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		Diakinesis and Metaphase: Cell with					Anaphase I and II: Cells with						Telophase I and II: Cells with		
			per cell	per bivalent	Abnorm.	Univalents	Quadrivalents	Stickiness	Chromosome mosaic	No abnor.	Laggards	Sticky bridge	Inversion bridge	Irregular spindle mechanism	More or less than 4 groups at A II	No. abnor.	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	3	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

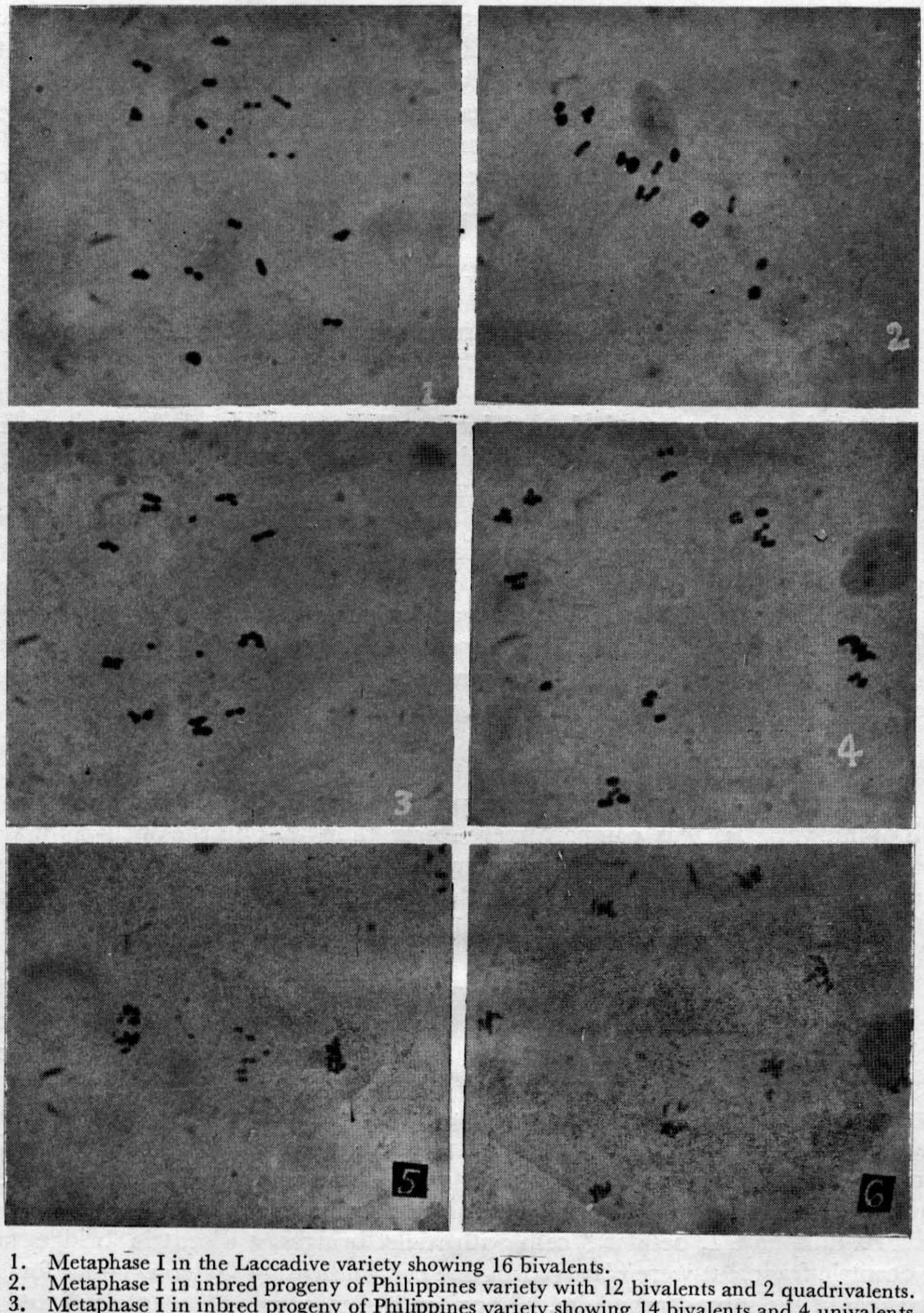

FIG. 1. Metaphase I in the Laccadive variety showing 16 bivalents.
FIG. 2. Metaphase I in inbred progeny of Philippines variety with 12 bivalents and 2 quadrivalents.
FIG. 3. Metaphase I in inbred progeny of Philippines variety showing 14 bivalents and 4 univalents.
FIG. 4. Pollen mother cell with 32 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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