plants. Müntzing and Akdik (1948) reported that pairing value of the I_1 and I_2 generation of inbred rye was lower than that of the parent population plants. Jain and Basak (1964) have stated that partial or complete failure of pairing and chiasma formation in *Delphinium* is brought about by inbreeding depression. An increase in the number of univalents has been observed in the inbred progenies of Philippines and Andaman varieties and also in dwarf plams. Reduced number of chiasmata observed in these palms, may be due to lack of terminal affinity or high terminilization at first metaphase and high terminilization may perhaps be due to increased homology.

One of the important features of the results of this investigation was the increase in the number of unpaired chromosomes at metaphase I in the dwarf as well as in the inbred progenies of Philippines and Andaman varieties. Most probably, metaphase I univalents tend to lag and divide equationally at anaphase I and therefore tend to be left in the cytoplasm as micronuclei at telophase. Lamm (1936) reported reduced pairing during meiosis in inbred rye plants and that those univalents which have divided during the first anaphase lag at the second anaphase. In the tetrads they then appear as micronuclei. Myers and Hill (1943) reported a similar behaviour in the inbred plants of *Dactylis glomerata*. The behaviour of univalents in the above inbred progenies of coconut is thus analogous to that described for inbred rye by Lamm (1936) and for *Dactylis glomerata* by Myers and Hill (1943). It was suggested by Myers and Hill (1943) that the unequal distribution and tendency for loss of the metaphase I univalents and their division products probably were more important factors in conditioning production of aneuploids and decreased fertility in plants of *Dactylis glomerata* than was the presence of quadrivalents. It seems probable that in the inbred palms of Philippines, Andaman and dwarf varieties, these meiotic irregularities have a major role in conditioning variations in fertility and the reduction in fertility which accompanies inbreeding.

It is interesting to note that the inbred progenies of Philippines and Andaman varieties and both inbred and open-pollinated progenies of Cochin China and New Guinea show comparatively higher frequency of chromosome aberrations and higher percentage of pollen sterility while in Laccadive variety, none of the selfed progenies so far examined show any indication of inbreeding depression as far as cytological behaviour is concerned. Inbreeding depression observed in Philippines and Andaman varieties and lack of inbreeding depression in Laccadive variety may suggest that either the Laccadive varieties are less sensitive to inbreeding or the difference in the intensity of inbreeding and selection in the geographically distinct varieties has resulted in the above situation. In Philippines, New Guinea and Cochin China, unlike in Laccadive, selection of planting material based on early germination and vigour of seedlings was in practice from the very early days. It is possible that generations of such selection based especially on early germination and early splitting of the leaves would have resulted in heterozygosity to varying extents in the progenies. Further selfing would naturally be expected to produce increased cytological instability in the progenies. These observations, therefore, support the views expressed by Swaminathan and Nambiar (1961) that the dwarf coconut occurring in different countries may be the products of inbreeding among different tall varieties.

Summary

Cytological consequences of inbreeding in a few geographically distinct exotic varieties of coconut palms available at Central Coconut Research Station, Kasaragod were studied. Inbred and open-pollinated progenies of Laccadive, Andaman, Philippine, New Guinea and Cochin China varieties and green, yellow and orange types of dwarf variety of indigenous and Malayan origin were screened for their meiotic behaviour.

Meiosis was comparatively more regular in both inbred and open-pollinated progenies of Laccadive variety.

Inbred progenies of Philippines and Andaman varieties and both inbred and open pollinated progenies of Cochin China and New Guinea show comparatively higher frequency of chromosome aberrations and higher percentage of pollen sterility. It is suggested that inbreeding depression observed in

Philippine, Andaman and Cochin China varieties and lack of inbreeding depression in Laccadive varieties is either due to the difference in the intensity of inbreeding and selection between these geographically distinct varieties or to the Laccadive genotypes being comparatively less sensitive to inbreeding.

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CENTRAL PLANTATION CROPS RESEARCH INSTITUTE

KASARAGOD-670 124, KERALA

RPF III

Project No. Gen IV (231)

Date of Start: 1970

I. Technical Programme:

1. Cytological screening of Exotic and Indigenous cultivars
2. Meiotic studies in selfed and open pollinated progenies of germplasm accessions
3. Cytological investigations on TxD hybrids with known parentage
4. Pachytene analysis in Dwarf (CDG &CDO) and Tall (WCT) cultivars
5. Characterisation of the chromosomes of Tall and Dwarfs by karyotypic features
6. Standardisation of fluorescent banding technique for somatic chromosomes in coconut.

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The project title was changed in Fourth Annual Research Council (Feb.2 to 6 1976) as:

CYTOLOGICAL INVESTIGATIONS IN DIFFERENT VARIETIES AND FORMS OF <u>COCOS NUCIFERA</u>

RPF III
Project No.
12. Final Report 19 —19 Date of Start:

Gen.IV(231): CYTOLOGICAL INVESTIGATIONS (Final Report)

This project was started during the year 1970. Earlier studies on coconut cytology were mainly concerned with the chromosome number in different cultivars (Santos, 1929; Janaki Ammal, 1945; Sharma and Sarkar, 1956; Nambiar and Swaminathan, 1960; and Ninan et al., 1960). Nambiar and Swaminathan (1960) studied the chromosome morphology of Tall coconut palms and reported that the satellite bearing chromosomes (nucleolar organisers) occupied the 6th and 9th position in the order of relative length of the chromosomes in the complement. Nambiar and Swaminathan (1960) and Swaminathan and Nambiar (1961) studied the meiotic behaviour of WCT and CDG palms and reported that meiosis in Tall was normal while chromosomal rearrangements like translocations and inversions were noticed in the Dwarf. Ninan and Satyabalan (1963) also observed several irregularities at different stages of meiosis in spicata palms.

The present study was mainly concerned with the meiotic behaviour of different Tall and Dwarf cultivars (both indigenous and exotic), and hybrids. Pachytene analysis and chromosome handing were also attempted.

1. <u>MEIOTIC BEHAVIOUR IN TALL AND DWARF CULTIVARS</u>:

Meiotic behaviour of five Talls (three exotic, namely Borneo Tall, Seychelles Tall, Jamaica Tall and two indigenous Kappadam and Spicata) and four Dwarfs (MDG, MDY, Laccadive Dwarf and CDG) and Natural Cross Dwarf (NCD) was studied. The data are presented in Table 1.

In the Tall cultivars the chiasma frequency ranged between 1.74 to 1.76 per bivalent. The percentage of aberrant cells was also very low in all the cultivars excepting Spicata in which 3 to

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18 per cent of the cells showed abnormalities. The pollen stainability ranged between 91 to 95 per cent. Among the Dwarfs the chiasma frequency was very low in MDY (1.64/bivalent) while in Laccadive Dwarf it was 1.73 per bivalent. Maximum irregularities were noticed in MDY (10 to 22 per cent) and pollen stainability was about 60 x per cent. MDY also showed irregularities in about 7 to 21 per cent of the cells scored and the pollen stainability of 75 per cent. CDG had maximum irregularities at M-1(26.7%). However, in the Laccadive Ø Dwarf the aberrant cells were only 0.7 to 8 per cent. Meiosis in NCD was comparable to that of WCT palms. The frequency of chiasma per bivalent was 1.79 and the meiotic irregularities were minimal (0 to 4.85 per cent). The pollen stainability in acetocarmine was 94 per cent.

2. MEIOTIC BEHAVIOUR OF SELFED AND OPEN-POLLINATED PROGENIES OF EXOTIC AND INDIGENOUS CULTIVARS

The meiotic behaviour of self and open pollinated progenies in five exotic (Cochin, China, New Guinea, Philippines, SS Green and Fiji) and two indigenous (Laccadive Ordinary and Andaman Giant) cultivars was studied. The chiasma frequency per cell in the self-pollinated progenies was low in Andaman Giant, Philippines and Fiji, compared to the open-pollinated progeny palms, indicating possible inbreeding depression (Table 2). In rest of the cultivars, the chiasma frequency in both self- and open-pollinated progenies did not differ much. In Laccadive Ordinary the diffeence between the self-and open-pollinated progenies is much less and the meiosis was more regular with very low proportion of aberrant cells (below 3 per cent). Contrastingly in the other indigenous cultivar Andaman Giant, the differences between the self- and open-pollinated progeny palms were more pronounced. The meiotic irregularities ranged between 7 to 18 per cent in the selfed compared to 1.0 to 5 per cent in the open-pollinated progeny palms. The reduction in pollen stainability in selfed progenies was also noticed.

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Among the exotic introductions, the selfed progenies of Philippines and Fiji showed the higher proportion of cells with aberrant meiotic stages. The pollen stainability was over 95 per cent in open-pollinated progenies while the selfed progenies had less than 90 per cent pollen stainability.

3. MEIOTIC BEHAVIOUR IN HYBRIDS (T x D and D x T) AND THEIR PARENTS:

Meiosis in WCT, CDO and hybrid combinations CDO x WCT and WCT x CDO was studied. The chiasma frequency in WCT and hybrids was at par while the Dwarf showed reduced chiasma frequency (Table 3). In CDO the proportion of aberrant cells at all the stages of meiosis was comparatively much higher than that in the Talls and Hybrids. The aberrant meiotic stages recorded in the Dwarf were also reflected in the pollen stainability which was comparatively much lower.

4. PACHYTENE ANALYSIS

An improved acetocarmine staining technique for the study of the pachytene chromosomes in coconut was standardised and the procedure consists of the following steps:

1. fix the flower buds from the second or third (counting from the first unopened inflorescence) in 1:4 acetic alcohol preferably in the morning (10.00 to 11.00 a.m.). Change the fixative after every 24 hours for a week.

2. bring down to distilled water through alcohol series.
3. hydrolyse individual anthers in 1 N HCl at 60°C for 8 to 10 minutes.
4. allow the hydrolysed anthers to cool down to room temperature
5. wash in distilled water
6. squash in 45% acetic acid (this step was found to give good spread of chromosomes)

7. stain in 1% aceto carmine by smearing with iron needle.

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8. Seal and store at 10 to 14°C in refrigerator for 24 hours before analysis.

Pachytene chromosomes of Dwarf Green showed that the shortest chromosome in the complement is only 1/3 of the longest chromosome.(Fig 1) Five chromosomes have subterminal centromeres nine submedian and two median. The short arm of the sixth and the long arm of the twelth chromosome were found attached to the nucleolus. The pachytene morphology of the Dwarf Green palm conforms to type 2 b described by Stebbins (1958), since the ratio of the longest/shortest chromosome is 2.8:1 and 50% of the chromosomes in the complement have an arm ratio of over 2:1 (Table 4).

In the WCT complement, eight of the chromosomes could be identified by their markers.(Fig 2) Two of them were nucleolar chromosomes (1 & 6). One of them is a submedian chromosome (No.6) with a very long heterochromatic segment near centromere in the long arm, while in another (No.1) the heterochromatic segments on either side of the centromere were more or less equal. The centromere is median in the 15th and submedian in 16th which are the two shortest chromosomes in the complement. In another chromosome (No.2), two distinctly stained dark knobs are present in the long arm. Only one of the chromosomes (No.4) in the complement has a terminal knob. In another median chromosome (No.14) the short arm comprised of seven heterochromatic segments proximal to centromere and a short distal euchromatic segment. In one of the median chromsomes (No.7), the heterochromatic blocks were present only near the centromere and the rest of the chromosomal arms comprised of only euchromatin. Remaining eight chromosomes can be identified only by their arm ratios and relative lengths.

In one of the WCT palms(progeny of prepotent palm) three chromosomes 5, 6 and 9 have shown distinct unpaired segments consistently, thus indicating the non-recombination in these segments which may result in en-bloc transmission of genes. This

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could be one of the factors for the high frequency of yield transmission in prepotents.

5. CHROMOSOME BANDING:

Methodology to induce banding in the somatic chromosomes in the near-UV and visible light was standardised. Acridine Orange (1%) was used for staining near-UV, and Giemsa (1%) for visible light.

The technique for staining with acridine orange involves fixing in acetic methanol, denaturation in 0.1 X SSC at 100°C, reassociation in 2 X SSC at 55 to 60°C and staining in 1% acridine orange at pH 6.0. By this method, single and double stranded DNA could be distinguished by difference in colour. Single stranded DNA flucresces red, and double stranded DNA flucresces green.

The procedure standardized for giemsa staining is as follows.

1. fix in acetic alcohol for 6 hrs.
2. bring down to distilled water through alcohol series
3. hydrolyse in 1 N HCl at 60°C for 8 to 10 minutes
4. Wash throughly in distilled water
5. squash in 45% acetic acid
6. remove the cover glass after storing for 12 hrs.
7. wash the slides in 2 to 3 changes of distilled water
8. immerse in 4% formalin for a maximum of 10 seconds
9. wash in distilled water
10. stain in 1% giemsa prepared in 2 x SSC for 5 to 10 minutes
11. wash well in distilled water/running tap water
12. air dry in a jet of air at 40 to 50°C for 20 minutes
13. mount in DPX or other neutral media.

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Table 1. Meiotic behaviour in Tall and Dwarf Coconut Cultivars

Cultivar	X-ma frequency per cell	per bivalent	% cells with irregularities M-I	AI & II	Tel.I & II	Pollen stainability
Borneo	28.00	1.75	2.1	0.9	0.5	94
Seychelles	28.05	1.75	2.1	0.7	0.4	95
Jamaica	28.15	1.76	2.1	0.5	0.3	96
Kappadam	27.80	1.74	7.2	1.7	1.3	92
Spicata	27.81	1.74	18.5	4.4	2.9	91
CDG	26.7	1.7	29.2	9.6	7.7	69
MDG	27.17	1.69	22.4	12.1	14.3	60
MDY	26.28	1.64	21.9	10.3	7.1	75
Laccadive Dwarf	27.25	1.73	8.5	0.9	0.7	92
NCD	28.72	1.79	4.9	0.7	0.0	94

Table 2: Meiotic behaviour of selfed and open pollinated progenies of Exotic and indigenous cultivars.

Cultivar		X-ma frequency Per cell	per bivalent	% cells with irregularities M-I	AI & II	Tels I & II	Pollen stainability
Andaman Giant	OP	28.53	1.78	4.7	2.2	2.1	97
	SP	27.75	1.73	18.4	9.7	10.5	87
Cochin China	OP	28.24	1.76	5.4	7.5	4.3	88
	SP	28.00	1.75	3.6	7.1	6.7	89
Laccadive ordinary	OP	27.59	1.72	7.7	1.8	1.7	97
	SP	27.52	1.72	6.9	1.7	2.0	96
New Guinea	OP	28.60	1.79	12.3	7.2	5.3	88
	SP	28.40	1.77	16.7	5.6	6.1	92
Philippines Laguna	OP	28.30	1.77	10.4	2.0	1.8	94
	SP	27.61	1.72	17.4	6.5	10.2	89
SSG	OP	27.55	1.72	8.8	1.8	1.6	97
	SP	27.20	1.70	20.9	2.2	2.0	95
Fiji	OP	28.70	1.79	4.1	1.9	0.0	97
	SP	27.48	1.72	11.1	5.9	4.2	90

OP = Open pollinated SP = Self pollinated

Table 3. Meiotic behaviour in T x D, D x T and their parents

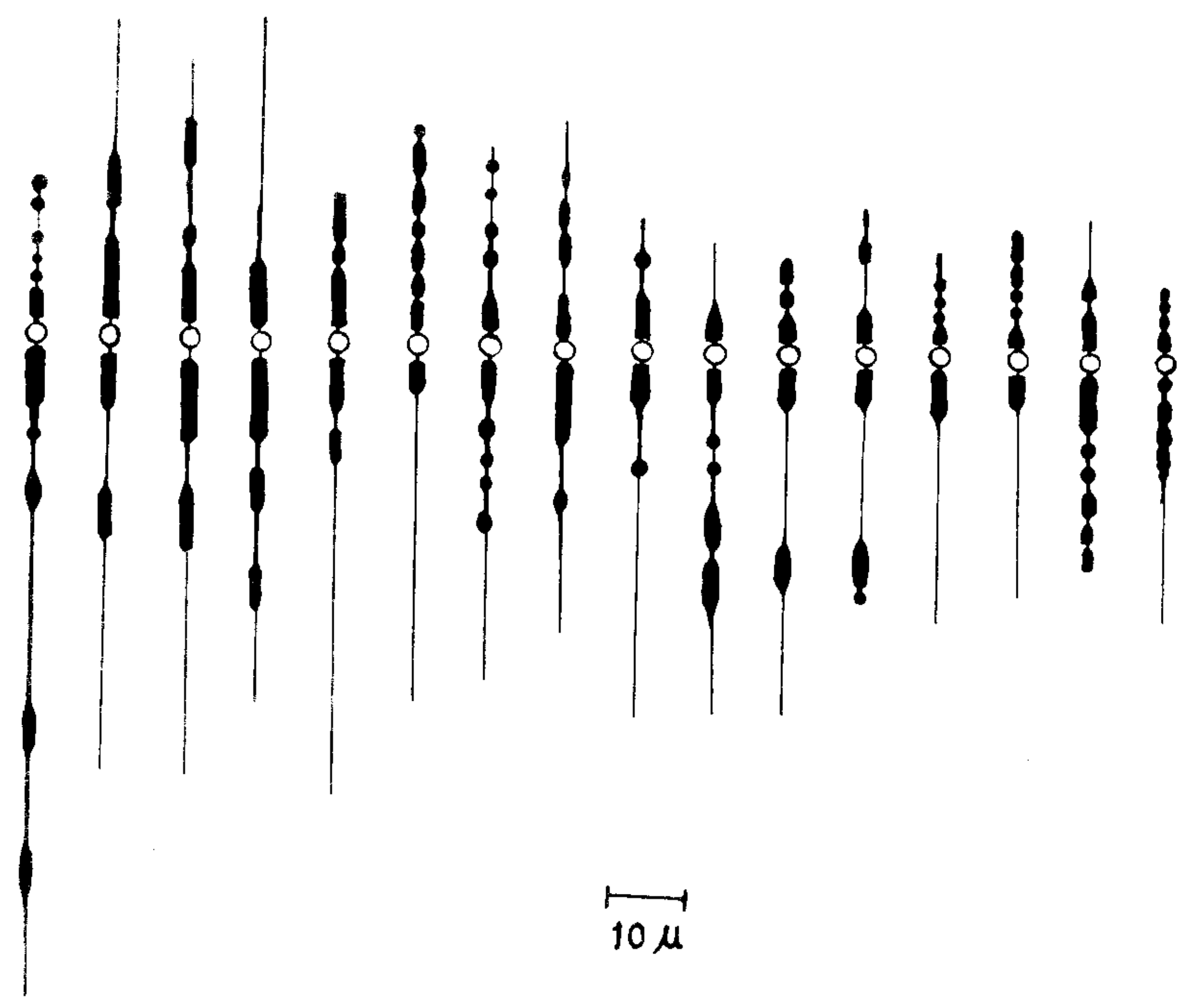
Plant type	X-ma frequency Per cell	per bivalent	% cells with irregularities M-I	AI & II	Tels I & II	Pollen stainability
WCT	28.9	1.8	6.4	1.9	0.3	96
CDO	27.1	1.7	24.4	7.9	5.4	78
WCT x CDO	28.5	1.8	6.8	1.8	1.3	97
CDO x WCT	27.6	1.7	10.3	2.8	2.6	92

Table 4. Pachytene analysis in WCT and CDG chromosome complements

Chromosome	WCT Absolute length (u)	WCT Relative length	WCT SA/LA ratio	WCT Centromere position	CDG Absolute length (u)	CDG Relative length	CDG SA/LA ratio	CDG Centromere position
1	137	9.26	0.57	Sm	159.99	10.94	0.16	St
2	127	8.59	0.86	m	131.34	8.98	0.71	Sm
3	120	8.11	0.50	Sm	124.67	8.52	0.62	Sm
4	114	7.71	0.80	m	121.33	8.30	0.89	m
5	109	7.37	0.81	m	107.11	7.32	0.12	St
6	109	7.37	0.34	Sm	93.33	6.38	0.47	Sm
7	102	6.90	0.88	m	92.00	6.29	0.58	Sm
8	94	6.35	0.67	Sm	90.00	6.15	0.80	m
9	94	6.35	0.84	m	86.00	5.88	0.32	Sm
10	93	6.29	0.63	Sm	82.67	5.65	0.15	St
11	78	5.27	0.65	Sm	74.66	5.10	0.26	Sm
12	78	5.27	0.41	Sm	65.33	4.46	0.58	Sm
13	74	5.00	0.80	m	64.66	4.42	0.61	Sm
14	70	4.43	0.94	m	60.66	4.15	0.18	St
15	48	3.24	0.84	m	56.67	3.87	0.73	Sm
16	31	2.09	0.55	Sm	55.34	3.78	0.19	St

St - Sub terminal m - mediam Sm - Sub mediam

CDG CHROMOSOME COMPLEMENT



WCT CHROMOSOME COMPLEMENT