INVESTIGATIONS ON CYTOGENETICS, FLOWERING AND SEEDSET IN GINGER *(Zingiber Officinale Rosc)*

By

SATHIABHAMA K. U.

THESIS

submitted in partial fulfilment of
the requirement for the degree

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

Department of Plantation Crops and Spices
COLLEGE OF HORTICULTURE
Vellanikkara - Trichur

1988

DECLARATION

I hereby declare that this thesis entitled "Investigations on Cytogenetics, flowering and seedset in ginger (*Zingiber officinale* Rosc)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
August, 1988.

Sathiabhama.
SATHIABHAMA K.U.

CERTIFICATE

Certified that this thesis entitled "Investigations on Cytogenetics, Flowering and seedset in ginger (*Zingiber officinale* Rosc)" is a record of research work done independently by Mrs. Sathiabhama K.U., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

G. Sreekandan Nair 10/8/88

(Dr. G. Sreekandan Nair)
Chairman
Advisory Board,
Professor,
College of Agriculture,
Vellayani.

Vellanikkara,
August, 1988.

CERTIFICATE

We, the undersigned members of the Advisory Board of Mrs. Sathibhama K.U. a candidate for the degree of Master of Science in Horticulture, agree that the thesis entitled "Investigations on cytogenetics, flowering and seedset in ginger (*Zingiber officinale* Rosc)" may be submitted by Mrs. Sathibhama K.U., in partial fulfilment of the requirement for the degree.

G. Sreekandan 3rd 8 88

Dr. G. Sreekandan Nair,
Chairman,
Advisory Board.

Smt. Prasannakumari Amma,
Member

Dr. P.A. Wahid,
Member.

Smt. Achamma Ommen,
Member.

ACKNOWLEDGEMENT

I am highly indebted to Dr. G. Sreekandan Nair, Professor, of Horticulture, College of Agriculture, Vellayani, formerly Head of Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara and Chairman of my advisory committee for suggesting the problem, stimulating encouragement and constructive criticism during the course of my research work and preparation of the thesis.

I am greatful to Dr. P.A. Wahid, Professor, Radiotracer and member of my advisory committee for his assistance in irradiation work and encouragement during the course of investigation and writing of the thesis.

Smt. Prasannakumari Amma, Asst. Professor (Hort.) Department of Plantation Crops and Spices, College of Horticulture and member of my advisory committee has been a source of inspiration and helping hand in the entire period of investigation. I express my thanks to her.

My thanks are also due, to Smt. Achamma Cherian, Department of Agricultural Botany, College of Horticulture and member of my advisory committee for her valuable suggestions and helps.

A name which should have been mentioned earlier is of Dr. C. Ramachandran, Asst. Professor (Hort.), Department of Plantation Crops and Spices, College of Horticulture and the former chairman of my advisory committee. He was responsible for formulating the research problem. He has worked and helped more than his officialdom. His constructive criticism during the preparation of the manuscript of the thesis was also very helpful. I express my deep sense of gratitude to him.

I express my sincere thanks to all the staff members of the Department of Plantation Crops and Spices, College of Horticulture for their valuable helps during the entire course of investigation.

I am thankful to Smt. N.V. Kamalam, Associate Professor, Radiotracer, Kerala Agricultural University for her helps in the irradiation work.

My gratitude and thanks are also due, to the Kerala Agricultural University Computer Centre, Vellanikkara for helping me in the statistical analysis.

I express my gratitude and thanks to my friends especially to Miss. Lekshmy, M.L. for their help during the entire course of investigation.

I am extremely thankful to my husband Sri. Ramdas and other family members for their help and moral support without which I would not have completed my thesis work.

Lastly I express my thanks to the Kerala Agricultural University for awarding me the Junior Research Fellowship which helped me financially to complete my M.Sc. (Hort.) programme.

Vellanikkara,
August, 1988.

SATHIABHAMA K.U.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

List of appendices

I. Weather data during the period (January to December, 1987).

Introduction

INTRODUCTION

Ginger (*Zingiber officinale* Rosc) belonging to the family Zingiberaceae is an important commercial spice throughout the world, esteemed for it's aroma, flavour and pungency. It was one of the earliest oriental spices introduced to the West and the demand for this spice is increasing ever since. The aromatic rhizomes of ginger find application both as a spice and in medicines. At present ginger is ranked as the third most important spice crop of India, standing next to pepper and cardamom. India is also the largest producer and exporter of ginger in the world, contributing to about 50 per cent of world production and export.

The increasing demand of ginger and it's suitability for varied preparations have put forth the need for promising ginger types having special quality attributes. Unfortunately, varieties having the twin advantages of high yield and special quality attributes are rare at present. Also the ginger crop in India is facing the increasing devastation of soft rot and bacterial wilt for which resistant types are yet to be developed. Some other bottlenecks such as high fibre content of varieties and high cost of production are also associated with ginger cultivation in India. Hence there is an imperative need for developing high yielding varieties with less fibre content and resistant to diseases.

if India has to keep up her position in the world market (Muralidharan and Kamalam, 1973; Nair et al., 1980).

The analysis of crop improvement programmes so far undertaken in ginger reveals that the major thrust has been given to selection of cultivars suited to local conditions. The advantage of genetic variability is to be tapped fully and exploited for the benefit of cultivars (Muralidharan and Sakunthala, 1975; Nair et al., 1980). Hybridization at interspecific or intervarietal levels has not been reported so far. Similarly the genetics of ginger is poorly understood. The greatest handicap which obstruct the hybridization and genetical studies, and for that matter breeding of ginger is the absence of fruitset and seedset. Added to this difficultly is the shy flowering nature of Zingiber spp. It has been observed that only a few species of Zingiber and a few varieties of Zingiber officinale flower, that too for a short period of the year. In fact, the physiology of flowering in ginger is poorly understood in this regard.

Ginger has been described by many as a species flowering very shyly and never setting seeds (Hooker, 1882; East, 1940). Several factors such as self incompatibility, chromosomal aberrations,

defects in micro and megasporogenesis, lack of suitable pollinating agents and failure of pollen germination on stigma were reported to be responsible for the absence of fruitset and seedset in ginger (Fryxell, 1957; Ramachandran, 1969; Pillai et al., 1978; Ratnambal, 1979). However, there is no consensus among the workers as to the real mechanism operating in ginger. Unfortunately none of the previous investigators used in vivo pollen germination studies for concluding to the mechanism of self-incompatibility in ginger. Ginger is propagated vegetatively by means of rhizomes and lack of seedset is not a problem for its multiplication (Anon, 1978). However, seed propagation may be economical as far as cultivation is concerned, since cost of planting materials (rhizomes) takes about 40 per cent of cost of cultivation in ginger. Moreover, seedset if possible in ginger, will help the breeder to shuffle the genome and utilise the inherent superiority in each variety. Also it will facilitate the mutagenesis programmes and genetical studies to a great extent.

The importance of cytogenetical studies to establish the phylogenetic relationship and to explain the mechanism of sterility in crops has been well emphasised (Levitsky, 1931; Stebbins, 1974). Furthermore, cytogenetical studies are considered as useful tools for the characteristion of intraspecific

classification (Brandenburg, 1984; Ramachandran et al., 1986). Eventhough cytogenetical studies have been made in the genus Zingiber the information available on cytogenetical polymorphism in ginger is very meagre. Also the relationship of meiotic abnormalities towards pollen sterility leading to problems in fruitset and seedset is poorly understood. Ginger has been described by many, as a species producing high amount of sterile grains (Pillai et al., 1979)Jayachandran and Vijayagopal, 1979; Usha, 1983). However, details on the extent of variability for pollen sterility between varieties of ginger, the effect of different media on the pollen germination and pollen tube growth of different varieties and the effect of irradiation on the germination of pollen grains in ginger are not available. These details are quite significant in understanding the factors limiting fruitset and seedset in ginger and also to devise methods for breaking these barriers. Taking into account of the afore mentioned gaps in the biology of ginger crop, the present investigation was undertaken with the following objectives.

1) To work out the karyomorphology of ginger varieties and to study the cytogenetical polymorphism in relation to plant morphology.

2) To study the meiotic irregularities in relation to pollen sterility in different varieties.

3) To study the effect of media and irradiation on the pollen germination and pollen tube growth in different varieties.

4) To work out the pollen-pistil interaction by fluorescence microscopy to decide on the presence of incompatibility mechanism in ginger.

5) To study the factors responsible for absence of fruitset and seedset in ginger.

Review of Literature

REVIEW OF LITERATURE

Ginger (*Zingiber officinale* Rosc) has been described by many, as a species producing flowers very scarcely and setting no seeds. Eventhough some scattered unsuccessful attempts on fruitset and seedset are available, concerted and comprehensive efforts on this aspect have not been made so far. Also a mutually agreed unequivocal reason for the absence of fruitset and seedset in ginger is not available. Ginger is also a less studied species cytogenetically. Hence the accumulated literature on various aspects relevant to the subject matter of the thesis are reviewed below.

1) Origin and distribution

Ginger has been used in India and China as spice and medicine since ancient times. It is not known in a wild state, nor is the country of origin known with certainity. However, Burkill (1966) suggested that Malaysia may not be the centre of origin of ginger. Rosengarten (1969) recorded that ginger was mentioned by Chinese philosopher Confucius (551-479 B.C.) in his *Analects*. According to Purseglove *et al.* (1981) ginger might have originated in South East Asia, but later introduced to the tropics and cultivated often in homegardens on a significant scale in countries like China, Japan, Sierra Leone, Nigeria, Australia, India and West Indies.

2) Species and varieties

Ginger (*Zingiber officinale* Rosc) is a monocotyledonous plant belonging to the family Zingiberaceae of the order Zingiberales. Burkill (1966) mentioned several species that were used in native medicine in South East Asia, of which the most important seem to be *Zingiber cassumunar* Roxb and *Z. zerumbet* L. According to Purseglove *et al.* (1981) the genus *Zingiber* Boehm has about 80-90 species of perennial rhizomatous herbs throughout South East Asia and extending to Queensland and Japan.

Ginger is always propagated vegetatively and hence the literature on varieties are limited. Several reports on the types noticed in different areas are available. Ridley (1912) reported two types available in Jamaica and three races in West Malaysia. Gollifer (1973) described five types of ginger in the British Soloman Islands. Paulose (1973) listed the names of several common cultivars of ginger grown in different parts of Indian subcontinent.

3) Cytogenetics

The chromosome number of 2n = 22 for *Z. officinale* Rosc has been confirmed by many cytogeneticists (Morinaga *et al.* 1929; Sugiura, 1936; Chakravorthi, 1948; Federov, 1969), eventhough Takahashi (1930)

reported a chromosome number of 2n = 24 for the species. Raghavan and Venkatasubban (1943) reported the cytology of three species of Zingiber viz. Z. officinale Rosc Z. cassumunar Roxb. and Z. zerumbet Sm. and all of them had the chromosome number of 2n = 22. They also found differences in the chromosome morphology in these species and concluded that the chromosome of Zingiber officinale were different from the other two species in morphology. Darlington and Janaki Ammal (1945) observed two B chromosomes in certain types of Z. officinale in addition to the normal complement of 2n = 22. Chakravorti (1948) concluded that in view of the bivalent association in diploid species, Z. cassumunar and Z. zerumbet, Z. mioga Rosc with 2n = 55 chromosomes, is to be considered as a pentaploid. Sato (1960) also concluded that Z. mioga with 2n =55 is to be considered as pentaploid with a basic number of x = 11, based on studies of karyotype of 13 genera including 24 species in Zingiberales.

Sharma and Bhattacharya (1959) reported widespread occurence of inconsistency in chromosome number in several species of Zingiberaceae including Z.officinale. They also observed high number of fragments, stickness, bridges, laggards, micronuclei and structural alterations in somatic chromosomes of Z. officinale treated with X-rays.

Ramachandran (1969) reported the cytology of five species of Zingiber including Z. macrostachyum Daiz. Z. roseum Rosc and Z. wightianum Thor for the first time, in addition to Z. officinale and Z. zerumbet. He found evidence for structural hybridity involving interchanges and inversions in Z. officinale. Mahanty (1970) in an extensive cytological investigation in the Zingiberales reported chromosome numbers of Z. spectabile Griff and Z. cylindricum both as 2n = 22.

Suzuka and Mitsuoka (1978) conducted cytological studies in Zingiber mioga and reported it as an autopentaploid with 2n = 55. Pillai et al. (1978) observed that meiosis is highly irregular in ginger with only 46.6 per cent of the PMCs showing bivalents and the rest showing univalents, trivalents, quadrivalents, etc.

Karyomorphology of 32 cultivars of Z.officinale and three species of Zingiber viz Z. zerumbet, Z.macrostachyum and Z.cassumunar were investigated by Ratnambal (1979). She found considerable differences in gross morphological characteristics of chromosomes. A classification of karyotypes according to the degree of asymmetry showed that karyotype 1b was represented in most of the cultivars of Z. officinale, while 1a was found in Z.zerumbet, Z.macrostachyum and Z.cassumunar. D^2 analysis in 32 cultivars of

Z. officinale and three wild species recognized eight clusters based on the generalised distance for chromosome attributes. It was concluded that classification of the cultivars based on the geographical distance will be difficult in ginger. Ratnambal (1983) observed the formation of quadrivalents in most of the cultivars of Z. officinale Rosc during meiosis. Intraspecific variability for meiotic behaviour was also observed in 25 cultivars of Z. officinale. She established significant linear regression between pollen sterility and chromosome aberrations at anaphase II and aberrant quartets. It was concluded that the structural chromosome aberrations had a significant influence in lowering the fertility in cultivars of Z. officinale.

Ramachendran (1983) found that tetraploids (2n = 44) can be produced in ginger by colchicine treatment. Beltrant and Kam (1984) reported the somatic chromosome numbers of 33 species including nine Zingiber species and noticed polyploidy (2n = 66) and presence of B chromosomes (2n = 22 + 2 B) in Z. officinale. They also remarked that Indian and Malayan Zingiber species are diploids (2n = 22), while the Japanese species, Zingiber mioga is a pentaploid with 2n = 55. Datta and Bhattacharya (1985) observed cytological irregularities such as fragments and ring formations in Z. officinale. Omana Kumari and Mathew (1985) presented a detailed Karyotypic data on Z. officinale, Z. zerumbet,

Z. wightianum and Z. macrostachyum, all having 2n = 22 chromosomes.

4) Flowering

a) Flowering behaviour: Hooker (1892) described ginger as a species with very rare flowering. Holttum (1950) stated that flowers are seldom seen in Malaysia but are produced in some other countries, thereby it appeared that flowering in ginger is observed under certain conditions only. Pillai et al. (1978) reported that, of the 35 germplasm collections maintained in CPCRI, Kasaragod all but, six flowered and that flowering started in the last week of October and lasted till early December, the peak being in November. Nybe (1978) noticed that ginger types valluvenad, Vengara, Ernad-Chernad, Ernad Manjeri, Wynad Kunnamangalam, Taiwan, Tafingiva, Sierra Leone, Rio-de-Janeiro, Utter Pradesh, Jorhat, Narasapattom, Nadia, China and Assam showed 0.52 - 11.71 per cent flowering as against no flowering in Thodupuzha, Thingpuri and Himachal Pradesh types under Vellanikkara conditions in 1977. Similarly flowering of Rio-de-Janeiro was recorded under the climatic conditions prevailing at Vellayani by Jayachandran et al. (1979).

b) Inflorescence: Nybe (1978), reported that the inflorescence in ginger developed from the rhizome along with scale leaf. It formed a bracteate spike or raceme, each bract subtending a single flower with a lateral or obliquely posterior bracteole. The bracts were spirally arranged. The length of the stalk varied from 15-30 cm. Pillai et al. (1978) stated that single flowers were borne in panicles directly arising from the rhizome and also on terminal spikes. Inflorescence in ginger is a scape and it produced on a special leaf bearing shoot springing from the rhizome or is terminal, the number of terminal inflorescence being two per cent and the flowers are subtended by a prominent, fertile bract (Jayachandran et al., 1979).

A detailed description of ginger inflorescence had been furnished by Purseglove et al. (1981). Accordingly spicate inflorescence arises directly from the root-stock and has got a slender scape of 10 to 20 m with or without short leaftips and a cylindrical cone-like spike (4 to 7 m in length and 1.50 cm to 2.50 cm in diameter) with eppressed, ovate or elliptic and green bracts (2 to 3 cm long and 1.50 to 2.00 m wide) with a pale submarginal band and incurved translucent margins, bearing a single fragile and short-lived flower in the axil of each leaf.

c) Morphology of flowers: The floral details of ginger has been given by Nybe (1978), Jayachandran (1979) and Purseglove (1978). According to their description the flowers are small as compared to those of the other genera and are borne on the axile of a bract and are yellowish green, zygomorphic, bisexual, epigynous and trimerous. The calyx is tubular or bell shaped dividing above into three short teeth and split on one side. The corolla is tubular below (2.00 to 2.5 cm long) with three yellowish lobes of which the dorsal lobe is curved over the another. Androecium consists of stamens of which the outer three are reduced to staminodes. The inner lateral stamens are united and showing to form a deep purple cloured labellum. The posterior stamen of the inner whorl is the only fertile stamen and is enclosed by the labellum. Stamen has got a short and broad filament with two prominent anther lobes. The style passes through the groove formed by the anther lobes and ends in a capitate stigma. Stigma has a circular apical aperture surrounded by stiff hairs and it protrudes just below the apex of the appendage. Pillai et al.(1978) described the ovule as bitegmic and anatropous. Ovary is inferior, trilocular with several ovules per ovary.

d) Floral biology: Jayachandran et al. (1979) reported that it took 20 to 25 days from the bud

initiation to full bloom and a period of 23 to 20 days was required for the completion of the blooming in an inflorescence. However, Usha (1983) estimated that it took 29 days from the bud initiation to full bloom and about 9 to 18 days for the completion of blooming in an inflorescence in ginger.

Jayachandran et al. (1979) based on their studies on Rio-de-Janeiro indicated that blooming takes place in an acropetal succession and the flower falls on the next day of blooming. Pillai et al. (1978) observed that anther dehiscence takes place simultaneously with the flower opening in ginger. Jayachandran and Vijayagopal (1979) noticed that pollen shedding almost coincided with the flower opening. But Usha (1983) observed that anther dehiscence took place 10 to 25 minutes after flower opening. Stigma receptivity of ginger is still in darkness owing to the failure of hand pollination to set seeds. Observation under hand lens by Jayachandran et al. (1979) indicated that stigma was receptive at the time of anther dehiscence. Usha (1983) reported that stigma remained receptive from one hour to five hours after flower opening.

5) Pollen studies

a) Pollen production and morphology: Pillai et al. (1978) reported that the pollen grains in ginger are hermaphrodite, round and with a diameter of 77 to 104 µm.

the average being 91 µm. A striking feature observed by these workers was the very thick exine of the pollengrains. Jayachendran et al. (1979) stated that pollen grains are spherical with the size ranging from 90 to 100 µm, the mean being 95.5 µm. Jayachendran and Vijeyagopal (1979) also mentioned that anther lobes in ginger are filled with plenty of pollen grains. However, Usha (1983) estimated pollen production in ginger to 1,87,500 in variety Rio-de-Janeiro and 1,91,500 in variety Maran. According to her, the availability of pollen grains is not a limiting factor in ginger breeding.

b) Pollen fertility and germination: A high percentage of pollen sterility has been reported in ginger. Generally the stainability of pollen grains obtained in acetocarmine method of fertility assessment was only 35 per cent (Pillai et al., 1978). Usha (1983) reported only 12.48 per cent fertile pollen grains in Rio-de-Janeiro and 16.42 per cent in Maran.

Germination of pollen grains in ginger has been found to be very low by many investigators. Nair et al. (1975) reported that pollen grains of Rio-de-Janeiro, Wynad Local, Maran, Burdwan and Assam germinated in a media containing 15 per cent sucrose, 300 ppm calcium nitrate, 100 ppm boric acid, 200 ppm potassium nitrate, 100 ppm magnesium nitrate and one per cent agar.

However, the germination was less than 1.60 per cent. Pillai et al. (1978) found that addition of boric acid is helpful to break the exine and thereby to achieve the germination of pollen grains. Of the different media tried, the one with 8 per cent sucrose, 3 per cent gelatin and 60 ppm boric acid in moist chamber operating at 26.5°C gave the maximum germination of 14.50 per cent. Jayachandran et al. (1979) have put pollen sterility as high as 76 per cent. In their study, only 2.50 per cent germination was obtained in dextrose agar media. They argued that high percentage of sterility may be one of the reasons for poor germinations of grains. Usha (1983) reported that growth regulators viz. IAA and GA were not having any positive effect on pollen germination. Among the various media tried, 8 per cent sucrose with 3 per cent gelatin and 60 ppm boric acid gave maximum germination of 6.20 per cent.

6) Pollination and seedset

Pillai et al. (1978) reported that flower structure of ginger manifests an adaption suitable for entomophily. Hand pollination using large quantities of pollen grains in variety Rio-de-Janeiro by Jayachandran et al. (1979) and Usha (1983) could not achieve seedset.

According to Hooker (1894), ginger is a species never setting seed. East (1940) and Fryxell (1957) suspected that the failure to set seeds may be due toself incompatibility. Ramachandran (1969) and Ratnambal (1979) remarked that chromosomal aberrations were responsible for lack of seedset in ginger. Pillai et al. (1978) suspected three possible reasons for the absence of seedset viz., defects in micro and megasporogenesis, lack of suitable pollinating agents and failure of pollen germination on stigma or due to incompatibility. Jayachandran and Vijayagopal (1979) reported that in the event of incompatibility the inhibitory action may not be located on the stigma surface. On the other hand, Usha (1983) was of the opinion that incompatibility reaction may not be the factor causing failure of seedset in ginger, as she failed to get seed by bud pollination or with stigma and style removal. The growth regulator application also could not achieve seedset in her study.

7) Methods to overcome barriers in seedset

The compatible mentor pollen effect, the capacity of compatible pollen, in a mixture of compatible and incompatible grains applied on stigmas, to stimulate the growth of the incompatible pollen present in the mixture, was first observed by Michurich (1950) and subsequently reported by Glendinning (1960), Tsitsin (1962), Grant et al. (1962) de Nettancourt and

Grant (1963), Miri and Bubar (1966 and Karpov (1966) The mentor pollen technique to overcome crossing barriers in certain interspecific hydridization was applied by Stettler (1968). Knox (1972) also successfully repeated the experiments of Stettler. Knowledge of the behaviour of irradiated pollen may be useful for the application of mentor pollen technique, (Pandey, 1975). Sree ramalu et al. (1979), reported that no seedset or fruitfication was observed in three genera of flowering plants viz. Nicotiana, Oenothera and Lycopersicon after mentor pollination. The effect of irradiation on the transfer of certain specific characters like disease resistance was tried in Cucumis (den Nijs, and Oost, 1980). Boom and den Nijs (1983) obtained seed set in Cucumis by the mentor pollination technique.

Using 20 different species Brewbaker and Emery (1962) estimated that X-ray doses of 220 kR or higher inhibited pollen germination. In contrast, Pfahler (1971) found an average LD 50 of 0.5 kgy for pollen germination in Maize, Stotz (1972) suggested the examinations of pollen tube growth as a direct assessment of compatibility. According to Mock and Loescher (1973) in wide crosses, the pollen tube may grow the length of the stylo and penetrated the ovule but fertilization may not occur. Oost and den Nijs (1979) reported the effect of storage conditions on in vitro germination of irradiated and non-irradiated pollen of several

Cucumis species. Irradiated mentor pollen proved to be capable of occupying a certain proportion of ovules, depending on irradiation dose and time between subsequent pollinations (Boom and den Nijs, 1983; Visser et al. 1983). Dennissen and den Nijs (1987) based on their study on Cucumis sp., reported that pollen germination and pollen tube growth were reduced by increasing the irradiation dose for all species.

In vivo pollen germination in the pistils using fluorescece microscopy (Kho and Baer, 1968; Kho et al. 1982) to establish whether incompatibility system is operating or not in ginger has not been ascertained in ginger.

Materials and Methods

MATERIALS AND METHODS

Investigations on 'Cytogenetics, flowering and seedset in ginger' were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the year 1987. The weather data during the period (January - December, 1987) are given in Appendix-1.

1) Morphological observations

Nine varieties of ginger representing different geographical regions of India including a variety from Brazil were used for the study (Table 1).

Table 1. Source of different varieties of ginger

Sl. No.	Variety	Source
1.	Arippa	Andhra Pradesh
2.	Bajpai	West Bengal
3.	Burdwan	West Bengal
4.	Kuruppampady	Kerala
5.	Maran	Assam
6.	Nadia	Assam
7.	Narasapattam	Andhra Pradesh
8.	Rio-de-Janeiro	Brazil
9.	Valluvanad	Kerala

These varieties were raised in pots and normal cultural operations based on package of practices (Kerala Agricultural University, 1986) were followed.

observations on the following morphological characters were recorded from five plants in each variety. The mean values were then calculated and presented.

a) Number of tillers: The number of tillers per pot were counted after six months of planting rhizomes.

b) Plant height (cm): The height of the main tiller in each pot was measured from soil level to the tip of the highest leaf after six months of planting rhizomes.

c) Length of leaves (cm): The length of 10 leaves in each pot was measured after six months of planting rhizomes.

d) Breadth of leaves (cm): The breadth of 10 leaves in each pot was measured after six months of planting rhizomes.

e) Number of leaves per pot: The number of leaves per pot was counted six months after planting.

f) Days for appearance of flowers: The number of days for the appearance of first inflorescence after planting was recorded in each pot.

g) Number of flowers per scape: The number of flowers per scape were determined by counting the number of flowers that were opening in each inflorescence.

h) Number of inflorescence per pot: The number of inflorescence per pot were determined by counting

inflorescences produced during the entire period of life.

i) Number of inflorescence coming through heart: The number of inflorescence coming through the heart was counted and recorded.

j) Rhizome yield (g/pot): The yield was recorded by taking the fresh weight of rhizomes in each pot.

2) Cytogenetical studies

a) Standardization of time of collection of roots for mitotic studies: During the course of present investigation, ginger roots were found to give very low mitotic index as compared to many plant species. Also the usual time of collection of roots between 9-11 a.m. was giving few dividing cells in the preparations. In order to standardize the time of collection of roots for getting high mitotic index, rhizomes of the variety Rio-de-Janeiro were germinated in petridishes. The roots were collected at two hour intervels throughout a full day cycle of 24 hours. The squashing technique used for the analysis of mitotic index in root tips was based on a procedure by Ramachandran et al. (1985), the details of which are presented in the next section on 'mitotic chromosomes'. The number of dividing cells in 30 fields were counted and the mitotic index was calculated by the following formula:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

b) Mitotic chromosomes: Karyomorphology of the above nine varieties were analysed using root tip squashes. For this the following method which was essentially a modification of the procedure by Ramachandran et al. (1985) was adopted

i) Germinate the rhizomes in petridishes

ii) Collect the roots when they attain 1 to 2 cm long at 5-6 a.m.

iii) Pre-treat with saturated solution of paradichlorobenzene at room temperature for four hours.

iv) Wash with distilled water and fix the roots in Carnoys II fluid (6 ethyl alcohol: 3 chloroform: 1 glacial acetic acid) at room temperature for 48 hours.

v) Wash with distilled water and soften the roots in an enzyme solution containing 1 per cent cellulase and 0.5 per cent pectinase in 0.01 molar citrate buffer (pH 4.0) at 37°C for 30-45 minutes

vi) Wash with citrate buffer and then with distilled water.

vii) Hydrolyse the softened roots in 1 N HCl at 60°C for 15 minutes.

viii) Wash with distilled water and stain with Feulgen stain for 45 minutes until the meristematic tips turn magenda coloured.

ix) Squash the stained meristematic tips in 0.5 per cent acetocarmine.

x) Seal with paraffin wax and screen the slide for mitotic chromosomes.

Observations

Data were recorded from temporary slides only since excessive staining of cytoplasm reduced the clarity of preparations in permanent mounts. Karyomorphological measurements were recorded from five cells at metaphase stage in each variety. Care was taken to measure the chromosomes from cells at identical stages.

Measurements of length of long arm (l), short arm (s) and satellite of individual chromosomes were tabulated and homologous pairs were identified. All measurements were converted into micrometres and arm ratios (l/s) for each of the homologous pairs were calculated. The chromosomes were classified into the following groups according to Giorgi and Bozzini (1969) based on the position of the centromere and presence or absence of satllites.

SAT: Satellited chromosomes, the arm ratio of which is calculated leaving out the satellite length.

M: Median chromosomes, the arm ratio of which is between 1.00 and 1.25

SM: Submedian chromosomes, the arm ratio of which is between 1.26 and 1.75

ST: Subterminal chromosomes, in which the arm ratio is 1.76 and above.

The total chromatin length (TCL) of the haploid complement and also the relative chromosome length (RCL) which is expressed as the percentage of individual chromosome length, over the total length of haploid complement were estimated. Average chromosome length (ACL) in each variety was also calculated.

Total chromosome volume of the haploid complement was also computed for each species. For this the width of five chromatids in each cell was measured at random and mean chromatid width was estimated. Then the volume of each chromosome was calculated based on the total chromatid length (2 x chromosome length) and average chromatid width assuming the chromatids to be cylindrical (Ramachandran and Narayan, 1985).

Photographs were taken from temporary slides using on Olympus PM 6 manual camera unit. Idiograms were drawn for all the varieties whose karyomorphology were analysed. Categorisation of karyotype asymmetry of somatic complement hasbeen made according to the method of Stebbins (1958) who classified the karyotype under 12 Classes (1A - 4C) taking into account both the position

of the centromere in the chromosomes and the degree of difference between the largest and smallest chromosomes of a complement (Table 2). Total form per cent (TF%) was also calculated following the formula given by Huziwara (1962).

$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total chromosome length}} \times 100$$

Table 2. Stebbins (1958) classification

Largest chromosome length	Proportion of chromosomes with arm ratio more than 2 : 1			
Smallest chromosome length	0.00	0.01 - 0.5	0.5 - 0.99	1.00
2 : 1	1a	2a	3a	4a
2 :1 - 4 :1	1b	2b	3b	4b
4 : 1	1c	2c	3c	4c

c) Meiotic studies

Plants of all varieties were raised in pots keeping five pots for each variety during March - December 1987 and normal cultural operations were followed based on package of practices (Kerala Agricultural University, 1986).

Ginger produces bisexual flowers only. Hence bisexual flower buds of size 0.2 - 0.4 mm were fixed in Carnoys I fluid (acetic alcohol = 1:3) mixed with two to three drops of ferric acetate during 9-11 a.m. After 48 hours of fixation buds were transferred to 70% alcohol. Squashing the buds the next day of fixation gave better spread where as prolonged preservation led to chromosome clumping. A small bit of each anther was cut out and smeared well in a drop of one per cent acetocarmine. Cover slip was placed, the slide warmed gently and pressed strongly under filter paper to remove the excess stain. The preparations were sealed with paraffin wax. The slides were screened after 15-20 minutes when chromosomes have taken optimum stain and differentiated.

Observations

The following observations at metaphase I, Anaphase I and Telophase I were recorded.

i) Number of bivalents, trivalents, quadrivalents, pentavalents, hexavalents etc. per pollen mother cell (PMC)

ii) Total chiasmata per PMC

iii) PMCs with normal and abnormal anaphase I

iv) PMCs with laggards and bridges at anaphase I

v) PMCs with normal and abnormal telophase I

Chiasma frequency of meiotic chromosomes was calculated in each variety and subjected to 'F' test ((Panse and Sukhatme, 1976). Photographs of meiotic chromosomes were taken in an Olympus PM 6 manual camera unit.

3. Pollen morphology

a) Pollen fertility (%): Pollen grains were collected for each variety on the day of anther dehiscence. They were then stained with one per cent acetocarmine on slides and the fertility (%) was estimated as the percentage of stained over the total number of grains (Shivanna and Johri, 1985).

b) Pollen diameter (µm): The diameter of pollen grains (µm) was measured from 25 grains and the average value was worked out for each variety. Additionally the presence or absence of pores, was recorded.

c) Pollen shape: Shape of the pollen grains in all the varieties were noticed.

d) Pollen germination (%): Pollen grains from all nine varieties were collected and kept for germination in the following media for 24 hours in a BOD incubator at 25°C.

Media: 1. Distilled water

2. 8% sucrose + 1% 60 ppm boric acid

3. 15% sucrose + 60 ppm boric acid

4. 30% sucrose + 60 ppm boric acid

5. 8% sucrose + 60 ppm boric acid + 1% gelatin

6. 15% sucrose + 60 ppm boric acid + 1% gelatin

7. 30% sucrose + 60 ppm boric acid + 1% gelatin

The germinated grains were counted in 15 fields in different slides and the percentage of germination was calculated in the following way.

$$\text{Percentage of germination} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

The length of pollen tubes after 24 hours of germination were also measured in different media and mean values calculated.

e) Germination of irradiated grains: The pollen grains of variety Maran were irradiated with different doses of gamma rays i.e. 0 kR, 0.5 kR, 1 kR, 5 kR, 10 kR, 25 kR, 50 kR and 100 kR and kept for germination in the above seven media for 24 hours in a B.O.D. incubator at 25°C and germinated grains were counted in 15 fields in different slides and the relationship of irradiated doses and pollen germination (%) was recorded.

4. Pollen-pistil interaction

In order to study the pollen pistil interaction the following method of Kho and Baer (1968) was used. The flower buds of variety Maran were fixed in FAA (Formalin 5.0 : acetic acid 5.0 : 90 ethyl alcohol) at 0, 2, 4, 8, 12 and 16 hours after pollination for a period of 24 hours. The flowers were found to remain on the plant for less than 16 hours after opening. The fixed buds were transferred to 1 N NaOH for 7 to 12 hours at room temperature. They were washed thoroughly with distilled water and stained with 0.1 per cent aniline blue in 0.1 N K_3PO_4 for about 18 hours. The pistil was later macerated in 80 per cent glycerol. In vivo pollen tube growth in the pistils was then examined in a Leitz microscope under UV light.

Photomicrographs of the preparations were later taken in an Leitz automatic camera.

5. Overcoming the barriers in seedset

Ginger never set seeds. In order to overcome the barriers in seed set, the following techniques were attempted in two varieties of ginger. viz. Rio-de-Janeiro and Maran. Twenty five flowers were used in both cases.

a) Artificial self pollination: Pollination was carried out by using the pollen from the same flower.

b) Artificial sibbing: Pollination was done by using pollen from separate flowers of the same variety.

c) Artificial cross pollination between varieties: Pollinating flowers after anthesis with pollen from other varieties.

d) Bud pollination: Pollination carried out one day prior to flower opening.

e) Mentor pollination: Pollination was carried out by using mixture of irradiated (0.5 Kr) and normal pollen grains.

f) Chemically aided pollination: Stigmatic surface was smeared with germination media (8% sucrose + boric acid) and pollination was carried out with pollen grains of different variety.

g) Removal of stigma and artificial pollination: The stigma was removed using a scapel and the pollen grains were applied on the cut end of style at the time of receptivity.

Twenty five flowers were randomly selected and used for each pollination technique. Percentage of fruit set was recorded during subsequent days of pollination.

RESULTS

1) Morphological characters of varieties

Mean values of 10 morphological characters in nine varieties are presented in Table 3 and plate 1. These included both vegetative and floral characters viz. number of tillers per plant, height of plant (cm), length of leaves (cm), breadh of leaves (cm), number of leaves per pot, days for appearance of flowers, number of flowers per scape, number of inflorescences per pot, number of inflorescences coming through the heart and rhizome yield (g/pot). Statistical analysis of the data furnished in Table 4 did not show any significant difference between varieties with regard to any of the morphological characters at five per cent and one per cent levels. Incidently the variation observed within a variety was equal or higher than that between varieties. These traits were in general influenced by environmental factors.

2) Cytogenetical studies

a) Standardization of time of collection of roots:

The fixation of roots during the morning hours was found to give less number of dividing cells in the root tip squashes. In order to standardize the time of collection, mitotic index (%) was estimated at two hours

Table 3 Mean of morphological characters in nine varieties of ginger

Sl. No.	Variety	Characters									
		No.of tillers	Plant height (cm)	No.of leaves	Length of leaves (cm)	Breadth of leaves (cm)	Days for appear-ance of flowers	No.of flo-wers scape	No.of scape/ pot	No.of inflo-resce-nces coming through heart	Rhizome yield/ pot (g)
1.	Arippa	33.24	66.0	259.00	19.15	2.24	186	3.33	8.2	0	400.00
2.	Bajpai	24.00	89.2	168.4	23.78	2.54	189	3.20	7.60	1	525.00
3.	Burdwan	20.50	60.0	149.24	19.58	2.37	185	3.00	8.60	0	381.25
4.	Kuruppampady	26.00	76.5	191.25	21.14	1.99	178	4.00	8.90	2	418.75
5.	Maran	27.66	82.66	207.66	25.12	2.67	176	11.00	9.10	4	558.33
6.	Nadia	23.50	76.00	232.25	26.75	2.40	185	6.50	9.12	2	468.75
7.	Narasapattam	28.60	71.40	189.80	19.94	2.12	177	5.00	9.53	1	490.00
8.	Rio-de-Janeiro	28.20	66.80	202.20	19.15	2.46	175	9.20	8.54	5	460.0
9.	Valluvanad	25.00	67.25	162.5	22.73	2.41	184	3.00	8.95	1	312.5

Plate Ia-Ie Plant morphology in different varieties of ginger.

Plate I.

ARIPPA

BAJPAI

Plate Ib

BURDWAN

KURUPPAMPADY

Plate Ic

MARAN

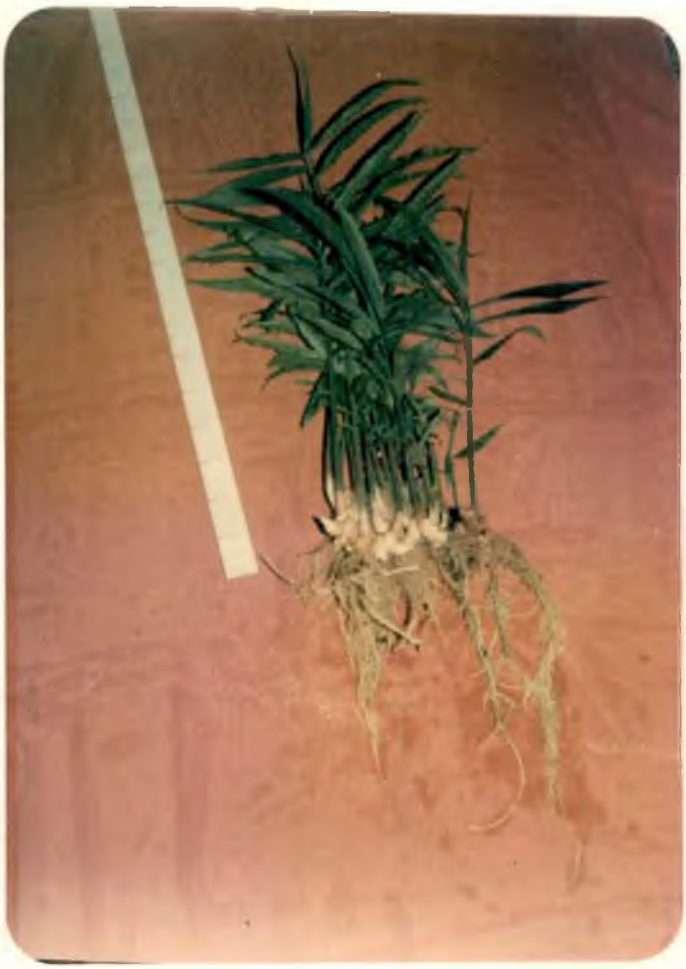
NADIA

Plate Id

NARASAPATTAM

RIO-DE-JANEIRO

Plate Ie



VALLUVANAD

Table 4 Analysis of variance for different morphological attributes in ginger

Characters df = 8	MSS	EMSS	F.value
No.of tillers	93.000	64.889	1.433 NS
Plant height	273.873	89.189	3.071 NS
No. of leaves	5990.688	3639.813	1.646 NS
Length of leaves (cm)	8.858	2.110	4.198 NS
Breadth of leaves (cm)	0.159	0.085	1.870 NS
Days for appearance of flowers	78.797	7.817	10.081 NS
No. of scape/pot	35.550	7.878	4.513 NS
No. of flowers/scape	11.100	9.434	1.177 NS
Presence and appearance of scape coming through heart	0.589	0.211	2.789 NS
Rhizome yield/pot (g)	75547.623	28205.836	2.678 NS

NS Not significant at 5% and 1% level

intervals and the data are presented in Table 5 and Fig.1. It was seen that the number of dividing cells as indicated by mitotic index increased during the night hours from 6 PM to 6 AM. Consequently a decrease in the mitiotic index was noticed in the day time from 6 AM to 6 PM. The maximum value of mitotic index (41.03 per cent) was observed in the roots collected at 6 AM and the minimum at 12 noon (2.89 per cent).

Table 5 Mitotic index in ginger var. Rio-de-Janeiro during a 24 hour cycle.

Time of collection of roots	Mitotic index (%)
6 AM	41.03
8 AM	4.73
10 AM	5.13
12 AM	2.89
2 PM	6.47
4 PM	7.09
6 PM	10.18
8 PM	11.31
10 PM	19.23
12 PM	20.85
2 AM	30.48
4 AM	25.46

b) Mitotic chromosomes

The karyomorphology of nine varieties of ginger was analysed in detail in the present study. In all nine varieties, a stable somatic chromosome count of 2n = 22 was observed in the root tip cells. The morphological characteristics of individual chromosomes with respect to length of long arm, short arm and satellite, arm ratio, type of chromosome, total chromatin length, relative chromosome length and total chromosome volume were determined. These observations are furnished in Tables 6 to 17 and plate 2. The idiograms of chromosomes drawn in the decreasing order of length are presented in Fig.2. Accordingly the karyotypes of different varieties are described below:

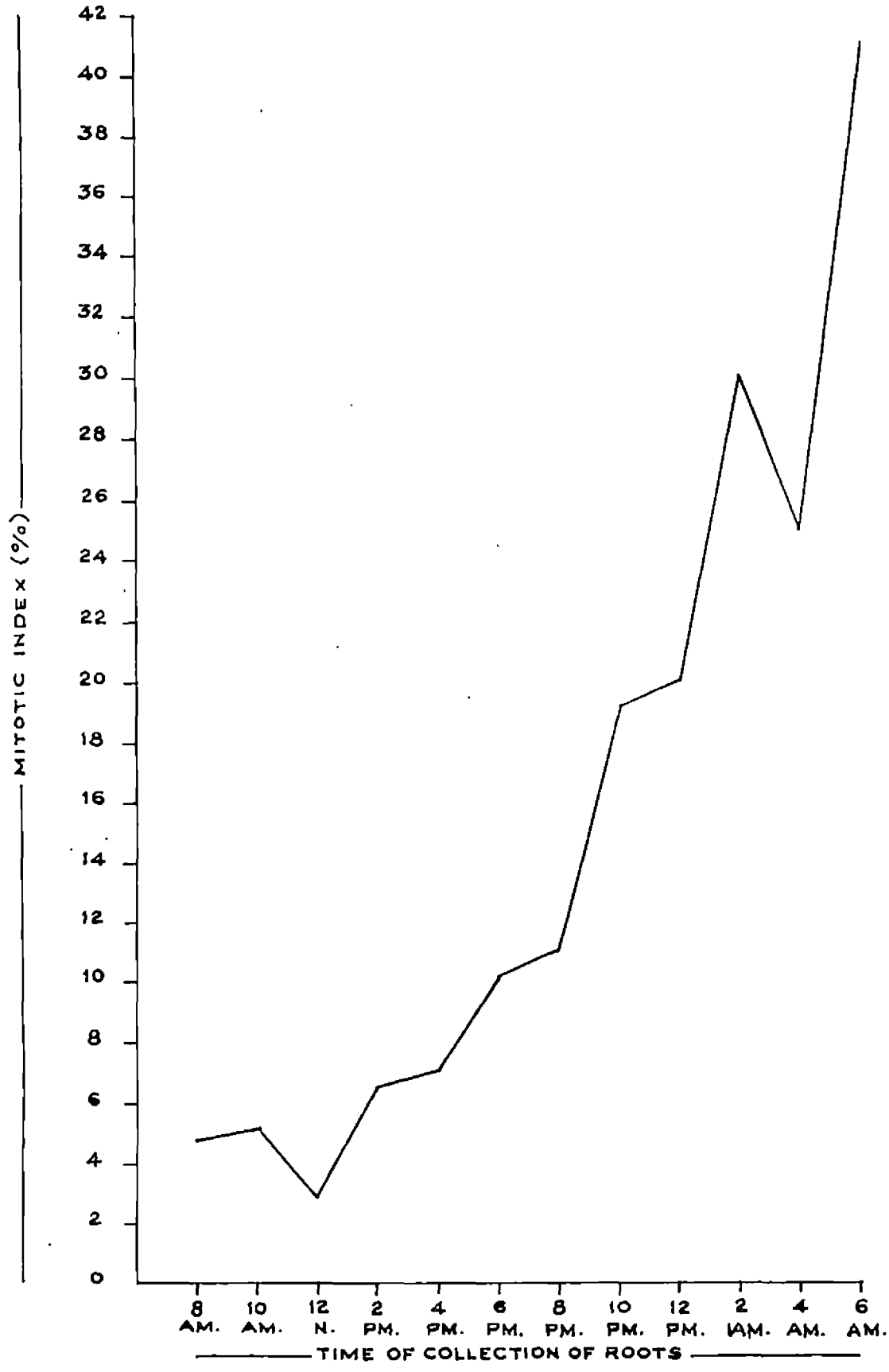


FIG. 1. MITOTIC INDEX (%) IN GINGER VARIETY - RIO-DE-JANEIRO.

i) Arippa: The chromosome complement in this variety is characterised by nine pairs of median and two pairs of submedian chromosomes (Table 6, Fig.2, Plate 2a). The length of individual chromosomes varied from 1.40 μ m to 3.55 μ m and the total chromatin length of the haploid complement was 25.27 μ m. The relative length of chromosomes ranged from 5.54 per cent to 14.06 per cent. The volume of individual chromosomes ranged from 3.18 μ m^3 to 1.26 μm^3 with a total of 21.76 μm^3 for the haploid complement.

ii) Bajpai: The karyotype analysis for this variety is given in Table 7, Fig.2. Out of the 11 pairs of chromosomes, ten were belonging to median and one to subterminal category in this variety. The individual length of chromosomes ranged from 1.60 μm to 4.01 μ m for the entire haploid complement. The volume of individual chromosomes showed a variation from 1.60 to 4.03 μm^3 and the total chromosome volume was 30.03 μm^3.

iii) Burdwan: The karyomorphology of Burdwan is presented in Table 8, and the ideogram in Fig.2 and Plate 2b. It was having 10 pairs of median and one pair of submedian chromosomes. The chromosomes ranged in length from 1.70 μ m to 3.30 μ m and the relative length from 6.41 per cent to 12.43 per cent. The total chromatin length of the chromosomes ranged from 1.96 μ m^3 to 3.82 μ m^3.

iv) Kuruppampady: The absolute length of individual chromosomes ranged from 1.50 μ m to 3.78 μm. The total chromatin length of the haploid complement was 27.99 μ m

Table 6 Karyotype analysis in ginger variety Arippa. (2n = 22)

Chromosome No.	Mean length (µm)			Arm ratio (1/3)	Type	Relative Chromosome length (%)	Volume (μm^3)
	Long arm	Short arm	Total length (µm)				
1.	1.80	1.75	3.55	1.03	M	14.06	3.18
2.	1.58	1.45	3.03	1.09	M	12.00	2.72
3.	1.50	1.33	2.83	1.13	M	11.21	2.54
4.	1.33	1.28	2.61	1.04	M	10.34	2.36
5.	1.20	1.10	2.30	1.09	M	9.11	2.08
6.	1.10	1.00	2.10	1.10	M	8.32	1.98
7.	1.00	1.00	2.00	1.15	M	8.51	1.94
8.	1.00	1.00	2.00	1.00	M	7.92	1.80
9.	1.00	0.80	1.50	1.25	M	7.13	1.62
10.	0.95	0.55	1.50	1.73	SM	5.94	1.36
11.	0.90	0.50	1.40	1.80	SM	5.54	1.26
			25.27				

M: Median SM. Submedian

Table 7 Karyotype analysis in ginger variety Rajpai (2n = 22)

Chromosome No.	Mean length (SM)			Arm ratio (l/s)	Type	Relative chromosome Length (%)	Volume (μm^3)
	Long arm	Short arm	Total length (SM)				
1	2.08	1.93	4.01	1.08	M	13.37	4.03
2	1.88	1.73	3.61	1.09	M	12.04	3.62
3	1.73	1.60	3.30	1.08	M	10.99	3.30
4	1.58	1.45	3.03	1.09	M	10.09	3.03
5	1.55	1.33	2.88	1.17	M	9.59	2.88
6	1.45	1.28	2.73	1.13	M	9.09	2.74
7	1.35	1.13	2.48	1.19	M	8.23	2.48
8	1.20	1.15	2.35	1.04	M	7.83	2.36
9	1.10	1.10	2.20	1.00	M	7.32	2.00
10	1.00	0.80	1.80	1.25	M	5.94	1.80
11	1.00	0.60	1.60	1.67	ST	5.33	1.60
			29.99				

M: Median SM: Submedian ST: Subterminal

Table 8 Karyotype analysis in ginger variety Burdwan (2n = 22)

Chromosome No.	Mean length (µ m)			Arm ratio (l/s)	Type	Relative Chromosome length (%)	Volume (μm^3)
	Long arm	Short arm	Total length				
1	1.80	1.50	3.30	1.20	M	12.43	3.82
2	1.60	1.50	3.10	1.07	M	11.09	3.60
3	1.48	1.43	2.91	1.03	M	10.97	3.36
4	1.40	1.25	2.65	1.12	M	9.99	3.08
5	1.35	1.20	2.55	1.13	M	9.61	2.96
6	1.30	1.05	2.35	1.24	M	8.80	2.72
7	1.15	1.00	2.15	1.15	M	8.11	2.50
8	1.00	0.98	1.98	1.13	M	7.46	2.30
9	1.00	0.96	1.96	1.11	M	7.06	2.20
10	1.00	0.85	1.85	1.18	M	7.01	2.14
11	1.00	0.70	1.70	1.42	SM	6.41	1.96
			26.54				

M: Median SM: Submedian

(Table 9, Fig. 2). Out of the 11 pairs, 10 have median and one has subterminal centromere. The volume of individual chromosomes in this variety ranged from 1.89 μm^3 to 4.76 μm^3 with a total estimate of 38.39 μm^3 for the haploid complement.

v) Maran: The chromosomes of this variety ranged in length from 1.40 μ m to 3.71 μ m with a mean total chromatin length of 28.02 μ m (Table 10, Fig. 2, Plate 2c). The complement was also chracterised by five median and six submedian pairs of chromosomes. The volume of chromosomes ranged from 2.02 μm^3 to 5.34 μm^3 with a total estimate of 40.82 μm^3 for the haploid complement.

vi) Nadia: The karyotype analysis of this variety is presented in Table 11, Fig. 2 and Plate 2d. It has shown the variation in length from 1.70 μ m to 3.70 μ m between different pairs. The total chromatin length of haploid complement was 38.02 μ m. This variety also showed five pairs of median and six pairs of submedian chromosomes in its complement. The volume of individual chromosomes ranged from 2.94 μm^3 to 6.40 μm^3 with a total chromosome volume of 56.85 μm^3 for the haploid complement.

vii) Narasapattam: The length of individual chromosomes in this variety ranged from 1.70 μ m to 3.96 μ m with a total chromatin length of 30.23 μ m for the haploid complement. The classification of

Table 9 Karyotype analysis in ginger variety Kuruppampady (2n = 22)

Chromosome No.	Mean length (μm)			Arm ratio (L/S)	Type	Relative chromosome length (%)	Volume (μm^3)
	Long arm	Short arm	Total length (μm)				
1	2.05	1.73	3.78	1.18	M	13.49	4.76
2	1.80	1.65	3.45	1.09	M	12.32	4.35
3	1.55	1.50	3.05	1.03	M	10.89	3.84
4	1.45	1.45	2.90	1.00	M	10.36	3.65
5	1.38	1.38	2.76	1.00	M	9.85	3.48
6	1.28	1.13	2.41	1.13	M	8.60	3.04
7	1.20	1.08	2.28	1.11	M	8.14	2.87
8	1.05	1.05	2.10	1.00	M	7.50	2.65
9	1.03	0.93	1.96	1.11	M	6.99	2.47
10	1.00	0.80	1.80	1.25	M	6.43	2.27
11	1.00	0.50	1.50	1.82	ST	5.36	1.89
			27.99				

M = Median, ST = Subterminal

Table 10 Karyotype analysis in ginger variety Maran (2n = 22)

Chromosome No.	Mean length (μ m)			Arm ratio (l/s)	Type	Relative chromosome length (%)	Volume (μ m^3)
	Long arm	Short arm	Total length (μ m)				
1	2.09	1.63	3.71	1.28	SM	13.24	5.34
2	1.88	1.56	3.46	1.19	M	12.35	4.98
3	1.78	1.33	3.11	1.34	SM	11.10	4.48
4	1.50	1.40	2.90	1.07	M	10.35	4.16
5	1.50	1.15	2.65	1.30	SM	9.43	3.82
6	1.43	1.13	2.56	1.27	SM	9.11	3.69
7	1.25	1.95	2.30	1.19	M	8.19	3.32
8	1.20	0.95	2.15	1.26	SM	7.65	3.10
9	1.05	0.90	1.95	1.17	M	6.94	2.80
10	0.95	0.80	1.75	1.19	M	6.24	2.52
11	0.90	0.50	1.40	1.80	SM	4.98	2.02
			28.02				

M = Median, SM = Submedian

Table 11 Karyotype analysis in ginger variety Nadia (2n = 22)

Chromosome No.	Mean length (µm)			Arm ratio (l/s)	Type	Relative chromosome length (%)	Volume (μm^3)
	Long arm	Short arm	Total length (µm)				
1	2.10	1.60	3.70	1.31	SM	12.01	6.40
2	2.00	1.63	3.63	1.23	M	11.68	6.28
3	1.98	1.47	3.45	1.35	SM	11.19	5.97
4	1.85	1.45	3.30	1.28	M	10.71	5.71
5	1.80	1.45	3.25	1.24	M	10.55	5.62
6	1.78	1.00	2.78	1.78	SM	9.02	4.81
7	1.55	1.10	2.65	1.41	SM	8.60	4.58
8	1.43	0.93	2.56	1.54	SM	7.66	4.08
9	1.05	1.00	2.05	1.05	M	6.65	3.55
10	1.00	0.95	1.95	1.05	M	6.33	3.37
11	1.00	0.70	1.70	1.43	SM	5.52	2.94
			30.82				

M = Median, SM = Submedian

chromosomes according to arm ratio has shown 10 pairs of median and one pair of submedian chromosomes in the complement. The volume of individual chromosomes has shown a variation of 2.97 μm^3 to 6.98 μm^3 and the total chromosome volume amounted to 52.04 μm^3 (Table 12, Fig. 2).

viii) Rio-di-Janeiro: The karyotype of this exotic variety was characterised by 11 pairs of chromosomes which varied in length from 1.86 μ m to 3.68 μ m and the total chromatin length of the haploid complement is 28.67 μ m (Table 13, Fig. 2, Plate 2e). The chromosome complement was also characterised by a distinct pair of satellites on the short arm of the longest pair of chromosomes. Out of the 11 pairs, four were belonging to median, six to submedian and one to subterminal category. The volume of individual chromosomes in this variety ranged from 3.20 μm^3 to 6.27 μm^3 with a total estimate of 44.48 μm^3 for the entire haploid complement.

ix) Valluvanad: The karyotype analysis presented in Table 14, Fig. 2 and Plate 2f, showed that the length of chromosomes in this variety ranged from 1.50 μ m to 4.03 μ m with a total chromatin length of 29.44 μ m for the haploid complement. It has eight pairs of median, two pairs of submedian and one pair or subterminal chromosomes according to arm ratio grouping. The longest chromosome pair in this variety also showed a

Table 12 Karyotype analysis in ginger variety Narasapattam (2n = 22)

Chromosome No.	Mean length (µm)			Arm ratio (l/s)	Type	Relative chromosome length (%)	Volume (μm^3)
	Long arm	Short arm	Total length (µm)				
1	1.98	1.98	3.96	1.00	T	13.11	6.96
2	1.83	1.78	3.61	1.03	M	11.95	6.36
3	1.65	1.53	3.18	1.08	M	10.52	5.58
4	1.50	1.50	3.00	1.00	M	9.43	5.28
5	1.48	1.48	2.96	1.00	M	9.79	5.20
6	1.35	1.35	2.70	1.00	M	8.94	4.76
7	1.28	1.28	2.56	1.00	M	8.47	4.50
8	1.15	1.11	2.26	1.04	M	7.48	3.98
9	1.00	1.00	2.00	1.00	M	6.62	3.52
10	1.00	1.00	2.00	1.00	M	6.62	3.52
11	1.00	0.70	1.70	1.43	SM	5.63	2.98
			30.23				

M = Median, SM = Submedian

Table 13 Karyotype analysis in ginger variety Rio-de-Janeiro (2n = 22)

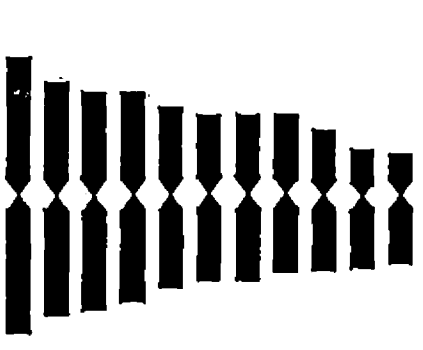
Chromosome No.	Mean length (µm)			Arm ratio (l/s)	Type	Relative chromosome length (%)	Volume (μm^3)
	Long arm	Short arm + Satellite	Total length (µm)				
1	1.59	(1.09+1.00)	3.68	1.46	SAT	12.83	6.27
2	1.79	1.46	3.25	1.22	M	11.33	5.54
3	1.92	1.17	3.09	1.64	SM	10.78	5.27
4	1.75	1.17	2.92	1.50	SM	10.18	4.98
5	1.50	1.17	2.67	1.28	SM	9.31	4.55
6	1.50	1.09	2.59	1.38	SM	9.03	4.41
7	1.50	1.00	2.50	1.50	SM	8.72	4.26
8	1.13	1.04	2.17	1.04	M	7.57	3.69
9	1.00	1.00	2.00	1.00	M	6.98	3.41
10	1.00	0.92	1.92	1.09	M	6.69	3.27
11	1.25	0.63	1.88	1.98	ST	6.56	3.20
			28.67				

M = Median, SM = Submedian, SAT = Satellited chromosome, ST = Subterminal

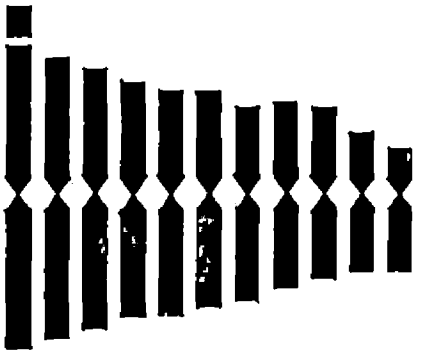
Table 14 Karyotype analysis in ginger variety Valluvanad (2n = 22)

Chromosome No.	Mean length (µm)			Arm ratio (l/s)	Type	Relative chromosome length (%)	Volume (µm^3)
	Long arm	Short arm	Total length (µm)				
1	2.08	1.95	4.03	1.07	SAT	13.69	3.10
2	1.93	1.78	3.71	1.08	M	12.59	2.86
3	1.85	1.50	3.35	1.23	M	11.38	2.58
4	1.55	1.45	3.00	1.07	M	10.19	2.32
5	1.45	1.45	2.90	1.00	M	9.85	2.24
6	1.45	1.20	2.65	1.21	M	9.00	2.04
7	1.40	1.00	2.40	1.40	SM	8.15	1.84
8	1.10	1.00	2.10	1.10	M	7.13	1.60
9	1.10	1.00	2.10	1.10	M	7.13	1.60
10	1.00	0.70	1.70	1.43	SM	5.78	1.30
11	1.00	0.50	1.50	2.00	ST	5.09	1.16
			29.44				

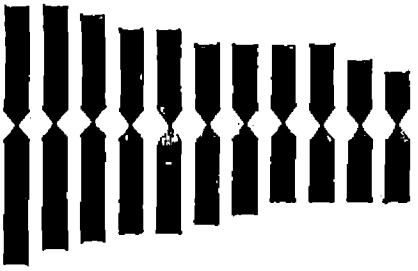
M = Median, SM = Submedian, ST = Subterminal, SAT = Satellited chromosome



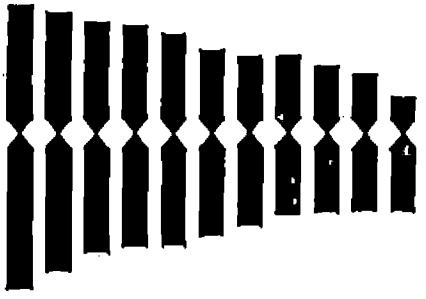
ARIPPA



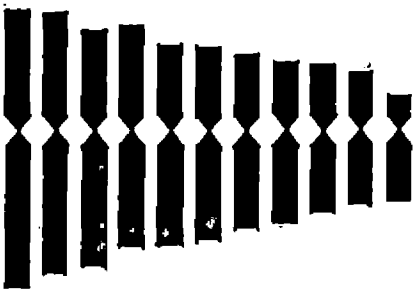
BAJPAI



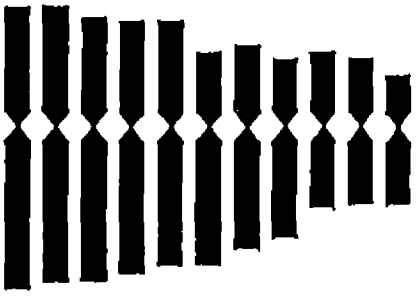
BURDWAN



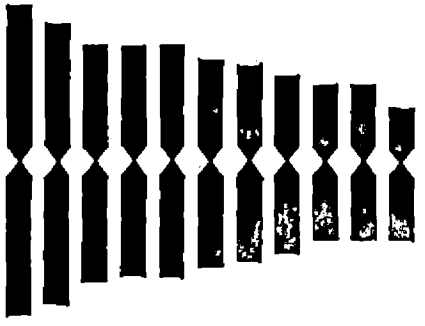
KURUPPAMPADI



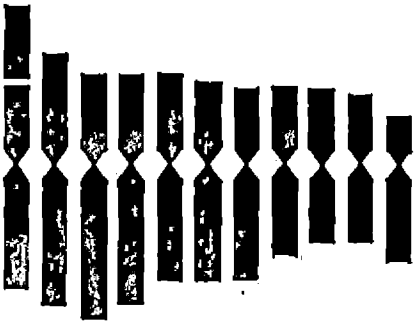
MARAN



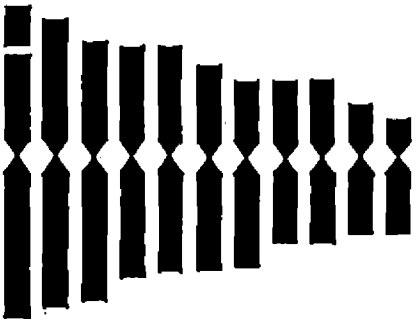
NADIA



NARASAPATTAM



RIO-DE-JANEIRO



VALLUVANAD

FIG. 2. IDIOGRAMS OF DIFFERENT VARIETIES OF GINGER.

Plate II. Mitotic chromosomes in some varieties of ginger (x5000)

a. Aripps

b. Burdwan

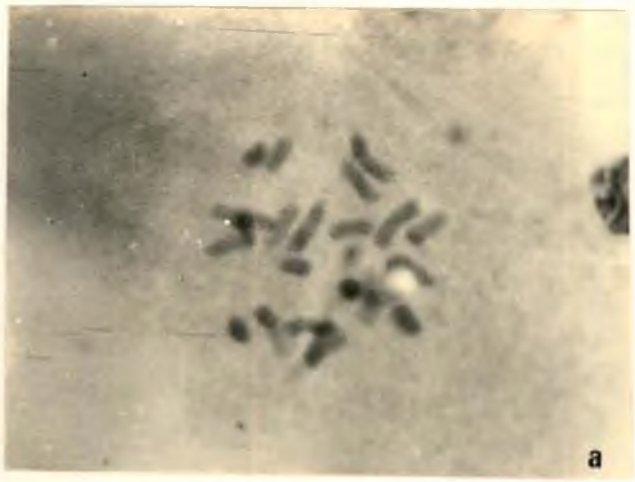
c. Maran

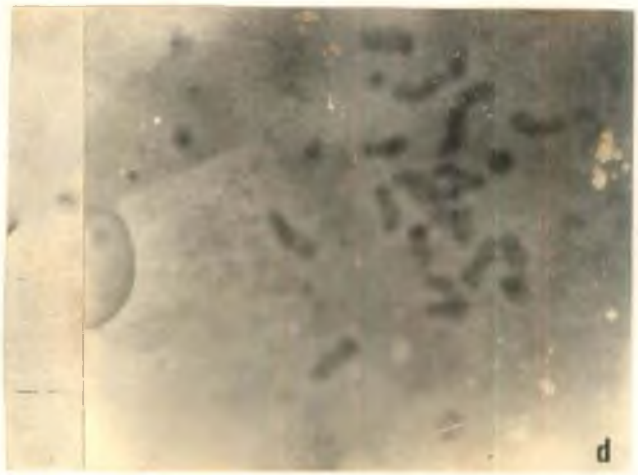
d. Nadia

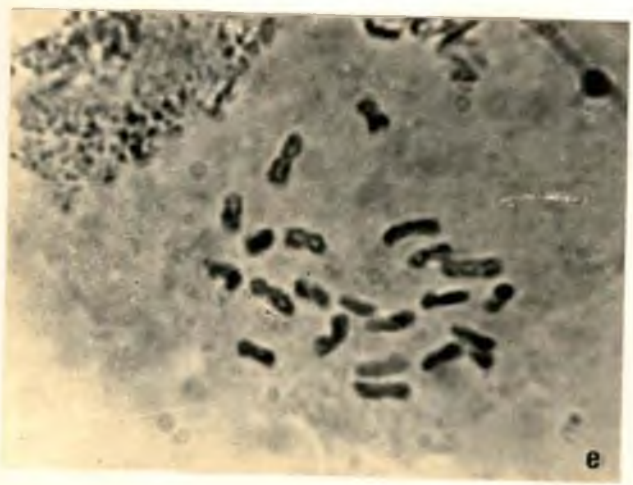
e. Rio-de-Janeiro

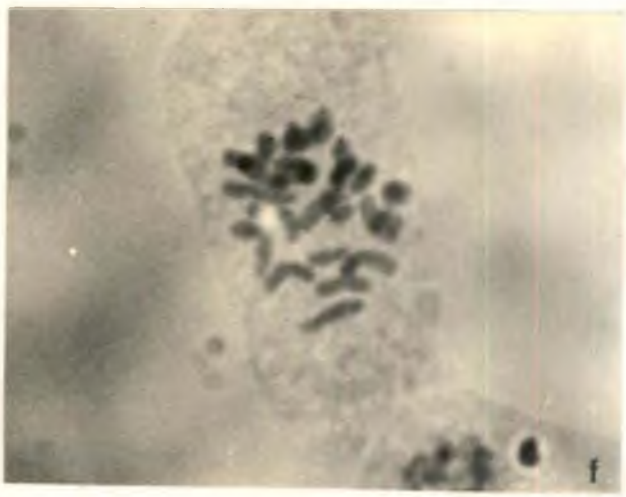
f. Valluvanad

Plate II









distinct satellite on the short arm. The volume of chromosomes ranged from 1.16 μm^3 to 3.10 μm^3 with a total estimate of 22.64 μm^3 for the entire haploid complement.

The karyotype differences between the varieties are summarised in Table 15. It be seen that the total chromatin length among the varieties ranged from 25.27 μ m for Arippa to 30.82 μ m for Nadia (Fig. 3). On the other hand, the total chromosome volume among the varieties ranged from 21.76 μm^3 for Arippa to 56.05 μm^3 for Nadia (Fig. 4). The maximum difference of chromosome length (1.50 - 4.03 μ m) was observed in Valluvanad and minimum in Burdwan (1.70 to 2.20 μ m).

b) Classification of karyotype according to Stebbins classification

The classification of karyotype according to the degree of asymmetry was also worked out for nine varieties of ginger using Stebbins classification is presented in Table 16. In general ginger varieties belong to the symmetrical groups 1a or 1b. However, the slightly asymmetrical nature of karyotype (between these two) as represented in 1b was observed in seven out of nine varieties excepting Burdwan and Rio-de-Janeiro. Those seven varieties belonging to 1b category had ratios of largest/smallest chromosomes between 2:1 and 4:1 and no chromosome with arm ratio

Table 15 Variation in length and volume of chromosomes in different varieties of ginger

	Variety	Range in Chromosome length (µm)	Average chromosome length (µm)	Total chromosome length (µm)	Total chromosome volume(μm^3)
1.	Arippa	1.40 - 3.55	2.29	25.27	21.76
2.	Bajpai	1.60 - 4.01	2.73	29.99	30.03
3.	Burduan	1.70 - 3.30	2.41	26.54	30.12
4.	Kuruppampady	1.50 - 3.78	2.54	27.99	35.39
5.	Maran	1.40 - 3.71	2.55	28.02	40.82
6.	Nadia	1.70 - 3.70	2.80	30.82	56.85
7.	Narasapattam	1.70 - 3.96	2.75	30.23	52.04
8.	Rio-de-Janeiro	1.88 - 3.68	2.61	28.67	44.48
9.	Valluvanad	1.50 - 4.03	2.68	29.44	22.64

Table 16 Karyotype symmetry/asymmetry in different varieties of ginger

	Variety	Proportion of chromosomes with arm ratio more than 2:1	Largest/smallest chromosome ratio	Stebbins classi-fication	Total form % (Huziwara 1962)
1.	Arippa	0.00	2.50 : 1	1b	46.54
2.	Bajpai	0.00	2.50 : 1	1b	48.63
3.	Burdwan	0.00	1.94 : 1	1a	45.44
4.	Kuruppampady	0.00	2.52 : 1	1b	47.48
5.	Maran	0.00	2.65 : 1	1b	44.14
6.	Nadia	0.00	2.18 : 1	1b	43.08
7.	Narasapattam	0.00	2.33 : 1	1b	48.66
8.	Rio-de-Janeiro	0.00	1.96 : 1	1a	41.44
9.	Valluvanad	0.00	2.69 : 1	1b	45.96

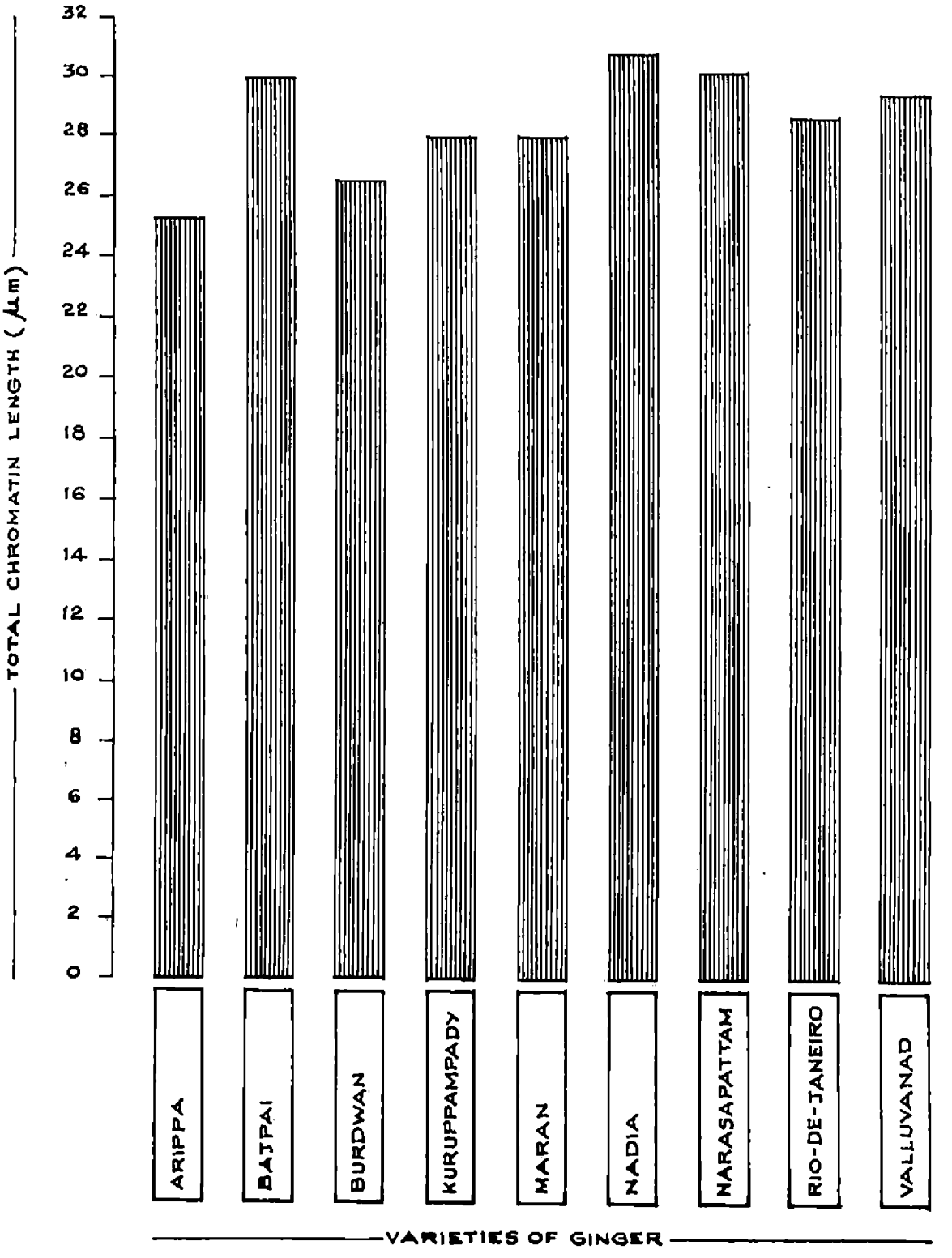


FIG. 3. TOTAL CHROMATIN (CHROMOSOME) LENGTH IN DIFFERENT VARIETIES OF GINGER.

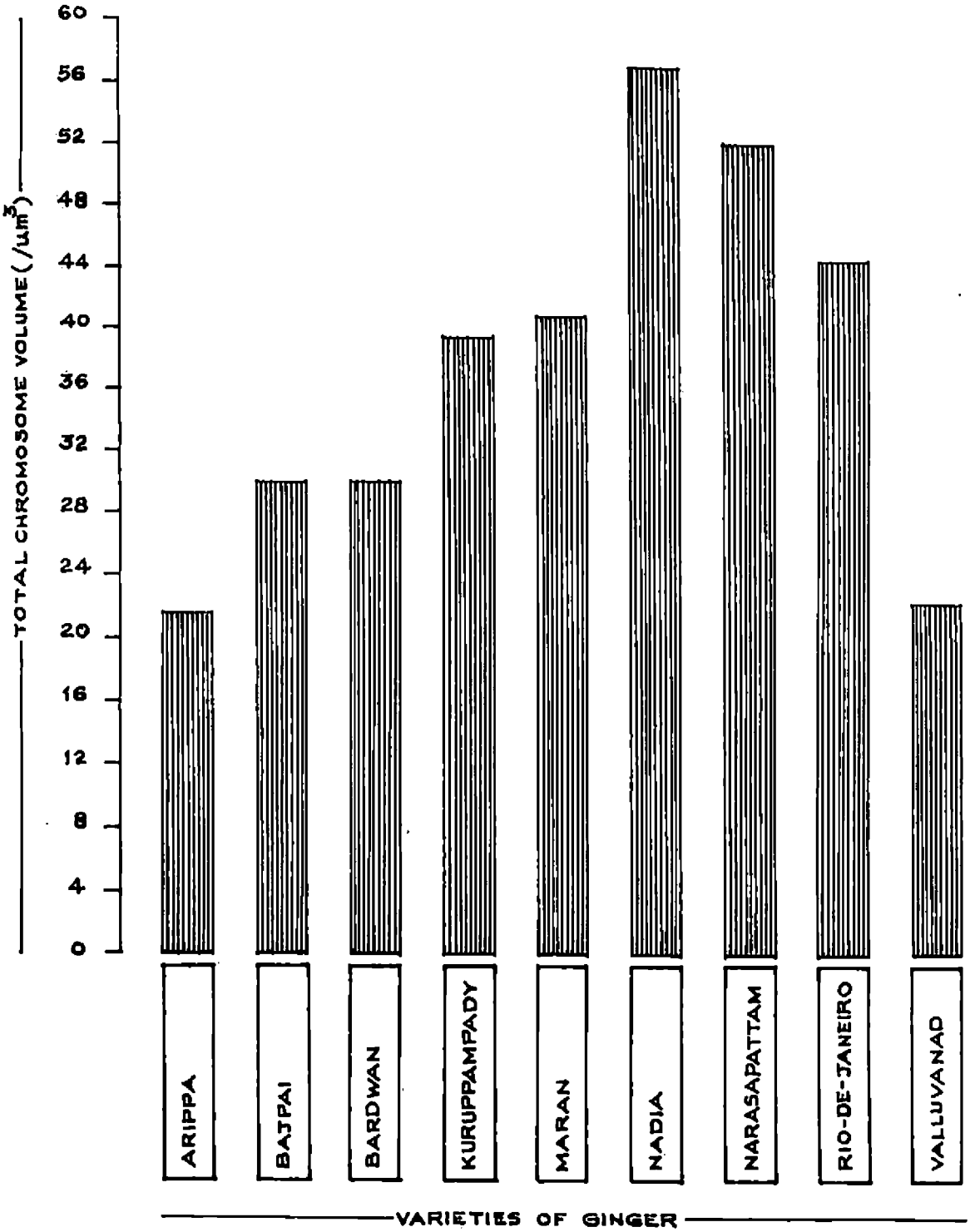


FIG. 4. TOTAL VOLUME OF CHROMOSOMES IN DIFFERENT VARIETIES OF GINGER.

171153

KERALA AGRICULTURAL UNIVERSITY LIBRARY THRISSUR 680 654

more than 2:1. On the other hand Burdwan and Rio-de-Janeiro were having largest/smallest chromosome ratio less than 2:1 and no chromosomes with arm ratio more than 2:1. The total form percentages of different varieties furnished in Table 16, exhibited a general trend in the evolution of karyotype. The values ranged from 41.44 per cent for Rio-de-Janeiro to 48.66 per cent for Narasapattam.

d) Variation in the number of median submediain and subtermenal chromosomes

The number of median, submedian and subterminal chromosomes in different varieties are presented in Table 17. Four varieties viz. Bajpai, Kuruppampady,

Table 17 Type of chromosomes in different varieties of ginger

	Variety	2n	Type of chromosome		
			Median	Submedian	Subterminal
1	Arippa	22	9	2	-
2	Bajpai	22	10	-	1
3	Burdwan	22	10	1	-
4	Kuruppampady	22	10	-	1
5	Maran	22	5	6	-
6	Nadia	22	5	6	-
7	Narasapattam	22	10	1	-
8	Rio-de-Janeiro	22	4	6	1
9	Valluvanad	22	8	2	1

Rio-de-Janeiro and Valluvanad exhibited a pair of subterminal chromosomes in the complement. The varieties Rio-de-Janeiro and Valluvanad have shown a distinct pair of satellited chromosomes also in the complement. The varieties Bajpai, Burdwan, Kuruppampady and Narasapattam were having more number of median chromosomes compared to other varieties.

e) Meiotic Studies

Ginger plants were found to flower very shyly. This was well illustrated in the morphological observations on flowering. The plants started flowering from middle of August and continued only untill middle of October. Only Maran, Rio-de-Janeiro and Valluvanad produced moderate number of scapes per pot. In all other varieties, many pots remained unflowered throughout the growing season. Therefore, defaulted meiotic studies were carried out only in Maran, Rio-de-Janeiro and Valluvanad. Also some flowers were available for meiotic studies from variety Nadia, which gave configurations at Anaphase I and Telophase I stage of meiosis. The meiotic configurations at Metaphase I stage of meiosis alongwith chiasma frequency are given in Table 18 and plate 3. The abnormalities at Anaphase I and Telophase I stage of meiosis in four varieties alongwith pollen sterility are furnished in Table 19.

In the variety Maran, out of 17 PMCs observed, one cell showed normal bivalent pairing. At Metaphase I mean bivalent frequency was more (5.41 per cell) which was followed by quadrivalents (1.59 per cell) univalents (1.47 per cell), trivalents (0.71 per cell), pentavalents (0.18 per cell) and hexavalents (0.06 per cell) in the order (Table 18). This variety showing the highest number of univalents also produced the lowest chiasma frequency (16.35 per PMC). At later stages of first meiotic division also, abnormalities were observed in the variety Maran (Table 19; Plate 3). In fact, normal Anaphase I was noticed only in 61.76 per cent of PMCs where as 20.5 per cent showed bridges and 17.64 per cent showed laggards. At Telophase I stage, 93 per cent PMCs showed normal division, 7 per cent produced abnormal division by producing micronuclei and formation of more than two groups of chromatin. Consequent to abnormalities, this variety produced a high percentage of sterile pollen grains (73.17 per cent).

Rio-de-Janeiro also showed abnormalities during meiosis. At Metaphase I, bivalents were more frequent (6.17 per cell), which was followed by quadrivalents (1.67) univalents (0.5), trivalents (0.5) and hexavalents (0.17). This exotic variety having the lowest number of univalents among the three varieties compared, exhibited the highest chiasma frequency (19.35 per PMC). Anaphase I abnormalities were also expressed in the form of bridges

Table 18. Chromosome configuration at Metaphase I during meiosis in different varieties of ginger

Variety	No.of cells observed	I	II	III	IV	V	VI	Chiasma frequency Mean ± S.E.
Maran	17	25 (1.47)	92 (5.41)	12 (0.71)	27 (1.59)	3 (0.18)	1 (0.06)	16.35±0.46
Rio-de-Janeiro	6	3 (0.50)	37 (6.17)	3 (0.50)	10 (1.67)	0 (0.00)	1 (0.17)	19.00±0.75
Valluvanad	5	4 (0.80)	33 (6.60)	4 (0.80)	7 (1.40)	0 (0.00)	0 (0.00)	17.4± 1.37

Values in parenthesis indicate mean frequency per cell.

Table 19. Number of cells showing abnormality at Anaphase I and Telophase II in different varieties

Variety	Total cells observed	Anaphase I			Telophase I			Pollen Sterility (%)
		Normal	Abnormal		Total cells observed	Normal	Abnormal	
			Bridge	Laggard				
Maran	68	42 (61.76)	14 (20.5)	12 (17.64)	172	160 (93.0)	12 (7.0)	73.17
Nadia	23	18 (78.26)	2 (8.69)	3 (13.04)	59	57 (96.61)	2 (3.38)	60.33
Rio-de-Janeiro	42	32 (76.19)	6 (14.28)	4 (9.52)	48	42 (87.5)	6 (12.5)	73.50
Valluvanad	29	17 (58.62)	5 (17.24)	7 (24.31)	30	25 (83.33)	5 (16.66)	75.48

Values in parenthesis indicate percentages.

Plate III. Meiotic configurations of ginger variety Maran

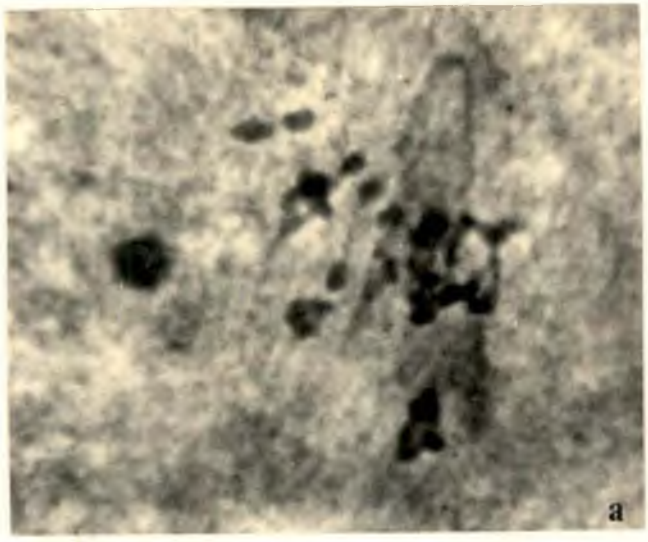
a: Diakinensis in Maran showing univalents and multivalents

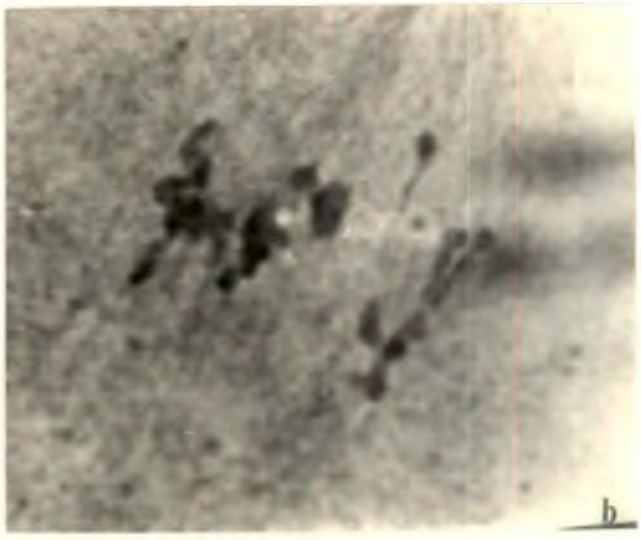
b: Metaphase I in Maran showing univalents and multivalents

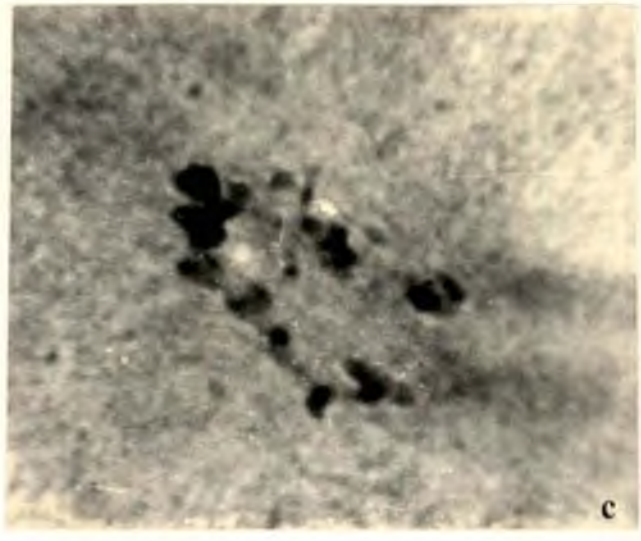
c: Anaphase I showing laggards.

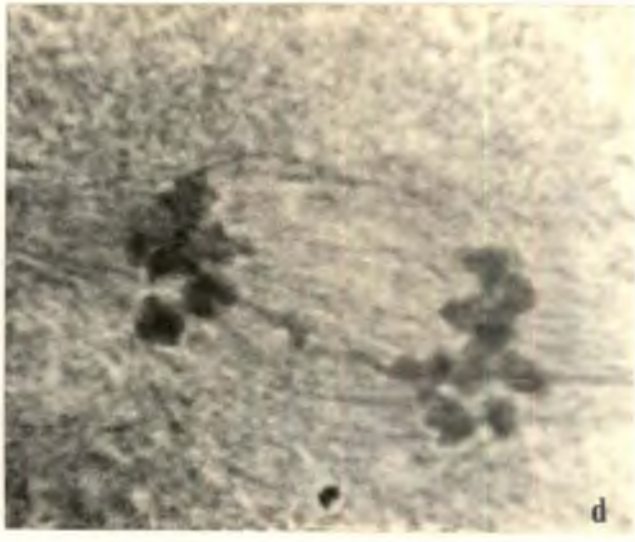
d: Anaphase I showing bridges.

Plate III









and laggards in 14.28 per cent and 9.52 per cent of the PMCs respectively. At Telophase I also 12.5 per cent PMCs showed abnormalities in the form of micronuclei and chromatin blocks. This exotic variety also produced 73.50 per cent sterile pollen grains.

In valluvanad, only five cells were obtained to score for Metaphase I configuration, which showed a bivalent frequency of 6.60 per PMC. Mean quadrivalents per cell was 1.40 and mean univalents and trivalents were 0.80 per PMC each. The mean chiasma frequency was 17.4 per PMC. At Anaphase I, bridges were observed in 17.24 per cent of PMCs and laggards in 24.31 of the cases. Telphase I abnormality was noticed in 16.66 per cent of the PMCs. This variety also produced a large proportion of sterile grains (75.48%). Both Anephase I and Telophase I abnormalities were frequent in variety Nadia, which produced 60.33 per cent sterile grains. In fact, Nadia has recorded the lowest Anaphase I and Telophase I abnormalities among the four varieties and produced the lowest percentage of pollen sterility.

3. Pollen morphology

The single bilocular anther of ginger contained a large amount of sticky pollen grains. The morphological features of pollen grains are presented in Table 20 and plate 4. The pollen grains were heterogeneous in size

Table 20. Pollen morphology in different varieties of ginger

	Variety	Average diameter (µm)	Shape	Presence of pores	Sterility(%)
1	Arippa	98.30	Round	Mono-sulcate	73.73
2	Bajpai	104.50	Round	Mono-sulcate	77.83
3	Burdwan	94.75	Round	Mono-sulcate	84.42
4	Kuruppampady	96.76	Round	Mono-sulcate	67.73
5	Maran	85.16	Round	Mono-sulcate	73.17
6	Nadia	106.00	Round	Mono-sulcate	60.33
7	Narasapattam	94.50	Round	Mono-sulcate	75.73
8	Rio-de-Janeiro	110.60	Round	Mono-sulcate	73.50
9	Valluvanad	93.75	Round	Mono-sulcate	75.48

Plate IV. Morphology of pollengrains in different varieties of ginger

A: Arippa

B: Bajrai

C: Burdwan

D: Kuruppampady

E: Maran

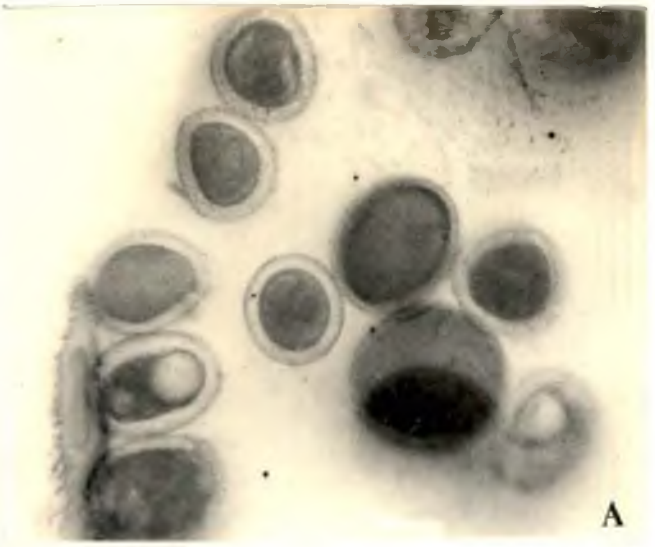
F: Nadia

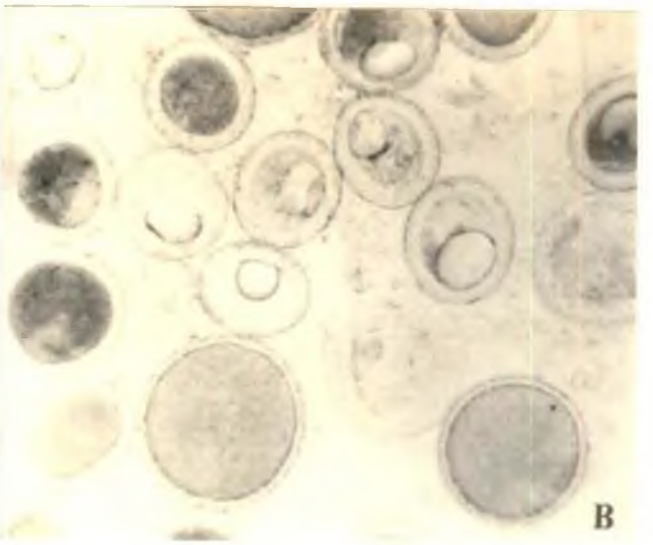
G: Narasapattam

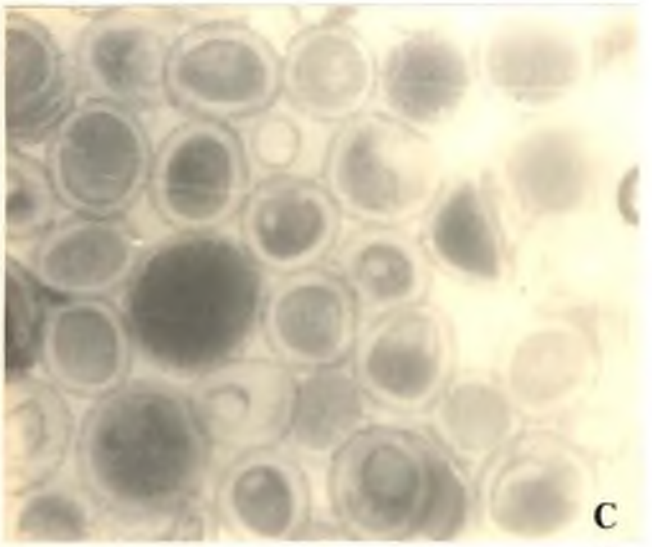
H: Rio - de - Janeiro

I: Valluvanad

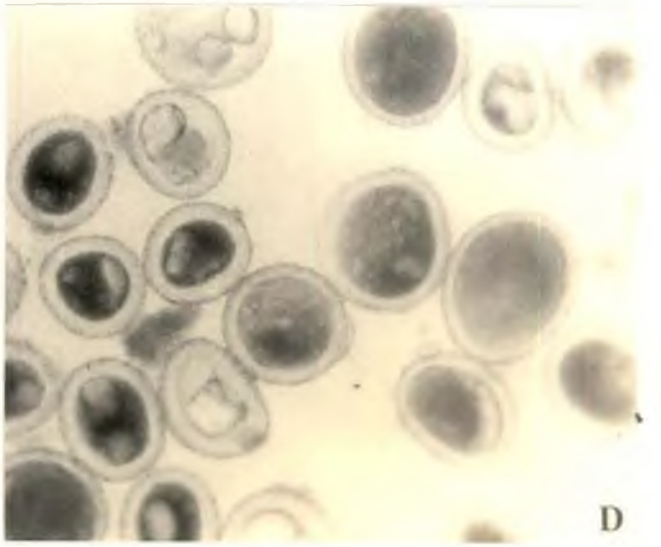
Plate IV

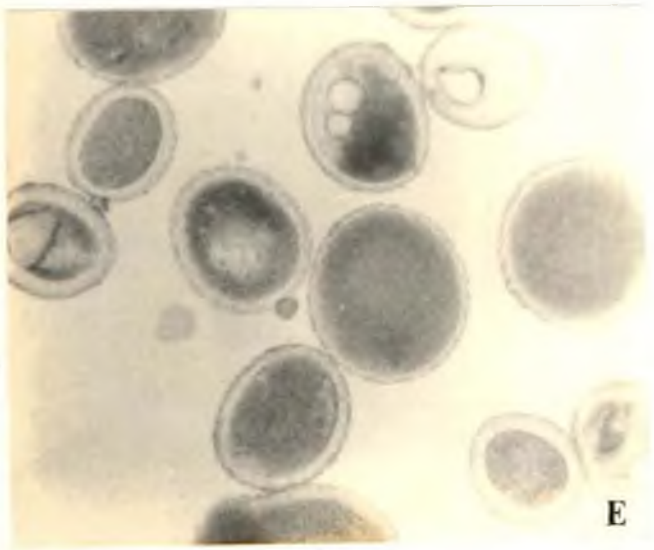

A

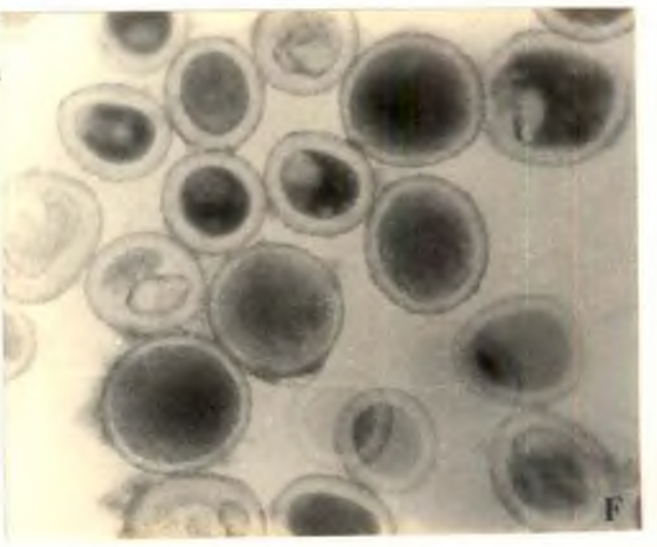

B

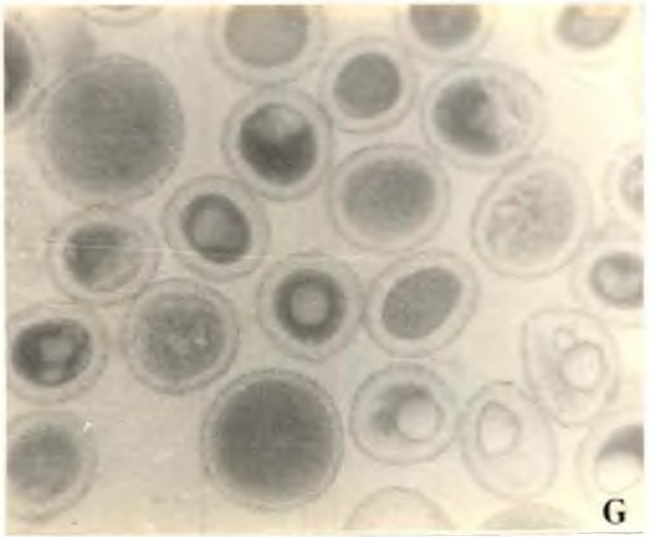

C

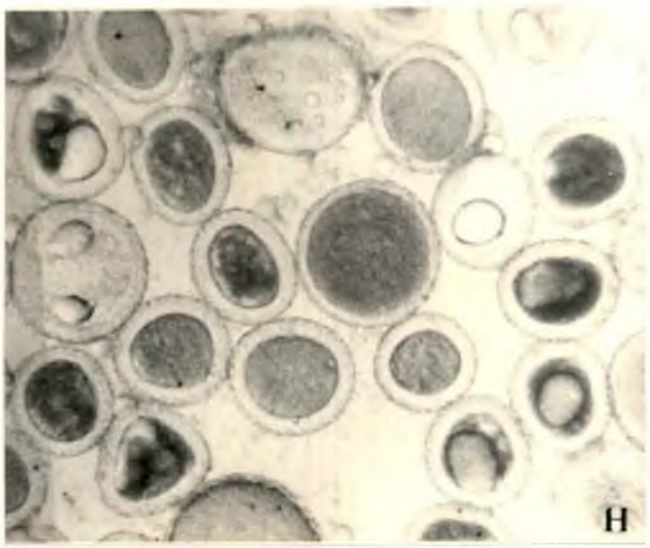
C

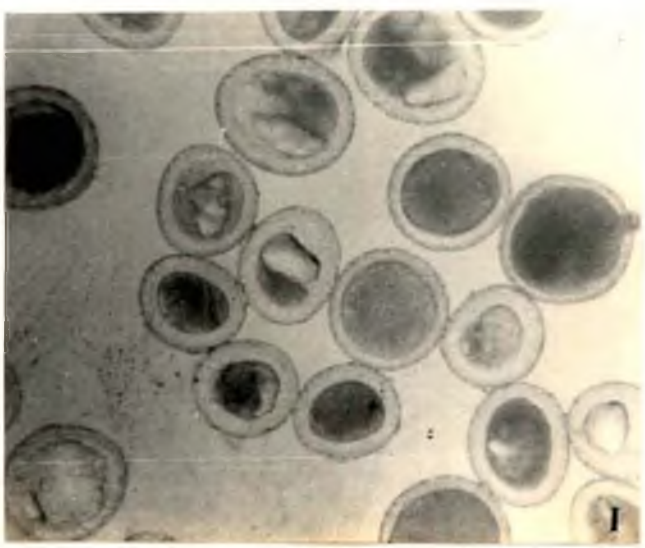

D


E


F


G


H


I

and were limited by a thick exine. They were round in shape and were monosulcate as far as pores are concerned.

a) Pollen fertility

All the ginger varieties used in the present study produced large amount of sterile grains (Table 20). Pollen fertility among varieties as assessed by stainability in acetocarmine ranged from 15.58 per cent in Burdwan to 39.67 per cent in Nadia. Excepting Nadia, all varieties produced high percentage of sterile grains (70.80 per cent). The mean diameter of pollen grains ranged from 85.16 μ m for Maran to 110.60 μ m for Rio-de-Janeiro. However, within a variety, the variation in the diameter of pollen grains is quite high.

b) Pollen germination

Pollen grains of all varieties were collected at the time of anther dehiscence and percentage of germination in different media were determined. Mean percentages of germination of pollen grains are presented in Table 21 and the analysis of variance is given in Table 22. It was observed that all nine varieties have poor percentage of germination of grains in distilled water and the mean values ranged from 1.97 per cent to 6.71 per cent. The medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin produced the highest percentage of germination (11.81%). This was followed by the medium having 30% sucrose + 60 ppm boric acid + 1% gelatin (8.63%) and subsequently by the

Table 21. Mean pollen germination (%) of ginger varieties in different media

Varieties	Medium 1 Distilled water	Medium 2 8% S+60 ppmB	Medium 3 15%S+60 ppmB	Medium 4 30%S+60 ppmB	Medium 5 8% S+60 ppmB+1%G	Medium 6 15%S+60 ppmB + 1% G	Medium 7 30%S+60 ppmB + 1% G
Arippa	3.97	4.09	6.42	4.91	12.34	7.08	6.46
Bajpai	4.00	5.26	3.51	2.49	11.12	8.62	7.87
Burdwan	1.97	3.07	3.19	2.20	7.46	4.80	6.99
Kuruppampady	6.506	9.23	9.36	7.53	13.97	10.73	12.75
Maran	3.87	5.68	5.29	4.32	11.95	7.41	8.17
Nadia	5.4	7.14	4.53	5.16	14.61	9.35	11.31
Narasapattam	4.0	6.02	4.88	5.29	10.59	6.62	7.98
Rio-de-Janeiro	6,74	4.52	7.34	4.35	11.77	7.57	8.78
Valluvanad	3.87	5.03	7.21	5.33	12.5	10.9	7.37
Mean	4.48	5.56	5.75	4.62	11.81	8.12	8.63

C D for media = 0.31 C D for variety = 0.27

S = Sucrose G = Gelatin B = Boric acid

Table 22. Analysis of variance for germination of Pollengrains of different varieties of ginger under different media

Source	df	S.S	M.S.S	F Value
Media	8	77.342	9.668	7.369**
Variety	6	271.252	45.209	34.457**
Media x Variety	48	42.570	0.887	0.676
Error	88.2	1157.207	1.312	

** Significant at 5% level

CD for media = 0.31 CD for variety = 0.27

medium having 15% sucrose + 60 ppm boric acid + 1% gelatin (8.12%). It was also noticed that boric acid had some positive influence on the germination of pollen grains. The microphotographs of the germinated pollen grains after 24 hours of incubation in some varieties of ginger under certain media are presented in Plate 5. On comparing the varieties and media together, it was found that the variety Nadia has recorded the highest percentage of germination in medium having 8% sucrose + 60 ppm boric acid + 1% gelatin (14.61%).

The correlation coefficient between pollen sterility and pollen germination was calculated. The correlation coefficient was significant and negative (0.92). Also

Plate V. Pollen germination in some varieties of ginger.

A: Bajpai

B: Kuruppampady

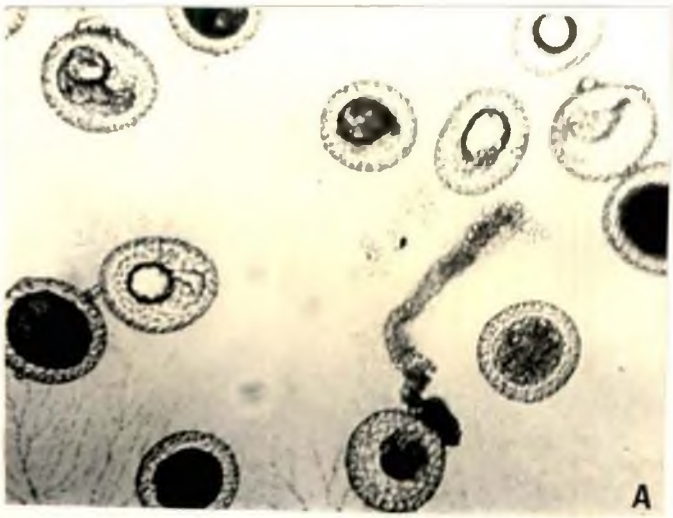
C: Maran

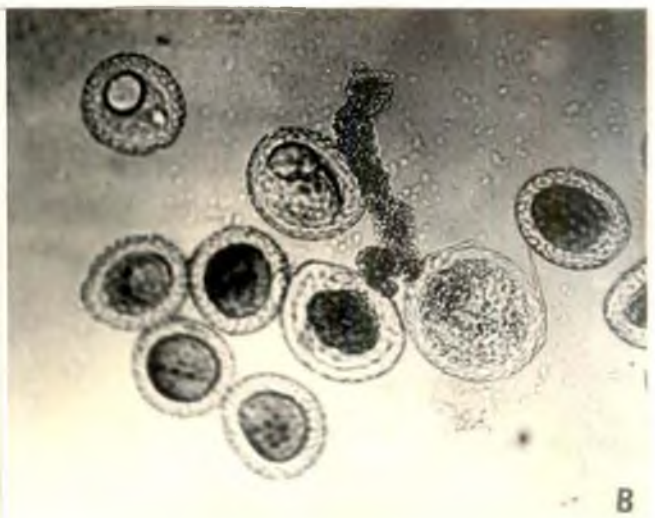
D: Nadia

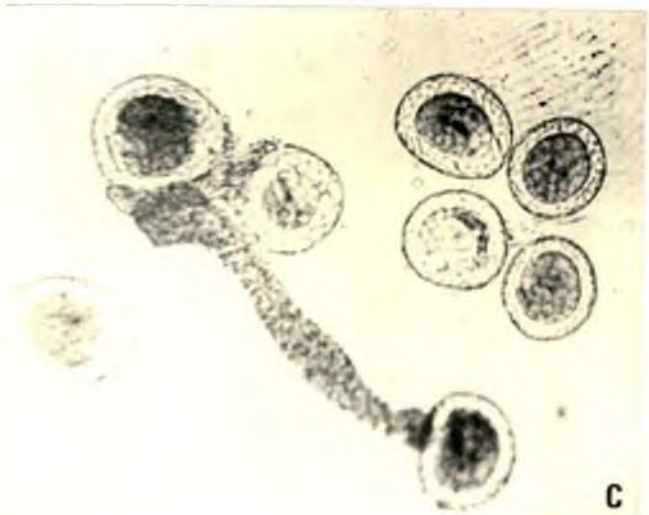
E: Narasapattam

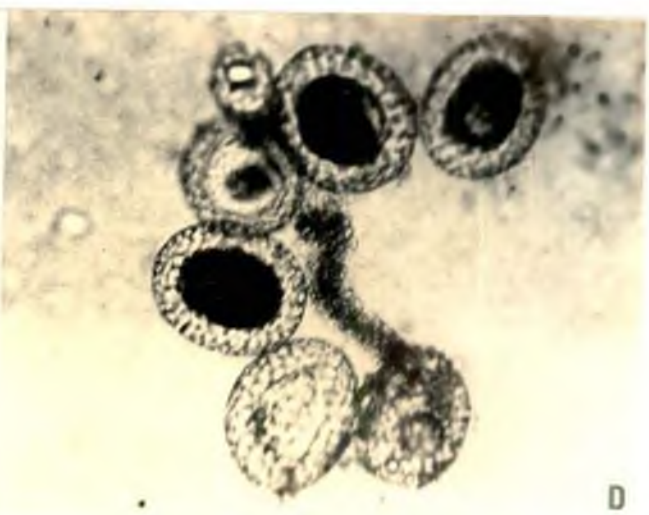
F: Rio - de - Janeiro

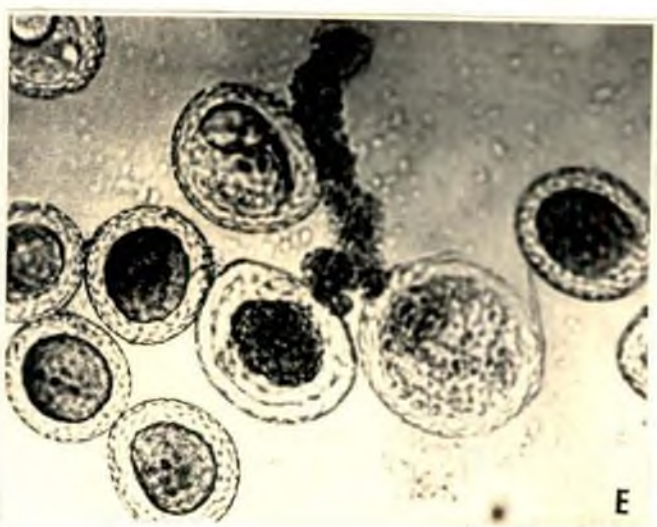
Plate V

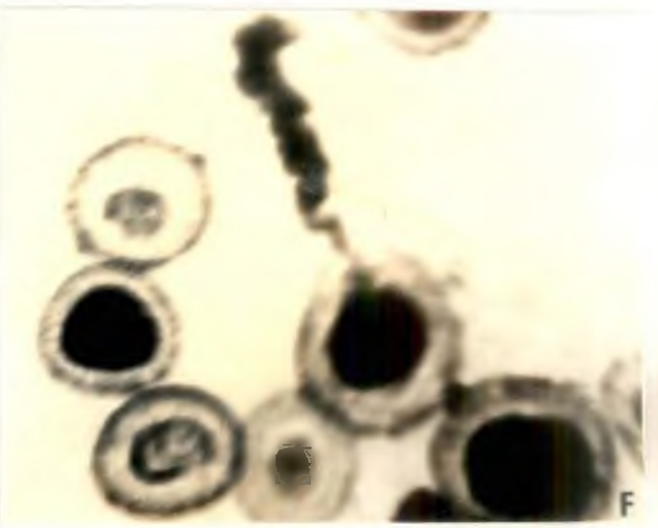

A


B


C


D


E


F

the variety having the lowest pollen sterility (60.33%) among the varieties used in the present study, exhibited the highest percentage of germination (14.61%). Conversely, Burdwan having the highest pollen sterility (%) showed the lowest percentage of germination of pollen grains (7.46%). The pollen grains after 24 hours of incubation in B.O.D. incubator produced pollen tubes of varying lengths in different media. The mean pollen tube lengths in different varieties under different media are compiled in Table 23. It was found that the variety Valluvanad had the longest pollen tubes if all the media are considered. But, Nadia has recorded the maximum pollen tube length of 108 μ m in the most suitable medium noticed in the study. Considering all media and all varieties together, the in vitro pollen tube length after 24 hours of incubation in ginger is only 60.01 μ m. On comparing the media alone, the medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin which gave the highest percentage of germination ranked first with regard to pollen tube length (83.33 μ m). This was followed by the medium having 30% sucrose + 60 ppm boric acid + 1% gelatin. The media having gelatin in all cases produced a better growth of pollen tubes.

c) Germination of irradiated pollen grains

The pollen grains of the variety Maran were irradiat with varying doses of gama rays. The germination capacit of the grains were later determined by keeping in differe media for 24 hours in a B.O.D. incubator. The mean perce age of germination of irradiated grains is given in Table

Table 23. Average length of the pollen tube (μ m) in different media in different varieties

Sl. No.	Variety	Medium 1 Distilled water	Medium 2 8% S+60 ppm	Medium 3 15%S+60 ppm	Medium 4 30%S+60 ppm	Medium 5 8%S+60 ppmB+1%G	Medium 6 15%S+60 ppm+1%G	Medium 7 30%S+60 ppmB+1% G	Mean
1.	Arippa	48	57	43	38	65	58	62	53
2.	Bajpai	44	53	41	42	92	54	58	54.85
3.	Burdwan	39	48	46	51	68	49	64	54.85
4.	Kuruppampady	38	44	51	48	70	45	48	49.14
5.	Maran	44	52	58	60	84	48	72	59.71
6.	Nadia	51	39	52	58	108	62	73	63.28
7.	Narasapattam	42	56	62	68	88	74	82	67.42
8.	Rio-de-Janeiro	38	48	50	62	84	92	87	65.85
9.	Valluvanad	44	38	59	48	91	78	68	72.0
	Mean	43.11	48.33	51.33	52.77	83.33	62.22	68.22	

S = Sucrose B = Boric acid G = Gelatin

Table 24 Mean germination(%) of irradiated pollen grains of variety Maran in different media

Media	Germination percentage								
	0 KR	0.5 KR	1 KR	5 KR	10 KR	25 KR	50 KR	100 KR	Mean
Medium 1 (Distiller water)	3.87	3.22	3.12	2.91	2.85	2.45	1.90	0	2.54
Medium 2 (8% Sucrose + 60 ppm Boric acid)	5.68	4.02	3.82	3.65	3.28	3.12	2.43	0	3.24
Medium 3 (15% Sucrose + 60 ppm Boric acid)	5.29	4.62	3.52	3.16	3.04	2.56	2.34	0	3.06
Medium 4 (30% Sucrose + 60 ppm Boric acid)	4.32	3.73	3.33	3.20	3.14	3.03	2.50	0	2.90
Medium 5 (8% Sucrose + 60 ppm Boric acid + 1% gelatin)	11.95	10.38	7.74	5.33	5.03	4.58	3.04	0.61	6.08
Medium 6 (18% Sucrose + 60 ppm Boric acid)+ 1% gelatin	7.41	6.12	5.41	4.76	4.47	4.16	3.18	0.50	4.50
Medium 7 (30% Sucrose + 60 ppm Boric acid + 1% gelatin)	8.17	7.10	6.33	5.42	5.12	4.57	3.10	0.59	5.05
Mean	6.67	5.59	4.75	4.06	3.84	3.49	2.64	0.24	

CD for media = 0.35, CD for irradiation = 0.30

and Fig. 5. The statistical analysis is furnished in Table 25. It was observed that germination was more in the non-irradiated grains than irradiated grains, in all media. The pollen germination (%) decreased with increase in irradiation dose (Fig. 5). The medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin was found to be the best even for irradiated grains. Earlier this medium has given the highest germination percentage for non-irradiated grains in all varieties of ginger.

4. Pollen pistil interaction

The technique of fluorescence microscopy was found to be quite suitable for studying the pollen-pistil interaction in ginger. Ginger plants flowered

Table 25. Analysis of variance for germination of irradiated pollen grains variety Maran

Sucrose	df	MSS	F.Value
Media	6	16.974	8.804**
Irradiation doses	7	42.141	21.858**
Media x dose	42	0.865	0.448
Error	784	1.928	

** Significant at 1% level

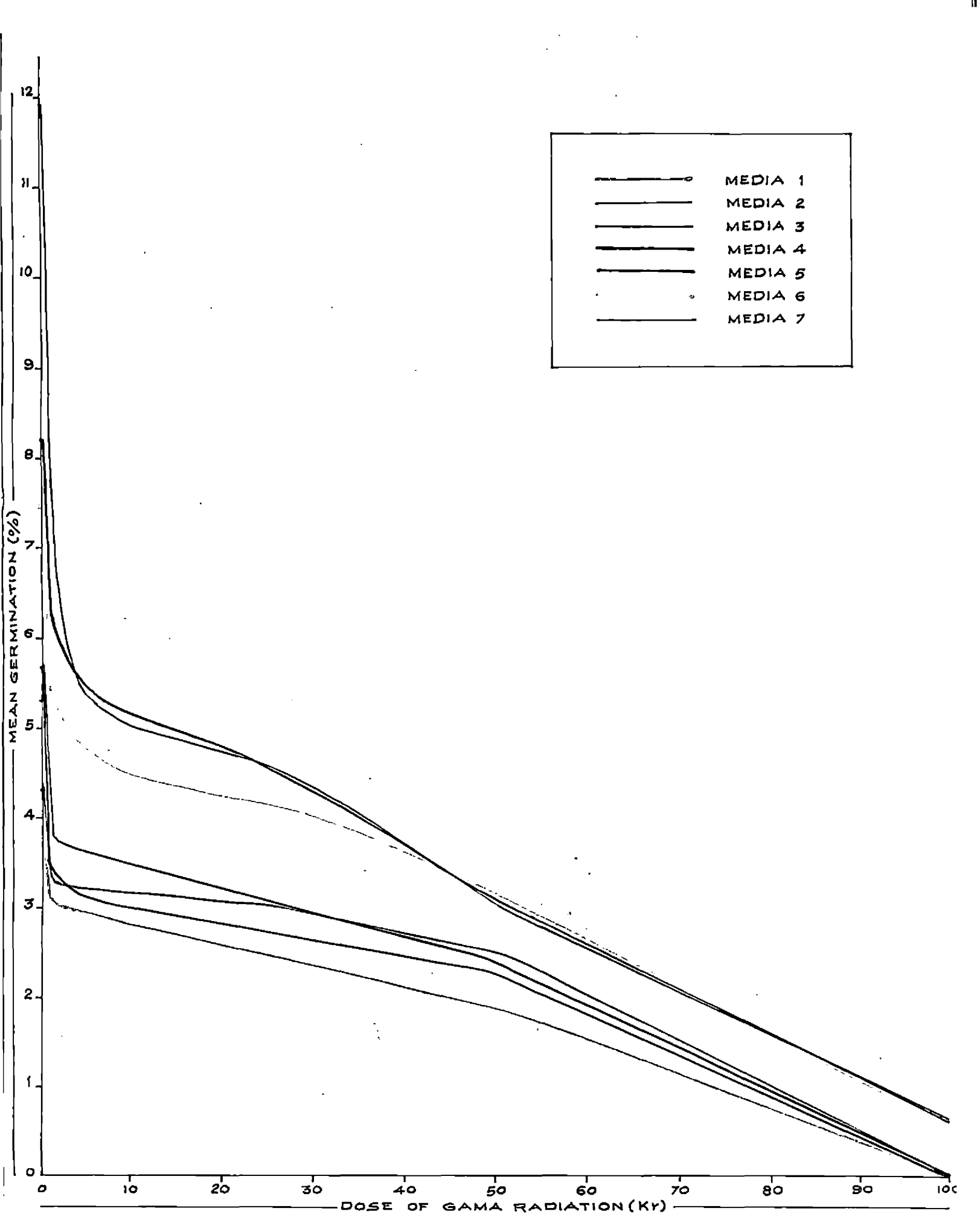


FIG.5. MEAN GERMINATION (%) OF IRRADIATED POLLEN GRAINS OF VARIETY MARAN IN DIFFERENT MEDIA.

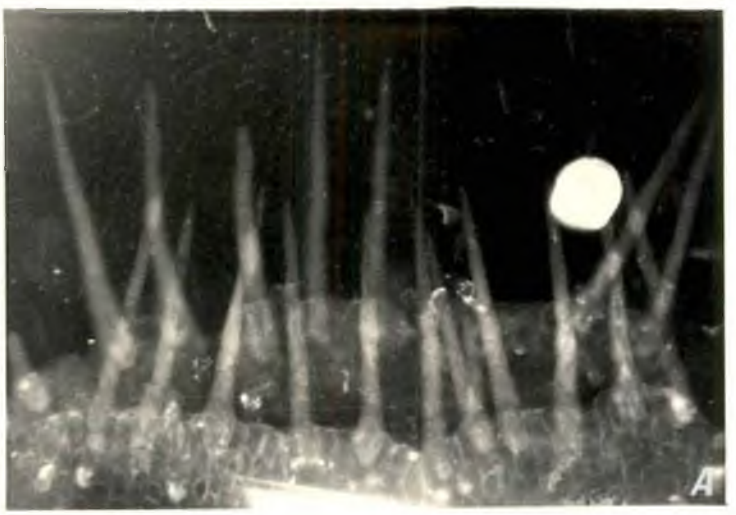
during the middle of August to the middle of October and the time of anthesis was between 3.30 to 4.30 PM. In the present study of pollen pestil interaction, artificial pollination was performed as a precaution to ensure enough pollen grains on the stigma. The pollen grains were sticky and the stigma at the time of receptivity produced an ooze which favoured the adherence of pollen grains on the stigma. It was also noticed that the flowers of ginger abscise in the stylar region before 12 hours of anthesis. However, with strenuous efforts, pistils were collected at 0, 2, 4, 8 and 12 hours after pollination (extending to night hours) and analysed microscopically under UV. It was found that the stigma of ginger flower is highly spiny in nature, the number of spines was about 26-30 per stigma (Plate 6A). Also the spines were closely arranged on the stigmatic surface. The ovules of ginger are lemon-shaped and were highly luminous with fluorescence staining (Plate 6B). The pollen grains applied on the stigma were either sticking to the spine tips or were damaged by the spines. Because of the closeness of spines, it was quite difficult for the pollen grains to gain contact with the stigmatic surface. At 2 hours after pollination, the stigma has shown enough fertile and sterile grain on the stigma in all the pollinated pistils, but none of them germinated

Plate VI. Appearance of Pistil in ginger variety Maran

A. Lemon shaped and luminous stigma covered with spines.

B. Ovules of ginger under UV.

Plate VI



(Plate 7A). However, examination of the pistils at 4 hours of pollination revealed two pollen grains out of twenty observed on the stigma were germinating in two slides. This contributes to about 10 per cent germination, which compared very well with *in vitro* germination of grains. The length of the pollen tube was about 70 µ m (Plate 7B), but the pollen tube was not penetrating the stigma. One of the germinated pollen grains on the stigma also showed coiled pollen tube growth, which was also noticed in the *in vitro* pollen germination (Plate 7C). Unfortunately the pistiles after 8 and 12 hours of pollination and also the stigma of shed flowers did not reveal any germination of grains on the stigmatic surface. The average length of style in Maran was 3.9 cm (39,000 µ m).

Overcoming the barriers in fruitset and seedset

In order to overcome the barriers in seedset, various pollination techniques such as artificial self pollination, artificial cross pollination, artificial sibbing, artificial cross pollination between varieties, bud pollination, mentor pollination with a mixture of normal and irradiated pollen grains, mixed pollination with pollen grains of different varieties, and chemically aided pollination were carried out in two varieties viz. Maran and Rio-de-Janeiro.

Plate VII. In vivo pollen pistil interaction in ginger variety

A: Ungerminated pollen grains 2 hours after pollination.

B: Germinated pollen grain with pollen tube grows between two spines on stigma.

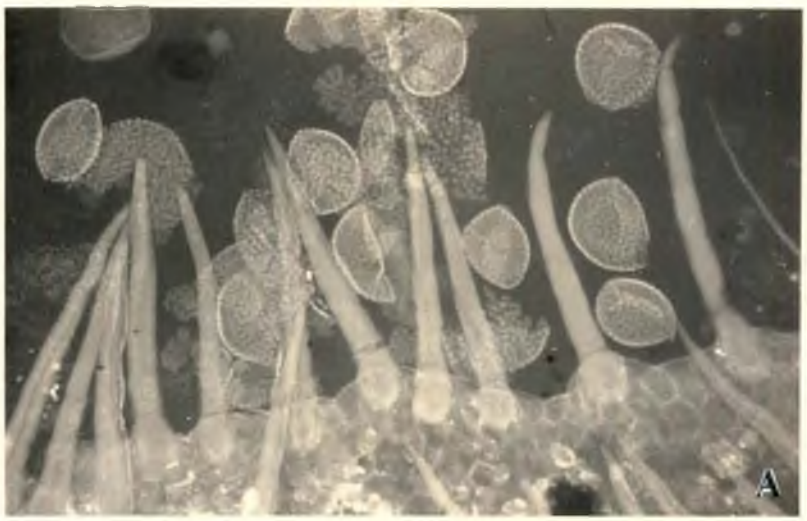
C: Coiled pollen tube growth.

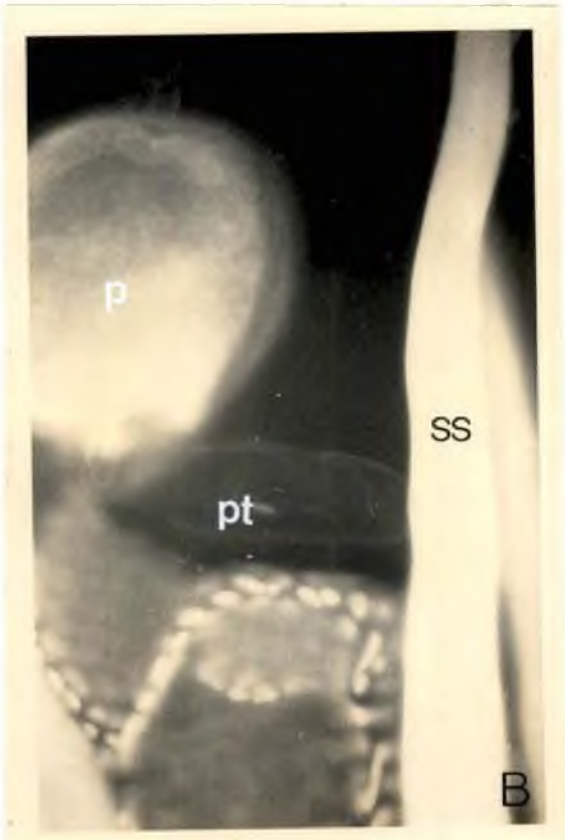
P = Pollengrain

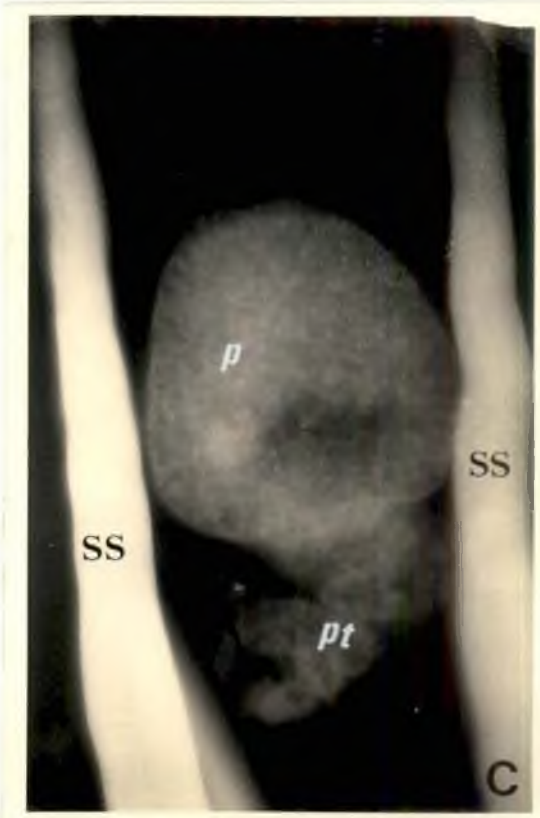
SS = Stigma spine

Pt = Pollen tube

Plate VII







Both these varieties produced enough flowers and hence twenty five flowers each were used for different types of pollination. Even the stigma and style were removed at different heights in these varieties and artificial pollination was employed. Unfortunately none of these pollination techniques was effective for the setting of fruits or seeds in ginger.

Discussion

DISCUSSION

Ginger (Zingiber officinale Rosc.), the only cultivated species of the genus Zingiber (Family Zingiberaceae) is distributed throughout tropical South East Asia extending to Queensland and Japan, tropical Africa and Central America. It is valued as an important spice, outsteemed for its aroma, flavour and pungency. In recent years, the use of ginger and its products have considerably increased. Hence there is good demand for this spice in the world market. The increasing demand and its suitability for varied preparations have put forth the need for promising ginger types having special quality attributes. Unfortunately, the types having the twin advantages of high yield and special quality attributes are rare at present. Further, resistant types against the devastating diseases of ginger such as soft rot and bacterial wilt are yet to be developed. The crop improvement programmes carried out so far have been mainly to select the best suited cultivar for the local conditions. Hybridization between cultivars and between other species has not been reported so far. Hence the inherent variability is yet to be exploited for the benefit of ginger cultivation. The greatest handicap in the breeding of ginger is the lack of fruitset and seedset. Furthermore, only

a few species of Zingiber and a few cultivars of ginger flower, that too for a restricted period of the year.

The genus Zingiber has been adequately treated by taxonomists (Schumann, 1904; Burkill, 1966; Purseglove et al. 1981). However cytogenetical investigations in Zingiber are quite meagre. As in many crops like cereals and potato identification of varieties in ginger are quite difficult due to the lack of enough morphological markers. In many crop species cytogenetical, protein and nucleic acid polymorphisms have been suggested to be useful for the identification and registration of varieties (Beckmann and Soller, 1986; Evola et al., 1986). The present investigation was aimed to study the morphological, cytological and pollen morphological variation in different varieties to find out reason(s) for the absence of fruitset and seedset and also to identify the means for achieving seedset in ginger.

1. Morphological studies

Zingiber officinale is a perennial rhizomatous herb, bearing leafy shoots close together, often with many leaves and robust branched rhizomes borne horizontally near the surface of the soil. Nybe (1978) reported that it was very difficult to classify the different types of ginger based on

morphological attributes. The identification of varieties based on morphological attributes is extremely strenuous in a standing crop. In the present investigation, the statistical analysis of morphological observations did not reveal any significant difference between varieties. This may be because of the prominent influence of environmental factors on morphological characters. Morphological characters are influenced to a great extent by soil and climatic factors (Friend, 1966). Hence their use as identifying characters are seldom recommended in cereals and potato. The results obtained in the present study also indicate that morphological characters cannot be relied upon for identifying varieties of ginger.

2) Cytogenetical studies

The use of cytogenetic investigations as an aid in establishing phylogenetic relationship and also in explaining the mechanism responsible for sterility has been recognized by many workers (Levitzky, 1931; Babcock et al., 1942; Stebbins, 1974). Absence of morphological difference between varieties adds to the use of cytogenetical investigations for the characterisation of varieties of ginger. In crops like wheat, rice, maize, potato, etc. biochemical and cytogenetical

techniques are used now-a-days to characterise and register different varieties. Isozyme markers, protein markers, restriction fragment length polymorphisms (RFLP), etc. have been ascribed as useful tools in this direction. The cytogenetical parameters for the identification and characterisation of varieties and cultivars of crops have been also mentioned (Brandenburg, 1984; Ramachandran et al., 1985).

a) Mitotic studies

In the present investigation, collection of roots in the morning hours repeatedly gave very low mitotic index in the root tip squashes. Hence a necessity was imposed to standardise the optimum time of collection of roots for mitotic studies in ginger. The mitotic index of roots collected at 2 hour intervals in a full day cycle of 24 hours, has shown clearly that cell division in roots is more in the night than in the day time (Fig. 1). Mitotic index increased from 6 PM. onwards and reached the maximum of 41.03 per cent by 6 AM. Hence optimum time for collection of roots for mitotic studies in ginger will be between 5-6 AM. Though it is inconvenient for a researcher to collect the root at such an early time, there is no other way as there is drastic reduction in mitotic index afterwards, as the day light comes in.

Incidentally it has gone to the lower level of 4.73 per cent by 8 AM. It may be worthwhile if efforts are made to unravel the physiological effect of day light on the reduction of mitotic index in ginger. Unfortunately, the physiology of ginger including flowering is seldom understood.

A number of report have appeared on the chromosome number of Zingiberaceae (Morinaga et al., 1926; Suguira, 1936; Raghavan and Venkatasubban, 1943; Chakrovorthi, 1948). Since then at least four extensive works on chromosome number and morphology have been reported in Zingiberaceae (Sato, 1960; Ramachandran, 1969; Mahanty, 1970; Ratnambal, 1979). In the present investigation, the somatic chromosome number was determined in nine varieties of ginger. It was found that 2n = 22 is constant among the nine varieties of ginger. Ratnambal (1984) also did not observe any variation in the number of chromosomes between varieties of ginger. Hence numerical variation in the evolution of different varieties of ginger would not have occured.

The nine varieties of ginger used in the present investigation did not exhibit any significant morphological difference. It is also quite difficult to distinguish and characterise the different varieties based on morphological attributes as they are influenced

to a great extent by soil and climatic factors. In this context, karyotype details may be useful for the characterisation of individual varieties of ginger either alone or in combination with biochemical attributes and morphology (Brandenburg, 1984). Cytogenetical parameters are seldom influenced by environment. The karyotype of nine varieties of ginger studied in the present investigation showed considerable differences in their morphological features such as length of chromosomes, centromere position, total chromatin length and total chromosome volume. Such differences would have occured through translocation, inversion and deletion of chromosome segments (Stebbins, 1950; 1958). In fact, ginger plant has the ability to sustain these chromosomal mutations by vegetative propagation. The varieties Arippa, Burdwan, Kuruppampady and Narasapattam having the largest number of median chromosomes and no subterminal chromosomes can be considered as primitive varieties. However, the varieties Rio-de-Janeiro, Maran, Nadia and Valluvanad were having more submedian and one subterminal chromosomes. These karyotypic changes would have occured through gross chromosomal mutations such as translocations inversions and deletions. Such changes are quite possible in ginger, as enough meitotic abnormalities have been observed in different varieties (Ratnambal, 1979; present investigation).

Stebbins classification (1958) recognize three grades of differences between the largest and the smallest chromosomes of the complement, and four degrees with respect to proportion of chromosomes which are acro, telo or metacentric. The classification of the Karyotype of different varieties showed that the varieties, in general have a symmetrical karyotype, since they fell into 1a and 1b group. Thus it can be inferred that Zingiber officinale belong to the primitive species of angiosperms (Stebbins, 1958). However, slightly asymmetrical karyotype represented in 1b as compared to 1a was observed in seven out of nine varieties. The karyotype of the varieties of Z.officinale has remained relatively symmetrical without much large changes, due to the lack of recombination and evolution by sexual process. This was also favoured by the vegetative method of propagation in ginger.

b) Meiotic studies

The analysis of microsporogenesis in four varieties of ginger carried out in the present investigation has shown some interesting features. The genome of ginger is highly unstabilized as far as meiosis is concerned. Out of the three varieties that could be compared at Metaphase I, Maran having the highest mean frequency of univalents showed the lowest chiasma frequency.

Rio-de-Janeiro, on the other hand produced the highest number of bivalents and chiasma frequency. However all three varieties produced multivalents and univalents in addition to bivalents in pollen mother cells (PMCs) at Metaphase I stage. The presence of quadrivalents and hexavalents in PMCs indicated that at least four to six chromosomes are involved in translocations in these varieties (Sybenga, 1975). The variation in chromosome association of different varieties of ginger, which consisted of univalents, trivalents, quadrivalents, pentavalents and hexavalents besides bivalents was possibly the outcome of irregular pairing of chromosomes due to translocations (Katiyar, 1978). The presence of univalents at Metaphase I has been variously attributed to failure of chromosomes to pair at zygotene or failure to form chaisma among paired partners. Un equal and delayed separation of multivalents may also give rise to univalents (Ratnambal, 1979).

Abnormalities were also observed as bridges and laggards at Anaphase I and as micronuclei formation at Telophase I stages of meiosis. The four varieties of ginger viz., Maran, Nadia, Rio-de-Janeiro and Valluvanad which could be studied in this respect showed varying degrees of abnormalities. The inter-varietal difference in the degree of abnormalities can be attributed to the role played by meiotic genes

(Grant et al., 1962; Rees, 1961) or may be the outcome of irregular pairing by transolocations (Katiyar, 1978). The variety Valluvanad having the highest percentages of abnormal Anaphase I and Telophase I PMCs produced the highest percentages of sterile grains (vide Table 19). On the other hand Nadia having the lowest percentages of abnormal Anaphase I and Telophase I PMCs produced the lowest percentage of sterile pollen grains. Even though the number of varieties analysed is admittedly small, there seems to exist a direct relationship of Anaphase I and Telophase I abnormalities with pollen sterility in ginger. Analysis of meiosis in a number of varieties may strengthen this finding. The presence of bridges in PMCs during meiosis I may be due to paracentric inversion of chromosomes segments (Sybenga, 1975). Ratnambal (1983) observed intraspecific variability for meiotic behaviour in ginger, and also suggested that pollen sterility is due to chromosomal aberrations.

A direct relationship between structural hybridity and pollen sterility has been established in Tulipa (Upcott, 1937) and Pongamia pinnata (Sarbhoy, 1977). It is well-known that structural heterozygotes for translocation and inversion contribute directly to alter the normal course of meiosis at later stages (Burnham, 1956). Chromosome bridges, fragments, micronuclei at Anaphase I etc. are common feature in these cases. Pollen mother cells showing chromosome bridges,

laggards and micronuclei give rise to unequal distribution of chromosomes and consequently to microspores with deficient or duplicate chromosome segments. Because of their non-disjunctional orientation and unequal separation, multivalents may also give rise to gametes with deficiency and/or duplication of chromosome segments or chromosomes. Thus in Zingiber officinale translocation and inversions which in turn will result in the formation of univalents, multivalents, bridges, laggards and micronuclei formation etc. during meiosis, contribute to pollen sterility. The varieties having the highest percentage of these abnormalities produce the highest percentage of pollen sterility.

3) Pollen studies

a) Pollen morphology

Ginger flowers carry enough pollen grains in the single bilobed anther. Anther dehiscence occurs immediately after flower opening and sticky pollen grains are dispersed from the ventral sides of the anther lobes. The pollen grains are round, highly heterogeneous in size and filling, and are limited by a very thick exine. A well defined pore is absent in the pollen grains and the conditions is discribed as monosulcate (Zavada, 1983). The fertility of the

grains as assessed by acetocarmine stainability, was very low in almost all varieties. Incidentally, high amount of meiotic irregularities leading to high percentage of pollen sterility in ginger is already discussed. Pollen fertility varied from 15.52 per cent to 39.67 per cent between the nine varieties. The variety Nadia produced the highest percentage of fertile grains and Burdwan showed the lowest pollen fertility. This intervarietal difference in pollen fertility is accompanied by the variation in the extent of meiotic abnormalities consequent to genomic instability. The consequence of high sterility of pollen grains is quite obvious, as it reduces the chance of proper fertilization and seed-set. Hence high pollen sterility is one of factors limiting seedset in ginger. Pillai et al., (1978) reported 35 per cent of pollen fertility in ginger. Usha (1983) has obtained 12.48 per cent fertility in Rio-de-Janeiro and 10.42 per cent in Maran.

The pollen grains of different varieties were highly heterogeneous in size and filling. The mean diameter of pollen grains ranged from 85.16 μ m in Maran to 110.60 μ m in Rio-de-Janeiro. The size of the grains have been found to be positively correlated with the number of chromosomes they carry, in many crop species. The varieties of ginger show varying degrees of meiotic abnormalities and pollen grains may be difficient or excess for the number of chromo-

somes or segments of chromosomes. This might affect adversely their formation and result in the varying size and filling of pollen grains (Shivanna and Johri 1985).

3.2 Pollen germination

Information on the germination of pollen grains under *in vitro* conditions are quite significant to understand the mechanisms limiting fruitset in ginger. In the present investigation, out of the seven different media tried, the medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin produced the highest percentage of germination of pollen grains (11.81%). Pollen germination was always found to be better in a B.O.D. incubator kept at 25°C than under room temperature conditions. Pillai *et al.*, (1978) reported 14.5 per cent germination of pollen grains in a medium containing 8% sucrose + 60 ppm boric acid + 3% gelatin. The highest percentage of germination of 14.61 per cent has been obtained for Nadia variety in the present study. However, all other reports indicate lower percentages of germination of pollen grains in ginger (Jayachandran *et al.*, 1979; Usha, 1983).

In ginger, initiation of pollen germination was marked by exine bursting and the extension of the initine as the pollen tube, typical of monoculcate

condition. The variety Nadia, having the highest percentage of germination of pollen grains. The correlation coefficient between pollen sterility and pollen germination (%) was also negetively significant (-0.92). This indicates that the varieties having higher pollen fertility will have higher percentage of pollen germination, which is quite logical. On comparing the varieties with regard to the length of pollen tubes after 24 hours of incubation in the media, Valluvanad recorded the maximum length which was followed by Narasapattam and Rio-de-Janeiro. Out of the different media compared, the one containing 8% sucrose + 60 ppm boric acid + 1% gelatin induced the growth of pollen tubes to the maximum extent (83.33 µm). Infact, the media containing gelatin in all cases produced a higher rate of growth of pollen tubes. However, the longest pollen tube recorded was only 109 µ m for Nadia variety in medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin. Interestingly, most of the pollen tubes towards the end of their 24 hours of growth, produced conspicuous coiling. Usha (1983) has observed coiling of pollen tubes in Maran and Rio-de-Janeiro. Investigations on this coiling of pollen tubes will be worthwhile to understand better the reproductive biology of ginger.

Irradiation of pollen grains has been suggested to be effective in breaking the barriers in fertilization (Stettler, 1968; Knox, 1972; Pandey, 1975). The behaviour of irradiated pollen grains expecially germination and growth under *in vitro* conditions has to be ascertained before the technique is used to break the barriers in seedset. The germination of irradiated grains of varying doses under different media carried out in the present investigation have shown that irradiation is always detramental for the germination of grains in ginger. As the irradiation dose increased, the germination percentage also decreased simultaneously. The germination percentage was less than one per cent at an irradiation dose of 100 kR. irradiation. The inhibitory effect of irradiation on pollen germination in plants has been reported by many investigators (Brewbaker and Emery, 1962; Pfahler, 1971). Membrane damage is mentioned as a possible cause for the inhibited cell elongation in irradiated pollen grains (Visser and Oost, 1981). Boom and den Nijs (1983) suggested that cell elongation, the most important process in pollen germination and tube growth may only be disturbed at higher doses of irradiation. The adverse effect of irradiation on the highly sterile and genomically unstable pollen grains of ginger may not be only disturbance at cell elongation and membrane levels, but may also be at cell division and genome levels.

4) Pollen-pistil interaction

In higher plants, proper interaction between pollen and pistil is quite significant for the normal functioning of microspores and seed production. The concept of pollen-pistil interaction depends upon the combination of stigmatic and pollen molecules at complementary sites (Sampson, 1962). In the present study, the fluorescence microscopic procedure of Kho and Baer (1968) for studying pollen-pistil interaction has been found to be quite suitable in ginger.

Pillai *et al.* (1978) suggested that failure of pollen germination on stigma or incompatibility may be one of the factors limiting seedset in ginger. Jayachandran and Vijayagopal (1979) reported that in the event of incompatibility, the inhibitory action may not be located on the stigma surface. On the other hand, Usha (1983) was of the opinion that incompatibility reaction may not be the factor causing failure of seedset in ginger. Eventhough present studies in this respect are admittedly inconclusive, some useful inferences can be drawn from the results of UV microscopy, which may serve as guidelines for future comprehensive investigations. The stigma of ginger flower is highly spiny for proper adherence, contact and germination of pollen grains. The pollen grains are highly sterile (73.17 per cent in Maran) and even under *in vitro* conditions gave only up to 11.95 per cent germination in Maran. The pollen

grains of the variety Maran was able to produce the maximum tube length of only 84 μ m, after 24 hours of incubation. In the *in vivo* pollen studies of stigma, a major percentage of pollen grains did not germinate probably due to sterility and inherent inability. However, some pollen was capable of germination on the stigma, as was revealed in 4 hours after pollination. As in the case of *in vitro* conditions pollen tube growth was slow and reached only 70 μ m after 4 hours. The penetration of pollen tube in the stigma was not observed. Also the removal of stigma at different heights and pollination did not facilitate fruitset and seedset. Hence, the present study can neither refute fully nor support the existence of incompatibility system in ginger. Existence of two prefertilization barriers such as sterility and incompatibility within a crop species that too within a variety is seldom noticed. In addition, ginger has a number of barriers limiting seedset which will be discussed later. Further, it is hard to see a plant species operating self and cross incompatibility at the same level. In the present investigation many techniques breaking incompatibility (if existing) such as bud pollination, artificial sibbing, artificial cross pollination between varieties, chemically aided pollination, mixed pollination, mentor pollination, stigma removal and artificial pollination etc. failed to produce fruitset and seedset. Hence based on the results, the existence of

incompatibility mechanism operating in ginger is quite doubtful. The unequivocal conclusion in this respect is possible through extensive fluorescence studies on *in vivo* germination on pollen grains on stigma under different techniques of pollination (to break incompatibility) using a profuse flowering variety with high pollen fertility.

5) Reasons for the absence of fruitset and seedset in ginger

Ginger has been described by many, as a species producing flowers very shyly and never setting seeds (Hooker, 1894; East, 1940; Fryxell, 1957; Ramachandran, 1969; Ratnambal, 1979). Every efforts to produce seeds in ginger has failed so far (Nair *et al.*, 1980). Also many conflicting surmises have been put forward by different workers with regard to the mechanism limiting fruitset and seedset in ginger. These include defects in micro and magasposogenesis, lack of suitable pollinating agents and failure of pollen germination on stigma or due to incompatibility (Pillai *et al.*, 1978, Usha, 1983). In the present investigation different pollination techniques were employed to overcome the barriers in fruitset and seedset. Among the stages of flower utilized for pollination, bud stage was unsatisfactory as stigma was not receptive at this stage and no set was obtained. Artificial self

pollination, artificial sibbing, artificial cross pollination between varieties, mentor pollination using normal and irradiated pollen grains, mixed pollination and chemically aided pollination failed to give any positive results on fruitset. Removal of stigma as well as partial and complete removal of style and artificial pollination also failed to give any success in fruitset. If the inhibitory substance present at the stigmatic surface or stylar neck as suspected in incompatibility system was the reason for the failure of fruitset and seedset, success could have been obtained through the removal of stigma or style. In short, it was not possible to overcome the long prevailing problem of absence of seedset in ginger. But the present investigation was rewarding in identifying the limiting factors for the absence of fruitset and seedset, which are summarised below:

a) Unstable genome and consequent abnormalities: As discussed earlier, structural chromosomal aberrations such as translocations, deletions inversions etc. are expressed in the form of multivalents, bridges, fragments, laggards etc. during meiosis. These abnormalities lead to the sterility of microspores and megaspores. Eventhough PMCs were analysed only, one can expect such abnormalities in the egg mother cells (EMCs) and sterility of megaspores. Hence even

after fertilization is achieved in ginger with much efforts, one should be prepared to overcome the possible embryo breakdown and seed sterility.

b) Pollen sterility: High pollen sterility ranging from 60-84 per cent might be one of the serious limitations for the setting of fruits and seeds in ginger. It is interesting to analyse how a diploid species like Zingiber officinale acquired itself such complicated meiotic system and consequent sterility. The one propagation method known in ginger is through rhizomes. Their rhizomes can be carried for considerable distance by man, animals and many other agencies and as a whole is immune to environmental changes. Hence it is possible that many individual cultivars of ginger found at present were probably the same ones in existence hundreds of years before. The age of clones in a vegetatively propagated crop like ginger is impossible to estimate (Stebbins, 1960). Probably due to continuous vegetative propagation, the species might have lost its need for sexual reproduction (Ratnambal, 1979). A parallel case has been reported in literature namely that of saffron (Crocus sativus). This species is highly sterile as to pollen and seed and is normally propagated by corms (Stebbins, 1950). In such perennial plant with efficient vegetative propagation, complete sterility is not creating any barrier for its survival.

c) Spiny stigma: The stigmatic surface of ginger flowers have spine like structures numbering to 26-30 per stigma. These spines are found to damage the pollen grains or prevent them to get attached to the stigmatic surface during the act of pollination.

d) Low percentage of pollen germination: From the correlation analysis it was seen that sterility is positively correlated with pollen germination. Even in the variety Nadia having the highest pollen fertility (39.67%), the maximum pollen germination noticed was only 14.61 per cent. Thus poor germination of pollen grains might be one of the reasons for failure of fruitset.

e) Slow germination of pollen grains: Initiation of pollen germination was marked by exine bursting and the extension of the intine as pollen tube. Even though some pollen grains are germinating under *in vitro* and *in vivo* conditions the process is taking place very slowly so that flowers are withered before the pollen tube could reach the ovules.

f) Coiling of pollen tube: Coiling of pollen tubes during the advanced steges of pollen germination might be another limiting factor for the failure of fertilization in ginger.

g) Long style length: The mean length of style in ginger is 3.9 cm (39,000 µm). The pollen tube attained only upto 108 µm in the best case under *in vitro* conditions after 24 hours. In the *in vivo* conditions also the pollen tubes measured only about 95 µm after 4 hours of pollination. So the possibility of pollen tube reaching the ovule which is 39,000 µm below is quite remote; by this time flower will be withered and dehisced.

h) Retention of opened flowers for a short period of upto 12 hours on the plant: The flowers were found to remain on the plant for less than 12 hours after anthesis. By the time, pollen grains germinate and grow abscision layer is formed in the stylar region and flowers are shed.

In essence, the absence of fruitset and seedset in ginger does not seem to be controlled by a single factor; but an array of factors make this challenging problem more complex.

Summary

SUMMARY

Ginger (Zingiber officinale Rosc) belonging to the family zingiberaceae is an important commercial spice throughout the world. The aromatic rhizomes of ginger find application both as a spice and in medicines. At present ginger is ranked as the third important spice crop of India, standing next to pepper and cardamom.

Through the demand for ginger is increasing varieties of ginger having high yield and special quality attributes are rare at present. Also the crop is facing the increasing devastation by soft rot and bacterial wilt for which resistant types are yet to be developed. The crop improvement programmes undertaken in the crop so far confined only to selection of cultivars suited to local conditions. The advantage of gentic variability is yet to be tapped and exploited fully in the crop.

The greatest handicap which obstruct the hybridzation and genetical studies and for that matter breeding of ginger is the absence of fruit set and seed set. Earlier investigators have proposed various conflicting surmises such as self incompatability, chromosomal aberrations, defects in micro and megasporogenesis, lack of suitable pollinating

agents and failure of pollen germination on stigma for the absence of fruitset and seedset in ginger. But none of the previous authors have used *in vivo* pollen germination studies for concluding to the mechanism of self-incompatibility in ginger. Even though cytogenetical studies have been made in the genus *zingiber* the information available on cytogenetical polymorphism in ginger is very meagre. Also the relationship of meiotic abnormalities towards pollen sterility leading to problems in fruitset and seedset is poorly understood. Ginger has been described by many as a spicies producing high amount of sterile pollen grains. Details on the extent of variability for pollen sterility between varieties of ginger, the effect of different media on the pollen germination and pollen tube growth of different varieties and the effect of irradiation on the germination of pollen grains in ginger are quite significant in understanding the factors limiting fruitset and seedset and also to devise methods for breaking these barriers. Considering these gaps in the biology of ginger crop, the present investigation namely 'Cytogenetics, flowering and seedset in ginger' was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara.

The study was carried on nine ginger varieties representing different geographical regions in India. These varieties were raised in pots and normal cultural operations based on package of practices (Kerala Agricultural University, 1986) were followed. Both morphological observations and cytological characters were recorded. Cytogenetical studies covered both mitotic and meiotic stages. Studies were also conducted on pollen morphology, pollen pistil interaction and methods to overcome the barriers in seedset.

The different morphological characters which include both vegetative as well as floral characters viz. number of tillers per plant, height of plant (cm), length of leaves (cm), breadth of leaves (cm), number of leaves per pot, days for appearance of flowers, number of flowers per scape, number of inflorescence per pot, number of inflorescance coming through the heart, rhizome yield (g/pot) etc., did not reveal any significant difference. Morphological characters are influenced to a great extent by soil and climatic factors. Hence their use as identifying characters are seldom recommended. The results obtained in the present study also indicate that morphological characters alone cannot be relied upon for identifying varieties of ginger.

Absence of morphological difference between varieties adds to the use of cytogenetical investigations for the characterisation of varieties of ginger. It was revealed that collection of roots in the day time gave very low mitotic index in the root tip squashes and the mitotic index was found to increase during the night hours. The optimum time for collection of roots for mitotic studies in ginger will be between 5-6 AM.

During the investigation, the somatic chromosome number was determind in nine varieties of ginger. It was found that 2n = 22 is constant in the nine varieties of ginger. Since the nine varieties did not exhibit any significant morphological differences, karyotype details was useful for the characterisation of individual varieties of ginger. The karyotype of nine varieties of ginger studied showed considerable difference in their morphological features such as length of chromosomes, centromere position, total chromatin length and total chromosome volume.

The classification of the karyotype of different varieties of ginger showed that Zingiber officinale belong to the primitive species of angiosperms. However slightly asymmetrical karyotype represented in 1b as compared to 1a was present in seven out of nine varieties

The analysis of microsporogeneis in four varieties of ginger showed that the genome of ginger is highly unstablized as far as meiosis is concerned. The variation in chromosome association of different varieties of ginger, which consisted of univalents, trivalents, quadrivalents, pentavalents and hexavalents besides bivalents were seen in ginger. Abnormalities were also observed as bridges and laggards at Anaphase I and as micronuclei formation at Telophase I stages of meiosis.

Ginger flowers carry enough pollen grains in the single bilobed anther. Pollen grains are round and monosulcate. The pollen grains of ginger are having high percentage of pollen sterility due to high amount of meiotic irregularities. Pollen fertility varied from 15.58 per cent to 39.67 per cent among the nine varieties. The mean diameter of pollen grains ranged from 85.16 μ m in Maran to 110.60 μ m in Rio-de-Janeiro

The study conducted on pollen germination have revealed that out of the seven different media tried, the medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin produced the highest percentage of germination of pollen grains (11.81%). By correlation coefficient analysis it was seen that the variety Nadia, having the highest percentage of pollen fertility has produced the highest percentage of germination of pollen grains.

Irradiation of pollen grains was done for breaking the barriers on fertilization. The germination of irradiated grains of varying doses under different media carried out have shown that irradiation is always detremental for the germination of grains in ginger. The germination percentage was less than one per cent at 100kR irradiation. It was seen that sterility and pollen germination was correlated.

The study of pollen-pistil interaction using flourescenee microscopy revealed that the stigma of ginger is highly spiny for proper adherence, contact and germination of pollengrains. In the *in vivo* pollen studies of stigma, a major percentage of pollen grains did not germinate probably due to sterility and inherent inability. Further, it is hard to see a plant species operating self and cross incompatibility at the same level. Many techniques for breaking incompatibility (if existing) such as bud pollination, artificial sibbing, artificial cross pollination between varieties, chemically aided pollination, mixed pollination, mentor pollination, stigma removal and artificial pollination failed to produce fruit set and seed set.

Many conflicting surmises have been put for ward by different workers with regard to the mechanism limiting fruitset and seedset in ginger. It was possible to identify some of the limiting factors for

the absence of fruitset and seedset in ginger. Structural chromosomal aberrations such as translocations, deletions, inversion etc. leads to the sterility of microspores. The pollen sterility ranges from 60-84 per cent might be one of the serious limitations for the setting of fruits and seeds. The spines present on the stigmatic surface of ginger flowers found to damage the pollen grains or prevent them to get attached to the stigmatic surface during the act of pollination. The poor germination of pollengrains might be one of the reasons for failure of fruitset. Eventhough some pollengrains are germinating the process is taking place very slowly so that flowers are withered before the pollen tube could reach the ovules. Coiling of pollen tubes during the advanced stages of germination might be another limiting factor for the failure of fertilization in ginger. The mean length of style in ginger is 39,000 μ m. The pollen tube attained the maximum of 108 μ m only in the best cases of pollen germination under <u>in vitro</u> conditions after 24 hours. So the possibility of pollen tube reaching the ovule which is 39,000 μ m below is quite remote by this time the flower will be withered and dehised. The ginger flowers were found to remain on the plant for less than 12 hours after anthesis. By the time, pollen grains germinate and grow, aabscisian layer is formed in the stylar region and flowers are shed.

The present studies revealed that the absence of fruitset and seedset in ginger does not seems to be controlled by a single factor, but an array of factors make this challenging problem more complex.

Future lines of works suggested

1) It will be worthwhile if efforts are made to reveal the physiological effect of day length on the reduction of mitotic index in ginger.

2) It was also revealed that boric acid had some positive influence on the germination of pollen grains, which may be further studied.

3) The media having gelatin in all cases produced a better growth of pollen tube. This also needs further extensive studies.

4) Medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin was found to be the best for germination of pollen. This medium may be further enriched and studies conducted to get a better pollen germination and pollen tube growth.

5) It has been revealed that the sterility and pollen germination are correlated. This may be further investigated.

References

REFERENCES

Anonymous, 1978. All India Co-ordinated Spices and Cashewnut Improvement Project: Progress 1976-'77. Central Plantation Crops Research Institute, Kasaragod, pp. 54.

*Babcock, E.B., Stebbins, G.L. and Jenkins, J.A. 1942. Genetic evolutionary process in Crepis. Amer. Nat., 76 : 337-363.

Beckmann, J.S. and Soller, M. 1986. Restriction fragment length polymorphisms in plant improvement. Oxford surveys of plant Molecular Biology, 3 : 196-250.

Beltran, I.C. and Kam, Y.K. 1984. Cytotaxonomic studies in the Zingiberaceae. Notes from the Royal Botanical Gardens, 41 : 3.

Boom, J.M.A.V. and Den Nijs, A.P.M. 1983. Effects of r-radiation on vitality and competitive ability of Cucumis Pollen. Euphytica, 32 : 677-684.

Brandenburg, W.A. 1984. Biosystematics and hybridization in horticultural plants. In: Grant, W.F. (ed) Plant Biosystematics. Academic Press, Canada. pp. 617-632.

* Brewbaker, J.L. and Emery, G.C. 1962. Pollen radiobotany. Radiation Botany, 1 : 101-154.

Burkill, I.H. 1966. A dictionary of the economic products of the Malay Peninsula. Min. of Agric. and Co-operatives, Kulalampur, pp. 2443.

Burnham, C.R. 1956. Chromosomal interchanges in plants. Bot. Rev., 22 : 419-552.

Chakravorti, A.K. 1948a. Theory of fragmentation of chromosomes and evolution of species. Sci. Cult., 13 : 309-312.

Chakravorti, 1948b. Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. Sci. Cult., 14 : 137-140.

Darlington, C.D. and Janakiammal, E.K. 1945. Chromosome atlas of cultivated plants. George Allen & Unwin Ltd., London, pp. 397.

Datta, A.K. and Biswas, A.K. 1985. EMS induced mitotic consequences in three rhizomatous spice yielding plants. Chromosome Information Service, 39 : 25-26.

Dennisen, C.J.M. and den Nijs, A.P.M. 1987. Effects of gamma irradiation on in vitro pollen germination of different Cucumis species. Euphytica, 36 : 651-658.

den Nijs, A.P.M. and Cost, E.H. 1980. Effect of mentor pollen on pistil pollen incongruities among species of Cucumis L. Euphytica, 29 : 207-271.

de Nettancourt, D., and Grant, W.F, 1963. The cytogenetics of Lotus II. A diploid interspecific hybrid between L. tenuis and L. filicaulis. Can. J. Genet. Cytol, 5 : 338-347.

* East, E. 1940. The distribution of self sterility in the flowering plants. Proc. Amer. Phil. Soc., 82 : 449-518.

Evola, S.V., Burr, F.A. and Burr, B. 1986. The suitability of restriction fragment length polymorphisms and genetic markers in maize. Theor. Appl. Genet., 71 : 765-771.

* Federov, A. 1969. Chromosome numbers of flowering plants. (Z. Bulkhosish, V. Grif, T. Matvijeva, O. Zakharyeve, Leningrade.

Friend, D.J.C. 1966. The growth of cereals and grasses. Butterworths, London, pp. 359.

Fryxell, P.A. 1957. Mode of reproduction in higher plants. Bot. Rev., 23 : 156-233.

Giorgi, B. and Bozzini, A. 1969. Karyotype analysis of I. turgidum (L.) and related tetraploid wheats. Caryologia, 22 : 249-259.

Gollifer, D.E. 1973. The introduction of spice crops into the British Solomon Islands. Proceedings of the conferance on spices. Tropical Products Institute, London.

Glendinning, D.R. 1960. Setting of self incompatible cocoa. Nature 187 : 170.

* Grant, J.E., Pandey, K.K. and Williams, G. 1962. Pollen nuclei after ionising irradiation for egg transformation in Nicotiana. New Zealand J. Bot., 18 : 339-341.

* Holttum, R.E. 1950. The Zingiberaceae of Malay Peninsula. Gardens Bull. Singapore, 13 : 1-249.

Hooker, J.D. 1892. Flora of British India VI. London pp. 792.

Huziwara, Y. 1962. Chromosomal evolution in the subtribe Asterinae. Evolution, 13 : 188-193.

Jayachandran, B.K. and Vijayagopal, P., 1979. Attempts on breaking self incompatibility in ginger (Zingiber officinale R.) Agri. Res. J. Kerala, 17 : 256-257.

Jayachandran, B.K. and Sedthumadhavan, P. 1979. Floral biology of Zingiber officinale R. Agri. Res. J. Kerala, 17 : 93-94.

* Kannan, K. and Nair, K.P.V. 1965. Zingiber officinale (Ginger) in Kerala. Madras Agric. J., 52 : 168-176.

* Karpov, G.K. 1966. Remote hybridization in the works of the I.V. Michurin Central Genetical Laboratory. Soviet Genet., 2 : 102-106.

Katiyar, R.B. 1978. Radiocytogenetical studies on Capsicum I. Meiotic abnormalities. Cytologia, 43 : 415-421.

Kerala Agricultural University, 1986. Package of practices recommendations, 1985. Extension Division, Mannuthy, Kerala, India.

Kho, Y.O.; and Baer, J. 1968. Observing pollen tubes by means of fluorescence. Euphytica, 17 : 298-300.

Kho, Y.O.; den Nijs, A.P.M. and Franken, J. 1980. Interspecific hybridization in Cucumis L. II. The crossability of species: an investigation of in vivo pollen tube growth and seed set. Euphytica, 29 : 661-672.

Knox, R.B.; Willing, R.R. and Ashford, A.E. 1972. Pollen wall proteins role as recognition substances in interspecific incompatibility in poplars. Nature, 237 : 381-383.

*Leviksky, G.A.1931. The karyotypes in systematics. Bull.Appl. Bot. Plant Breed, 27: 220-240.

Mahanty, H.K. 1970. Acytological study of the Zingiberales with special reference to their taxonomy. Cytologia 35 : 13-49.

*Michurich, L.V. 1950. Selected works. Moscow (English Edition).

Miri, R.K. and Bubar, J.S.1966. Self incompatibility mechanism as an out crossing mechanism in bird foot trace foil (Lotus Carniculatus). Can. J. Plant Sci., 46 : 411-418.

Morinaga, I.; Fukushima, E.; Kano, T. and Yamasaki 1926. Chromosome numbers of cultivated plants. Bot. Mag. (Tokyo), 43 : 589-594.

Mock, J.J. and Loescher, W.H. 1973. Incompatibility of maize and Sorghum manifest in failure of pollengrowth. Egypt. J. Genet. Cytol, 2: 338-344.

Muralidharan, A. and Kamalam, N. 1973. Improved ginger means more foreign exchange. Indian Emg, 22 : 37-39.

Muralidharan, A. and Sakunthala, B. 1973. Variability in different clones of ginger. Indian Spices 11 : 2-5.

Nair,M.K., Nambiar, M.C. and Ratnambal, M.J. 1980. Cytogenetics and crop improvement of ginger and turmeric. Proceedings of the National Seminar on ginger and turmeric pp. 15-23.

Nair,M.K., Ravindran, P.N. and Pillai, P.K.T. 1975. Cytological studies in ginger with special reference to sterility and induced polyploidy. Annual Report. CPCRI, Kasaragod.

*Nakahara, K. and Komoto, Y. 1957. The effect of high temperature on the meiotic figures of Tradescantia Paludosa. Cytologia, 22 : 1-11.

Nybe, E.V. 1978. Morphological studies and quality evaluation of ginger (Zingiber officinale Rosc) types. M.Sc. Thesis. College of Horticulture, Kerala Agricultural University, Vellanikkara, Trichur. pp. 107.

Omanakumari, N. and Mathew, P.M. 1985. Karyomorphological studies on four species of Zingiber. Adns. Cytologia, 50 : 445.

Oost, E.H. and den Nijs, A.P.M. 1979. Storage and irradiation of Cucumis pollen and their influence on pollen vitality. 11 : 63-67.

Pandey, K.K. 1977. Mentor pollen. Possible role of wall-held pollen growth promoting substance in overcoming intra and interspecific incompatibility. Genetica, 47 : 219-229.

* Parry, J.W. 1962. Spices: their morphology, histology and chemistry. Chemical Publishing Co., New York, pp. 45-59.

Paulose, T.T., 1973. Ginger cultivation in India. Proceedings of the conference on spices. Tropical Products Institute, London. pp. 14-16.

Panse, V.G. and Sukhatme, P.V. 1978. Statistical methods for agricultural workers. ICAR, New Delhi, 3rd ed. pp. 343.

* Pfahler, P.L. 1971. In vitro germination and pollen tube growth of maize (Zea mays L.) pollen V. Gamma irradiation effects. Radiation Botany, 11 : 233-238.

Pillai, P.K.T.; Vijayagopal, G. and Nambiar, M.C. 1978. Flowering behaviour, cytology and pollen germination in ginger (Zingiber officinale Rosc). J. Plant. Crops, 6 : 12-13.

Purseglove, J.W. 1978. Tropical crops - Monocotyledons Longman Group Ltd., London, pp. 533-540.

Purseglove, J.W.; Brown, E.G.; Green, C.L. and Robbins, S.R.J., 1981. Spices Longman, London, Vol. 2 pp. 447-531.

Raghavan, T.S. and Venkatasubban, K.R. 1943. Cytological study in the family Zingiberaceae with special reference to chromosome number and cytotaxonomy. Proc. Ind. Acad. Sci., 17B : 118-132.

Ramachandran, K., 1969. Chromosome numbers in Zingiberaceae. Cytologia, 34 : 213-221.

Ramachandran, K., 1983. Polyploidy induced in ginger by colchicine treatment. Curr. Sci., 51 : 6 : 288-289.

Ramachandran, C., Brandenburg, W.A. and den Nijs, A.P.M., 1985. Intraspecific variation in C-banded karyotype and chiasma frequency in C. sativus. Pl. Syst. Evol., 151 : 31-41.

Ramachandran, C. and Narayan, R.K.J. 1985. Chromosomal DNA variation in Cucumis. Theor. Appl. Genet., 69 : 497-502.

Ramachandran, C. Seshadri, V.S. and Pai, R.A. 1985. Cytogenetical studies on dessert and non-dessert forms of muskmelon (Cucumis melo L). Cytologia, 50 : 631-641.

* Ramachandran, C. and Seshadri, V.S. 1986. Cytological analysis of the genomes of cucumber and muskmelon. Z. plansenzuchtg, 96 : 25-30.

Ratnambal, M.J. and Pillai, P.K.T. 1978. Cytological studies in ginger with special reference to sterility and induced polyploidy. Annual Report, CPCRI, Kasaragod, pp. 128.

Ratnambal, M.J., 1979. Cytogenetical studies in ginger (Zingiber officinale Rosc.) Ph.D. Thesis. University of Bombay, India. pp. 145.

Ratnambal, M.J. and Nair, M.K. 1983. Micro-sporogenesis of ginger (Zingiber officinale R.) Placrosym IV. CPCRI, Kasaragod.

Ratnambal, M.J. 1984. Somatic chromosomes of Zingiber officinale R. and related species. Nucleus, 27 : 198-202.

Rees, H. 1961. Genotypic control chromosome form and behaviour. Bot. Rev. 27 : 288-318.

Ridley, H.N. 1912. Spices MacMillan, London. pp. 389-421.

Rosengarten, F. 1973. The Book of Spices. Pyramid communication Inc. USA. pp. 475.

Sampson, D.R. 1962. Interspecific pollen stigma incompatibility in Cruciferae. Can. J. Genet. Cytol., 4 : 38-49.

Sarbhoy, R.K. 1977. Cytological studies in Pongamia pinnata (L.) Pierre. Cytologia, 42 : 415-423.

Sato, D. 1960. The karyotype analysis in Zingiberales with special reference to the proto-karyotype and stable karyotype. Scientific papers of the College of General Education, Univ. of Tokyo, 10 : 225-243.

* Schumann, K. 1904. Zingiberaceae. In Englers. Pflanzenreich., 4 : 458.

Shivanna, K.R. and Johri, B.M. 1985. The Angiosperm pollen: structure and function Wiley Eastern Ltd., New Delhi, pp. 365.

* Sharma, A.K. and Bhattacharya, N.K. 1959a. Cytology of several members of Zingiberaceae and a study of the inconsistancy of their chromosome complement. Lacellule, 59 : 279-349.

Sharma, A.K. and Bhattacharya, N.K. 1959b. Experimental study to compare the effect of X-ray on the somatic chromosomes of a few species of monocotyledons. Cytologia, 24 : 244-258.

Smith, P.M. 1979. Minor crops. In. N.W. Simmonds (ed). Evolution of crop plants, Longman, London, pp. 301-324.

Sreeramulu, K., ZJER, G.M.M.B. and Dijkhuis, p. 1979. Mentor pollen effect on gametophytic incompatibility in Nicotiana, Oenothera and Lycopersicon. Theor. Appl. Genet., 54 : 215-218.

Stebbins, G.L. 1945. The cytological analysis of species hybrids. Bot. Rev., 11 : 463-486.

Stebbins, G.L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York. pp. 645.

* Stebbins, G.L. 1958. Longivity, habitat and release of genetic variability in higher plants. Cold spring Harb. Symp. Quant. Biol., 23 : 365-378.

Stebbins, G.L. 1974. Variation and evolution in flowering plants. Edward Arnold (Publishers) Ltd.,London, pp. 399.

Stettler, R.F. 1968. Irradiated mentor pollen, its use in remote hybridization of block cotton wood. Nature, 19 : 746-747.

Stott, K.G. 1972. Pollen germination and pollen tube characteristics in a range of apple cultivars J. Hort. Sci., 47 : 191-198.

Sugiura, T. 1936. Studies in the chromosome numbers in higher plants. Cytologia, 7 : 544-595.

* Suzuka, O. and Mitsuoka, S. 1978. Zingiber mioga Rosc. a sterile plant. Rep. Kihar. Inst. Biol. Res. 20 : 103-107.

Sybenga, J. 1975. Meiotic configurations. Springer-Verlag, New York. pp. 251.

Takahashi, K. 1931. In Darlington, C.D. and Janaki Ammal, E.K. 1945. Chromosome atlas of flowering plants. George Allen and Unwin Ltd., London. pp. 397.

* Tsitsin, N.V. 1962. Wide hybridization of plants (Ed. Tsitsin, N.V.) Israel Prog. Sci. (Transl) pp. 2.

Upcott, M. 1937. The genetic structure of Tulipa-structural hybridity. J. Genet., 34 : 339-398.

Usha, K. 1983. Effect of growth regulators on flowering, pollination and seedset in ginger. (Zingiber officinale R). M.Sc. Thesis submitted to Kerala Agricultural University, Vellanikkara, Kerala. pp. 87.

Visser, T. and Oost, E.H. 1981. Pollen and pollination experiments-III. The viability of apple and pear pollen as affected by irradiation and storage. Euphytica, 30 : 65-70.

Visser, T.; Verhaegh, J.J.; Marcucci, M.C. and Uijtewal, B.A. 1983. Pollen and pollination experiments VIII. The effect of successive pollination with compatible and self incompatible pollen in apple and pear. Euphytica, 32 : 57-64.

Zavada, M.S. 1983. Comparative morphology of Monocot pollen and evolutionary trends of apertures and wall structures. Bot. Rev., 49 : 331-332.

* Originals not seen

Appendices

APPENDIX-I

Weather data during the period (January-December, 1987)

Month	Total rainfall mm	Sunshine hours		Temperature		Mean relative humidity (%)
		Total	Mean	Mean X	Mean N	
January	0	299.0	9.6	33.2	22.7	52
February	0	285.0	10.1	35.0	22.4	52
March	0	305.0	10.2	36.4	22.2	55
April	131.3	236.0	7.8	36.2	25.3	64
May	95.0	279.0	9.0	36.1	24.7	66
June	837.7	126.0	4.2	30.7	23.7	83
July	336.5	176.0	5.7	30.3	23.5	84
August	388.4	113.5	3.7	29.6	23.5	87
September	174.0	222.9	7.4	31.5	23.9	79
October	280.4	193.3	6.2	31.9	23.9	79
November	224.4	200.8	6.7	31.6	22.8	77
December	64.6	250.4	8.1	31.6	23.3	77

INVESTIGATIONS ON CYTOGENETICS, FLOWERING AND SEEDSET IN GINGER *(Zingiber Officinale Rosc)*

By

SATHIABHAMA K. U.

ABSTRACT OF A THESIS

submitted in partial fulfilment of
the requirement for the degree

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

Department of Plantation Crops and Spices
COLLEGE OF HORTICULTURE
Vellanikkara - Trichur

1988

Abstract

Investigation on Cytogenetics, flowering and seedset in ginger was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara with the following objectives.

1. To workout the karyomorphology of ginger varieties and to study the cytogenetical polymorphism in relation to the plant morphology.

2. To study the meiotic irregularities in relation to pollen sterility in different varieties.

3. To study the effect of media and irradiation on the pollen germination and pollen tube growth in different varieties.

4. To work out the pollen-pistil interaction by flourescence microscopy to decide on the presence of incompatibility mechanism in ginger.

5. To study the factors responsible for absence of fruitset and seedset in ginger.

The study was carried on nine ginger varieties representing different geographical regions in India. Both morphological and cytogenetical characters were recorded. Studies were also conducted on pollen morphology, pollen-pistil interaction and on methods to over come the barriers in seed set. Absence of

morphological difference between varieties adds to the use of cytogenetical investigations for the characterisation of varieties of ginger. The miotic index was found to be maximum during 5-6 AM. All the nine ginger varieties studied showed a chromosome number of 2n = 22. The karyotype of nine varieties of ginger studied showed considerable difference in their morphological features such as length of chromosomes, centromere position, total chromatin length and total chromosome volume. Such differences could have occured through translocation, inversion and deletion of chromosome segments. Classification of karyotypes in ginger varieties fell in to primitive 1a and 1b group. During meiotic studies it was seen that the genome of ginger is highly unstable. Abnormalities like bridges and laggards were also present which will lead to the formation of micronuclei.

Ginger flowers carry enough pollen grains in the single bilobed anther. But 60-84 per cent of the pollen grains were sterile. The high amount of meiotic irregularities may be leading to high percentage of pollen sterility. Out of the seven different media tried for pollen germination, the medium containing 8% sucrose + 60 ppm boric acid + 1% gelatin produced the highest percentage of germination of pollen grains (11.81%). The sterility and pollen germination was also correlated.

Irradiation of pollen grains has been suggested to be effective in breaking the barriers in seedset. But it was seen that the irradiation of pollen grains was deteremental for the germination of pollen grains in ginger. Proper interaction between pollen and pistil is quite significant for the seed production. The study by using uv microscopy revealed the presence of spiny stigmatic surface which will prevent proper adherence, contact and germination of pollen grains. It is hard to see whether self and cross incompatibility is operating in ginger. In the present investigation many techniques for breaking incompatibility (if existing) such as bud pollination, artificial sibbing, artificial cross pollination between varieties, chemically aided pollination, mixed pollination, mentor pollination, stigma removal and artificial pollination failed to produce fruitset and seedset. Hence, based on the result the existence of incompatibility mechanism operating in ginger is quite complicated. Structural chromosomal aberrations such as translocation, deletions, inversions etc. leads to the sterility of microspores and megaspores. Pollen sterility ranging from 60 - 84 per cent might be one of the serious limitation for the setting of fruit and seeds in ginger. The spines present on the stigmatic surface prevent the pollen grain to get attached to the stigmatic surface during the act of pollination.

Sterility is correlated with pollen germination and it is only upto 14.61 per cent in variety having maximum fertility. The germination of pollen grains was taking place very slowly so that flowers are withered before the pollen tube could reach the ovules. Coiling of pollen tube was also noticed during the advanced stages of pollen germination. The style length was very long (39,000 μ m) in ginger. But the pollen tube attained only 108 μ m in the best case under *in vitro* condition after 24 hours. So the possibility of pollen tube reaching the ovule is remote by the time flower will be withered and dehisced. The flowers were found to remain on the plant for less than 12 hours after anthesis.

In essence, the absence of fruit set and seed set in ginger dose not seem to be controlled by a single factor, but an array of factors make this challenging problem more complex.