MORPHOLOGICAL, CYTOLOGICAL AND GENETICAL INVESTIGATIONS ON INDUCED AND SPONTANEOUS MALE STERILE CLONES OF PARA RUBBER TREE - *HEVEA BRASILIENSIS* (Willd. ex Adr. de Juss.) Muell. Arg.

THESIS
SUBMITTED TO THE UNIVERSITY OF KERALA
IN PARTIAL FULFILMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN THE FACULTY OF SCIENCE
(BOTANY)

BY

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MARCH 1990

Dedicated to my

beloved Father

CERTIFICATE

This is to certify that the Ph.D. Thesis entitled **Morphological, Cytological and Genetical Investigations on Induced and Spontaneous Male Sterile Clones of Para Rubber Tree - Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.** is an authentic record of original research work carried out by Smt. C.K. Saraswathy Amma under our joint supervision and guidance during the period May 1984 to March 1990. We further certify that no part of this work has previously formed the basis for the award to the candidate of any Degree, Diploma, Associateship, Fellowship or other similar titles of any University or Society.

Smt. C.K. Saraswathy Amma has passed the B.Sc. and M.Sc. degree (Botany) in First Class and First Rank in the State (University of Kerala). She is the winner of the National Merit Scholarship for securing First Position in B.Sc.(Botany) and also the first recipient of Prof. Raman's Shastabdyapoorti Memorial Gold Medal for securing First Rank in M.Sc. Botany. She has also passed Ph.D. qualifying examination of University of Kerala in First Class held during May 1988.

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DECLARATION

I hereby declare that the thesis entitled **Morphological, Cytological and Genetical investigations on induced and spontaneous male sterile clones of Para Rubber tree - Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.** submitted by me for the Degree of Doctor of Philosophy in Botany, of the University of Kerala, embodies the results of original research work carried out by me at the Rubber Research Institute of India, under the joint supervision of Prof. Dr. A.N. Namboodiri, Director, Tropical Botanic Garden and Research Institute, Trivandrum and Dr. A.O.N. Panikkar, Deputy Director (Botany), Rubber Research Institute of India, Kottayam. I further declare that this thesis has not previously formed the basis for award of any degree or diploma or other title.

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C.K. SARASWATHY AMMA

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INTRODUCTION

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg., the Para rubber tree, is a native of Brazil, South America, primarily Amazon Valley and 99% of natural rubber produced in the world exclusively comes from this species. This species was introduced to tropical Asia during 1876 by Sir Henry Wickham through Royal Botanic Gardens, Kew, England. The genus Hevea belongs to the family Euphorbiaceae. Besides the Amazon, it also grows in Bolivia, Columbia, Ecuador, Guyana, Peru, Surinam and Venezuela (Wycherley, 1977). The centre of genetic diversity of the genus is Rio Negro. Early history and taxonomy of the genus are given by Schultes (1956, 1970, 1977) and Polhamus (1962). Eventhough the taxonomy of the genus was dubious, Schultes (1980, 1987) recognised ten species of Hevea:-

Hevea benthamiana Muell. Arg.

Hevea camargoana N.C. Bastos

Hevea camporum Ducke.

Hevea guianensis Aubl var. lutea (Spruce ex Benth)

Hevea guianensis Aubl var. marginata (Ducke)

Hevea microphylla Ule

Hevea nitida Mart. ex Muell. Arg.

Hevea pauciflora (Spruce ex Benth) Muell. Arg.

Hevea rigidifolia (Spruce ex Benth) Muell. Arg.

Hevea spruceana (Benth) Muell. Arg.

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.

Among the ten species of Hevea, natural rubber production in the world is exclusively dependent on H. brasiliensis. Natural rubber is one of the most versatile vegetable products having manifold uses. More than 35,000 articles are manufactured from this plant and there is hardly any segment of life which does not make use of rubber-based products. Rubber plantations have profound influence on the economic and social life of the people of several countries. Over thirty million people in the world are dependent on natural rubber for their livelihood. World natural rubber production during 1987 was 47,05,000 tonnes and total area under rubber cultivation is about 77,00,000 hectares (Anonymous, 1989a). In India, the production of natural rubber during 1988-89 is estimated to be 2,60,000 tonnes (Anonymous, 1989b). The total area under rubber cultivation during 1987-88 has been 3,97,700 hectares (Anonymous, 1989a).

Hevea brasiliensis is a perennial tree having an economic life span of 25-30 years. It is a sturdy, quick-growing tree which attains a height of 25 to 30 m and is the tallest species of the genus. The tree attains a height of over 40 m in wild condition and lives for a century. Though latex is present in all parts of the plant, the bark of the trunk alone is usually exploited commercially. The young plants show characteristic growth pattern of alternating periods of rapid elongation and consolidation. As a result, leaves are produced in flushes during periods of active growth. Within each flush there is a cluster of spirally arranged trifoliate glabrous leaves. Oldest leaves are larger with longer petioles than those produced subsequently within a flush. Normal annual leaf fall, known as wintering, occurs in the case of mature trees during the period from December to February in South India. Refoliation and flowering follow wintering. In Malaysia, there are two flowering seasons (Ghandimathi and Yeang, 1984) and only one flowering season in Nigeria (Omokpise, 1976). Some trees may show off-season flowering during September-October in India. Extra-floral nectary glands are present at the distal end of petioles where the leaflets join, and these glands produce honey during the flowering season. The inflorescence is a panicle having many branches, which bear short stalked pubescent flowers of both sexes (Morris, 1929). Pistillate flowers are large and borne at the tip of the main axis and major branches. Female flower has

one whorl of bell shaped perianth with five yellow lobes and also possesses a greenish disc below the perianth lobes. Gynoecium is tricarpellary syncarpus with single ovule in each locule. Stigma is short and trilobed. The male flowers are far more in number compared to female flowers. They are yellow and smaller in size having five perianth lobes. They possess ten stamens. The anthers are sessile and are arranged in two whorls of five each, on a central staminal column. Each anther contains two pollen sacs which dehisces longitudinally liberating the pollen grains. The pollen grains are yellow in colour.

Hevea brasiliensis is largely cross pollinated. Pollination is mainly by insects. The morphological characters of the flower, especially the sticky stigmatic surface and pollen confirm the entomophilous nature. Webster and Paardekooper (1989) reported over thirty species of insects visiting the flowers. The pollination is almost entirely effected by midges and thrips (Warmke, 1951; Rao, 1961). Fertilization takes place within 24 hours after pollination. Only a small proportion of the female flower set fruit and a good number are shed during tender stage. In Puerto Rico, Warmke (1951, 1952) reported 5% or less success after natural pollination in field. But Rao (1961) found only 0.3 to 1.6% fruitset in Malaysia. Details regarding the factors influencing fruitset in Hevea following hand pollination were given by Ghandimathi and

Yeang (1984). From among the initial fruitset about 30 to 50% fall off after a month. Fruit wall attains full size in about three months, when the embryo is still microscopic. It ripens five to six months after fertilization. Mature fruit is large, composed of three lobes, having a single seed in each locule. Fruit is regma. Mesocarp is thin, coriaceous. The endocarp is woody and dehisces explosively and noisely with endocarp breaking into six pieces. The seeds are thrown to a distance of about 15 m (Radhakrishna Pillai, 1980). There is clonal variation in fruit and seed characteristics (Polhamus, 1962; Saraswathy Amma et al. 1981). The seeds are large and have fairly thick testa with smooth shiny surface having characteristic mottlings. Endosperm is white in viable seeds. The seeds retain viability only for a short period (Dijkman, 1951; Edgar, 1958; Polhamus, 1962; Joseph et al., 1980; Webster and Baulkwill, 1989) and therefore under normal practice the seeds are put for germination as quickly as possible after collection. H. brasiliensis can also be propagated by vegetative means. The method of vegetative propagation is by patch grafting. During the initial years of rubber plantation industry, only seeds were available as material for propagation and the earlier plantations had only seedling trees. With the perfection of vegetative method of propagation, clonal propagation had gradually become established in plantation practice and seeds are now almost replaced by budgrafts in raising rubber plantations.

Since H. brasiliensis is a perennial tree, the crop is not easily amenable to genetic studies. Moreover, the species is highly heterozygous and the trees normally require about five years for normal flowering. Cytological studies are also very meagre, due to small size and stickiness of chromosome which render difficulty in obtaining good cytological preparations.

In early days, the chromosome count made by various workers in Hevea spp. showed variations as 2n = 16, 34 and 36 (Heusser, 1919; Bangham, 1931; Perry, 1943; Paddock, 1943). Subsequent cytological studies carried out by several investigators (Ramaer, 1935; Baldwin, 1947; Majumder, 1964; Senanayake, 1968; Ong, 1976; Saraswathy Amma et al., 1984a) have confirmed that the chromosome complement of Hevea is 2n = 36. An attempt had been made to study the pachytene chromosomes of seven species of Hevea (Ong, 1981). Metaphase chromosomes of somatic cells of H. brasiliensis differed from one another in size and shape. The chromosomes are small and varied in length from 1.50 to 3.60 µm. All the three types of chromosomes metacentric, sub-metacentric and acrocentric are present. The total chromosome length of the species is 89.7 µm. Meiotic division is normal. The usual configuration is eighteen bivalents at metaphase I. Of these ten are reported to be ring shaped and the other eight rod shaped. Of these two were long, four medium and the remaining two short (Ong, 1981).

Literature is replete with reports and reviews on male sterility in annual crops (Correns, 1928; Allen, 1940; Edwardson, 1956, 1977; Duvick, 1959; Filian and Christie, 1966; Laser and Lersten, 1972; Gottschalk and Kaul, 1974; Frankel and Galun, 1977; Pearson, 1981; Kaul, 1988). But studies pertaining to details regarding male sterility is very meagre in tree crops in general. However, spontaneous male sterile clones had been observed and the meiotic irregularities reported in H. brasiliensis and the hybrids of H. spruceana and H. brasiliensis (Ramaer, 1935; Majumder, 1964, 1967; Annamma et al., 1980; Leconte and Nicolas, 1985).

Ramaer (1935), who studied the cytology of Hevea, found partially or completely male sterile clones of H. brasiliensis and in the hybrid of H. spruceana x H. brasiliensis. He concluded that irregular meiosis of the pollen mother cells was responsible for the sterility. Majumder (1964) carried out detailed studies on male sterile clones and reported that meiosis appeared to be normal and about 80 to 95% of the pollen grains were found to be empty and deformed. He further reported male sterility in the clone GT 1 during 1967. Leconte and Nicolas (1985) reported male sterility in the clone GT 1 and had given an account of microsporogenesis in this clone stating that the microspores aborted while they were still encased in tetrads and subsequent formation

of pollen grains was suppressed. Male sterility in two clones of H. brasiliensis namely RRII 15 and RRII 17 was also reported (Annamma et al., 1980). All these investigators had only mentioned about the spontaneous male sterility in H. brasiliensis.

Cytological and genetical studies in H. brasiliensis, as well as in other species of the genus, are meagre. A perusal of the available literature has elucidated some information on spontaneous male sterility in H. brasiliensis, but detailed studies on the nature and cause of male sterility have not been reported. Moreover, induced male sterility in this crop has not so far been reported. Cytological and genetical studies are difficult in this crop due to the nonamenability of the materials to cytological techniques and also the long breeding cycle. Detailed cytogenetical investigations are, however, very important for a thorough understanding of the genetic system, essential in chalking out meaningful breeding programmes. As the genetic variability in the crop is narrow, any newly evolved cytotypes and sterile clones will enrich the genetic reservoir. Moreover, the induction of male sterility and evaluation of progenies from the male sterile clones have not been previously reported in this species and the present study has been taken up in view of the dearth of information in these important aspects. In this background, an attempt has been made to study the morphology, cytology and

genetics of spontaneous as well as induced male sterile clones and their utility from the practical point of view, the results of which are incorporated in this thesis.

MATERIALS

Location:-

The studies were carried out at two experimental stations of the Rubber Research Institute of India, one at the headquarters (9° 32' N, 76° 36' E) in Kottayam district and the other, the Central Experiment Station (9° 22' N, 76° 50' E) at Chethackal, Ranni in Pathanamthitta district of Kerala State. The materials for the present investigations were generated and collected from these locations.

Experimental materials:-

The general information about the tree Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg is depicted in Figs. 1 to 11. The materials for the studies comprised of both exotic and indigenous clones of H. brasiliensis showing male sterility. In the course of regular cytological screening of different clones male sterility was observed in two exotic and three indigenous clones. Induction of male sterility was attempted employing different techniques like application of Colchicine, Ethyl methane sulphonate and

gamma irradiation. Male sterility was found induced in four clones resulting the above treatments. Both induced and spontaneous male sterile clones were investigated further.

Induction of polyploidy

RRII 105, evolved by the Rubber Research Institute of India through hybridization and selection with Tjir 1 x Gl 1 as parents, was used for polyploidization. Plants raised in budwood multiplication nurseries were cut back and at the time of bud break, the buds were swabbed with cotton. Aqueous colchicine 0.75% was dropped on to the swabs so that the young bud sprouts were in contact with the solution. The treatment was continued twice daily for two weeks. The basal buds of those sprouts which showed morphological variations were utilised for vegetative multiplication. Subsequent vegetative generations were raised employing the basal buds of respective shoots upto VM_7 generation for stabilizing the character. Cytological studies were carried out to ascertain ploidy level.

Induction of mutation

For induction of mutation, gamma rays and chemical mutagen EMS (Ethyl methane sulphonate) were used. Both seeds and

vegetative buds were treated with gamma rays. The optimum dose for seed irradiation was 2000-3000 rads, whereas 4000 rads was found lethal (Markose et al., 1974; Saraswathy Amma et al., 1983, 1985). For vegetative buds, application of gamma rays at a dose of above 2000 rads was lethal and resulted in poor budding success and rate of survival. Radiation induced sterile plant from a population resultant of 3000 rads and mutagen induced sterile plant from EMS treated population were studied.

Synthesis of Triploid

An induced triploid was evolved (Text. Fig. I) by crossing diploid (Gl 1, 2x) with induced tetraploid RRII 105 (4x). Vegetative progenies were established and were utilised for the studies.

Progenies of male sterile clones

From the male sterile clones, those having fruitset were chosen for the studies on progenies. These included clones GT 1, Ch 2 and RRII 35. Open pollinated seeds were collected from these as well as a fertile clone Mil 3/2 for comparative observations as control.

Methods

Morphological studies

The budgrafted plants of induced as well as spontaneous male sterile clones were raised in polybags (65 x 35 cm) and planted in nursery in three replications, each having five plants per type. The morphological characters like height, girth, number of flushes, number of leaves in a flush, specific leaf weight, stem index, petiolar index and foliar index were observed. Foliage characters were recorded fro· middle leaflets of 100 mature leaves selected at random. From each flush of growth three leaves were selected from top middle and bottom. Stem index, petiolar index and leaf index were determined following Mendes (1969) and specific leaf weight as suggested by Chatterton et al. (1972) as shown below:-

$$\text{Petiolar index} - \frac{\text{Diameter of the petiole at its half length}}{\text{Length of petiole}}$$

$$\text{Foliar index} - \frac{\text{Diameter of the principal vein at its half length}}{\text{Length of vein}}$$

$$\text{Stem index} - \frac{\text{Thickness of stem at its half length}}{\text{Length of whorl}}$$

Budgrafted plants of these materials were induced to flower early, at the age of 30 months by ring barking (Saraswathy Amma, 1975). Flower size, ie. length and breadth of both male and female flowers, just prior to anthesis were recorded. The data were subjected to statistical analysis. In the case of spontaneous male sterile clones flowers were collected from mature trees. Seeds were collected and the morphology and seed size were noted. Observations were taken from 100 flowers and seeds selected at random. Correlation of characters were also calculated in the case of growth attributes of polybag plants of male sterile clones.

Cytological studies

Mitosis:

For mitotic studies tender leaves were pretreated with saturated aqueous solution of Paradichlorobenzene (PDB) and kept at 10°C for 2.5 to 3.0 h. The pretreated leaves were thoroughly washed in water and preserved in alcohol-acetic acid (3:1). Leaf-tips were washed and hydrolysed in 1 N HCl for 20 to 25 min at 60°C. After thorough washing in water they were kept overnight in 2% acetocarimine. Squash preparations were made in 45% acetic acid and the slides were made permanent by acetic-butanol series.

Meiosis:

For meiotic studies, male flower buds, at the appropriate stage of development, from the male sterile clones as well as control were collected and fixed in modified Carnoy's fluid (3:1:1), alcohol, acetic acid, chloroform. After 24 h, the materials were transferred to 3:1 alcohol-acetic acid. Staminal columns were dissected out and stained overnight in 2% acetocarmine. Preparations were made in 45% acetic acid and observations were taken from 100 pollen mother cells, selecting at random 10 cells from 10 slides from temporary mounts.

Pollen and cytological studies were repeated for three consecutive flowering seasons.

Palynological studies

For morphological studies flowers were collected and preserved in 70% alcohol. Acetolysis was done by the standard procedure (Erdtman, 1952; Nair et al., 1977). Pollen grains were examined and measurements of equatorial diameter, polar diameter, exine thickness and pore diameter were taken at a magnification of 400 X by means of an

ocular micrometer using light microscope. A total of 100 pollen grains, 10 each from 10 slides, selected at random were used for measurements of pollen characteristics.

SEM studies were carried out at the National Botanical Research Institute, Lucknow. The acetolysed pollen grains were placed on adhesive tape attached to an aluminium stub. The samples were coated with gold (200 A°) JEOL ION sputter using a Coater (JFC 1000) and observed with a JEOL JSM 35 C Scanning Electron Microscope and photographed at 2000 X and 6000 X. Five samples were observed from each clone.

The methods and terminology used for the morphological studies of pollen grains were those followed by Nair (1961, 1970).

For the assessment of pollen stainability as an index of pollen sterility, mature male flowers just prior to anthesis were collected and treated with (1:1) acetocarmine glycerine mixture. For studies on pollen germination, male flowers were collected just prior to anthesis and pollen grains were dusted in 20% to 25% sucrose solution with 0.01% boric acid and germinated by the "hanging drop" technique. The concentration of sucrose solution was selected after preliminary studies. Germination percentage was assessed by scoring 100 pollen grains from ten microscopic

fields after three hours of incubation. For pollen production studies (Mathur and Mohan Ram, 1986) mature flower buds just prior to anthesis were collected at random and the anther columns from five flowers were dissected out. Anthers were homogenized in 2 ml of 10% aqueous glycerine, using a glass homogenizer. Pollen counts were made using a haemocytometer and compound research microscope. Seven replicates were taken for each homogenate and the number estimated twice from which average per anther per flower was taken.

Male flower buds of male sterile, triploid and tetraploid along with control were collected at different stages of development. The length of flower buds was measured. Microsporocytes or microspores contained in each bud were stained with 1% aceto-carmine solution and their diameter were measured. In each bud, the mean value of diameter of fifty sporocytes or spores was calculated. The relationship between flower size and size of the pollen mother cells was ascertained.

For studying the microsporogenesis the materials were fixed vacuum applied in 3:1 alcohol:acetic and preserved in 70% alcohol. Paraffin blocks of the materials were prepared (Johansen, 1940) and sections were cut at 10-12 μm. Staining was done with Delafields hematoxylin, safranine and fastgreen. Sections were

made permanent and mounted in Euparel and observations and photomicrographs were taken from the permanent slides.

Estimation of DNA

Young shoot tips were collected from diploid, triploids and tetraploid and pretreated with saturated solution of Para-dichlorobenzene (PDB) for 2.5 to 3.0 h at 10°C. The leaf tips were washed thoroughly with water and fixed in (3:1) alcohol-acetic acid for 24 h and preserved in 70% alcohol. The samples were hydrolysed in 1 N HCl for 20 to 25 min and washed thoroughly in water and kept in leuco-basic fuchsin at pH 3.6 overnight at low temperature. The samples were washed with SO_2 water for 30 min with three changes of 10 min each. The root tip of Allium cepa was also treated in the same manner as control. Squash preparations were made in a drop of glycerine. Photometric measurements were taken on Vickers Scanning Cytophotometer M 85a at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow.

Genetical studies

Mature dry fruits resulting from open pollination before dehiscence, were collected from trees of GT 1, Ch 2, RRII 35 and

Mil 3/2 and the seeds were taken by opening the fruits. They were germinated in germination beds in the normal manner (Jopseph et al., 1980) and the rate of germination was assessed from the seventh day onwards up to the 19th day. From among the seeds which sprouted from 7th to 13th day of sowing, a nursery was established, adopting RBD* with five replicates of forty seedlings per plot. The planting was done at a spacing of 30 cm between plants in four rows of ten seedlings each, the distance between rows being 30 cm. Observations were recorded from the sixteen inner plants in each plot. Height and basal girth of the seedlings were recorded at 12 and 24 months growth. At the age of 30 months, test tapping was done adopting half spiral alternate daily (S/2 d/2) system. Tapping was commenced at 15 cm height from the ground level and girth was also recorded. First ten tappings were to initiate and regulate flow. After ten days tappings, yield of the next five consecutive tappings was collected as cup lumps and oven dried at 60°C. The dry weight of the lumps was recorded when the moisture was removed. Yield recordings were repeated during two subsequent quarters also. The seedlings were stimulated by applying 0.1% of Ethrel (2 Chloro ethyl phosphonic acid), on the bark just below the tapping cut with a brush. Yield after stimulation was also recorded for five days. Girth after tapping was also measured after the third quarter. From among the seedlings based on yield and secondary characters forty one progenies

*Randomized block design

were selected. The growth attributes and yield of these selections were also recorded.

The male sterile clone GT 1 was crossed with four male fertile clones (RRII 105, RRII 118, RRIC 100 and RRIM 600). Hand pollinations were also carried out incorporating the fertile clones RRII 105 and PR 107 as female and male parent. The details of hand pollinations attempted are given in Table 2. The seedlings were multiplied vegetatively and ten budgrafted plants from each combinations were planted in the nursery. At the age of 24 to 30 months early flowering was induced by ring barking (Saraswathy Amma, 1975). The nature of sterility in the F_1 progenies was studied employing cytological and palynological techniques.

STATISTICAL ANALYSIS

Analysis of variance

Analysis of variance in the morphological characters and growth attributes was done as suggested by Panse and Sukhatme (1957). The manifestation of genotypic (G) and environmental (E) effects on the observed value of a character was partioned by the method of analysis (Kempthorne, 1957):

$$V(X) = V(G) + V(E) \quad \text{or}$$

$$\sigma^2 P(X) = \sigma^2 (g)(X) + \sigma^2 e(X)$$

where $\sigma^2 P(X)$ is the phenotypic variance of character X, $\sigma^2 g(X)$ is the genotypic variance of X_1 abd $\sigma^2 e(X)$ is the variance due to environment.

The extent of covariance between x and y, due to genetic and environmental factor, was partioned using the formula:

$$Cov\ (xy) = Cov\ G(xy) + Co\ E(xy) \quad \text{or}$$

$$\sigma\ p(xy) = \sigma\ G(xy) + \sigma\ E(xy)$$

where G(xy) is the covariance between x and y attributable to genotypes and E(xy) that due to environment.

Correlation

The phenotypic correlation coefficients were estimated as:

$$r\hat{p}\ (xy) = \frac{\sigma\ \hat{p}\ (xy)}{\sigma\ \hat{p}\ (x) \cdot \hat{p}\ (y)}$$

where p (x) and p (y) are the estimated phenotypic standard deviation of x and y.

Co-efficient of variation

The co-efficient of variation for phenotypic and genotypic traits were estimated as below:

Phenotypic coefficient of variation:

$$C.V.p\ (x) = \frac{\sigma p\ (x) \times 100}{\bar{x}}$$

and genotypic coefficient of variation:

$$C.V.g\ (x) = \frac{\sigma g\ (x) \times 100}{\bar{x}}$$

Heritability (H2), general combining ability (GCA), genetic advance (GA) were calculated following the methods suggested by Singh and Chowdhery (1979). Simple and multiple correlations were also worked out with regard to yield and secondary characters.

Photomicrographs of cytological and palynological preparations were taken from suitable slides using Orthoplan large field Microscope (Leitz-Wetzlar, Germany) with WILD MPS 12 (Heerbrugg, Switzerland) attachment.

Table 1. Details of male sterile materials.

Clone	Origin	Cytotype	Nature of sterility
(a) Spontaneous sterility			
RRII 15	Indian	2n = 3x = 54	Spontaneous triploid
RRII 17	India	2n = 2x = 36	Male and female sterile
RRII 35	India	2n = 2x = 36	Complete male sterility
Ch 2	Malaysia	2n = 2x = 36	Complete male sterility
GT 1	Indonesia	2n = 2x = 36	Complete male sterility
(b) Induced sterility			
RRII 105 Polyploid	Colchiploidy	2n = 4x = 72	Tetraploid partial sterility
Induced triploid	Diploid x tetraploid	2n = 3x = 54	Partial sterility
Mutagen induced	EMS induced	2n = 2x = 36	Complete male sterility
Radiation induced mutant	Gamma rays 3000 r seed treatment	2n = 2x = 36	Complete male sterility

Table 2. Details of hand pollinations.

Sl. No.	Combinations	Hand pollinations done	Final success %	Seeds obtained
1.	GT 1 x RRII 105	200	2.5	15
2.	GT 1 x RRIC 100	200	2.7	18
3.	GT 1 x RRII 118	200	3.0	18
4.	GT 1 x RRIM 600	215	2.3	15
5.	RRII 105 x PR 107	200	2.5	18

OBSERVATIONS

SECTION 1. MORPHOLOGY

Spontaneous male sterile clones:-

Among the five spontaneous male sterile clones there is distinct morphological variations in colour and texture of leaves (Figs. 13 and 14). Mean leaf area and range are depicted in Table 3. In GT 1 and Ch 2 leaves are comparatively average in size. In the former, leaves are dark green while in Ch 2 the leaves are pale green in colour, whereas in RRII 35 the leaves are large and dull green in colour. The leaves of RRII 17 are comparatively small in size with dark green colour. In RRII 15, leaves are large with dark green colour having very prominent veins.

Induced male sterile clones:-

In radiation induced male sterile clone, the leaves are found to be comparatively very small with dark green colour (Fig. 12b).

In mutagen induced male sterile clone, the leaves are large (Fig. 15) more over similar to that of RRII 105. The leaves of control clone RRII 105 are large (86.77 cm^2) and glossy with dark green colour. The leaves of induced triploid show average size, green colour and prominent veins (Fig. 16). In tetraploid leaves are large, thick and dark green in colour having very prominent veins (Fig. 17). The number of leaflets show variations during the young stages of growth in induced triploid (Figs. 18 and 19) and tetraploid and the number varies from two to five. The control clone RRII 105 has only three leaflets. Among the ten clones, the radiation induced mutant showed the lowest (26.67 cm^2) value and tetraploid showed the highest (107.12 cm^2) value for leaf area.

Growth attributes of 30 months old polybag plants of male sterile clones and control are depicted in Table 4. With regard to plant height there was no significant difference between the clones compared to that of RRII 105. The height of RRII 35, Ch 2, tetraploid and RRII 15 were, however, numerically more compared to that of the control. Plant height was comparatively less for the radiation induced mutant (Fig. 20) and triploid. When the height of the radiation induced mutant was taken for comparison, significant differences were noted among the clones.

There was no correlation between specific leaf weight to any other character.

Correlation matrix of growth attributes of Ch 2 is given in Table 6. Significance at 1% level was noted for height with diameter and number of flushes. Diameter was highly correlated to number of flushes and leaves per flush. There was considerable correlation for stem index and foliar index. The specific leaf weight was related to stem index($P<0.05$) and foliar index($P<0.01$).

For RRII 35 (Table 7) high correlation was noted for diameter and leaves per flush, stem index and number of flushes. Correlation at 5% level was noted for diameter and stem index, number of flushes and leaves per flush and leaves per flush and stem index. There was negative correlation ($P<0.01$) between number of flushes and leaves per flush to specific leaf weight.

The growth attributes of RRII 17 (Table 8) showed that there was maximum correlation between height to diameter and number of flushes. Highly significant correlation was observed for diameter to number of flushes and leaves per flush, number of flushes to petiolar index. Considerable correlation was noted for stem index to height and diameter. Negative correlation was

In the case of diameter, there was significant differences between the control RRII 105 and Ch 2, RRII 15, RRII 35 and tetraploid. The radiation induced mutant showed the lowest value for diameter followed by the triploid. With regard to number of flushes there was no significant difference among the clones compared to that of the control except that for triploid. Tetraploid, triploid, Ch 2 and radiation induced mutant showed significant differences in petiolar index. Significant difference in foliar index was noted for all the clones studied except for GT 1. In the case of stem index, triploid, tetraploid and radiation induced mutant showed significant differences. With regard to specific leaf weight, significant difference was noted for all the clones except GT 1, and EMS treated. However, the specific leaf weight was numerically more for RRII 15, RRII 17 and RRII 35 compared to the control RRII 105.

For clone GT 1 (Table 5), the maximum correlation is attributed between diameter and leaves per flush. It is also observed that height is highly correlated with diameter and number of flushes, diameter is highly correlated with leaves per flush and stem index. It is again clear that leaves/flush and stem index, stem index and petiolar index are highly correlated characters. There is considerable correlation between diameter with number of flushes and foliar index. Leaves per flush and foliar index.

noted for leaves per flush to petiolar index. The specific leaf weight was not related to any other character in this clone.

The clone RRII 15 (Table 9) showed significant correlation for diameter to height (r = 0.5656), diameter to number of flushes (r = 0.7585), petiolar index to foliar index (r = 0.5814) and specific leaf weight (r = 0.4982). There was negative correlation for diameter and petiolar index and stem index and foliar index. There was correlation at 5% level for height to number of flushes and number of flushes to stem index.

In the mutagen induced male sterile clone (Table 10) there was maximum correlation for height to diameter, number of flushes, leaves per flush, petiolar index and foliar index. Diameter showed correlation to number of flushes (r = 0.7613), leaves per flush (r = 0.3731), petiolar index (r = 0.5318) and foliar index (r = 0.5051). Number of flushes was related to leaves per flush (0.7363) and to petiolar index (r = 0.4819), stem index and petiolar index were correlated to foliar index at 1% level. There was significant correlation between foliar index to specific leaf weight ($P < 0.01$).

Maximum correlation was noted between height to diameter and number of flushes, diameter to number of flushes. Leaves per flush and specific leaf weight are highly correlated in the

induced triploid (Table 11). Negative correlation at 1% level was observed for leaves per flush to foliar index. Significance at 5% level was noted between number of flushes to petiolar index as well as foliar index to specific leaf weight.

In the radiation induced mutant (Table 12), maximum correlation is attributed to number of flushes to petiolar index, foliar index and specific leaf weight and also petiolar index to foliar index and specific leaf weight and foliar index to specific leaf weight, diameter to stem index. Specific leaf weight was related to stem index, petiolar index and foliar index. Highly significant correlation was also noted for height and leaves per flush. Petiolar index was negatively ($P<0.05$) correlated to diameter. There was negative correlation between height to number of flushes, stem index, petiolar index, foliar index and specific leaf weight; specific leaf weight and leaves per flush, stem index and leaves per flush.

In the induced tetraploid (Table 13) there was significant correlation between height and diameter, stem index and number of flushes. Negative correlation was noted for petiolar index to stem index ($r = -0.3600$) and number of flushes ($r = -0.6169$).

The control clone RRII 105 (Table 14) showed maximum significant correlation for height to diameter, number of flushes, leaves per flush, foliar index and diameter to leaves per flush and foliar index. Stem index was related to foliar index and specific leaf weight, leaves per flush to stem index and foliar index. Specific leaf weight was also related to foliar index. There was negative correlation at 5% level between the petiolar index and leaves per flush.

Flower morphology

Studies on the morphology of flowers of male sterile clones, both induced and spontaneous, showed variations in colour and size (Figs. 21 to 32) of flowers. In GT 1, there was only very poor development of male flowers (Fig. 23). They were reduced in size and fallen by abscission before full development. Perianth was light yellow in colour and flowers did not open. The anther column was completely dry in the almost mature male buds. The F_1 progenies of the cross having GT 1 as the female, also showed flower morphology and development just as the male flowers of GT 1.

In Ch 2 morphology of the flower was similar to that of the normal fertile clone. There were ten fully developed anthers

and normal dehiscence was also noted. But 30% of anthers showed partial dehiscence. Flowers are yellowish in colour and comparatively large in size.

In RRII 35, the male flowers did not attain full size. Before attaining complete maturity they were found to fall off. Anther development was also not complete and there was only poor development of anther column. In all the three male sterile clones mentioned above, the female flowers were not affected by male sterility. They were quite normal and had normal fruitset as observed in the case of fertile clone.

The flowers of the triploid and the tetraploid were bigger compared to those of diploid (control) (Figs. 26 to 29). The anthers, anther column and gynoecium were also larger in size. The tetraploid showed bright yellow flowers. The spontaneous triploid, RRII 15 also showed bigger flowers with deep yellow colour. The development of male flower, however, was not complete. Only a very few per cent (10) attained full development. The anther columns were shrivelled and dried. But in the induced triploid the morphology of the flower was normal. There were ten fully developed anthers arranged on a fully developed anther column. Flowers were light yellow. In RRII 17, the flowers were comparatively smaller. But in this case also the development

of male flower was not normal. They did not reach full size but were shed in the developing stage. When the buds were opened, there was a hard brittle pin in the centre of the flower or often seen loose on the receptacle. However, about 10% of flowers showed normal development.

In the radiation induced mutant there were smaller flowers. Male flowers possessed normal anther column having 10 anthers. In the mutagen treated plant, the morphology of the female flower was similar to that of control. But male flowers showed reduced size and did not attain normal size. RRII 105, the control, showed no abnormalities in flower morphology. In mutagen induced mutant and RRII 35 (Fig. 30), there was poor development of male flowers.

Length and breadth of both male and female flowers were given in Table 15. There was significant difference in flower size. Flowers were bigger in tetraploid, Ch 2 and the triploids - both spontaneous and induced. Flowers were very small in the radiation induced mutant and GT 1. Male flowers were also very large in the tetraploid, Ch 2 and triploids compared to those of the control. RRII 105, triploid, Ch 2, RRII 15 and the tetraploid showed significant difference in flower size.

Fig. 1 : A rubber estate

Fig. 2 : A young rubber tree

Fig. 3 : A seedling nursery

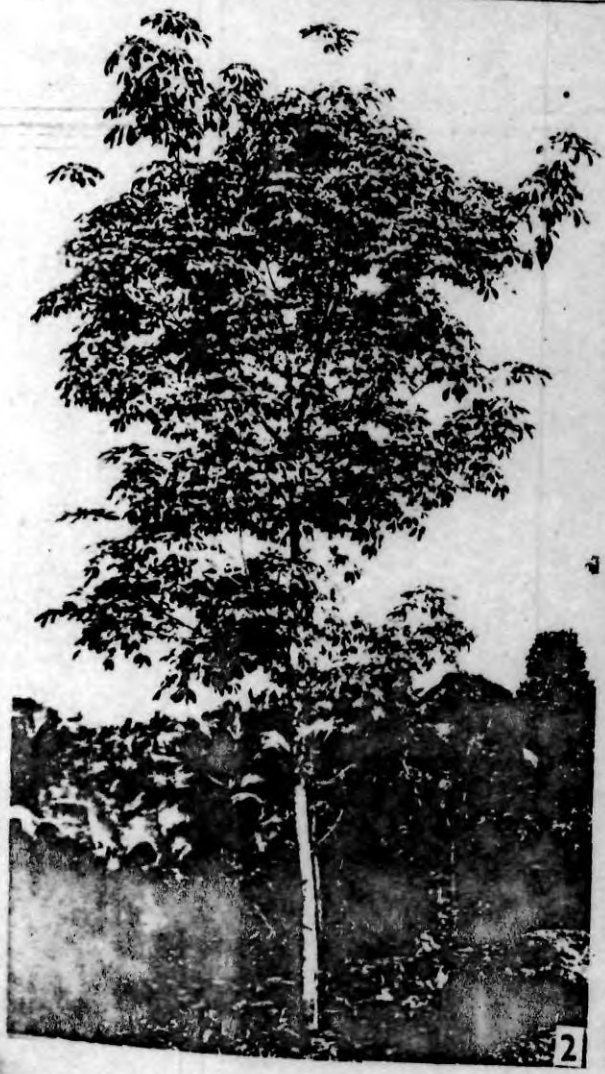

Fig. 4 : Polybag plant x 1/20

Fig. 5 : Budgrafted plant showing flushes of growth x 1/2

Fig. 6 : Leaf showing nectary glands x 1/4

Fig. 7 : Inflorescence x 1/5

Fig. 8 : Terminal female flower x 1/5

Fig. 9 : Male flower showing anther column x 2

Fig. 10 : Fruit x 1/2


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Fig. 11 : Seeds

Fig. 12 : a) Leaf of RRII 105

b) Leaf of radiation induced mutant

Fig. 13 : a) Leaf of RRII 17 x 1/5

b) Leaf of RRII 15

Fig. 14 : a) Leaf of RRII 17 x 1/5

b) Leaf of Ch 2

c) Leaf of RRII 35

Fig. 15 : Flush of EMS treated plant x 1/4

Fig. 16 : A flush of induced triploid showing leaves with four leaflets x 1/5

Fig. 17 : A flush of induced tetraploid x 1/5

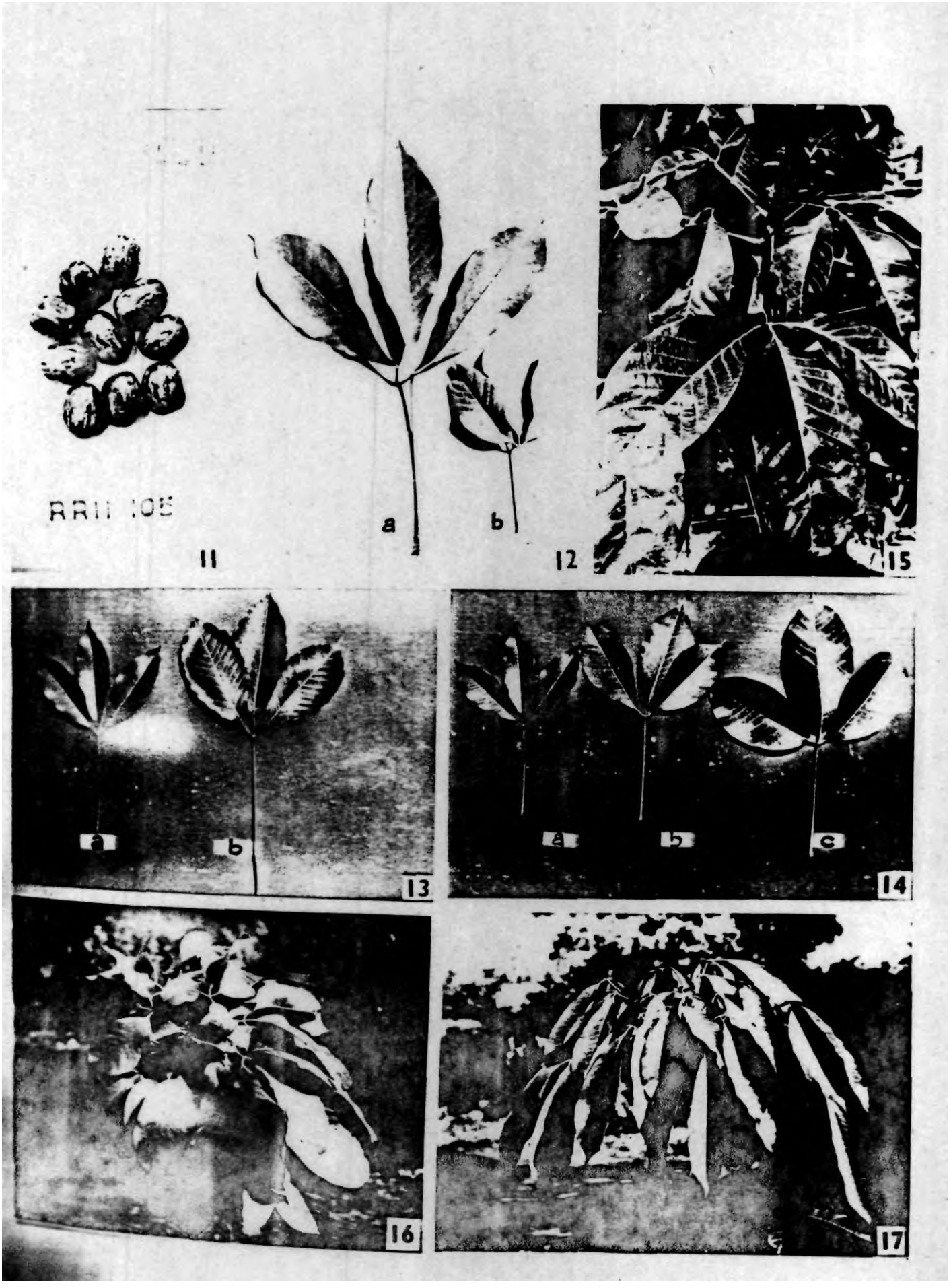
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Fig. 18 : Leaf of induced triploid x 1/7

Fig. 19 : Leaf of induced tetraploid x 1/7

Fig. 20 : Radiation induced mutant plant showing compact leaves x 1/11

Fig. 21 : Development of flowers in male fertile clones x 1/

Fig. 22 : Inflorescence of mutagen induced mutant x 1/3

Fig. 23 : a) Inflorescence of GT 1 showing very poor development of male flowers and normal development of female flowers

b) Inflorescence of control clone x 1/4

Fig. 24 : a) Inflorescence of RRII 15

b) control x 1/4

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Fig. 25 : Inflorescence of RRII 17

Fig. 26 : a) Female flowers of triploid

b) Female flowers of tetraploid

Fig. 27 : a) Male flower of triploid

b) Male flower of tetraploid

c) Male flower of control

Fig. 28* : a,a1) Gynoecium and androecium of polyploid.

b,b1) Gynoecium and androecium of control

Fig. 33 : Seeds of GT 1 in different views

Fig. 34 : Seeds of Ch 2 in different views

Fig. 35 : Seeds of RRII 35 in different views

Fig. 36 : Seeds of Mil 3/2 in different views

*Figs. 29 to 32 incorporated in the next plate

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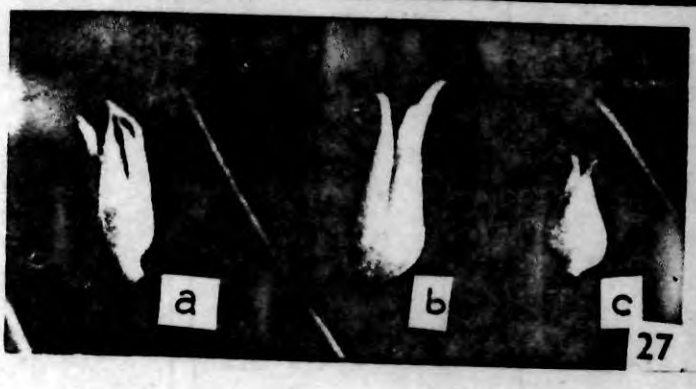

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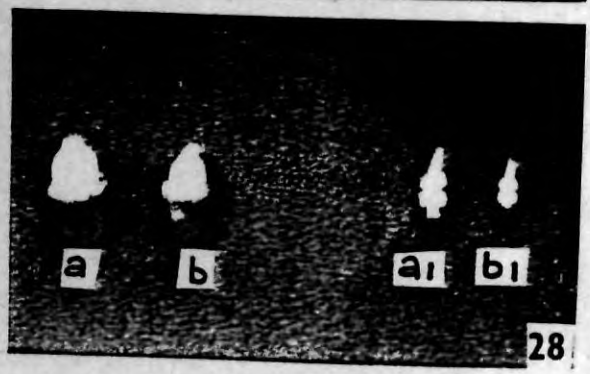

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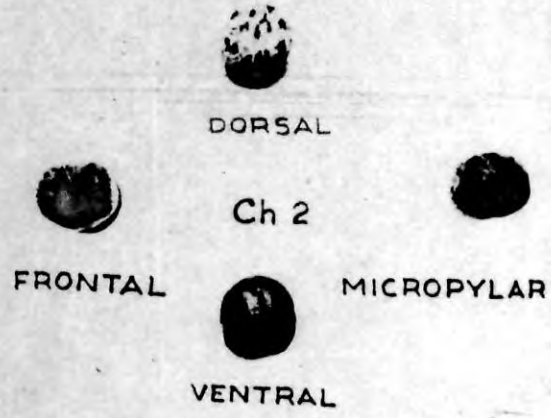

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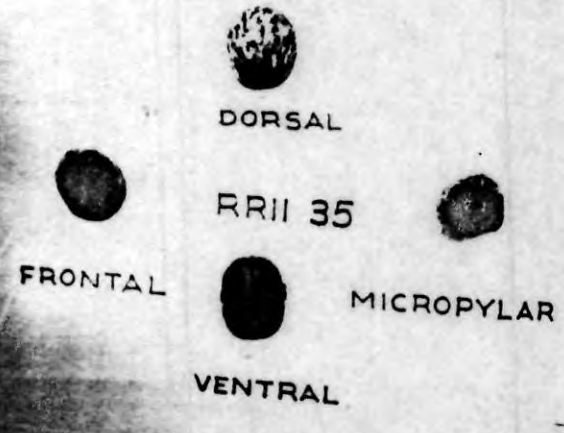

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Fig. 29 : Inflorescence of tetraploid x 1/2.5

Fig. 30 : Inflorescence of RRII 35 x 1/2.5

Fig. 31 : Inflorescence of control clone x 1/2.5

Fig. 32 : Inflorescence of Ch 2 x 1/2.5

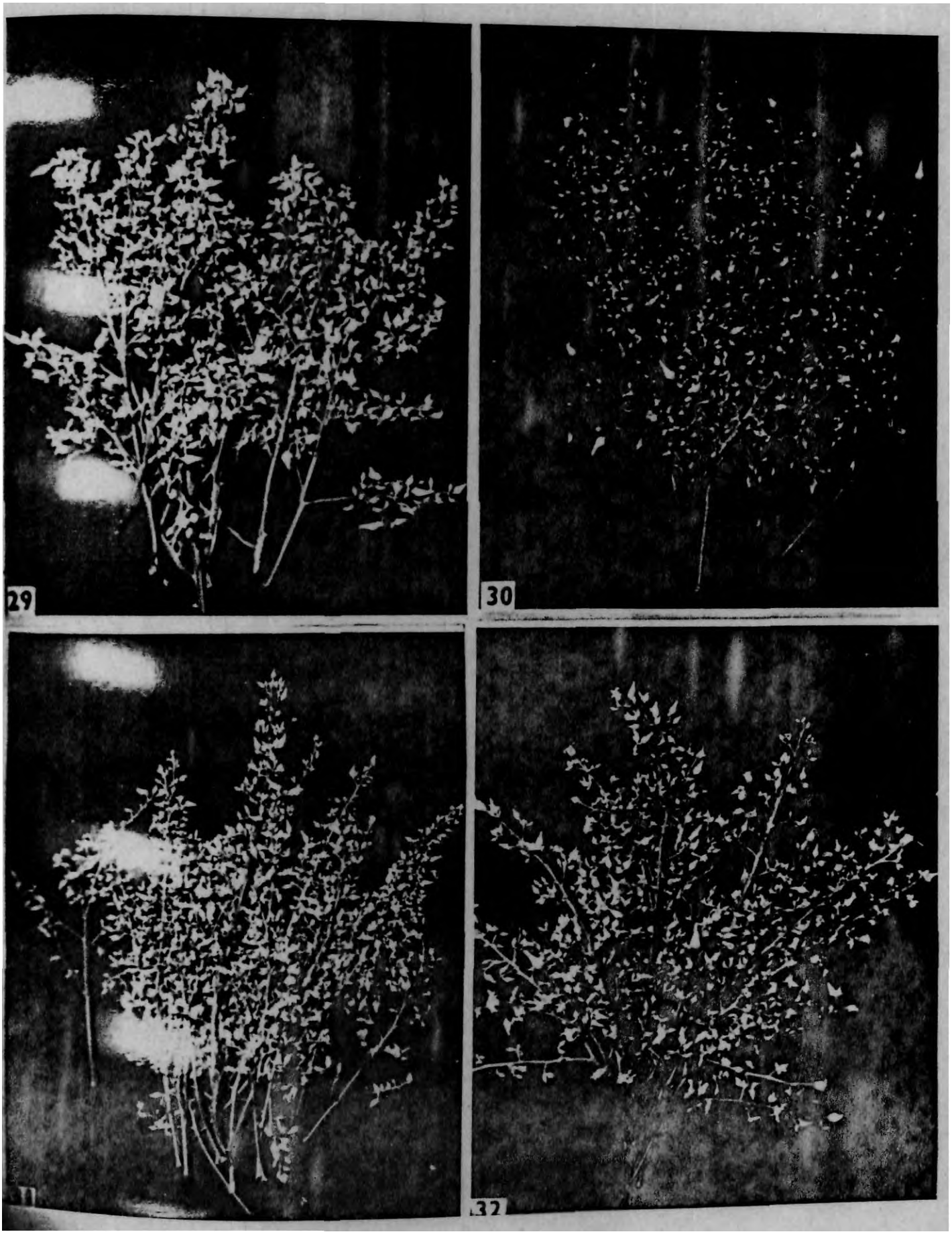
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Table 3. Leaf area of male sterile and control clone of Hevea brasiliensis.

Sl.No.	Clone	Leaf area (cm^2)	Range (cm^2)
1.	GT 1	69.23 ± 37.92	12.01 to 162.11
2.	Ch 2	70.11 ± 21.42	35.00 to 111.09
3.	RRII 15	84.06 ± 33.86	35.98 to 166.09
4.	RRII 17	59.36 ± 15.88	27.07 to 93.03
5.	RRII 35	80.05 ± 30.49	29.02 to 145.13
6.	Mutagen induced mutant	85.00 ± 33.16	32.50 to 175.25
7.	Triploid	75.29 ± 24.74	36.00 to 133.24
8.	Tetraploid	107.12 ± 31.04	56.74 to 155.46
9.	Radiation induced mutant	26.67 ± 14.60	4.07 to 53.91
10.	RRII 105	86.77 ± 40.02	18.99 to 186.20

$>$ 80 = Large

$>$ 60 = Average

$<$ 60 = Small

Table 6. Growth attributes of polybag plants of male sterile clones and control.

Sl. No.	Clone	Height (cm)	Diameter (mm)	No. of flushes	Leaves/ flush	Stem index	Petiolar index	Foliar index	Specific leaf weight ($g\ cm^{-2}$)
1.	GT 1	202.86	17.83	6.21	16.16	0.0517	0.0123	0.0034	0.0062
2.	Ch 2	228.68	19.29	6.82	15.23	0.0493	0.0173	0.0046	0.0067
3.	RRII 15	216.45	20.57	5.82	16.00	0.0530	0.0135	0.0043	0.0069
4.	RRII 17	198.57	16.69	6.08	13.97	0.0519	0.0122	0.0045	0.0069
5.	RRII 35	228.73	19.71	5.23	15.65	0.0463	0.0120	0.0039	0.0068
6.	EMS treated	184.82	17.56	6.05	16.85	0.0512	0.0147	0.0038	0.0066
7.	Triploid	169.80	15.68	4.73	11.20	0.0654	0.0168	0.0056	0.0079
8.	Tetraploid	216.80	20.83	6.20	16.40	0.0703	0.0181	0.0068	0.0092
9.	Radiation induced mutant	90.67	10.12	4.80	13.47	0.0937	0.0168	0.0077	0.0085
10.	RRII 105	199.65	16.10	5.90	15.22	0.0471	0.0171	0.0036	0.0066
	SE	11.53	0.71	0.26	0.54	0.010	0.001	Negligible	Negligible
	CD (5%)	34.27	2.10	1.11	1.60	0.012	0.002	0.001	0.001

Table 5. Correlation matrix of growth attributes of GT 1.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.6726**	0.6132**	0.5920**	0.2776	0.2737	0.2451	-0.0727
2.	Diameter		1.0000	0.4651**	0.8970**	0.6176**	0.3192	0.5429**	-0.0178
3.	No. of flushes			1.0000	0.3179	0.0284	0.3036	0.0319	-0.1310
4.	Leaves/flush				1.0000	0.7146**	0.2765	0.4133*	-0.1310
5.	Stem index					1.0000	-0.2400	0.5057**	-0.1694
6.	Petiolar index						1.0000	0.1064	-0.3004
7.	Foliar index							1.0000	0.1321
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 6. Correlation matrix of growth attributes of Ch 2.

Characters	1	2	3	4	5	6	7	8
1. Height	1.0000	0.4970**	0.6346**	0.2961	-0.2872	0.2149	-0.2334	0.0332
2. Diameter		1.0000	0.7264**	0.6444**	0.3164	0.3417	0.1527	0.1312
3. No. of flushes			1.0000	0.2371	0.0765	0.0733	-0.1918	0.0538
4. Leaves/flush				1.0000	0.3266	0.3543*	0.4263*	0.2573
5. Stem index					1.0000	0.0439	0.2694	0.3654*
6. Petiolar index						1.0000	-0.3845*	-0.2935
7. Foliar index							1.0000	0.7360**
8. Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 7. Correlation matrix of growth attributes of RRII 35.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	-0.0483	-0.0397	0.0051	-0.2117	-0.0147	-0.5298**	-0.4676**
2.	Diameter		1.0000	0.1337	0.4891**	0.3923*	0.1368	0.1589	0.0810
3.	No. of flushes			1.0000	0.3916*	0.4580**	0.2915	-0.0912	-0.5351**
4.	Leaves/flush				1.0000	0.3552*	0.0598	0.3104	-0.5433**
5.	Stem index					1.0000	0.4726**	0.1589	-0.1478
6.	Petiolar index						1.0000	-0.1795	-0.2409
7.	Foliar index							1.0000	0.2362
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 8. Correlation matrix of growth attributes of RRII 17.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.8655**	0.8074**	0.3379	0.4410*	0.3008	0.1085	0.1642
2.	Diameter		1.0000	0.5865**	0.4568**	0.3934*	0.1137	-0.0431	0.2215
3.	No. of flushes			1.0000	0.1017	0.5074**	0.6984**	0.0226	-0.0812
4.	Leaves/flush				1.0000	0.0704	-0.4842**	-0.2174	0.1141
5.	Stem index					1.0000	0.3919*	0.4813**	-0.0297
6.	Petiolar index						1.0000	0.0301	-0.0061
7.	Foliar index							1.0000	-0.1729
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 9. Correlation matrix of growth attributes of RRII 15.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.5656**	0.7585**	0.0943	0.2473	-0.1695	0.0321	0.0152
2.	Diameter		1.0000	0.3133	0.3394	0.1740	-0.5862**	-0.3516*	-0.2695
3.	No. of flushes			1.0000	-0.2870	0.4054*	0.0668	0.0152	0.0494
4.	Leaves/flush				1.0000	-0.1761	-0.1913	-0.0349	0.1456
5.	Stem index					1.0000	-0.1252	-0.5374**	-0.0984
6.	Petiolar index						1.0000	0.5814**	0.4982**
7.	Foliar index							1.0000	0.1504
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 10. Correlation matrix of growth attributes of mutagen (EMS) induced plants

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.8478**	0.8124**	0.4574**	0.1275	0.5777**	0.6266**	0.3025
2.	Diameter		1.0000	0.7613**	0.3731*	-0.0902	0.5318**	0.5051**	0.2161
3.	No. of flushes			1.0000	0.7363**	-0.0243	0.4839**	0.2602	0.1715
4.	Leaves/flush				1.0000	0.4298*	0.1514	0.2082	0.2584
5.	Stem index					1.0000	-0.2073	0.4612**	0.1163
6.	Petiolar index						1.0000	0.5495**	0.2875
7.	Foliar index							1.0000	0.4467*
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 11. Correlation matrix of growth attributes of induced triploid.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.7416**	0.7610**	-0.0540	-0.2374	-0.2429	-0.2033	-0.0252
2.	Diameter		1.0000	0.6590**	0.2040	-0.1695	-0.1505	-0.0749	0.0771
3.	No. of flushes			1.0000	0.2776	0.0788	-0.4159*	-0.2149	0.0271
4.	Leaves/flush				1.0000	0.2089	0.0099	-0.4866**	0.5277**
5.	Stem index					1.0000	-0.4450*	-0.1007	0.2750
6.	Petiolar index						1.0000	0.3775*	-0.2378
7.	Foliar index							1.0000	-0.4109*
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 12. Correlation matrix of growth attributes of radiation induced mutant.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	-0.1498	-0.8629**	0.4420*	-0.5371**	-0.8482**	-0.8519**	-0.8667**
2.	Diameter		1.0000	-0.3090	-0.4495**	0.6715**	-0.3506*	-0.2811	0.0421
3.	No. of flushes			1.0000	-0.2109	0.2434	0.9800**	0.9215**	0.8471**
4.	Leaves/flush				1.0000	-0.8464**	-0.2297	-0.3161	-0.5435**
5.	Stem index					1.0000	0.2349	0.3022	0.6705**
6.	Petiolar index						1.0000	0.9672**	0.8522**
7.	Foliar index							1.0000	0.8671**
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 13. Correlation matrix of growth attributes of induced tetraploid.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.5730**	0.0295	0.1839	-0.0563	0.0016	0.2767	-0.0044
2.	Diameter		1.0000	0.1955	0.0774	-0.1966	-0.0535	0.2487	0.2067
3.	No. of flushes			1.0000	-0.3409	0.6271**	-0.3600*	-0.0949	0.1768
4.	Leaves/flush				1.0000	-0.1432	0.2992	0.1745	-0.1820
5.	Stem index					1.0000	-0.6169**	0.1979	-0.0647
6.	Petiolar index						1.0000	-0.0132	-0.2882
7.	Foliar index							1.0000	-0.2647
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 14. Correlation matrix of growth attributes of RRII 105.

	Characters	1	2	3	4	5	6	7	8
1.	Height	1.0000	0.6900**	0.7000**	0.7530**	0.1525	-0.1033	0.5108**	0.0001
2.	Diameter		1.0000	0.2265	0.6062**	0.2929	0.0658	0.4699**	0.2697
3.	No. of flushes			1.0000	0.2296	-0.1642	0.0333	0.0343	-0.3433
4.	Leaves/flush				1.0000	0.5479**	-0.3915*	0.7665**	0.1916
5.	Stem index					1.0000	-0.1714	0.5466**	0.4665**
6.	Petiolar index						1.0000	-0.0768	-0.2158
7.	Foliar index							1.0000	0.4196*
8.	Specific leaf weight								1.0000

* Significant at 5% level.

** Significant at 1% level.

Table 15. Morphological characters of female and male flowers of male sterile and control clones.

Clone	Female		Male	
	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)
GT 1	8.22	2.36	3.38	1.40
Ch 2	12.90	3.28	7.36	2.81
RRII 15	11.93	5.94	7.81	3.81
RRII 17	9.42	2.13	5.62	1.88
RRII 35	9.79	1.95	4.78	2.42
EMS treated	10.55	3.40	3.44	2.11
Triploid	10.97	3.06	7.82	2.91
Tetraploid	12.99	3.68	8.06	3.33
Radiation induced mutant	5.77	2.77	3.72	1.89
RRII 105	10.65	3.32	4.39	2.69
SE	0.06	0.07	0.07	0.04
CD	0.19	0.20	0.21	0.12

SECTION 2. CYTOLOGY AND PALYNOLOGY

Cytology of normal clone of Hevea brasiliensis:-

Cytological studies both meiosis and mitosis in Hevea brasiliensis are very difficult due to very small size and stickiness of chromosomes.

MITOSIS:-

Mitotic studies of tender leaf tips of control clone, RRII 105 have showed that the chromosome complement is 2n = 2x = 36 (Fig. 37). At metaphase, 36 bivalents are seen. There are distinct prophase, metaphase, anaphase and telophase resulting in two qualitatively and quantitatively identical daughter cells.

MEIOSIS:-

Meiotic division is also normal in the control clone. The prophase (Fig. 38) of reduction division begins with leptotene stage followed by zygotene, pachytene diplotene, and diakinesis (Fig. 39). During metaphase I (Fig. 40), the chromosomes scatter

through the spindle which has made its appearance and afterwards orient themselves in the equatorial plane. In H. brasiliensis exact metaphase stage is of very short duration and hence it is very difficult to trace this stage in detail (Ramaer, 1935). Anaphase I is perfectly normal in the control clone. Eighteen chromosomes are clearly seen in each pole. First meiotic division is followed by the second meiotic division (Fig. 41) which is also normal in the control clone resulting in tetrads which gives rise to four haploid microspores (Fig. 42) and giving rise to stainable pollen grains (Fig. 43) in the usual manner typical to dicotyledonous plant. Thus the meiosis in the control clone is normal without any abnormalities.

Cytology of spontaneous male sterile clones

In the three spontaneous male sterile clones GT 1, Ch 2 and RRII 35, meiotic division was found to be normal up to the formation of haploid microspores. Soon after the formation of tetrads, there was complete degeneration (Fig. 44) cytoplasm and nuclei in the microspores resulting in sterility. In the clone GT 1 and RRII 35 at the maturity of male flowers, no pollen grains were observed in the pollen sac (Figs. 45 and 46). However, in Ch 2, very few pollen grains could be observed, which were sterile (Table 16).

In H. brasiliensis, microsporogenesis was perfectly regular and confirmed to the classical pattern described for angiosperms (Maheswari, 1950; Laser and Lerston, 1972).

In GT 1 and RRII 35 during the tetrad stage, obstruction of microsporogenesis was observed and the callose wall uniting the microspores did not dissolve. Consequently the microspores were not found liberated in the locule and had undergone rapid cytoplasmic degeneration. The anthers also showed complete degeneration. Cells of tapetum manifested some sort of hypertrophy and then degenerated after the abortion of microspores. In Ch 2, the tapetum showed abnormal behaviour and was persistent (Fig. 47). Development of anther was more or less the same in both fertile and sterile clones with the exception of the behaviour of tetrads and tapetal cells after the beginning of meiosis. The anther wall at the tetrad stage consisted of four layers: an epidermis, an endothecium, a middle layer and tapetum (Fig. 48). The tapetal cells showed multinucleate condition. At many places the tapetal cells were seen to have undergone a periclinal division, the tapetum consequently becoming two layered at these points (Fig. 49). In fertile clone, the tapetal layer showed degeneration (Fig. 50) as the microspore mother cells progressed towards completion of meiosis. Furthermore, the tapetal layer in sterile clone persisted for longer period. Average radial width (μm) of tapetum of two

[redacted]

male sterile clones, GT 1 and Ch 2, along with fertile clone are depicted in Text. Fig. II. It may be seen that tapetum was persistent and showed an average 20-30 μm whereas, in the fertile clone it showed a maximum width of 8 μm and absent at dehiscing stage.

Spontaneous triploid

The chromosome complement of the somatic cell was found to be 2n = 54 (Fig. 51). Meiotic division exhibited a wide range of abnormalities in the spontaneous triploid. At metaphase I, (Fig. 52) various chromsome associations like trivalents, bivalents and univalents were observed (Table 17). The range of trivalents was 9 to 18, bivalents 0 to 10 and univalents 0 to 10. Precocious separation was also noted (Fig. 53). The average occurrence of chromosome association per cell was 15.16 ± 0.33 for trivalents, 2.38 ± 0.30 for bivalents and 3.76 ± 0.34 for univalents. There was formation of 18 trivalents in 5% of PMCs while 15% cells showed the maximum of 10 univalents and 2% cells showed 10 bivalents which was the maximum.

There was some abnormalities in the formation of microspores in a tetrad. Instead of formation of four microspores in a tetrad, there was formation of five and six microspores. Irrespective

of the number of microspores, there was complete degeneration of cytoplasm and nuclei resulting in total sterility. The production of even sterile pollen grain was practically absent. Even the male flowers did not attain normal maturity.

Induced triploid

The mitotic studies of the somatic cell of the induced triploid also showed the chromosome complement to be 2n = 54 (Fig. 54).

Details of chromosome association at metaphase I are given in Table 18. The triploid exhibited univalent formation (1 to 17) followed by bivalents (1 to 10) and trivalents (7 to 17) (Figs. 55 and 56). The mean occurrence of chromosome associations per cell was 10.36 ± 0.68 for univalents 6.71 ± 0.23 for bivalents and 10.07 ± 0.24 for trivalents. During anaphase I unequal segregation and formation of laggards (Fig. 57) were also noted. Tetrads with microspores having unequal size were visible and very small microspores were also seen. After the liberation of microspores from the tetrad, only a very few developed into stainable pollen.

Induced tetraploid

Mitotic studies of leaf tip from the tetraploid have shown 72 chromosomes (Fig. 58). Meiotic irregularities were noted in the PMCs of induced tetraploid of RRII 105. At metaphase I, besides predominant bivalent formation, univalents, trivalents and quadrivalents were also observed (Figs. 60 and 61). Univalents, bivalents, trivalents and quadrivalents showed a range of 1 to 9, 21 to 32, 0 to 4 and 0 to 4 respectively. Details of chromosome association at metaphase I are given in Table 19. The average occurrence of chromosome association per cell was 5.90 ± 0.38 for univalents, 26.50 ± 0.46 for bivalents, 1.70 ± 0.21 for trivalents and 2.00 ± 0.18 for quadrivalents. Anaphase I showed unequal segregation, formation of laggards, formation of micronuclei, etc. After telophase II, microspores were formed in groups consisting of 2 to 6 numbers and they were of different sizes. After the liberation of microspores from the tetrad, development of fertile as well as sterile pollen grains were noted.

RRII 17

Meiotic studies of male flower buds from RRII 17, which exhibited male and female sterility, had shown that there was a wide spectrum of abnormalities. Normal meiotic behaviour, with

formation of 18_{II} was never observed in this sterile clone. There was predominant formation of univalents at metaphase I (Fig. 62) ranging from 8 to 32 and bivalents 2 to 14 (Table 20). There was poor spindle formation, non-orientation of the univalents and clumping of chromosomes at equatorial plate. Anaphase I was highly irregular with unequal distribution of chromosomes, absence of active polar movement and varying number of laggards (Fig. 63). In telophase II, the number of sporads ranged from 3 to 9 (Fig. 64) the highest frequency (44%) being six (Fig. 65). Cells showing five and seven microspores was 17 and 16% respectively. 14% cells exhibited four and three microspores were noted in 5% of the cells. The occurrence of eight and nine microspores were observed in 3 and 1% of cells. However, after the microspores were liberated there was complete degeneration of cytoplasm and nuclei resulting in the formation of sterile pollen grains of varying size and shapes. The smallest pollen grains had an average size of 21.38 x 18.93μ m and the largest had 55.33 x 49.23μ m. There were about 41% of sterile pollen grains having an average size of 31.23 x 28.48μ m and 38.5% had 41.88 x 37.50μ m mean size. The normal size of pollen grain in the fertile control clone was 39.00 x 36.00μ m.

Radiation induced mutant

Cytomixis had been noted in the induced mutant. About 30% of the PMCs examined revealed this aberration. Cytoplasmic connections between PMCs were observed at all stages of meiosis (Fig. 66). Chromatin materials were also seen passing from one cell to the other from early prophase (Fig. 67) to telophase II (Figs. 68 to 71). The frequency of cells showing cytomixis is given in Table 21. At telophase II, 55% cells showed cytoplasmic connections. In some cases most of the pollen mother cells in a flower were found to be involved in cytomixis, while the adjacent flowers were normal without any aberrations. During prophase I, 1 to 15 cells, at metaphase I, 2 to 4 cells, at telophase II, 2 to 8 cells in a field were observed to show cytoplasmic connections. Direct fusion as well as connecting cytoplasmic strands were observed among the pollen mother cells. Due to the transfer of chromatin materials, pollen mother cells with more or reduced number from the normal number n = 18 was observed (Fig. 72). At anaphase I bridge formation with and without laggards was seen in 3% cells. After telophase II aberrant cytokinesis was noted resulting in total sterility.

In the mutagen induced sterile clone also the meiosis was normal up to the formation of tetrads. After that complete degeneration was noted resulting in sterility.

Cytokinetic aberrations

In normal clones of H. brasiliensis cytokinesis occurred after telophase II resulting in tetrad. Wall formation was of simultaneous type. Instead of normal tetrad formation cytokinetic aberrations, like variation in the formation of microspores were observed in the male sterile clones. In RRII 17 due to abnormal cytokinesis, microspores of varying size and shape were formed. Due to cytokinetic aberrations the distribution of nuclei showed wide variations in radiation induced mutant. There were different types of abnormalities in cleavage of cytoplasm. The distribution of nuclei in the tetrad, is diagramatically depicted in Text. Fig. III. The nuclei were distributed in 2 + 1 + 1, 2 + 2 and the details are furnished in Table 22. In the first type, four microspores were formed as in the case of normal cells. In the second case, there was central cleavage of cytoplasm resulting in the union of microspores. In the third instance, there was no cleavage of cytoplasm and the nuclei were distributed in the common cytoplasm. In the fourth case, there was two nuclei in one cell and one each in the others. There were microspores connected with cytoplasmic strands (Fig. 73) formed as a result of incomplete cleavage of cytoplasm. Normal microspores as well as microspores with divided nuclei (Fig. 74) were also observed. Megapollen were noted in 0.5% cells (Fig. 75). Pollen conglomerates were also observed

(Fig. 76). Whatever may be the abnormalities, all these ultimately resulted in the production of sterile pollen grains (Fig. 77) (Table 23).

Palynology

The pollen grains of Hevea are yellow and powdery. The pollen of all the species are 3 zonocolporate (Fig. 77). The shape varies from oblate spheroidal to prolate spheroidal. Amb is triangular with convex mesocolpium. The apertures are tenumarginate. In a few acetolysed grains the three apertures were found united to form parasyncolpate nature. In vitro and in vivo germination of pollen grains are normal in the control clone RRII 105 (Figs. 78 to 80).

Light microscopic observations

The control clone RRII 105 showed 92.8% pollen stainability and pollen germination was 80%. Fertile pollen grains were comparatively absent in GT 1, RRII 35, RRII 17, RRII 15 and in the mutagen induced mutant, although 0.5% of stainable pollen grains were observed in the radiation induced mutant. Sterile pollen grains showed wide range of variations in size and shape in RRII 17 and the radiation induced mutant. Pollen stainability in the induced

tetraploid was 80% (range 77 to 82%). Among the stainable pollen 30% (28 to 33%) had three pores and 50% (47 to 52%) had four pores. In the induced triploid the pollen stainability recorded was 5.5%. There were pollen grains with four and three pores (Fig. 81).

The pollen morphological characters of diploid, triploid and tetraploid are given in Table 24. In the control clone RRII 105, the pollen grains were 3 zono-colporate and showed an average size of 34.98 x 28.61 μ m (Figs. 82 and 83). In the triploid and tetraploid the size of pollen grains was 46.50 x 37.90 μ m and 49.20 x 42.48 μ m respectively. Exine thickness in the control clone was 3.00 μ m whereas, the triploid and tetraploid recorded 3.33 and 5.54 μ m respectively. The ora diameter also showed variation in size and the average measurements for this parameter was 4.00 μm for diploid, 4.50 μ m for triploid, 5.82 μ m for tetraploid. The biggest pollen grains in each cytotype recorded 36.30 x 31.55 μ m, 62.50 x 55.00 μ m, 52.50 x 45.00 μ m respectively for diploid, triploid and tetraploid.

Text. Fig. IV shows the relationship between the size of flower buds and that of microspore mother cells and microspores of the clone RRII 105. Pollen mother cells were about 25 μ m in diameter during very early prophase stage. They grew larger

to about 50 μm at tetrad stage. Microspores within the tetrad were small (15 μm). But soon on release they enlarged and attained a diameter of about 35 to 40 μm and their development was very rapid. After attaining the maximum size, the microspores maintained more or less the same volume up to anthesis. Flowers having a length of about 3 mm showed the tetrads after which there was considerable increase in length resulting in mature male flowers having a length of 5 mm.

The relationship between the size of flower buds and that of microsporocytes or microspores in induced triploid is depicted in Text. Fig. V. Compared to the diploid RRII 105, the microspore mother cells of the triploid were larger in size (35 μm). At the tetrad stage, they showed a size of about 54 μm. The microspores within the tetrad were small, mean being 20 μm and the range 5 to 25 μm. But microspores in free stage showed comparatively larger size (33 μm). The pollen grains showed marked variation in size (10 μm to 55 μm). The percentage of fertile pollen grains were comparatively less, which however also showed wide variation in size.

In the tetraploid (Text. Fig. VI) also the size of pollen mother cells and microspores were related to flower size. The microspore mother cell showed an average diameter of about 45 μm

and the tetrad showed 72 μm. The microspores within the tetrad were very small, the mean being 25 μm and the range 2 to 30 μm. But when they were released from the tetrad the average size of free microspores was about 33 μm. The microspores gradually developed into mature stainable and sterile (Fig. 84) pollen grains. Pollen grains showed wide range of variation 3 μm to 62 μm.

In the male sterile clones studied there was a direct relationship between flower size and developing microspores. Pollen mother cells of the clone Ch 2 (*Text. Fig. VII*) showed an average diameter of about 35 μm and the tetrad showed 45 μm. Since there was degeneration of cells, further development after the tetrad stage was arrested and the resulting sterile pollen grains were poorly developed.

SEM Studies

Scanning electron micrographs of the diploid and tetraploid are given in Figs. 85 to 90. The polar view of pollen grains of RRII 105 and an enlarged view of the exine are shown in Figs. 85 and 86. The pollen grains having three and four pores are depicted in Figs. 87 to 89 and a portion of exine taken at higher magnification is shown in Fig. 90.

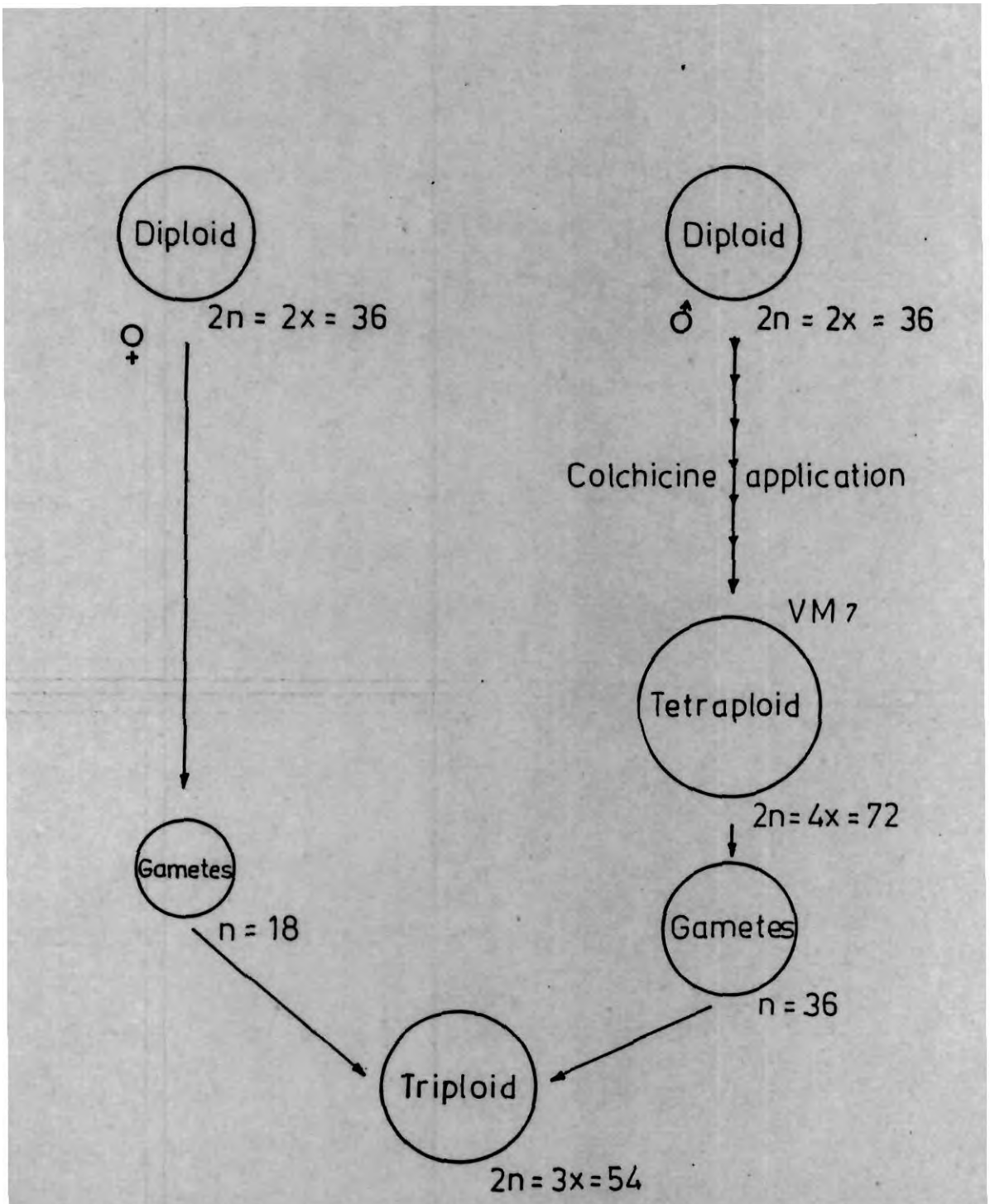
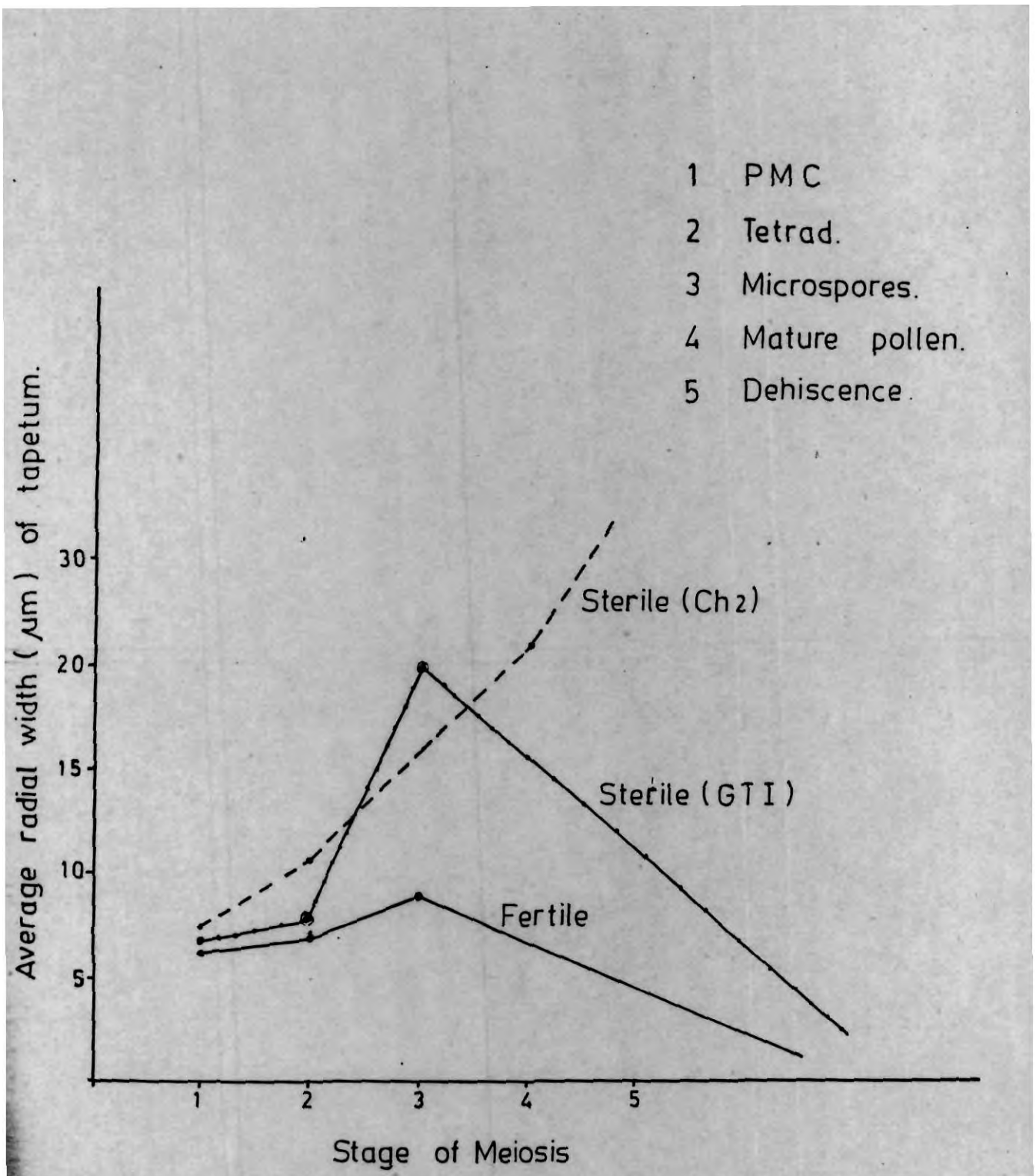
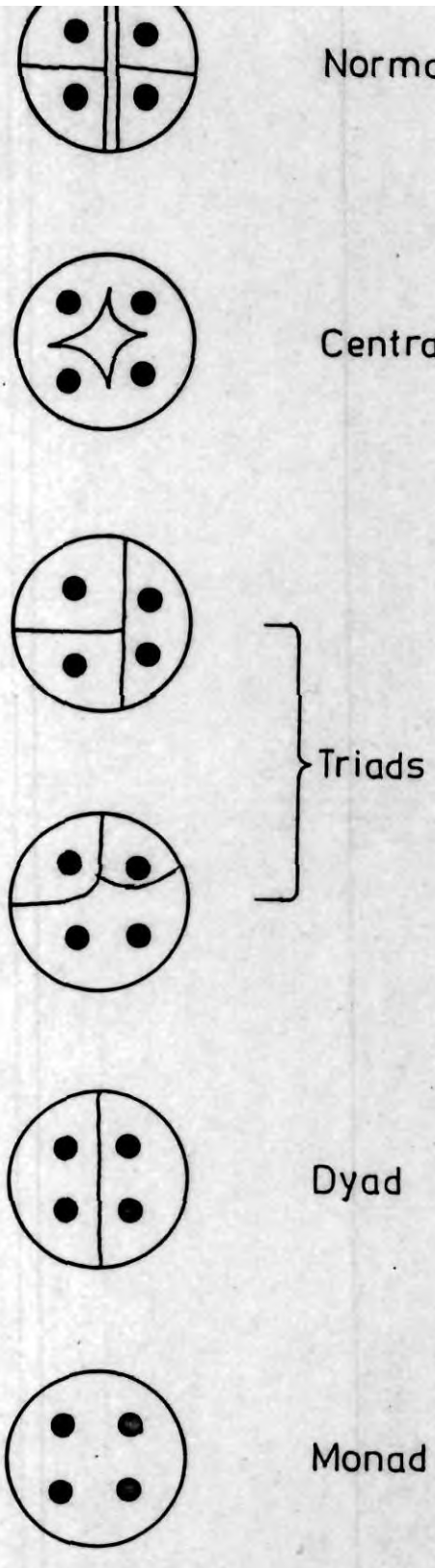


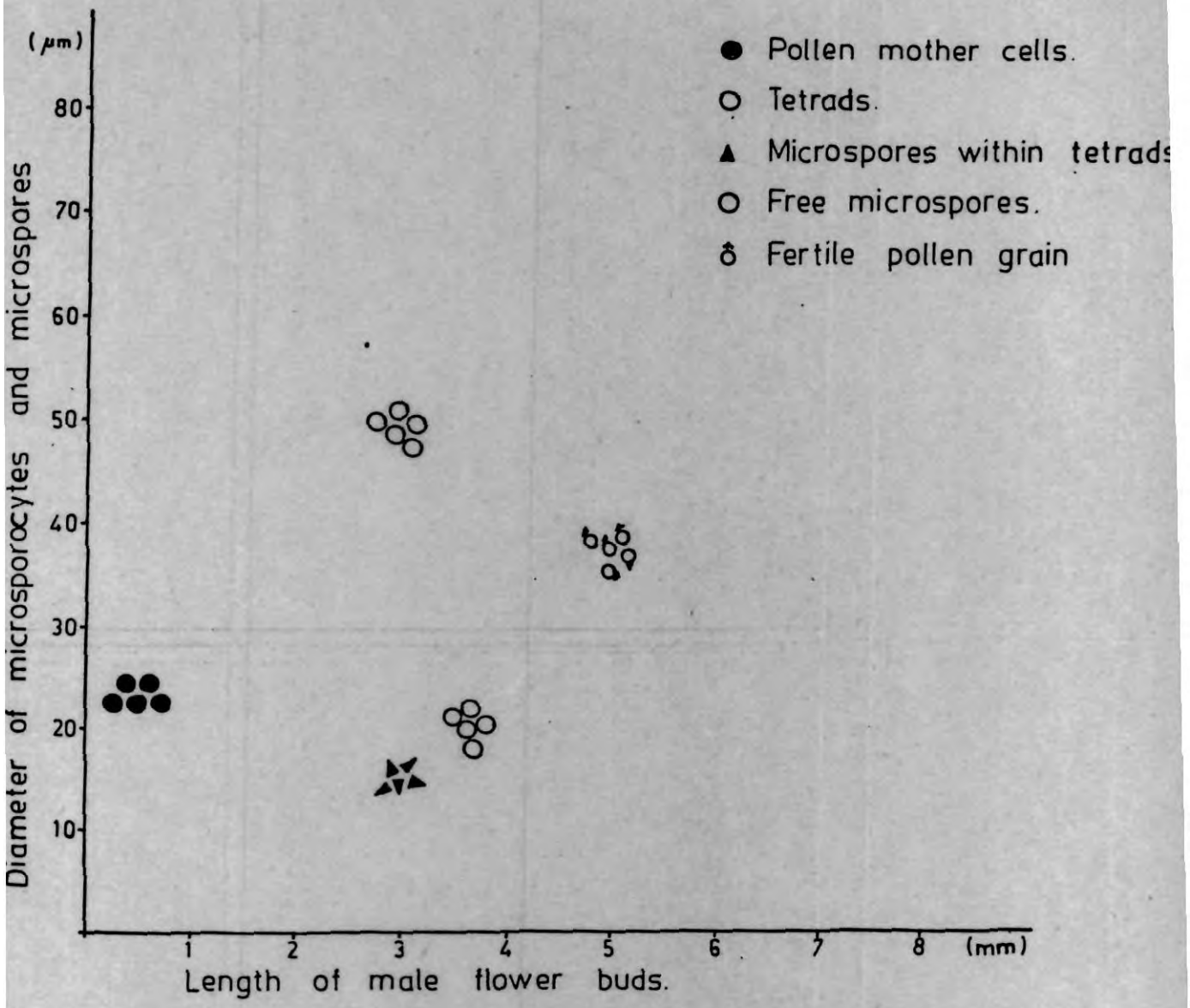
Fig. I : Synthesis of triploid



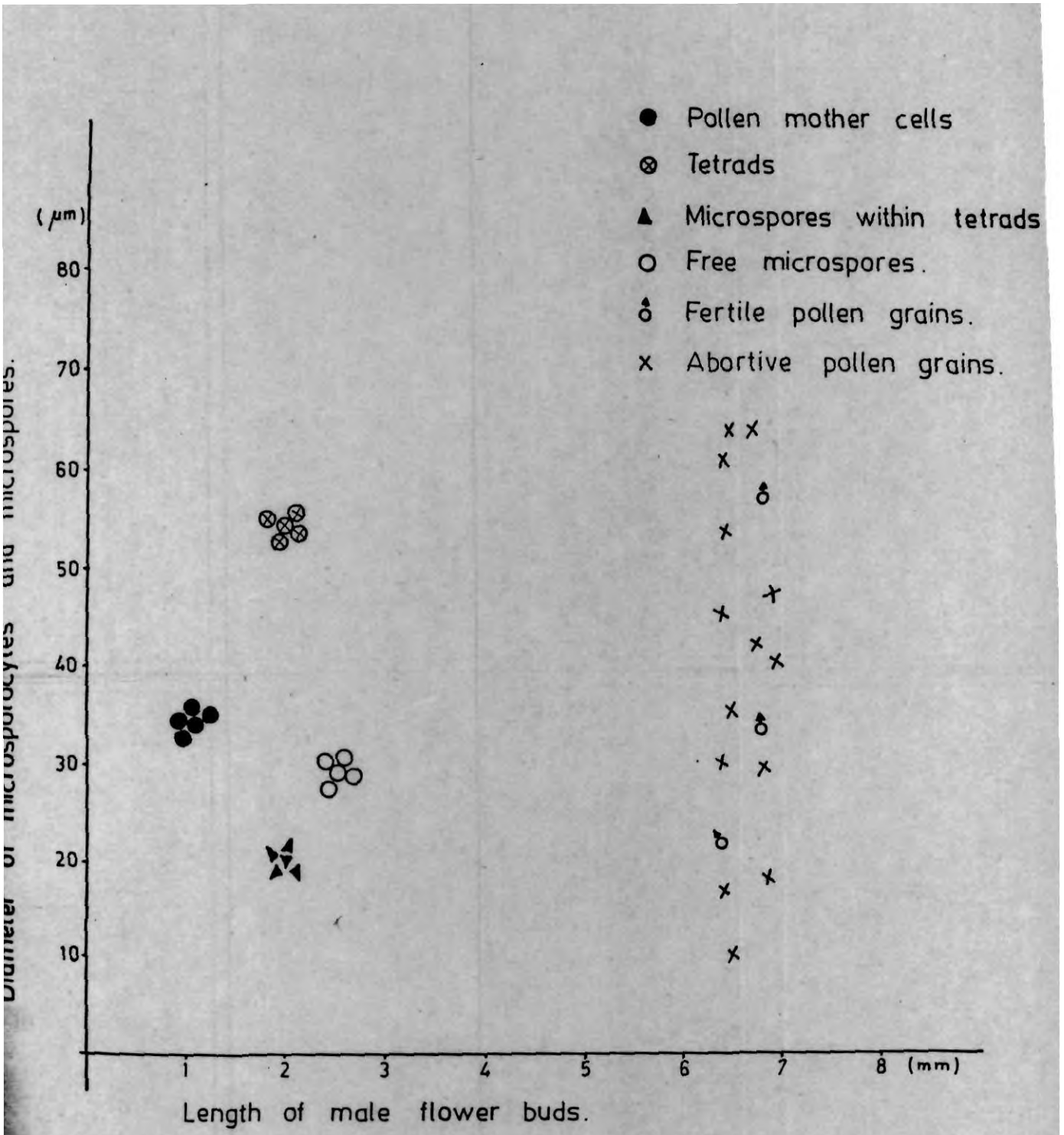
Text. Fig. II : Average radial width (µm) of the tapetum at different meiotic stages of male fertile and sterile clones.



Text. Fig. III : Diagramatic representation of different Types of abnormalities observed at the Tetrad stage in radiation induced mutant.



xt Fig: IV: Size of microsporocytes and microspores at different developmental stages of flower buds of RRII 105.



ext. Fig. V: Size of microsporocytes and microspores at different devolopmental stages of flower buds in triploid.

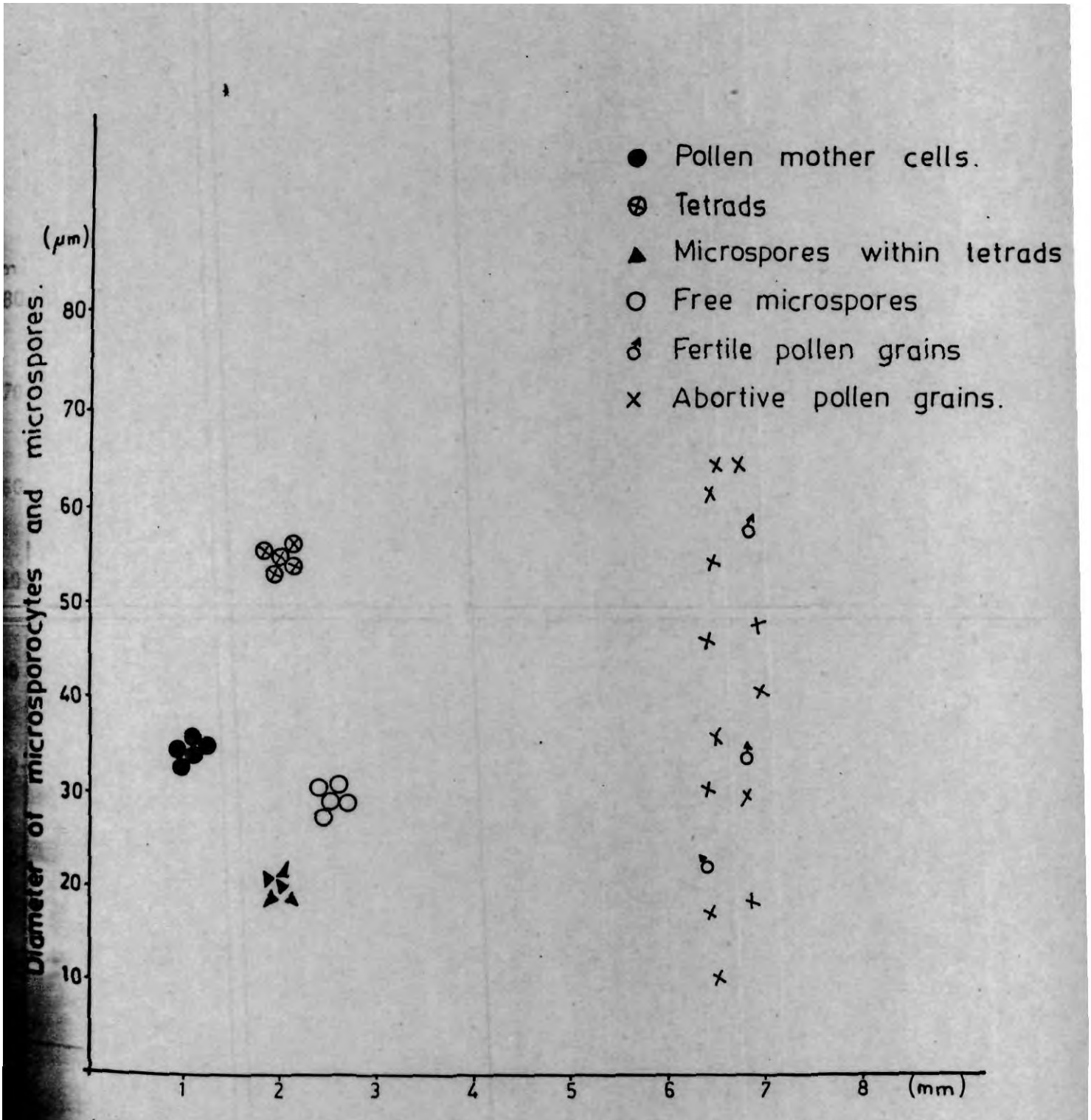


Fig. VI: Size of microsporocytes and microspores at different developmental stages of flower buds in tetraploid.

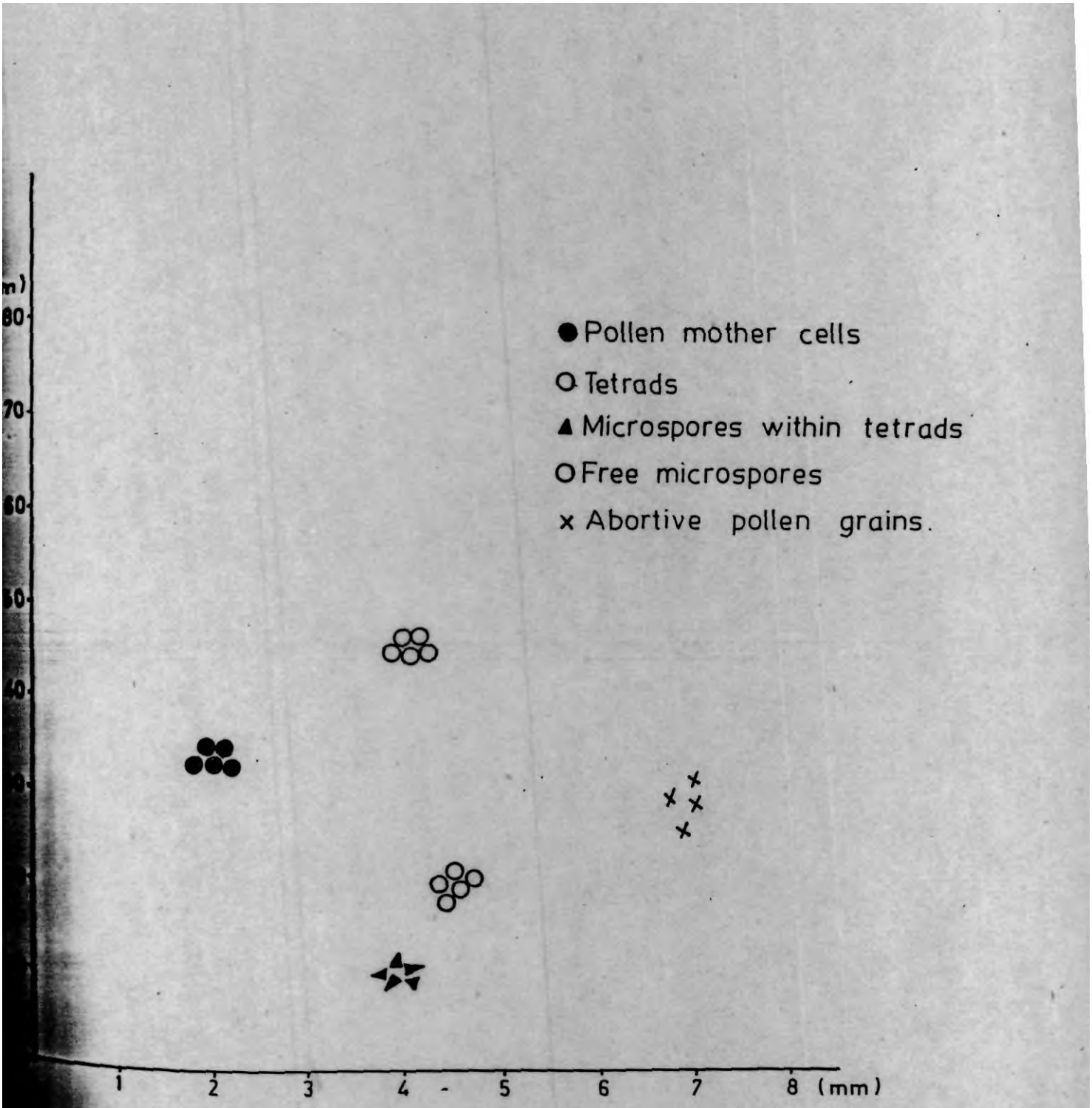


Fig. VII : Size of microsporocytes and microspores at different Developmental stages of flower buds in male sterile clone Ch 2.

zono-colporate condition with a peculiar (heteromorphic areoles) exine ornamentation. Almost all the areoles are united. Colpi membrane is crustate.

In RRII 17 (Fig. 91) there was no pore differentiation. Exine ornamentation is heteromorphic, with very large areoles. Moreover, the development of exine ornamentation is not complete (Fig. 92).

In Ch 2, the sterile pollen grains are 3 zono-colporate (Fig. 93). Colpi membrane is crustate. Bridges over the colpi membrane are seen (Fig. 94). It is clavate and columella tips are heteromorphic and united.

Cytophotometric determination of DNA

The data on relative nuclear DNA (4C) amount in diploid, spontaneous triploid, induced triploid and induced tetraploid are given in Table 25. There were significant difference in DNA content among the cytotypes. The relative DNA content of diploid is 44.29 pg. But the autotetraploid representing C_{20} generation showed an almost two fold increase. The triploid showed more or less thrice the volume of haploid nuclei. Among triploids, the spontaneous triploid showed numerically less value compared to that of the induced triploid.

RRII 105

Pollen grains are 3 zono-colporate crustate island along the length of colpi is visible. Areoles rugulate, surface island conical and very close with narrow depression. Areoles free or united into island. There are furrow island with crustation. Furrow bridge is also seen.

Tetraploid

Pollen grains are parasyncolporate. Free areoles less than rugulate. There are three colporate and four colporate grains. Areoles are united and they are of different size and shape. Exine ornamentation is also large in size.

Triploid

In the induced triploid also pollen grains with 3 and 4 germ pores are seen. The size of exine ornamentation is also large. The pollen grains are three and four zono-colporate.

Sterile pollen grains

Pollen from the radiation induced sterile clone showed three

Table 17. Chromosome association during metaphase I in the spontaneous triploid.

Chromosome association	Percentage of cells
$9_{III} + 9_{II} + 9_{I}$	11
$10_{III} + 10_{II} + 4_{I}$	2
$10_{III} + 8_{II} + 8_{I}$	1
$10_{III} + 9_{II} + 6_{I}$	3
$10_{III} + 7_{II} + 10_{I}$	9
$11_{III} + 6_{II} + 9_{I}$	6
$11_{III} + 7_{II} + 7_{I}$	2
$12_{III} + 6_{II} + 6_{I}$	10
$12_{III} + 5_{II} + 8_{I}$	7
$12_{III} + 9_{II} + -$	1
$12_{III} + 4_{II} + 10_{I}$	5
$13_{III} + 6_{II} + 3_{I}$	4
$13_{III} + 5_{II} + 5_{I}$	14
$14_{III} + 1_{II} + 10_{I}$	1
$14_{III} + 5_{II} + 2_{I}$	5
$15_{III} + 3_{II} + 3_{I}$	4
$15_{III} + 2_{II} + 5_{I}$	2
$16_{III} + 2_{II} + 2_{I}$	3
$17_{III} + 1_{II} + 1_{I}$	3
$17_{III} + - + 3_{I}$	2
$18_{III} + - + -$	5

Table 16. Production of pollen in male sterile and control clones.

	Clone	Pollen/anther	Stainability of pollen
1.	RRII 105	175 - 200	92.8
2.	Tetraploid	100 - 150	80.0
3.	Triploid (induced)	50 - 100	5.5
4.	Spontaneous Triploid RRII 15	Nil	-
5.	Ch 2	20 - 50	-
6.	RRII 17	Nil	-
7.	Mutagen induced	Nil	-
8.	Radiation induced	100 - 150	0.5
9.	GT 1	Nil	-
10.	RRII 35	Nil	-

Table 18. Chromosome association during metaphase I in the induced triploid.

Sl.No.	Chromosome association	Percentage of cells
1.	$7_{III} + 10_{II} + 13_{I}$	2
2.	$8_{III} + 9_{II} + 12_{I}$	2
3.	$8_{III} + 9_{II} + 14_{I}$	3
4.	$8_{III} + 7_{II} + 16_{I}$	3
5.	$8_{III} + 10_{II} + 10_{I}$	2
6.	$9_{III} + 7_{II} + 13_{I}$	4
7.	$9_{III} + 8_{II} + 11_{I}$	1
10.	$9_{III} + 6_{II} + 15_{I}$	2
11.	$9_{III} + 5_{II} + 17_{I}$	2
12.	$9_{III} + 9_{II} + 9_{I}$	5
13.	$10_{III} + 7_{II} + 10_{I}$	6
14.	$10_{III} + 8_{II} + 8_{I}$	3
15.	$10_{III} + 6_{II} + 12_{I}$	4
16.	$10_{III} + 10_{II} + 4_{I}$	1
17.	$11_{III} + 6_{II} + 9_{I}$	6
18.	$11_{III} + 5_{II} + 11_{I}$	2
19.	$11_{III} + 7_{II} + 7_{I}$	7
20.	$11_{III} + 4_{II} + 13_{I}$	1
21.	$12_{III} + 5_{II} + 8_{I}$	3
22.	$12_{III} + 6_{II} + 6_{I}$	9
23.	$12_{III} + 4_{II} + 10_{I}$	1
24.	$13_{III} + 5_{II} + 5_{I}$	9
25.	$13_{III} + 6_{II} + 3_{I}$	2
26.	$14_{III} + 3_{II} + 6_{I}$	2
27.	$14_{III} + 1_{II} + 10_{I}$	1
28.	$14_{III} + 5_{II} + 2_{I}$	6
29.	$15_{III} + 2_{II} + 5_{I}$	2
30.	$15_{III} + 3_{II} + 3_{I}$	2
31.	$17_{III} + 2_{II} + 2_{I}$	1
32.	$17_{III} + 1_{II} + 1_{I}$	3
33.	$17_{III} + - + 3_{I}$	3

Table 17. Chromosome association during metaphase I in the spontaneous triploid.

Chromosome association	Percentage of cells
$9_{III} + 9_{II} + 9_{I}$	11
$10_{III} + 10_{II} + 4_{I}$	2
$10_{III} + 8_{II} + 8_{I}$	1
$10_{III} + 9_{II} + 6_{I}$	3
$10_{III} + 7_{II} + 10_{I}$	9
$11_{III} + 6_{II} + 9_{I}$	6
$11_{III} + 7_{II} + 7_{I}$	2
$12_{III} + 6_{II} + 6_{I}$	10
$12_{III} + 5_{II} + 8_{I}$	7
$12_{III} + 9_{II} + -$	1
$12_{III} + 4_{II} + 10_{I}$	5
$13_{III} + 6_{II} + 3_{I}$	4
$13_{III} + 5_{II} + 5_{I}$	14
$14_{III} + 1_{II} + 10_{I}$	1
$14_{III} + 5_{II} + 2_{I}$	5
$15_{III} + 3_{II} + 3_{I}$	4
$15_{III} + 2_{II} + 5_{I}$	2
$16_{III} + 2_{II} + 2_{I}$	3
$17_{III} + 1_{II} + 1_{I}$	3
$17_{III} + - + 3_{I}$	2
$18_{III} + - + -$	5

Table 19. Chromosome association during metaphase I in the induced tetraploid clone of Hevea brasiliensis.

Sl.No.	Chromosome association	Percentage of cells
1.	$3_{IV} + 1_{III} + 26_{II} + 5_{I}$	15
2.	$1_{IV} + 1_{III} + 32_{II} + 1_{I}$	4
3.	$2_{IV} + 1_{III} + 27_{II} + 7_{I}$	9
4.	$1_{IV} + 2_{III} + 30_{II} + 2_{I}$	7
5.	$2_{IV} + - + 30_{II} + 4_{I}$	2
6.	$- + 1_{III} + 30_{II} + 9_{I}$	4
7.	$1_{IV} + 3_{III} + 26_{II} + 7_{I}$	1
8.	$2_{IV} + 3_{III} + 24_{II} + 4_{I}$	4
9.	$1_{IV} + 2_{III} + 27_{II} + 8_{I}$	5
10.	$2_{IV} + 1_{III} + 26_{II} + 9_{I}$	8
11.	$1_{IV} + 2_{III} + 28_{II} + 6_{I}$	5
12.	$1_{IV} + 1_{III} + 29_{II} + 7_{I}$	4
13.	$4_{IV} + 2_{III} + 22_{II} + 6_{I}$	9
14.	$2_{IV} + 2_{III} + 28_{II} + 2_{I}$	2
15.	$3_{IV} + 1_{III} + 22_{II} + 9_{I}$	8
16.	$3_{IV} + 4_{III} + 21_{II} + 6_{I}$	5
17.	$2_{IV} + 2_{III} + 26_{II} + 6_{I}$	2
18.	$1_{IV} + - + 32_{II} + 4_{I}$	3
19.	$4_{IV} + - + 25_{II} + 6_{I}$	2
20.	$4_{IV} + - + 28_{II} + 6_{I}$	1

Table 20. Chromosome association at metaphase I of RRII 17 of *Hevea brasiliensis*.

Chromosome association	Percentage of cells
$2_{II} + 32_{I}$	6
$3_{II} + 30_{I}$	12
$4_{II} + 28_{I}$	3
$5_{II} + 26_{I}$	4
$6_{II} + 24_{I}$	13
$7_{II} + 22_{I}$	3
$8_{II} + 20_{I}$	19
$9_{II} + 18_{I}$	3
$10_{II} + 16_{I}$	15
$11_{II} + 14_{I}$	3
$12_{II} + 12_{I}$	8
$13_{II} + 10_{I}$	9
$14_{II} + 8_{I}$	2

Table 21. Frequency of cells showing cytomixis/cytoplasmic connections in the radiation induced mutant clone of Hevea brasiliensis.

Meiotic stage	Total cells observed	Percentage
Prophase I	713	36.00
Metaphase I	100	28.00
Anaphase I	100	10.00
Telophase I	100	8.00
Telophase II	541	55.75
Microspore	100	2.00

Table 23. Size of sterile pollen grains in the radiation induced mutant clone of Hevea brasiliensis.

Size of pollen (µm)	Percentage observed
35.42 x 30.42	18.50
39.52 x 36.45	37.00
51.10 x 44.02	22.00
71.50 x 60.00	22.50

Table 22. Details of cells showing cytokinetic aberrations in radiation induced mutant clone of Hevea brasiliensis.

Distribution of nuclei in the tetrad stage	Percentage
2 + 1 + 1	20
2 + 2	15
1 + 3	10
4	5
1 + 1 + 1 + 1 (Normal)	50

Table 25. 4C nuclear DNA content in three cytotypes of *Hevea brasiliensis*.

Cytotype	2n	DNA (pg)
RRII 105	36	44.29
Triploid	54	62.43
Triploid	54	60.19
Tetraploid	72	89.37
SE		0.12
CD (5%)		0.32

Table 24. Morphological characters of stainable pollen from diploid, triploid and tetraploid clones of *Hevea brasiliensis*.

Parameters	Diploid Mean ± SE	Triploid Mean ± SE	Tetraploid Mean ± SE
Polar Diameter (μm)	34.98 ± 0.48	46.50 ± 2.04	49.20 ± 3.26
Range	(29.70 to 36.30)	(35.00 to 52.50)	(45.00 to 62.50)
Equatorial diameter (μm)	28.61 ± 0.49	37.90 ± 1.80	42.48 ± 2.37
Range	(24.75 to 31.55)	(30.00 to 45.00)	(30.00 to 55.00)
Exine thickness (μm)	3.00 ± 0.24	3.33 ± 0.98	5.54 ± 0.41
Ora diameter (μm)	4.00 ± 0.31	4.50 ± 0.63	5.82 ± 0.27

Fig. 37 : Metaphase of RRII 105 showing somatic chro
complement 2n = 36 x 3000.

Fig. 38 : Prophase I of control clone RRII 105 x 3000

Fig. 39 : Diakinesis x 3000

Fig. 40 : Metaphase I x 4000

Fig. 41 : Telophase II x 3000

Fig. 42 : Microspores x 2400

Fig. 43 : Stainable pollen grains x 190

Fig. 44 : Degenerating tetrads in the male sterile clone

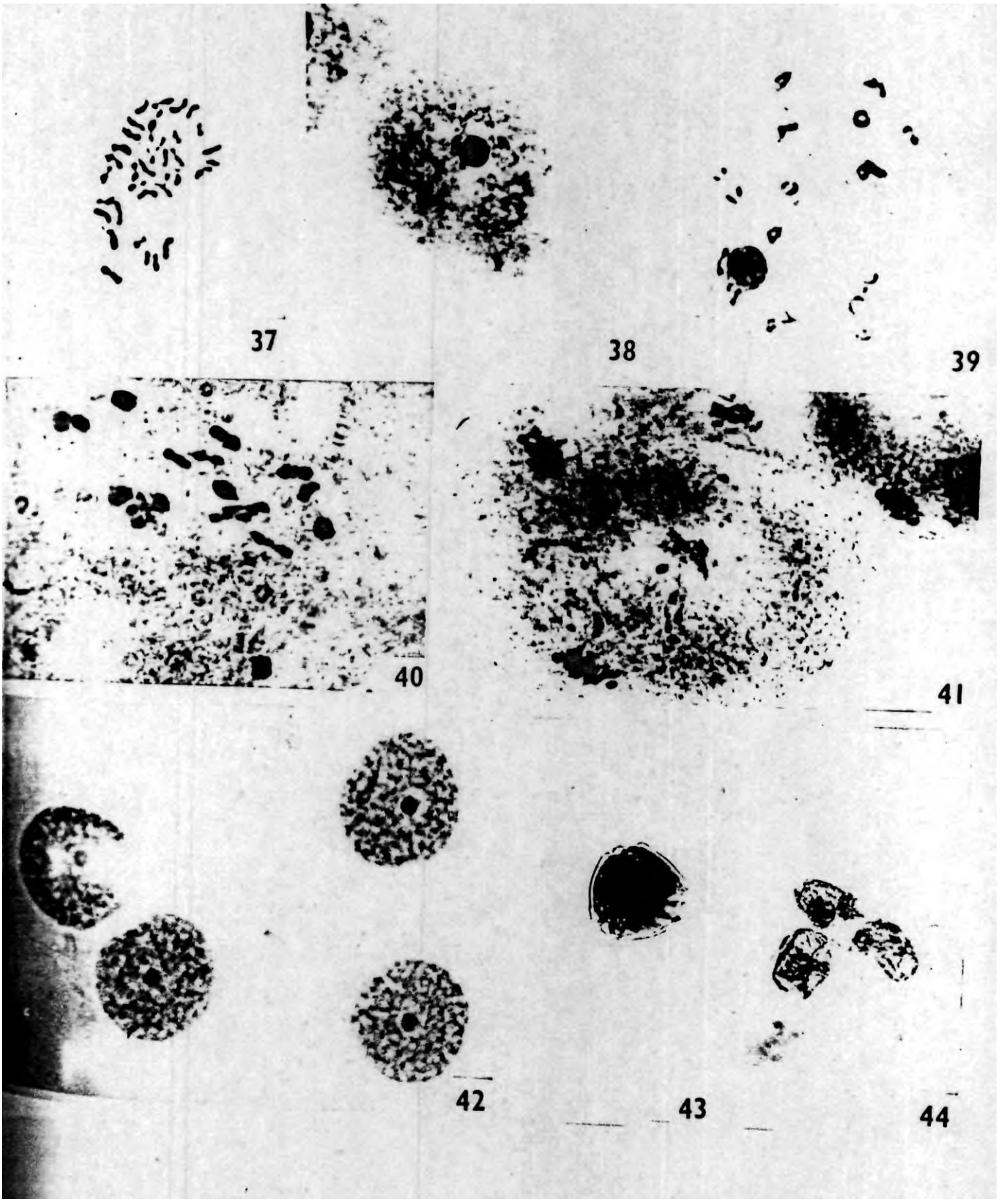

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Fig. 45 : Anther of RRII 35 devoid of pollen grains x 480

Fig. 46 : Anther of RRII 105 showing stainable pollen grains x 480

Fig. 47 : T.S. of anther of male sterile clone showing tapetum x 480

Fig. 48 : A portion of anther showing abnormal tapetum x 1200

Fig. 49 : T.S. of anther of male sterile clone GT 1 x 480

Fig. 50 : T.S. of anther of fertile clone showing complete degeneration of tapetum x 1200

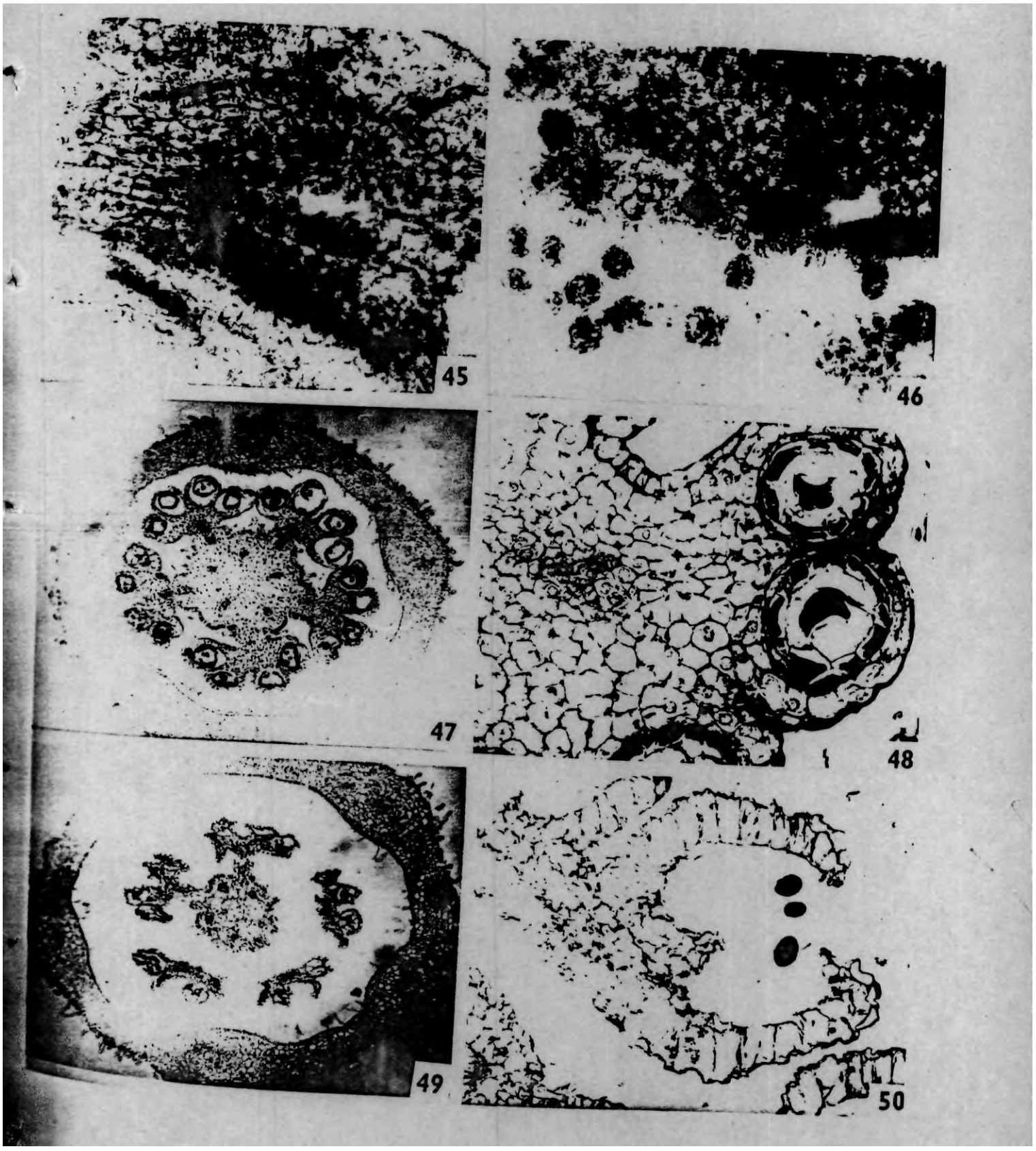
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Fig. 51 : Somatic chromosome complement of triploid showing 2n = 54 x 3000

Fig. 52 : Metaphase I x 3000

Fig. 53 : Anaphase I showing laggards x 3000

Fig. 54 : Somatic chromosome complement of induced triploid showing 2n = 54 x 3000

Fig. 55 : Metaphase I x 4000

Fig. 56 : Anaphase I x 3800

Fig. 57 : Late Anaphase I showing lagging chromosome x 4000

Fig. 58 : Chromosome complement of induced tetraploid 2n = 72 x 3500

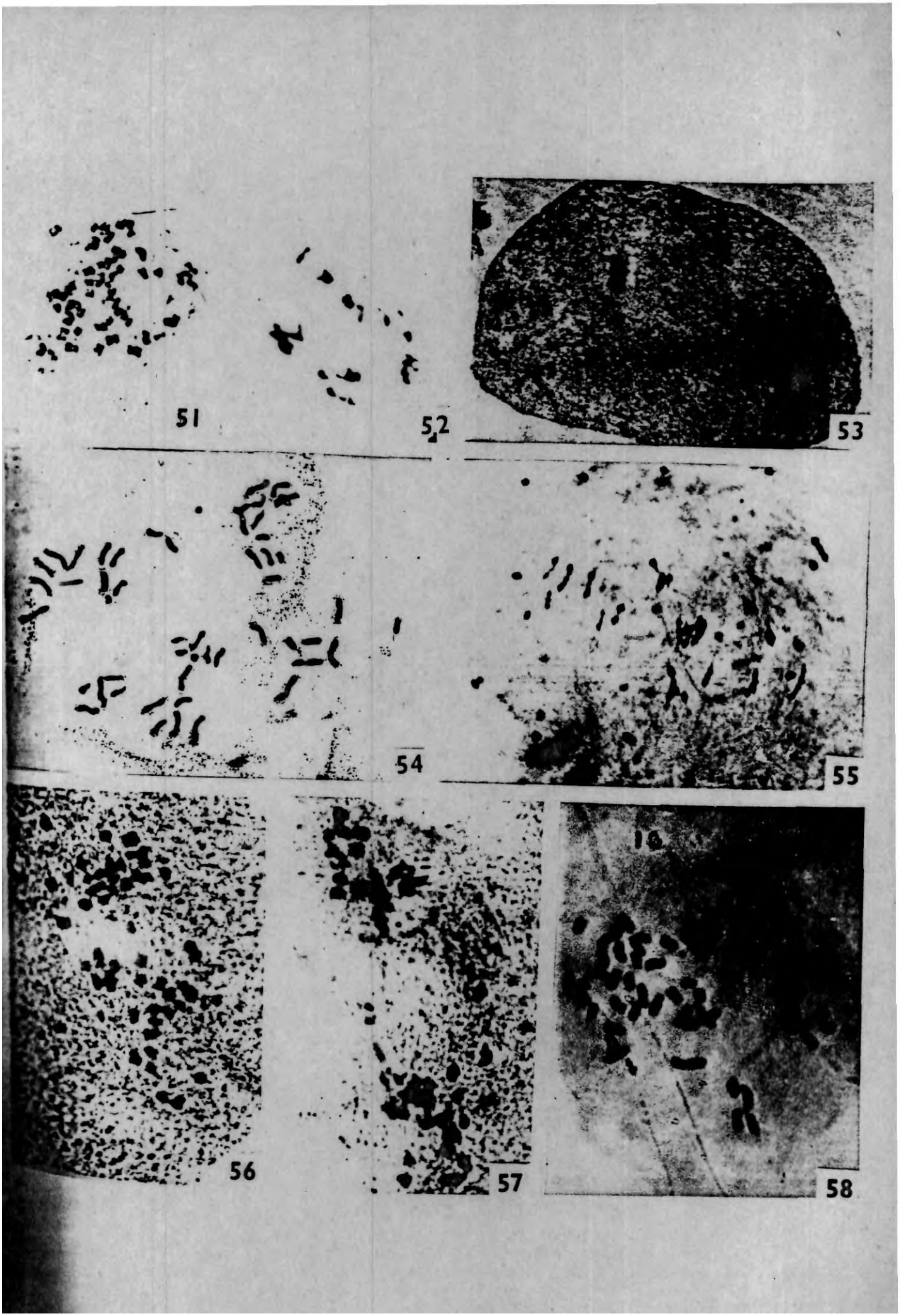
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Fig. 59 : Prophase I of induced tetraploid x 3000

Fig. 60 : Metaphase I x 3000

Fig. 61 : Metaphase I x 3000

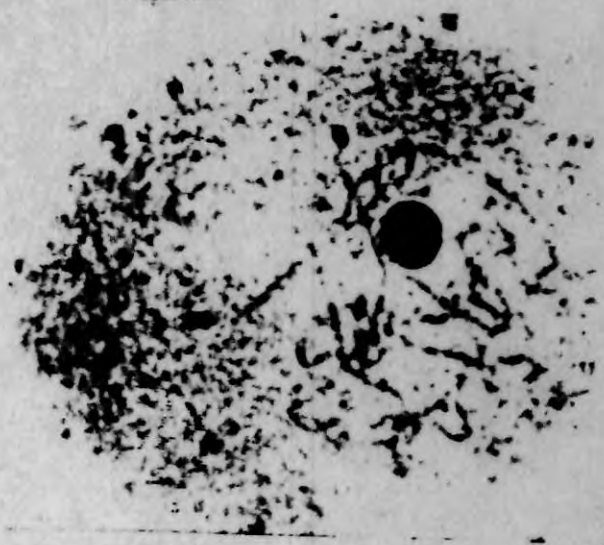
$3_{IV} + 4_{III} + 22_{II} + 4_{I}$

Fig. 62 : Metaphase I of RRII 17 x 3000

Fig. 63 : Anaphase I of RRII 17 showing laggards x 3800

Fig. 64 : Telophase II showing nine groups of nuclei x 3000

Fig. 65 : Tetrad showing six microspores x 1200



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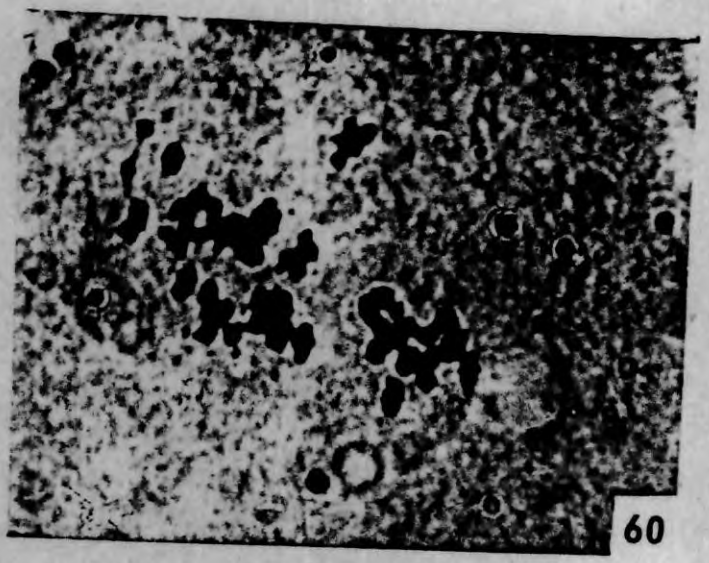

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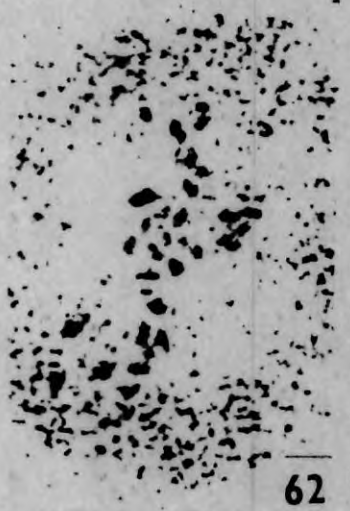

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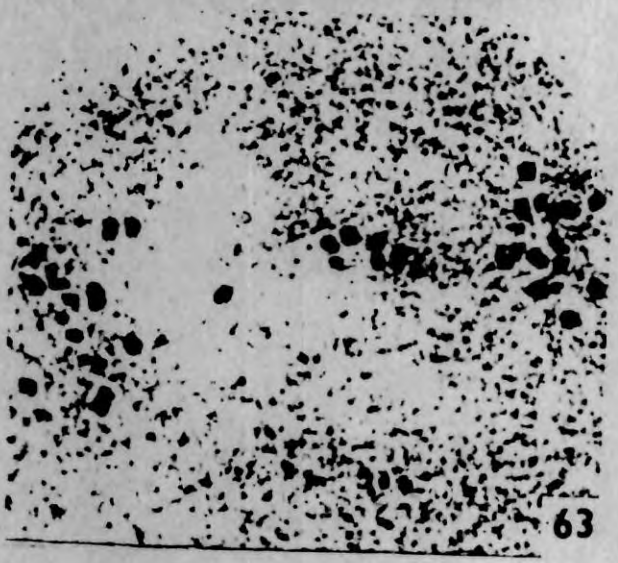

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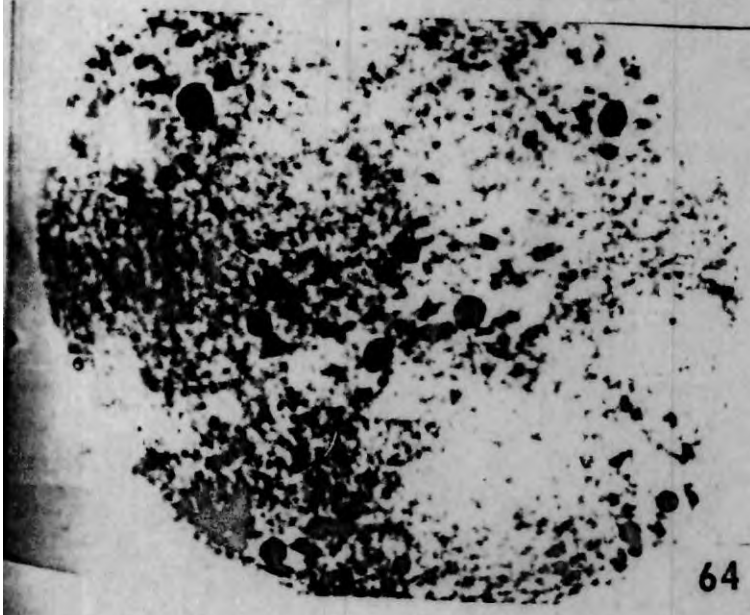

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Fig. 66 : A group of prophase cells from radiation induce mutant showing cytoplasmic connections x 190

Fig. 67 : Two prophase cells showing migration of chromati material x 3000

Fig. 68 : Late metaphase I - cytoplasmic connection showin the initiation of chromosome movement x 3000

Fig. 69 : Showing more than one cytoplasmic connections x 3000

Fig. 70 : Telophase II showing cytoplasmic connections x 30

Fig. 71 : Showing two cytoplasmic connections x 3000

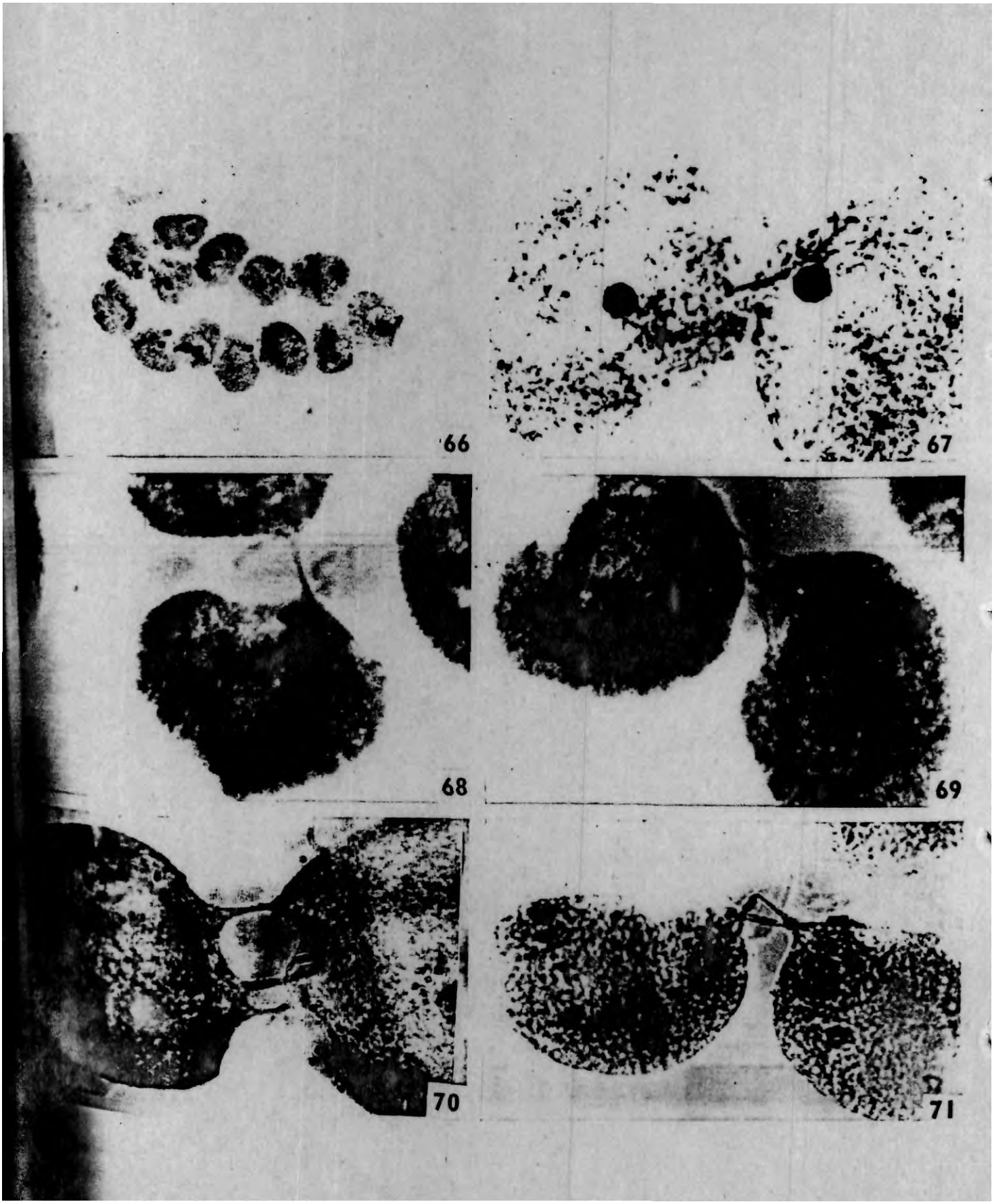
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Fig. 72 : Metaphase I showing reduced number of chromosomes x 3000

Fig. 73 : Microspores connected with cytoplasmic strands x 3000

Fig. 74 : Microspores with divided nuclei x 3000

Fig. 75 : Megapollen x 3000

Fig. 76 : Pollen conglomerate x 480

Fig. 77 : Stainable and sterile pollen grains of RRII 105 x 190

Fig. 78 : <u>In vitro</u> germination of pollen grain x 60

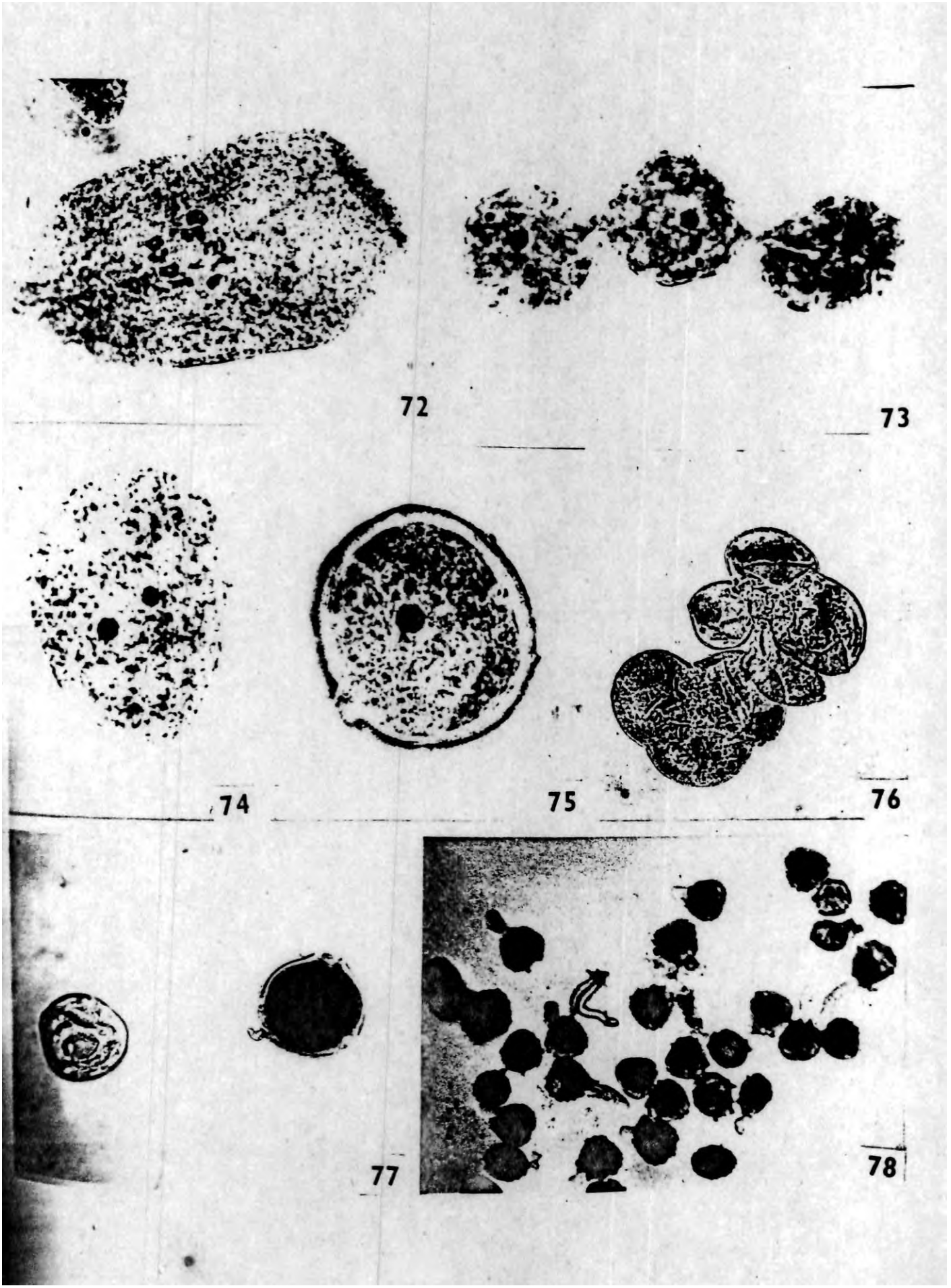

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Fig. 79 : L.S. of style and stigma x 190

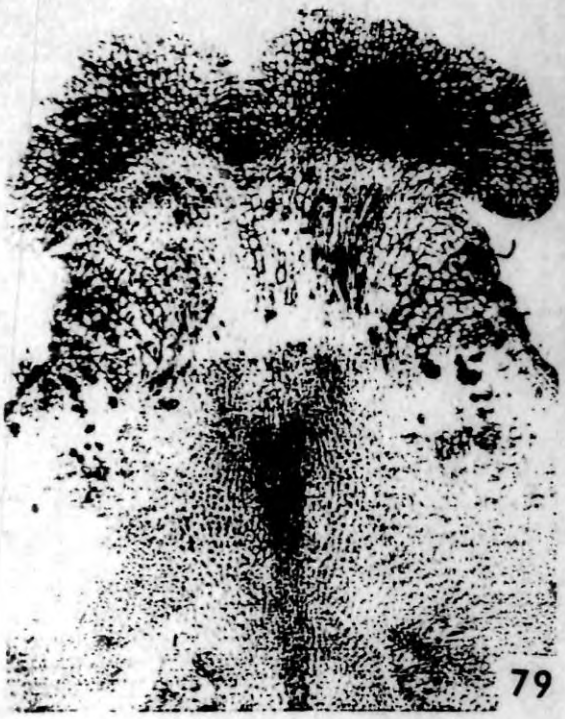
Fig. 80 : Germinating pollen grains on the stigma x 160

Fig. 81 : Pollen grains of tetraploid showing 4 and 3 pores x 190

Fig. 82 : Pollen grain - in polar view x 480

Fig. 83 : Pollen grain - in equatorial view x 480

Fig. 84 : Sterile pollen grain x 750



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SCANNING ELECTRON MICROGRAPHS

Fig. 85 : Pollen grain of RRII 105

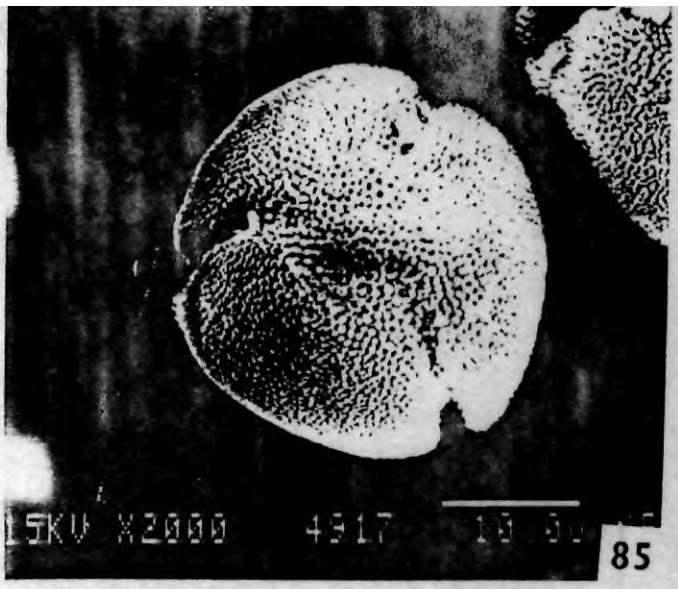
Fig. 86 : A portion of exine

Fig. 87 : Pollen grain of tetraploid having three pores

Fig. 88 : Pollen grain of tetraploid having four pores

Fig. 89 : Parasyncolpate grain

Fig. 90 : A portion of exine of tetraploid



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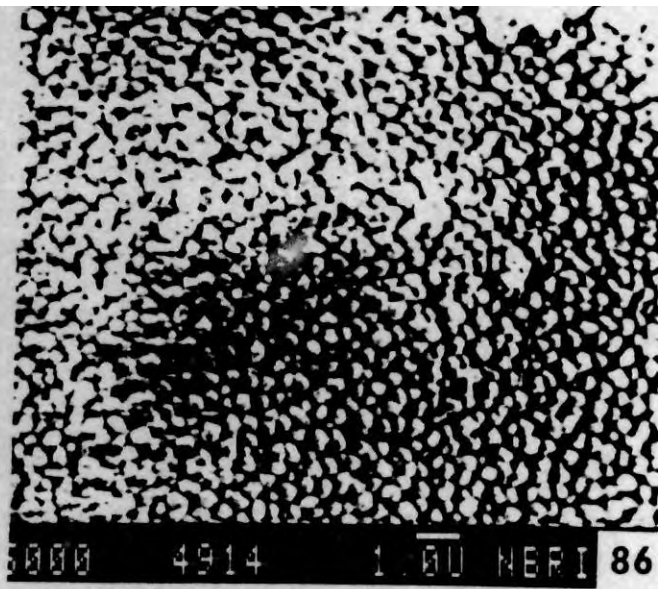

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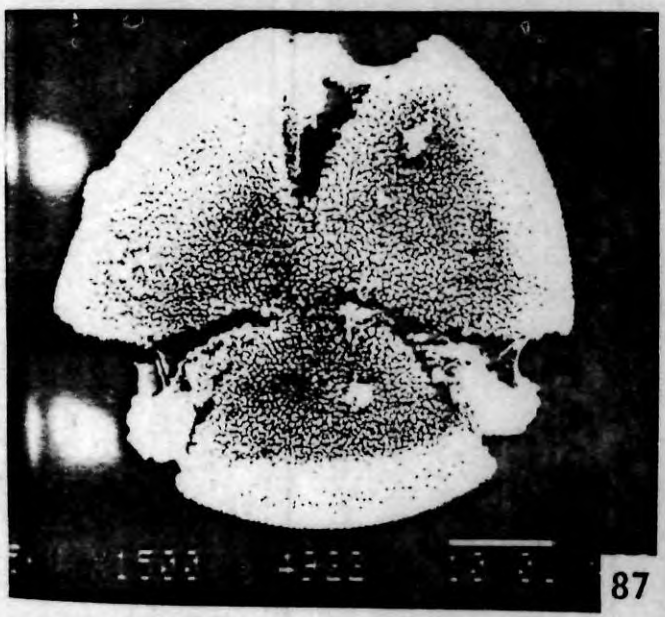

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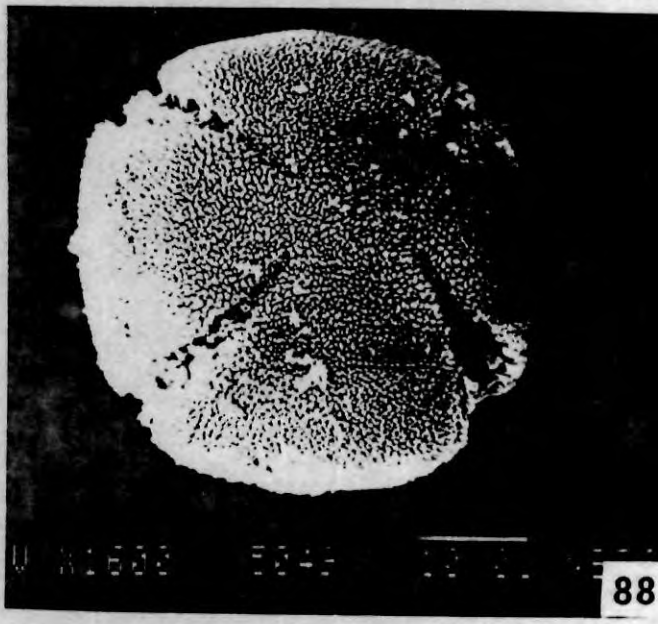

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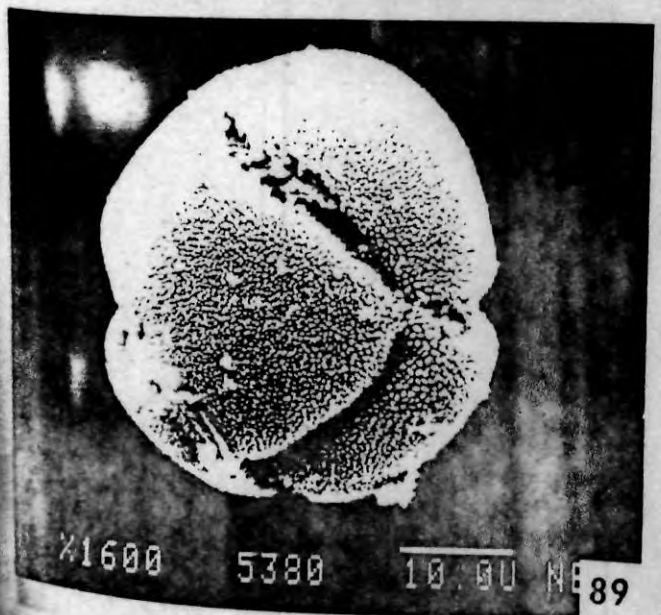

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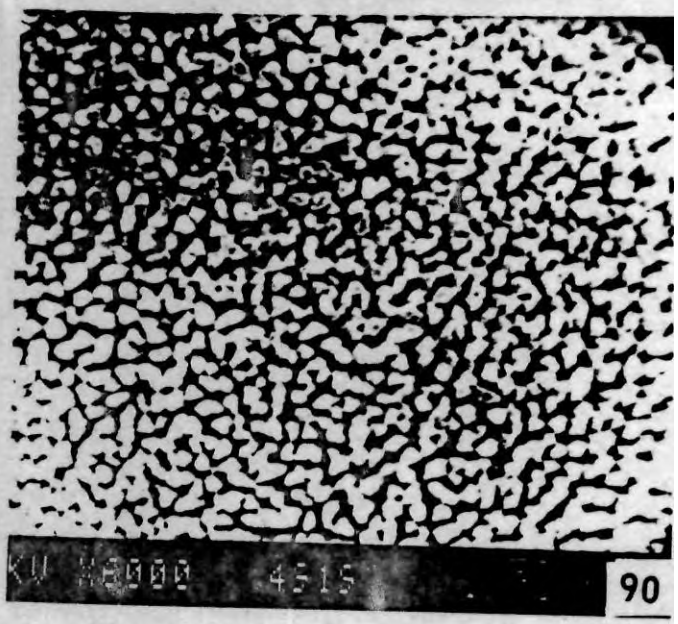

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SEM PHOTOGRAPHS

Fig. 91 : Sterile pollen grain of RRII 17

Fig. 92 : Portion of exine

Fig. 93 : Sterile pollen grain of Ch 2

Fig. 94 : Portion of exine

20KV X6000 0914 1.0U NBRI
92

20KV X6000 0913 1.0U NBRI
91

20KV X6000 0916 1.0U NBRIL
94

20KV X6000 0915 1.0U NBRI
93

Evaluation of seedling progenies of male sterile clones:-

The data on germination of seeds are given in Table 26. Compared to the seeds of the control clone, Mil 3/2, the seeds from the male sterile clones recorded early germination as well as higher percentage of germination. The male sterile clones RRII 35, Ch 2 and GT 1 recorded 84, 87 and 95% seed germination respectively whereas, the control clone showed only 70% seed germinability.

The growth attributes of the progenies of the male sterile clones and the control are shown in Table 27. All the progenies of male sterile clones recorded significantly more height and girth compared to those of the control at the age of one year. During the second year's growth also the male sterile clones recorded the same trend. With regard to height at 30 months growth there was no significant differences between the treatments (Table 28). For all other parameters significant differences were noted among treatments. In the case of yield on test tapping also the progenies of male sterile clones showed better performance than the control. Mean juvenile yield elucidated that GT 1 showed higher value for all the seasons. During January-February season GT 1 showed 0.3940, June-July season 0.5711 and October-November 1.3820 g dry rubber yield per tree per tap. In bark thickness also this clone

SECTION 3. GENETICS

Studies on F_1 progenies of GT 1:-

All the F_1 plants of cross combinations having GT 1 as the female and also the control, which were ringbarked, showed flower initiation after nine months of imposing the treatment. The male flowers of the F_1 clones having GT 1 as the female parent did not reach normal size as seen in F_1 of male fertile clone. They were reduced in size and fallen by abscission as seen in the case of GT 1. The perianth was light yellow in colour and flowers did not open. The anther column was completely dry and fertile pollen grains were totally absent. The mode of flower development was similar to that of GT 1. Flower development and pollen production were normal in the control clones. As in the case of GT 1 parent, the male flowers of the hybrids showed normal meiosis up to the tetrad stage. After the tetrad stage the microspores showed abortion and further development was completely blocked. The light yellow flowers did not even have sterile pollen.

showed higher value compared to other clones. During the first season the mean yield of the male sterile clones and the control had shown that GT 1 was followed by RRII 35 and Ch 2. But during the second season GT 1 was followed by Ch 2 and RRII 35 and during the third season also the same trend was seen in the case of yield. In the case of girth there was significant difference among the clones. GT 1 showed the highest girth followed by RRII 35 and Ch 2. The control clone Mil 3/2 showed comparatively less value for all these attributes including the juvenile yield for all the seasons.

Genetic parameters of growth attributes and juvenile yield are given in Table 29. The difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was wide for yield compared to other characters. Heritability and genetic advance had shown that during juvenile stage height and girth showed high value. But after 24 months growth, height showed low value for heritability. In the case of test tapping yield during the young stage heritability was less. But when the seedlings became older the value for heritability for yield was higher. Heritability was found to be high for yield during 3 years growth.

The general combining ability of four clones are given in Table 30. GCA for male sterile clones was more compared to that for the control. Among the male sterile clones GT 1 showed higher value for GCA, compared to Ch 2 and RRII 35. GCA for test tapping yield recorded higher value for GT 1 followed by RRII 35. For bark thickness also GT 1 itself showed higher value.

Regression analysis for juvenile yield (Y) on height (X_1) and girth (X_2) for three seasons are depicted in Tables 31 to 42. Simple and multiple regression are fitted to relate juvenile yield as independent variable, individually with height, girth during first and second seasons and bark thickness during the third season.

Table 31 shows the regression analysis for juvenile yield on height and girth of GT 1 during January-February season. The regression analysis revealed that 35% of total variation on yield was accounted for girth. During June-July season the regression analysis for yield on girth is given in Table 32. Highly significant correlation was recorded for yield and girth. Regression analysis for yield (Y) on bark thickness (X_3) and girth (X_2) is depicted in Table 33. About 48% of total variation on yield was due to girth and bark thickness.

In the case of the clone Ch 2 yield was related to girth only at 5% level (Table 34). During June-July season (Table 35) significance at 1% level was noted for yield and girth. 58% of total variation was due to girth. In the third quarter also significance at 1% level was noted for girth and yield (Table 36).

During the first and second quarter the clone RRII 35 showed significance at 1% level for girth and yield (Tables 37 and 38). 36% of total variation of yield was due to girth during January-February season. But during October-November there was correlation between girth and yield at 1% level.

The control clone Mil 3/2 showed no correlation for yield on height and girth. But during June-July season significance at 5% level for yield on girth and during October-November season there was significance at 1% level for yield on girth and 37% of total variation for yield was due to girth.

The pooled data on yield for three seasons were also subjected to analysis. The analysis of variance for the mean yield of three male sterile clones and control are given in Table 43. Significant differences among the clones were noted. The control clone showed an average yield of 0.27 per plant per tap. GT 1 showed the highest juvenile yield (0.79 g) followed by Ch 2

(0.69 g), RRII 35 (0.56 g). All the male sterile clones were significantly superior to that of the control.

From among the seedling progenies of male sterile clones 41 seedlings were selected based on juvenile yield and secondary attributes for further field evaluation. Yield after stimulation along with mean yield for the three seasons and girth increment (girth at the time of initiation of test tapping and girth after tapping for three consecutive seasons) on tapping are depicted in Table 44. Mean yield over three quarters was given as per plant per tap. Mean yield after stimulation is also given. The seedlings showing highest mean juvenile yield were from the progenies of the male sterile clone GT 1. Among the seedlings selected, 46.3% was from GT 1, 36.6% from Ch 2 and 17.1% from RRII 35. Among the male sterile clones GT 1 showed superiority over the others.

Table 26. Germination of seeds of male sterile clones and the control.

Days after sowing	GT 1	Ch 2	RRII 35	Mil 3/2
7th day	321	255	120	5
10th day	400	84	220	80
13th day	100	24	200	200
16th day	45	60	150	100
19th day	50	9	40	120
Total seeds germinated	916	432	730	508
Total seeds kept for germination	961	496	870	725
Percentage	95.32	87.10	83.91	70.07

Table 27. Growth attributes of the progenies of male sterile clones and the control.

Sl.No.	Clones	Mean height (cm)	1st Year Mean diameter (mm)	2nd Year Mean height (cm)	Mean girth (cm)
1.	GT 1	190.10	20.96	318.14	9.03
2.	Ch 2	180.42	20.34	286.51	8.10
3.	RRII 35	169.44	17.97	316.45	8.72
4.	Mil 3/2	149.11	14.11	206.86	5.53
	SE	5.80	0.29	7.87	0.31
	CD	17.87	0.89	24.24	0.95

Table 28. Yield and secondary characters of progenies of male sterile clones and the control.

Clones	January-February			June-July		October-November		
	Mean height (cm)	Mean girth (cm)	Mean yield g/tree/tap	Mean girth (cm)	Mean yield g/tree/tap	Mean girth (cm)	Mean bark thickness (mm)	Mean yield g/tree/tap
GT 1	489.60	13.93	0.3940	18.05	0.5711	21.38	4.08	1.3820
Ch 2	430.10	12.97	0.3485	17.48	0.5195	20.78	3.50	1.1893
RRII 35	495.23	13.57	0.3554	19.07	0.4496	22.87	3.62	0.9054
Mil 3/2	449.27	10.93	0.1901	15.83	0.1469	18.70	3.27	0.4397
SE	0.44	0.44	0.04	0.61	0.05	0.80	0.17	0.09
CD		1.34	0.12	1.84	0.15	2.40	0.51	0.28

Table 29. Genetic parameters of growth attributes and juvenile yield of progenies of male sterile clones.

Age	Characters	General Mean	PCV	GCV	H^2	GA
1st year	Height (cm)	172.27	12.23	9.64	0.62	26.97
	Girth (cm)	57.62	17.22	16.84	0.95	6.23
2nd year	Height (cm)	281.99	19.16	18.29	0.90	100.99
		7.85	21.78	19.87	0.84	2.94
After 2½ years	Height (cm)	458.55	9.05	4.45	0.24	20.68
	Girth (cm)	12.85	12.99	9.81	0.57	1.97
	Yield (g/t/tap)	0.32	37.50	25.63	0.43	0.11
After 3 years	Girth (cm)	17.61	6.87	10.91	0.39	1.56
	Yield (g/t/tap)	0.43	148.84	120.93	0.66	0.28
After 3½ years	Girth (cm)	20.93	11.85	7.31	0.38	1.95
	Bark thickness (mm)	3.60	14.17	8.33	0.34	0.36
	Yield (g/t/tap)	0.98	46.94	40.82	0.75	0.71

...general combining ability.

Characters	Age (year)	GT 1	Ch 2	RRII 35	Mil 3/2
Height	1	17.83	8.15	-2.83	-23.16
Diameter	1	2.62	1.99	-0.38	-4.23
Height	2	36.14	4.52	34.46	-75.13
Girth	2	1.19	0.25	0.88	-2.31
Height	2	31.05	28.45	6.68	-9.28
Girth	2½	0.07	0.02	0.03	-0.13
Yield	2½	0.14	0.08	0.01	-0.24
Girth	3	0.44	-0.12	1.46	-0.23
Yield	3	0.14	0.08	0.01	-0.24
Girth	3½	0.44	-0.15	1.93	2.23
Bark thickness	3½	0.46	-0.11	0	-0.35
Yield	3½	0.87	-0.21	0.40	-0.53

Table 31. Regression analysis for yield (Y) on height (x_1) and girth (x_2) of GT 1 (January-February)

Anova Table

Source	Df	SS	MS	F
Reg	2	0.9367	0.4683	7.4231**
Error	27	1.7034	0.0631	

$R^2 = 0.3548$

Variable	Reg. coe.	SE	T-value
x_1	-0.0005	0.0008	0.6900^{NS}
x_2	0.0712	0.0204	3.4831**
Intercept	0.3392		

N.S. Not significant

** Significant at 1% level.

Table 32. Regression analysis for yield (Y) of girth (x_2) of GT 1 (June-July)

Anova Table

Source	DF	SS	MS	F
Reg	1	1.0260	1.0260	15.6080**
Error	28	1.8406	0.0573	

R^2 = 0.3975

Variable	Reg coe	SE	T-value
x_2	0.0600	0.0152	3.9507**
Intercept	0.5131		

** Significant at 1% level.

Table 33. Regression analysis for yield (Y) on bark thickness (x_3) and girth (x_2) of GT 1 (October-November)

Anova Table

Source	DF	SS	MS	F
Reg	2	3.0179	1.5092	12.4110**
Error	27	3.2827	0.1216	

R^2 = 0.4790

Variable	Reg coe	SE	T-value
x_3	0.3687	0.1159	3.1822**
x_2	0.6070	0.1520	3.9943**
Intercept	0.5270		

** Significant at 1% level.

Table 34. Regression analysis for yield (Y) and height (x_1) and girth (x_2) of Ch 2 (January-February)

Anova Table

Source	DF	SS	MS	F
Reg	2	0.1537	0.0769	5.2081*
Error	27	0.3984	0.0148	

$R^2 = 0.2784$

Variable	Reg coe	SE	T-value
x_1	0.0002	0.0004	$0.4358^{N.S}$
x_2	0.0332	0.0128	2.6113*
Intercept	0.1506		

N.S. Not significant

* Significant at 5% level.

Table 35. Regression analysis for yield (Y) on girth (x_2) of Ch 2 (June-July).

Anova Table

Source	DF	SS	MS	F
Reg	1	2.0905	2.0905	39.1611**
Error	28	1.4947	5.3383	

$R^2 = 0.5831$

Variable	Reg coe	SE	T-value
x_2	0.1019	0.0169	6.2579**
Intercept	1.2627		

** Significant at 1% level.

Table 36. Regression analysis for yield (Y) on bark thicknes (x_3) and girth (x_2) of Ch 2 (October-November).

Anova Table

Source	DF	SS	MS	F
Reg	2	9.6960	4.8480	16.3076**
Error	26	7.7294	0.2973	

R^2 = 0.5564

Variable	Reg coe	SE	T-value
x_3	0.1209	0.1537	$0.7862^{N.S}$
x_2	0.1546	0.0372	4.1509**
Intercept	2.4701		

N.S. Not significant.

** Significant at 1% level.

Table 37. Regression analysis for yield (Y) on height (x_1) and girth (x_2) of RRII 35 (January-February)

Anova Table

Source	DF	SS	MS	F
Reg	2	0.2627	0.1314	7.5807**
Error	27	0.4678	0.0173	

R^2 = 0.3596

Variable	Reg coe	SE	T-value
x_1	0.0020	0.0004	$0.3409^{N.S}$
x_2	0.0342	0.0120	2.8607**
Intercept	0.1664		

N.S. Not significant

** Significant at 1% level.

Table 38. Regression analysis for yield (Y) on girth (x_2) of RRII 35 (June-July).

Anova Table

Source	DF	SS	MS	F
Reg	1	0.7165	0.7165	11.5048**
Error	28	1.7438	0.0623	

R^2 = 0.291

Variable	Reg coe	SE	T-value
x_2	0.0591	0.0174	3.3919**
Intercept	0.6766		

** Significant at 1% level.

Table 39. Regression analysis for yield (Y) on bark thickness (x_3) and girth (x_2) of RRII 35 (October-November)

Anova Table

Source	DF	SS	MS	F
Reg	2	1.5252	0.7626	15.0718**
Error	27	1.3661	0.5059	

$R^2 = 0.5275$

Variable	Reg coe	SE	T-value
x_3	0.4230	0.0495	0.8536
x_2	0.4689	0.9185	5.1047**
Intercept	0.8746		

** Significant at 1% level.

Table 40. Regression analysis for yield (Y) on height (x_1) and girth (x_2) of Mil 3/2 (January-February)

Anova Table

Source	DF	SS	MS	F
Reg	2	0.0091	0.0046	$1.1383^{N.S}$
Error	27	0.1083	0.0040	

$R^2 = 0.0778$

Variable	Reg coe	SE	T-value
x_1	0.0001	0.0002	$0.2843^{N.S}$
x_2	0.0139	0.0102	1.3571
Intercept	0.0119		

N.S. Not significant.

Table 41. Regression analysis for yield (Y) on girth (x_2) of Mil 3/2 (June-July)

Anova Table

Source	DF	SS	MS	F
Reg	1	0.0141	0.0141	4.9232*
Error	28	0.0805	0.0028	

$R^2 = 0.1495$

Variable	Reg. coe	SE	T-value
x_2	0.0158	0.0067	2.2188*
Intercept	0.0670		

* Significant at 5% level.

Table 42. Regression analysis for yield (Y) on bark thickness (x_3) and girth (x_2) of Mil 3/2 (October-November)

Anova Table

Source	DF	SS	MS	F
Reg	2	0.4688	0.2344	8.0782**
Error	27	0.7834	0.0290	

$R^2 = 0.3477$

Variable	Reg coe	SE	T-value
x_3	-0.1203	0.6863	1.7528
x_2	0.0682	0.0175	3.9086**
Intercept	0.4730		

** Significant at 1% level.

Table 43. Analysis of variance for yield.

Source	SS	DF	MSS	VR	F (5%)
Replicates	0.2097	5	0.0419	3.57*	2.90
Treatments	0.8991	3	0.2997	25.54**	3.29
Error	0.1760	15	0.0117		
Total	1.2849	23			

SE/PLOT = 0.11 CV = 18.81%

Mean Table

* Significant at 5% level.

** Significant at 1% level.

Treatment	Mean
	SE: 0.04
	CD: 0.12
T1	0.79
T2	0.69
T3	0.56
T4	0.27
GM	0.58

Table 44. Yield (juvenile) and secondary characters of selected seedling progenies from male sterile clones.

Sl. No.	Code No.	Mean yield over three seasons g/t/tap	Girth increment on tapping	Yield after stimulation g/t/tap
1.	a	1.0277	16.5	2.8982
2.	b	1.0322	13.0	1.3444
3.	c	0.9739	10.5	1.9770
4.	d	1.2278	13.0	1.7216
5.	e	2.3298	12.5	3.7930
6.	f	0.8334	9.5	1.4434
7.	g	0.8155	7.5	1.7730
8.	h	1.0514	11.0	2.8500
9.	i	0.9159	14.0	1.6408
10.	j	1.1767	15.0	2.7614
11.	k	0.8520	10.0	1.9806
12.	l	1.0360	10.0	0.9764
13.	m	0.9841	18.0	1.7198
14.	n	1.1459	11.0	1.6026
15.	o	1.0609	9.0	1.0778
16.	p	1.3973	9.0	2.9790
17.	q	1.1289	10.0	1.3876
18.	r	1.0794	11.0	3.4110
19.	s	0.9039	11.0	2.1636
20.	t	0.7464	9.0	1.0298
21.	u	1.2532	7.5	1.3908
22.	v	0.6234	13.0	1.3576
23.	w	1.0265	15.0	2.0998
24.	x	0.6781	13.0	1.6190
25.	y	2.3098	10.5	2.5436

(Contd.....)

Sl. No.	Code No.	Mean yield over three season g/t/tap	Girth increment on on tapping	Yield after stimulation g/t/tap
26.	z	0.8107	12.0	1.5092
27.	ai	0.7429	13.5	1.7684
28.	bi	0.7094	13.0	1.3410
29.	ci	0.7968	10.0	1.4504
30.	di	0.7960	10.5	1.6462
31.	ei	0.6241	14.0	1.8866
32.	fi	0.7585	11.5	1.0234
33.	gi	0.5723	12.5	0.8798
34.	hi	0.7645	10.0	2.2540
35.	ii	0.7255	11.0	2.2912
36.	ji	0.6234	13.0	1.3576
37.	ki	0.5559	12.5	1.0954
38.	li	0.6810	9.0	1.3284
39.	mi	0.7100	7.0	0.7104
40.	ni	0.7464	7.0	0.9764
41.	oi	0.6865	11.0	1.0052

1 - 19 : GT 1

20 - 34 : Ch 2

35 - 41 : RRII 35

DISCUSSION

The Para rubber tree, Hevea brasiliensis, having an economic life span of 30 to 35 years is the most important commercial source of natural rubber in the world. Only limited studies have been made on the morphology, cytology and genetics of Hevea. Investigations on male sterility in H. brasiliensis and the other nine species of the genus are meagre. Detailed knowledge on these aspects are important for increasing genetic variability by artificial methods and also to programme meaningful breeding strategy in this crop. An attempt has been made to have detailed investigations on the above aspects. Induction of male sterility and investigations on the induced male sterile clones have not so far been reported in Hevea.

Morphology

Distinct morphological variations in foliar, floral and growth attributes were observed among the male sterile clones investigated. When the leaf area was taken into consideration, the tetra-

ploid showed very large (107 cm^2) leaves and the radiation induced mutant showed very small leaves (26 cm^2). Leaf area was reported to be more for autotetraploid of Coleus (Bahl and Tyagi, 1988). The very large leaves of tetraploid is likely to be due to the effects of polyploidization whereas, the very small leaves of radiation induced mutant might be due to the effect of gamma irradiation. In the induced triploid and radiation induced mutant, the leaves are arranged very closely and their internodal length is considerably reduced resulting in semi-dwarf stature. The compact nature of the crown is of practical importance, if this character can be incorporated with moderate yield in Hevea. Boertjes and Dejong (1984) had also reported reduced plant stature for plants resultant of irradiation in Chrysanthemum.

Variations in the number of leaflets (2 to 5) were observed in the induced triploid and tetraploid. It is interesting to note that the expression of this character is more predominant during initial stages of growth of budded plants. But as it grows the variation in the number of leaflets is obscure. This variation was observed even upto VM_{20} generation with regard to tetraploid and VM_{15} generation to induced triploid. However, the spontaneous triploid do not exhibit any variation in the number of leaflets. But the leaves and floral parts are larger in spontaneous triploid.

Cytogenetic stability is an important aspect for the induction of polyploidy for further exploitation and crop improvement. It primarily depends on the propagation method of the species concerned. In Hevea, vegetative propagation through budgrafting is easy and difficulties have not been experienced in budtake on normal stocks. Even after twenty generations of vegetative multiplication, the tetraploid maintained its character. The triploids also maintained this character.

Dark green colour and prominent veins are reported for the polyploids in Hevea (Shepherd, 1969; Markose, 1975; Zheng Xuequin et al., 1980; Saraswathy Amma et al., 1984a, 1988a). The dark green colour of the polyploid leaves may be due to greater thickness and similar results had been reported in Ipomoea species (Vijayabai et al., 1976). Capsicum (Indira and Susan Abraham, 1977) and Matricaria (Arora and Madhusoodanan, 1981).

The clones Ch 2, RRII 15, RRII 35 and tetraploid showed significant variation in vigour. Mendes (1969) had reported more vigour in a polyploid Hevea clone of IAN-873 and Goncalves et al. (1983) also reported increase in vigour in IAN-717. Ong et al. (1984) also reported more vigour for the putative polyploids in Hevea clones. Aung and Walton (1987) had also reported more vigour for polyploids in Elymus.

The correlation between the different growth attributes showed wide variation with respect to clone and growth. Except RRII 35 and radiation induced mutant height is correlated to girth and vigour in turn is correlated to other attributes. From the physiological point of view Singh *et al.* (1985) reported that the sink demand in male sterile line is expected to be lower than in fertile counterparts. In addition, the energy required for the normal development of male gametophyte is also diverted to vegetative growth in male sterile lines. Vigorous male sterile mutant was also reported in *Capsicum* (Meshram and Narkhede, 1982). A general reference to the excessive vegetative growth for male sterile plants is made by Dawson (1962).

Triploids are reported to be vigorous in sugercane and *Ipomoea* (Frey, 1967), *Luffa* (Agarwal *et al.*, 1979). In the present investigation, the spontaneous triploid of *H. brasiliensis* (RRII 15) is vigorous. But the induced triploid is not found to be vigorous. Moreover, the vegetative growth is very poor compared to the control. The reason attributed to this may be due to the effect of back crossing. Induced triploid is evolved by crossing diploid Gl 1 and induced tetraploid of RRII 105. RRII 105 is a hybrid clone evolved by crossing Tjir 1 and Gl 1, of which Tjir 1 is an Indonesian clone and Gl 1 is a Malaysian clone. Both these clones are ortet selections.

Morphologically distinct variations in flower development are noted in GT 1, RRII 17, RRII 35, mutagen induced mutant and spontaneous triploid. But sterility in these clones can be ascertained only after flowering and microscopical observation of flowers. Hadley and Starnes (1964) had also reported similar observations in soybean. He opined that early drooping of flower was the first noticeable characteristics of sterile plant.

Flower size also showed significant differences among the clones studied. Compared to the control, RRII 105 triploids, tetraploid and Ch 2 show bigger flowers and GT 1 and radiation induced mutant show comparatively smaller flowers. Bigger floral size for triploids and tetraploids are reported (Dwivedi et al. 1988). Flowers of polyploids were reported to be bigger in Hevea (Ong, 1981; Tan, 1987; Saraswathy Amma et al., 1984a; Younglin et al., 1984). Seeds of GT 1, RRII 35, Ch 2 and the control Mil 3/2 also showed variation in shape, mottlings and ridges. All these clones have seeds of medium size.

The morphology, the external expression is only a measure of the genetical potential of the plant body as a whole. The use of morphological knowledge in applied botany has been demonstrated amply by the great strides made in agriculture and forestry (Nair, 1979). Hence, morphological studies are of paramount importance.

CYTOLOGY

Small size and stickiness of chromosomes are limiting factors in obtaining good cytological preparations in Hevea brasiliensis. Moreover, compared to any other annual crop, this perennial plant is not easily amenable to cytological techniques which is amply illustrated by the paucity of literature on this aspect.

A perusal of the available literature on cytology of H. brasiliensis shows that the haploid number is 18. However, whether this number is the basic number of the species is a matter of controversy and there are two schools of thoughts. Hevea species are diploid with a basic chromosome number n = 18 (Ramaer, 1935; Majumder, 1964; Ong, 1981; Saraswathy Amma et al., 1984a; Abbott and Atkin, 1987; Webster and Baulkwill, 1989). But Ong (1976) suggested segmental allopolyploid origin of the genus. The species has also been reported to be a probable amphidiploid with a basic chromosome number of nine (Low and Bonner, 1986). Most of the workers have confirmed that the chromosome count is 2n = 36. According to Ramaer (1935) 18 seems to be a high basic number. But a few plants in the family Euphorbiaceae like 19 in Smilax, 17 in Pyrus (Ramaer, 1935) and 17 in Euphorbia (Srivastava et al., 1987) have similar high basic number. Moreover,

in Hevea, a plant with 9 or 12 chromosomes has not yet been found. There were nowhere indication that 18 could be a secondary basic number. Eventhough, the cytology of Hevea is a conjecture, in the present investigation H. brasiliensis is regarded as diploid having a chromosome complement of 2n = 2x = 36. Results from the present investigations on the cytology have also confirmed this conclusion.

Spontaneous male sterile clones:-

In the spontaneous male sterile clones, GT 1, Ch 2 and RRII 35 meiosis was normal up to the formation of tetrad and after that there was complete degeneration resulting in male sterility. Literature pertaining to this aspect is quite meagre in perennial tree crops (Bensimon, 1985). But this type of male sterility was reported in annual crops like Allium (Monosmith, 1928), Brassica (Jones and Emsweller, 1937), Capsicum (Peterson, 1958), Hordium (Ahokas, 1978), Medicago (Davis and Greenblatt, 1967), Pelargonium (Tokumasu, 1974), Zea (Lee et al., 1979).

Proliferation of tapetum was noted in GT 1 and Ch 2. In these sterile clones, the tapetum was persistant and showed an average width of 20 to 30 μm, whereas, in the fertile clone, tapetum showed only a maximum width of 8 μm and totally absent

at the dehiscing stage. The observation is in conformity with the report of Leconte and Nicolas (1985) in GT 1. Similar types of abnormalities of tapetum were noted in Brassica (Chowdhury and Das, 1966), Allium (Saini and Davis, 1969) and Solanum (Sawhney and Bhadula, 1988). But endothecium was devoid of fibrous thicknening and there was early degeneration of tapetal cells in Nicotiana (Jagannadham, 1988).

According to Abdalla and Hermsman (1972) there are two types of male sterility in Solanum, one characterised by the formation of shrunken irregularly shaped pollen grains and the other (bubble sterility) wherein pollen grains degenerate. In the present investigations also both these types of male sterility were noted. The genetically conditioned male sterility in higher plants can also be due to genes which influence the functioning ability of the sex organs without influencing microsporogenesis (Van der Meer and Van Bennekom, 1970). In the present investigation microsporogenesis is normal except for the abnormal behaviour of tapetum and degeneration of tetrads.

The spontaneous male sterile clone, RRII 17 showed variable number of bivalents and univalents at metaphase I elucidating that the male sterility apparently is a case of desynapsis. The phenomenon in which the homologous chromosomes pair normally

at pachytene and fail to remain associated as bivalents at diakinesis and metaphase I due to lack of chaisma formation was termed as desynapsis (Desi et al., 1973). The congression of chromosomes was imperfect and the chromosomes rarely oriented themselves on metaphase plate. In some cells the univalents were distributed randomly throughout the pollen mother cell. The distribution of univalents at metaphase I may be either polar or more or less equatorial (Riley and Law, 1965). The unequal distribution of chromosomes at anaphase had resulted in the production of gametes with varying degrees of chromosomal imbalance consequently gametes with complete genome are not produced. At the end of meiosis the pollen mother cells produced varying number of microspores, which also were different in size and shape.

The present study is a first report of desynapsis in Hevea (Saraswathy Amma et al., 1990). Asynapsis was already reported in this crop (Ramaer, 1935). Prakken (1943) first proposed a classification of the synaptic mutant into weak, medium and strong types on the basis of frequencies of bivalents and univalents in meiocyte. In a weak type, only a few univalents are formed in the majority of meiocytes. Further, they are characterised by high chaisma frequency, low frequency of irregularities at M.I and A.I and relatively high fertility. In medium strong type, many univalents are formed in most of the cells. Meiotic irre-

gularities will be frequent, leading to relatively high sterility. In complete or strong mutants all chromosomes in most of the meiocytes show univalents very early in meiosis with high frequency of irregularities and almost total sterility. The observations in Hevea indicate strong desynapsis and total sterility. Analysis of early prophase cell is very difficult in this crop, hence pachytene analysis has not been attempted. A large number of meiotic abnormalities causing sterility had been described and discussed by Darlington (1932). Formation of polyspory in the male sterile clone might have resulted from irregular division during the first anaphase and forming more than two groups. This type of abnormalities were reported in other desynaptic plants (Ahloowalia, 1969; Misra and Shastry, 1969; Koduru and Rao, 1981; Singh and Gupta, 1981; Karihaloo and Koul, 1983).

In RRII 17, as in the case of PMCs, meiosis apparently did not appear to be normal in the megaspore mother cell and disintegrating embryosac was noted in the mature female flower. Absence of fruit set both under natural condition and artificial hybridisation indicate the possibility of desynapsis in the meagaspore. A similar situation was reported in Allium (Koul, 1975). However, detailed investigations of the megasporogenesis have not been incorporated in the present work. Since the desynaptic plant is showing tolerance to abnormal leaf fall disease (Markose,

1984) this can be utilised for crown modification in Hevea. Moreover, desynaptic plant may provide a valuable tool for experimental approach to the problems of chromosome pairing and chiasma formation.

Spontaneous triploid

Triploid reported in the present work is the first report in H. brasiliensis (Nazeer and Saraswathy Amma, 1987). A triploid plant (2n = 54) in H. guianensis and one haploid in H. pauciflora have only been reported earlier (Baldwin, 1947). But there is plenty of literature on triploids in annual crops (Belling, 1925; Karasawa, 1934; Binek and Bingham, 1970; Mehetre and Thombre, 1982). Gates (1908) discovered the phenomenon of triploidy in hybrid Oenothera and a large number of triploids have been described since. In the spontaneous triploid of H. brasiliensis at metaphase I, various chromosome associations like trivalents, bivalents and univalents were observed (Table 17). In 5% PMCs complete trivalents (18_{III}) were observed. From the cytological observation it is clear that the spontaneous triploid is an autotriploid characterised by the presence of a maximum number of 18 trivalents.

In nature, triploids may originate either due to crossing

between tetraploid and diploid taxa or by fusion of reduced egg cells with two male nuclei or fertilization of unreduced egg cell and a male nucleus. The cross between tetraploids and diploids seems to be hardly probable because spontaneous tetraploids do not occur among Hevea. Therefore, a cross between diploid can be hypothesised by assuming that fertilization of functional 2n egg by haploid sperm or of the haploid egg by a 2n sperm nucleus occurred. Fertilization of reduced and unreduced gametes might have resulted the formation of triploids as observed in Tulipa (Newton and Darlington, 1920), Petunia (Dermen, 1931), Allium (Khoshoo and Sharma, 1959), Cynodon (Gupta and Srivastava, 1970), Portulaca (Singh, 1979) and Crocus (Chichiricco, 1984).

Instead of formation of four microspores in a tetrad five and six microspores are formed. There was total degeneration of cytoplasm and nuclei resulting in total sterility.

The sterility of the triploid is due in part to the disharmonious gene combination. Triploids provide an important research tool for the production of aneuploid series. Such aneuploids will be of value in genetic investigations.

Induced triploid

In H. brasiliensis, artificial triploid was evolved by crossing diploid with colchicine induced tetraploids. In other annual crops, it is reported that allotriploids have evolved by crossing between tetraploid and diploid and also in intergeneric and interspecific crosses (Balog, 1984). Induced triploids are reported in other crops like Raphano-Brassica (Karpechenko, 1927), Solanum (Janaki Ammal, 1934), Pyrus (Darlington and Moffett, 1930), Luffa (Roy and Ghosh, 1971).

In the induced triploid the maximum of only 17 trivalents was observed at metaphase I. In other words 18 trivalents which is the maximum possibility was not observed in any of the microsporocytes. This may possibly be due to the genetic difference between the genomes involved in its origin or the result of precocious disjunction of trivalents. Most triploids are sterile because of the variable chromosome complements within the gametes, which results from the segregation of dyads from the trivalents.

In the induced triploid and spontaneous triploid no fruitset was observed. Lack of fruitset in Hevea is of special advantage since it reduces the incidence of abnormal leaf fall disease. It is known that fruits act as major source of inoculum for abnormal leaf fall disease (Radhakrishna Pillai et al., 1980).

Comparative studies on induced and spontaneous triploid:-

There is morphologically distinct variations between the two types of triploids. Cytologically, the spontaneous triploid shows autotriploid nature chracterised by the formation of 18 trivalents. Whereas, in the induced triploid complete trivalents is not observed. Moreover, in the spontaneous triploid there is complete male sterility. But in the induced triploid 5.5% stainable pollen grains were observed. Both the triploids are found to be fruitless. The development of male flower is very poor in the spontaneous triploid and it is quite normal in the induced triploid (Saraswathy Amma et al., 1988a).

Induced tetraploid:-

Attempts to induce polyploidy have been initiated by the various Rubber Research Institutes. Shepherd (1969) working in Prang Besar Estate, Malaysia made attempts to induce polyploization on rubber seeds and buddings. The Chinese workers (Zheng Xuequin et al., 1980) also reported successful production of polyploid plants with colchicine treatment. At Rubber Research Institute of Malaysia, Kuala Lumpur also (Ong et al., 1984) polyploidization was attempted but reported only putative polyploidy. It may be noted that induction of polyploidy in rubber is laborious and

time consuming. Moreover, due to chimeric problem and difficulty in stabilizing the polyploid character the success obtained is very low. But in India (Markose, 1975; Saraswathy Amma et al., 1980; 1984a) true polyploids of H. brasiliensis were evolved.

The induced tetraploid studied in the present investigation showed wide range of abnormalities. At metaphase I, predominant formation of bivalents (21-32), univalents (1-9), trivalents (0-4) and quadrivalents (0-4) were observed (Table 19). Some of the explanations put forth for low incidence of multivalents in autotetraploids are the small size of chromosomes, preponderance of submetacentric, sub telocentric and acrocentric chromosomes and presence of genes for low chiasma frequency (Darlington, 1965; Stebbins, 1971). Reduction in quadrivalent frequency in autotetraploids was reported in Cosmos (Mathew and Rajkumar Thomson, 1984) and Zinnia (Gupta and Rajini Koak, 1976). Small size of chromosome is reported to be responsible for low frequency of multivalents in these crops. But it is a matter of controversy as to whether size of chromosome plays any role in the formation of quadrivalents in autotetraploid. Pal and Pandey (1982) reported the influence of genes or gene complexes which suppressed the multivalent association of chromosomes in grain amaranths.

Polyploidy is one of the evolutionary mechanisms in producing radically different and well adapted genotypes (Stebbins, 1968). Polyploids can enrich the germplasm resource of Hevea, which can be utilised for future breeding programme. The performance of these newly generated materials has to be evaluated in the field trials. The field evaluation of Hevea, a perennial tree having an immaturity period of 6-7 years, is a very long term task.

Radiation induced mutant:-

Cytomixis:

In the radiation induced mutant, cytomixis was observed. This phenomenon has been reported in Hevea for the first time by Saraswathy Amma and Panikkar (1988c). Transmission of chromatin materials from one cell to another through cytoplasmic connections was first observed by Kornicke (1901) in Crocus. Gates (1911) studied this phenomenon in Oenothera gigas and Oenothera bienis and coined the term 'cytomixis'. Since then cytomixis has been recorded in a very wide range of taxa (Habib and Chennaveeraiah, 1976; Omara, 1976; Narain, 1979; Siddiqui et al., 1979; Jayabalan and Rao, 1987).

In the radiation induced mutant, cytomixis/cytoplasmic

connections were observed from early prophase to microspore stage. A similar case in which cytomixis/cytoplasmic connections were observed in all the meiotic stages was reported in Menha piperata (Kundu and Sharma, 1988). Passage of nuclear material was also reported in telophase II in Vigna (Sen and Bhattacharya, 1988). The origin and evolutionary significance of cytomixis are not precisely understood. Several suggestions had been put forward to explain the cause and probable origin of cytomixis (Maheswari, 1950; Heslop-Harrisons, 1966; Whealan, 1974; Rao, 1975; Narain, 1979; Mantu De and Sharma, 1983). Whealan (1974) suggested that these intermeiocyte connections might serve as channels in the exchange of nuclear material. Some cytologists consider cytomixis as an extremely abnormal phenomenon (Bauchan et al., 1987). Cytomixis in the present study appears to be spontaneous and expressed by an unbalanced genetic system resulting from gamma irradiation.

Cytomictic condition was found to be associated with high pollen sterility in Papaver (Bahl and Tyagi, 1988). However, cytomixis alone cannot account for the total pollen sterility. Where there was no cytomixis after the tetrad formation, there was complete degeneration of microspores resulting in sterility. Therefore, it can be concluded that the normal PMCs are unable to produce functional male gametes. This differential behaviour could

be due to differences in the degree of expressivity of the mutant gene.

Cells with reduced number of chromosome from the model number n = 18 were also noted in the radiation induced mutant. However, pollen mother cells with bivalents higher than 18 were not observed. It is not unlikely that degeneration of pollen mother cells with higher chromosome number has occurred. Such behaviour of chromosomes and degeneration of pollen mother cells were reported in Cissus (Agarwal, 1983). Usually high chromosome number in germinal line is an adaptive disadvantage (Bhattacharya, 1983).

Details of chromosome aberrations induced by radiations were described by Evans (1962). In the present investigation pollen conglomerates were noted in the mature anther of the radiation induced mutant. Formation of pollen conglomerates was also reported in Alnus (Bensimon, 1985).

Meiotic abnormalities are noted in RRII 17, the spontaneous sterile clone, radiation induced mutants, triploids and tetraploid in H. brasiliensis. Deviation from the normal meiotic process are significant from the evolutionary point of view. The behaviour of chromosomes at meiosis also controls distribution of genes but

boosts up the genetic variability compared to that of a conservative meiotic system (Padmaja, 1988).

Cytokinetic aberrations

In the radiation induced mutant, a wide spectrum of cytokinetic aberrations was noted which also led to the formation of sterile pollen grains (Saraswathy Amma et al., 1989b). The distribution of nuclei in the tetrad showed wide range of variations. Final products of meiosis not only depend on how the nucleus divides but also on how the cleavage occurs and microspore walls are formed in the meiocyte. In the normal course of meiosis, it is to be expected that there is coordination between karyokinesis and cytokinesis. Deviation in any of these can lead to sterility (Ramanna, 1974). Wide spectrum of cytokinetic aberrations associated with male sterility were reported in Alcopercurus (Johnson, 1944), Impatiens sultani (Tara and Namboodiri, 1974, 1976), Solanum (Abdalla and Hermsen, 1972) and in Medicago (Mecoy and Smith, 1983), leading to the formation of sterile pollen grains.

In the mutagen induced male sterile clone also meiosis was normal up to the formation of tetrads. Thereafter degeneration was noted resulting in sterility. Sterility induced by mutagen, Ethyl methane sulphate, has been reported in Nigella (Gilot-De

Ihalle, 1976), Hordeum vulgare (Kumar and Singh, 1983), Turnea ulmifolia (Tarar and Dnyansagar, 1978).

Palynology

Pollen grain, being a partner in the biology of reproduction in plants, has a vital role to play in crop improvement. Pollen grains possess a unique form and variation found in individuals of a population can provide a clue to the genetical make up of the plant (Nair, 1988). Hence palynological studies are of paramount importance in agricultural crops and its application has been stressed by several workers (Erdtman, 1952; Nair, 1961; 1970; Khoshoo, 1979). In Hevea information pertaining to morphology of pollen is very meagre (Markose and Nair, 1970; Saraswathy Amma et al., 1989a).

The control clone, RRII 105 showed 92.8% pollen stainability and 80% pollen germinated in vitro. However, in Ch 2, RRII 17 and mutagen induced mutant, complete pollen sterility was noted. Even sterile pollen grains are absent in RRII 35, RRII 15, GT 1 and mutagen induced mutant. The induced triploid showed 5.5% stainable pollen and the radiation induced mutant had 0.5% stainable pollen. The tetraploid with pollen grains having three and four pores showed 80% pollen stainability. There were pollen grains with three and four pores in the induced triploid.

The size of mature pollen grains in the triploid and tetraploid showed wide range of variation (Table 24. Variation in pollen size and number of germpores were noted in other crops and the details are discussed along with SEM studies of pollen grains of polyploids. The pollen grains are three and four colporate in the polyploid.

There was direct relationship between the size of male flower buds and that of microsporocytes and microspores. Pollen mother cells as well as microspores are comparatively small in RRII 105, but larger in the triploid and the tetraploid. It is interesting that the maximum size of the microsporocyte is noted at the tetrad stage (25 μm in RRII 105) and microspores within tetrad are very small (15 μm). As soon as they are released they enlarge and attain a definite size. The pollen grains show wide variation in size and shape for triploid and tetraploid. Such variation was absent in Pelargonium (Tokumasu, 1974). Light microscopic observations of pollen grain of male sterile clones have elucidated wide spectrum of variation with respect to sterility, shape and size of pollen grains.

The scanning electron microscopic studies have particularly given a better insight into the fine structure of the exine surface. At the same time the studies also provide a new parameter for

differentiation of genotypes. In RRII 105 the ornamentation is basically areolate. Furrow island and bridge are seen. In the tetraploid and triploid exine is large and areoles are united.

Varying degrees and combinations of apertural types were noted in different cytotypes of Sisymbium irio complex (Nair and Sharma, 1966-67). The exine pattern of the diploid and octoploid taxa of Gloriosa are reported to vary (Ravikumar and Nair, 1985). There is a general correlation between level of polyploidy or chromosome number and pollen size and literature is replete with examples (Gould, 1953; Kapadia and Gould, 1964; Laws, 1965; Sreerengaswamy and Raman, 1973; Nair et al., 1977; Medus, 1978). Taxonomists and cytologists have been using size of mature pollen from herbarium sheets as a method of surveying the extent of polyploidy within certain taxa (Stebbins, 1958). The increase in pollen size as observed for polar diameter and equatorial diameter as well as an increase in exine thickness and ora diameter (Table 24) in the tetraploid indicate an expression of genomic variation of the cytotypes in their pollen characteristics.

Sterile pollen grains:-

The sterile pollen grains showed normal exine formation, but exhibited a more or less aberrant pattern. In RRII 105, the

pollen grain is stainable and the ornamentation is with heteromorphic areoles. Investigations on the development of irregular pollen have shown that in reduced, unreduced and tetrakaryotic cells as well as in atypical and abortive spores, typical exine ornamentation is observed (Rogers and Harris, 1969; Banerjee and Barghoon, 1976; Heslop-Harrison, 1962; Nair, 1988). Exine formed around nonfunctional pollen grains with degenerating nucleus may exhibit normal stratification but more or less an aberrant gross pattern. According to De Vries and Ie (1970) exine formation appears to be complete, but intine of the sterile pollen grains is far thinner than that of the fertile one. Mepham and Lane (1968) also hold the view that the exine material is a secretory product of pollen protoplast based on observations with Tradescantia. Skvaria and Larson (1966) were of opinion that normal exine was laid out around aborted grains derived from the microspores irrespective of its chromosome complement. As exine formation is not damaged when the pollen grain is sterile, it could be gathered that the exine is produced before the pollen degeneration starts (Heslop-Harrison, 1968; Sreedevi and Namboodiri, 1976). According to Rogers and Harris (1969) the control of exine deposition is more closely correlated to the microspore cytoplasm than to enclosed genome. It is therefore evident that even if the pollen grain is sterile there is exine formation. In H. brasiliensis also exine ornamentation is noted in the sterile pollen grains, though there is difference in the exine pattern and size of columella, the exine ornamentation is not perfect as in the case of pollen grains from fertile clone.

DNA content

Among angiosperms DNA content per nucleus varies over a 100 fold range (Rees and Jones, 1972). Polyploidy is responsible for some of these variations (Sparrow et al., 1972). Quantification of DNA has become an important and interesting subject of research in recent years. In Hevea, a preliminary attempt has made to study the characterisation of nuclear genome of H. brasiliensis (Law and Bonner, 1986). But analysis of variation in nuclear DNA content at different ploidy level in this crop has not been reported so far.

Relative nuclear DNA content (4 C) in diploid, spontaneous triploid, induced triploid and induced tetraploid showed significant difference among the cytotypes. Cytotypes with higher ploidy levels did have corresponding higher DNA value. The same trend was reported in Betula (Schaefer and Miksche, 1977), Amaranthus (Ohri et al., 1981), Piper (Rosabelle et al., 1986). Triploids showed approximately 1:1.5 with respect to diploids. Similar results have been obtained in other plants (Verma and Rees, 1974; Bennett and Smith, 1971). The nuclear DNA content for annual species was significantly lower than that for perennial species (Price, 1976; Bennett et al., 1977). A slight variation in nuclear DNA content between the two triploids can be correlated with reshuffling and rearrangement in chromosomes.

GENETICS

The hybrid clones of H. brasiliensis in which GT 1 was the female parent and the male fertile normal clones RRII 105, RRII 118, RRIC 100 and RRIM 600 were the male parents, showed complete male sterility. Since all the F_1 clones were male sterile, the genetic control of male sterility could be totally determined by cytoplasmic factors which are transmitted through the egg. The cytoplasmic control of male sterility in H. brasiliensis was was first reported by Saraswathy Amma et al. (1988b)*.

Majumder (1967), Anon (1983) and Leconte and Nicolas (1985) reported GT 1 as a male sterile clone while Olopade and Salawu (1986) and Anon (1987) considered this to be male fertile. The difference in fertility in different geographic regions may be due to the influence of environmental factors. Kaul (1988) reported that the potentiality of sex supression is genetically controlled, but the degree of its expression is environmentally influenced. There have been numerous studies to correlate the influence of various environmental factors on anther development and male sterility in crops like beets (Owen, 1945; Cortessi, 1967), cotton (Meyer and Meyer, 1965; Sarvella, 1966; Meyer, 1969), barley (Batch and Morgan, 1974), corn (Duvick, 1965), beans (Estrada

* Please see 2nd reference (Page No. 172).

and Mutschler, 1984) and rape (Shiga and Baba, 1971; Fan and Stefanson, 1986). Peterson (1958) noted that in cytoplasmic male sterile Capsicum, sterility is accentuated at temperatures above normal and that certain environmental conditions act on some internal systems by either promoting or inhibiting auxin-like substances which influence pollen formation and breakdown of tapetum leading to total sterility. The effects of environment on cytoplasmic male sterility in Sorghum and Petunia were reported by Kidd (1961) and Van Marrewijk (1969).

Sexuality in higher plants is a delicate and fragile system susceptible to the influence of genes (nuclear and cytoplasmic), chemicals, environment and their interaction. Sex alterations induced by chemicals and environment are excellently reviewed by Frankel and Galun (1977). Ahokas (1978) reported a barley mutant which responded to photoperiod and physiography so that it was complete male sterile in Finland but partially male sterile in USA. All investigators revealed in general that temperature interacts strongly with fertility restoring mechanisms in male sterile plants although genetical and physiological factors affect male sterility. Since there is considerable variation in the expression of sterility in GT 1, environment may be acting on some system which triggers the production of either fertile or abortive pollen grains. However, studies on the F_1 hybrid clone of GT 1 have elucidated that there

is manifestation of cytoplasmic male sterility in this clone. Since the cytoplasm is derived entirely from the female gamete, the F_1 plants produced are male sterile. Cytoplasmic male sterility is determined by cytoplasmic factors (plasma genes) which are transmitted entirely through the egg. Hence there is cytoplasmic control as far as the male sterility in GT 1 is considered. In order to draw a conclusion, detailed genetic studies are inevitable. Since it is a time consuming task especially in a tree crop like H. brasiliensis, this aspect is beyond the scope of this thesis.

Male sterility in plants is of unusual interest not only because of the different causes that can give rise to it, but because of its intrinsic value in plant breeding programmes. The spontaneous male sterile clones identified and having good fruit set are GT 1, Ch 2 and RRII 35. All these clones are ortet selections.

In the male sterile clones, there is no chance for self fertilization as the pollen produced is totally nonviable. Hence the seeds collected are hybrid seeds. Whereas, in the control clone Mil 3/2, also an ortet selection, the male and female flowers are fertile, and there are chances for self pollination. In Hevea selfing has been reported by Edgar (1958), Polhamus (1962), Ong (1976) and Simmonds (1989).

The seeds from the male sterile clones recorded more per-

centage of germination compared to that of the control. The vigour of the progenies of male sterile clones was also significantly more compared to that of the control. During young stage both height and girth showed significant differences. But after 24 months of growth there was no significant differences with regard to plant height for any of the clones under study. Among the male sterile clones studied, GT 1 was significantly superior to Ch 2 and RRII 35 in all the growth attributes. The progenies of GT 1 were reported to be superior in vigour in the nursery stage (Saraswathy Amma et al., 1984b). In the case of juvenile yield the seedlings from the male sterile clones showed superiority over the control. A similar trend in yield has been reported in male sterile line of wheat (Miller et al., 1974).

Since the difference between GCV and PCV are more for yield profound environmental influence is implied. High genotypic variation and high heritability indicate involvement of additive gene action for these characters. The response of selection is expected to be the best in crosses involving high GCA effects (Singh and Singh, 1976). Among the three male sterile clones studied GT 1 is showing comparatively high GCA and this clone is a high yielding one with desirable secondary attributes. Gill et al. (1973) indicated that high yielding parents usually have high GCA.

Regression analysis for yield (y) on height (x_1), girth (x_2) and bark thickness (x_3) have revealed that 36% of the total variation on yield was accounted by girth alone. When girth and bark thickness were taken together 47% of the total variation on yield was reflected in these parameters. There was highly significant positive correlation between yield and girth.

In most crops selection at the nursery level for yield is virtually useless because of low heritability/repeatability of the character. There is correlation between nursery yield and field performance (Tan, 1987). Accordingly nursery seedlings are grown at close spacing and test tapping is carried out at 2-3 years old seedlings (Tan and Subramaniam, 1976). Hence these parameters can be utilised for nursery selection to a certain extent. However, cent per cent correlation between juvenile characters to mature tree has yet to be established.

Effective improvement by selection within a population depends on the presence of sufficient additive genetic variation. Progeny testing allows an accurate estimate of genetic value of each established clone and those that produce poor progeny could be eliminated. In other words, progeny tests are important to assess genetic variability (Farmer, 1970). The progress of breeding depends upon the magnitude of genetic variability in the population and the extent to which desirable attributes are heritable.

High degree of heritability of a character is an indication of the effectiveness of selection based on a phenotypic performance. Johansen et al. (1955) suggested that heritability estimates with genetic gain are more useful for effective improvement. Eldridge (1978) suggested individual tree selection among open pollinated seed source in Eucalyptus as a method for genetic improvement. In forest trees, the information from progeny test is used for predicting the genetic gain from seed orchards (Krusche et al., 1980; Sniezko and Zobel, 1988). In Hevea, seedling vigour and juvenile yield are considered for selection (Tan, 1978a, 1981). Tan (1978b, 1979) found some of the high GCA parents obtained from nursery seedlings to be in the high GCA parental group in the mature stage. Ng et al. (1982) reported that crossed seeds are better than monoclonal seeds in Hevea. Since all the three male sterile clones discussed in the present study are good seed bearers these can be utilised for seed gardens. In Hevea seeds are indispensable to raise stock plants for budding and they also constitute a reservoir of genetic variability.

SUMMARY AND CONCLUSIONS

Five spontaneous male sterile clones are identified from the existing exotic and indigenous populations of Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg. out of which two (Ch 2 and RRII 35) are reported for the first time in this crop.

Polyploidy was induced in a clone (RRII 105) and a tetraploid having a chromosome complement of 2n = 4x = 72 was evolved. An artificial triploid (2n = 3x = 54) was synthesised by crossing diploid with the tetraploid. A radiation induced mutant and mutagen (Ethyl methane sulphonate) induced mutant showing male sterility were evolved.

Comparative studies on the morphology, cytology and genetics of these male sterile clones were carried out. A fertile clone, RRII 105, was investigated for comparison. There is distinct morphological variations in foliar and floral characteristics. Significant differences are noted among the clones with regard to growth attributes. The spontaneous male sterile clones GT 1, Ch 2, RRII 35, spontaneous triploid and tetraploid had comparatively more vigour

in terms of diameter and height, while radiation induced mutant was comparatively less vigorous.

Deviating from the typical trifoliate condition of normal Hevea clone, variation in the number of leaflets (2 to 5) at the nursery stage is noted for induced triploid and tetraploid. Even though triploids are reported to be vigorous in the present investigation induced triploid is not vigorous. This may be due to the effect of back crossing.

The correlation of growth attributes of these clones at 30 months growth has shown significant correlation ($P<0.01$) between height and girth.

The dwarf stature exhibited by induced triploid and radiation induced mutant can be incorporated in the breeding programme of Hevea.

The spontaneous triploid and RRII 17 have no fruitset, hence the incidence of leaf diseases are reported to be comparatively less. They can be incorporated for the crown modification.

Significant variations in flower size is also noted among these clones. The flowers are bigger in size for tetraploid, tri-

ploids and Ch 2 and comparatively smaller in radiation induced mutant and GT 1.

Mitotic studies have confirmed that the chromsome complement of triploid and tetraploid is 2n = 3x = 54 and 2n = 4x = 72 respectively, whereas the diploid shows 2n = 2x = 36.

Meiosis is normal in the spontaneous male sterile clones GT 1, Ch 2, RRII 35 and EMS induced mutant up to the formation of tetrads after which complete degeneration of cytoplasm and nuclei resulted in total male sterility.

Abnormalities in the tapetum are noted in GT 1 and Ch 2. Proliferation and persistance of tapetum is noted in GT 1 and Ch 2 whereas, complete degeneration of tapetum is noted in the normal fertile clone.

In the spontaneous sterile clone, RRII 17 wide spectrum of meiotic abnormalities are noted. Desynapsis is also observed. Predominant formation of univalents (8 to 32) is seen during metaphase I. Anaphase I is also highly irregular. Due to unequal segregation microspores of varying number (3 to 9) and size are observed.

In the radiation induced mutant, cytomixis is observed in 30% of the meiocytes. Cytoplasmic connections are observed in all stages of PMCs ranging from early prophase to microspore stage. The male sterility in this plant is due to genetic imbalance caused by irradiation which result in wide range of meiotic abnormalities coupled with cytokinetic aberrations. Pollen conglomerates and megapollen are also observed.

Meiotic studies of triploid exhibited univalents, bivalents and trivalents. Anaphase I showed unequal segregation formation of laggards. The spontaneous triploid showed autotriploid nature since it showed 18_{III} at metaphase I. The tetraploid showed large number of bivalents, followed by less formation of univalents, trivalents and quadrivalents. The distribution of nuclei during tetrad stage showed wide variations (2 to 6). Tetraploid showed 80% stainable pollen, induced triploid showed 5.5% whereas the spontaneous triploid did not have any stainable pollen.

Remarkable variation in the production of pollen grains was noted. Pollen production was practically absent in GT 1, RRII 35, mutagen induced mutant and spontaneous triploid. RRII 105 clone showed 150-200 pollen per anther (Table 3).

Variations in the number and size of germpores as well as

shape of pollen grains are reported in tetraploid and triploid compared to that of the diploid. A general correlation between polyploidy, genome complement and pollen characteristics was observed in the different cytotypes of H. brasiliensis.

The size of microsporocytes and microspores at different developmental stages of flowers of control RRII 105, Ch 2, triploid and tetraploid had shown considerable variations. The maximum size was noted at tetrad stage and minimum for the microspores within tetrad.

Scanning electron microscopic observations have given a better understanding of exine ornamentation in polyploid and sterile pollen grains. The sterile pollen grains showed normal exine formation, but exhibited a more or less aberrant pattern. Exine ornamentation is comparatively large for polyploids. In the sterile pollen differences in the exine pattern and size of columella are noted. However, the exine ornamentation is not perfect as in the case of fertile clone.

Estimation of 4 C DNA content in the various cytotypes has shown that cytotypes with higher ploidy level have corresponding higher DNA level.

Manifestation of cytoplasmic male sterility in GT 1 was noted for the first time in Hevea. However, further detailed genetic studies are essential to draw a conclusion.

Evaluation of the seedling progenies of male sterile clones GT 1, Ch 2 and RRII 35 along with that of fertile clone Mil 3/2 showed that the progenies of male sterile clones showed significant difference in vigour and juvenile yield. They also showed high H^2, GCA, GA indicating that these characters can be used for early evaluation. Among the three male sterile clones, GT 1 is showing superiority in growth attributes and juvenile yield.

Multiple correlation studies have shown that about 54% of total variation of yield is due to girth. The male sterile clones having good fruitset can be used for designing seed orchards. These materials can enrich the genetic reservoir of Hevea.

NEW FINDINGS

REPORTED FOR THE FIRST TIME FROM THE PRESENT STUDY

1. Identification of spontaneous male sterility in clones, viz. Ch 2 and RRII 35.

2. Successful induction of male sterility

   (a) Radiation induced mutant with dwarf stature

   (b) Mutagen (EMS) induced mutant.

3. identification of spontaneous triploid (RRII 15).

4. Synthesis of artificial triploid by crossing diploid with tetraploid.

5. Desynapsis in RRII 17.

6. Cytomixis in radiation induced mutant.

7. Wide spectrum of cytokinetic aberrations in the radiation induced mutant.

8. SEM studies on pollen grains of tetraploid, triploid and sterile clones.

9. Identification of pollen conglomerates.

10. 4 C DNA estimation of polyploids.

11. Evaluation of progenies of male sterile clones.

12. Manifestation of cytoplasmic male sterility in the clone GT 1. The male sterile clones having good fruitset can be utilised for hybrid seed production. Among the male sterile clones GT 1 is a good combiner.

FURTHER SCOPE OF INVESTIGATIONS FROM THE RESULT OF PRESENT STUDY

1. Detailed studies on genetic mechanism underlying the male sterile clones incorporating F_1 and F_2 generation.

2. Breeding behaviour of tetraploid and triploids.

3. Incorporation of clones showing desirable secondary attributes in the breeding programme of Hevea.

4. Male sterile clones having good fruitset can be incorporated in designing seed gardens for the production of hybrid seeds.

5. Field evaluation of the selected genotypes from the progenies of male sterile clones with the ultimate aim of selecting clones with high yield and good secondary attributes.

6. Induction of polyploidisation in the male sterile clones to study the effect of genome multiplication.

7. Biochemical characterisation of male sterile and newly evolved materials.

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* Original not seen.

GLOSSARY

Ch 2	-	A primary clone from Chemara, Malaysia.
GT 1	-	A primary clone from Gondang Tapen estate, Indonesia.
Mil 3/2	-	A primary clone from Millakande, Ceylon.
RRII 35	-	A primary clone from the Rubber Research Institute of India.
RRII 105	-	The most promising clone developed from the Rubber Research Institute of India having Tjir 1 as female and Gl 1 as the male parent.
Gl 1	-	A primary clone from Glenshiel, Malaysia.
Tjir 1	-	A primary clone from Tjirandi, Indonesia.

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