EFFECT OF GROWTH REGULATORS ON FLOWERING, POLLINATION AND SEED-SET IN GINGER (*Zingiber officinale*, Rosc.)

By

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THESIS

Submitted in partial fulfilment of
the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "Effect of growth regulators on flowering, pollination and seed-set in ginger (*Zingiber officinale* Rose.) is a bonafide record of research work done by me during the course of research work and 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,
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CERTIFICATE

Certified that this thesis is a record of research work done independently by Smt. Usha, K. 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.

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We, the undersigned members of the advisory committee of Smt. Usha, K. a candidate for the degree of Master of Science in Horticulture with major in Horticulture agree that the thesis entitled "Effect of growth regulators on flowering, pollination and seed-set in ginger (*Zingiber officinale* Rosc.)" may be submitted by Smt. Usha, K. in partial fulfilment of the requirement for the degree.

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Introduction

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), all around the world, is an important commercial spice esteemed for its aroma, flavour and pungency. It was one of the earliest oriental spices introduced to the west and commands an enviable position as a commodity of dear demand of our times.

Aromatic rhizomes of ginger find application both as a spice and in medicine. The rhizomes are widely used in the preparations of ginger beer, ginger wine, gingerale, ginger bread, biscuit, cakes, pudding, soups, pickles and curry powder. It claims proven pharmaceutical properties as a carminative, rubefacient, stimulant and flavourant.

Ginger is rated as the third most important spice of India standing next to pepper and cardamom. Cultivation of this spice is undertaken in almost all the tropical and sub tropical areas of India covering Kerala, Tamilnadu, Karnataka, West Bengal, Orissa, Bihar, Himalchal Pradesh, Uttar Pradesh and Maharashtra. Kerala accounts for 70 per cent of total ginger production and is the renowned centre for quality ginger. In the world map of ginger producing countries, India enjoys the unique

position as the largest producer, its contribution being 50 per cent. Inspite of the tough competition from Jamaica, Nigeria, Sierra Leone, Brazil, China, Japan and Indonesia, India still occupies the top position in the ginger trade internationally. United Kingdom, United States and Saudi Arabia are the important buyers of Indian ginger in international market.

Our attempts to boost ginger export are not free from set backs. The major bottlenecks identified in this respect are the high fibre content of Indian ginger compared to its traditional rival-the Jamacian ginger, in international market and the comparatively high cost of production. As a remedy, it was suggested to develop high yielding varieties possessing low fibre content and high volatile oil and oleoresin (Pruthi, 1976). In line with this suggestion, the National Seminar on ginger and turmeric (1980) had stressed the need for evolving ginger varieties possessing high yield, low fibre content, high oleoresin and resistance to soft rot. Unfortunately, no systematic studies have so far been carried out on the crop improvement aspects of ginger to achieve the aforesaid targets. Tailor made ginger types suiting to various preparations have got real demand in world market. Ideo types oriented to a more efficient photosynthetic system and rapid development of rhizomes had been long sought for.

Vegetative means being the rule and the most popularly adopted method of propagation, attempts in crop improvement of ginger were so far confined to the collection of cultivars from different localities and their comparative yield evaluation. Each centre of production tends to produce a distinctive type and the variation among these types are attributed to soil, climatic and cultural differences as well as the method of preparation. Several cultivars that differ in size, fibre content, moisture content, yield, pest and disease resistance and adaptability are known in India and abroad. Inspite of these advantages, the long cherished dream to evolve superior types through breeding has not materialised till date. The breeding programme in ginger is handicapped by the shy and erratic flowering nature encountered and the failure to achieve seed-set in all the types. Various factors like incompatibility, defective micro/mega sporogenesis, presence of inhibitory substances at the stigmatic surface, occurrence of high percentage of sterility and the failure of pollen germination are suspected to be factors responsible for these drawbacks.

No systematic study has been carried out on the floral biology of ginger. Similarly, positive results on pollen germination and seed-set in ginger are yet to come out. However, preliminary studies have disclosed

some aspects of cytology and floral biology. But the details now available are not adequate enough to carry out a crop improvement programme. Therefore, there is an immediate need to take up detailed study in these lines. Such a study should be helpful to spot out the hinderances involved in seed-set and pave the path to chalk out a suitable breeding programme.

In this connection, it is thought that the inherent inability of ginger to set seeds can be overcome by proper manipulation of flowering behaviour and floral structures. Nair et al. (1980) have advocated to explore such a possibility using growth normones. Accordingly, an investigation was carried out with three growth regulators viz., Kinetin, Ethrel and NAA at varying concentrations and urea as an aid in hormone translocation. The objectives were:

(i) Assessing the available varieties of ginger for flowering behaviour and the extent of flowering under Vellanikkara condition and

(ii) Evaluating the effect of Kinetin, Ethrel and NAA with and without urea on flower induction, retention, pollen characteristics and seed-set on Rio-de-Janeiro variety of ginger, a commercial variety in Kerala.

Review of Literature

REVIEW OF LITERATURE

Ginger is generally known as a species very rarely flowering and never setting seeds. Recently, the nature of flowering and floral biology of ginger have attracted the attention of breeders and horticulturists. However, attempts to induce flowering and seed-set in ginger have not succeeded so far. Studies being still in its infancy, the literature available in this line is rather limited.

A review of the work done so far on the flowering behaviour, floral biology, pollination, induction of seed-set and role of growth regulators on flowering, pollination and seed-set in ginger is furnished in this chapter.

2.1. Flowering behaviour

According to Hooker (1894), ginger is a species very rarely flowering. Holttum (1950) stated that flowers are seldom seen in Malaysia but are produced in some other countries. Thereby it appears that flowering in ginger had been 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 Valluvanad (6.57 per cent), Vengara (2.49 per cent), Ernad Chernad (4.94 per cent), Ernad Manjeri (1.11 per cent), Wynad Local (11.71 per cent), Wynad Kunnamangalam (1.60 per cent), Bajpai (7.43 per cent), Karakal (6.03 per cent), Taiwan (0.53 per cent), Tafingiva (0.63 per cent), Sierraleone (2.92 per cent), Maran (1.19 per cent), Rio-de-Janeiro (10.14 per cent), Wynad Mannantody (6.06 per cent), Kuruppampady (5.30 per cent), Arippa (0.61 per cent), Uttar Pradesh (1.14 per cent), Jorhat (8.15 per cent), Narasapattom (1.14 per cent), Nadia (0.52 per cent), China (0.74per cent) and Assam (10.94 per cent) profusely flowered as against no flowering in Thodupuzha, Thingpuri and Himachal Pradesh types under Vellanikkara conditions in 1977. Similarly, flowering in Rio-de-Janeiro was recorded under the climatic conditions prevailing at Vellayani by Jayachandran et al. (1979).

2.1.1. Inflorescence

Nybe (1978), has sketched out that the inflorescence develops from the rhizome along with a scale leaf. It forms a bracteate spike or raceme, each bract subtending a single flower with a lateral or obliquely posterior

bracteole. The bracts are spirally arranged. The length of the stalk varies from 15 to 30 cm.

Pillai et al. (1978) stated that single flowers are borne in panicles directly arising from the rhizomes and also as terminal spikes. Inflorescence in ginger is a scape and is produced on a special scale 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 rootstock and has got a slender scape of 10 to 20 cm with or without short leafy tips and a cylindrical, cone-like (4 to 7 cm in length and 1.50 to 2.50 cm in diameter) spike with appressed, ovate or elliptic (2 to 3 cm long and 1.50 to 2.00 cm wide) and green bracts with a pale sub marginal band and incurred translucent margins, bearing a single fragile and short lived flower, in the axil of each bract.

2.1.2. Floral characteristics

The floral characteristics of ginger has been outlined by Nybe (1978). According to his description,

the flowers are small as compared to those of the other genera, 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, but separates above into three subequal oblong to lanceolate connate segments less than 2.50 cm long. The stamens are in two whorls of three each. The outer whorl is represented by three lateral staminodes. The inner staminal whorl is complete, the median (posterior) stamen is fertile, while the other two unite to form the labellum which forms the most conspicuous member in the flower because of its pink colour with yellow markings. Anther is two celled, the cells being contiguous, crowned with a long narrow curved tapering grooved crest. Ovary is inferior, tricarpellary, syncarpous, ovules arranged on axile placenta. Style is filiform and lies in a channel along the fertile stamen. The stigma (funnel shaped and ciliate) projects beyond the crest of the anther. Stigmatic hairs are present in groups.

According to Jayachandran et al. (1979), ginger flowers are purple coloured having a perianth, consisting of sepals and petals. Androecium consists of stamens of which the outer three are reduced to staminodes. The inner lateral stamens are united and showy to form a deep

purple coloured labellum. The posterior stamen of the inner whorl is the only fertile stamen and is enclosed by the labellum. The filament is flat and short with two prominent anther lobes. The style passes through the groove formed by the anther lobes and ends in a capitate stigma. Jayachandran and Vijayagopal (1979) have described the bisexual flowers of ginger having one fertile stamen with two large anther lobes filled with plenty of pollen grains.

According to Purseglove et al. (1981) the floral structure of ginger is as follows ; Flowers are borne in the axil of a bract, have got thin, tubular, spathaceous (1.00 to 1.20 cm long) and three toothed calyx. The corolla is fused to form a tube of 2.00 to 2.50 cm length with 3 yellowish lobes of which the dorsal lobe is curved over the anther. The labellum or lip is nearly circular 1.20 cm long and wide, dull purple with cream blotches at the base. The labellum corresponds to three stamens with ovate oblong side lobes and are free almost to the base. Stamen has a short and broad filament, a cream coloured anther and a slender beak like dark purple connective which contains the upper part of the style. Stigma has a circular apical aperture surrounded by stiff hairs and it protrudes just below the apex of the appendage. Two slender free styloids have been observed. Ovary is inferior, trilocular with several ovules per locule.

2.2. Floral biology

Published data on the floral biology of ginger is very limited except for the preliminary observations made by Jayachandran *et al.* (1979). Their study on flower bud development revealed that it took 20 to 25 days from the bud initiation to full bloom and a period of 23 to 28 days was required for the completion of the blooming in an inflorescence.

2.2.1. Anthesis

In ginger, flower starts opening in the afternoon by about 3 P.M. (Pillai *et al.*, 1978). A spread out period of anthesis ie. between 1.30 P.M. to 3.30 P.M. has been reported under Vellayani conditions by Jayachandran *et al.* (1979).

2.2.2. Flower retention and anther dehiscence

Jayachandran *et al.* (1979) based on their studies on Rio-de-Janeiro indicated that blooming takes place in an acropetal succession and the flower fades and falls on the next day of blooming. Purseglove *et al.* (1981) opined that ginger flower is fragile and short lived.

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.

2.2.3. Pollen characteristics

2.2.3.1. Pollen production

No literature is available on the pollen production in ginger except the general observation reported by Jayachandran and Vijayagopal (1979) that anther lobes are filled with plenty of pollen grains.

2.2.3.2. Pollen morphology

According to Pillai et al. (1978) pollen grains are heteromorphic and round with a diameter of 77 to 104 µ the average being 91 µ. A striking feature observed by these workers was the very thick exine of the pollen grains. Jayachandran et al. (1979) stated that pollen grains are spherical with the size ranging from 90 to 100 µ, the mean being 95.50 µ.

2.2.3.3. Pollen fertility

A high percentage of pollen sterility has been reported in ginger. Stainability of pollen obtained in acetocarmine method of fertility assessment was only 35 per cent (Pillai et al., 1978).

2.2.4. Stigma receptivity, style length and ovary length

Stigma receptivity of ginger is still in darkness owing to the failure of hand pollination to set seeds. Observations under hand lens by Jayachandran *et al.* (1979) indicated that stigma was receptive at the time of anther dehiscence.

No mention is available in literature on style length and ovary length.

2.2.5. Pollen germination

Poor percentage of pollen germination has been reported by various authors. 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/air condition room of 25°C gave the maximum

pollen 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 argue that high percentage of sterility may be one of the reasons for poor germination.

From the foregoing, it appears that inspite of the best attempts, the maximum pollen germination obtained so far in ginger is only 14.50 per cent.

2.3. Pollination

Pillai et al. (1978) reported that flower structure of ginger manifests an adaptation suitable for entomophily.

Hand pollination using large quantities of pollen in ginger var. Rio-de-Janeiro was tried by Jayachandran et al. (1979). The procedures tried by them were (i) pollinating flowers at anthesis with mature pollen of the same flower (ii) pollinating flower buds to be opened within 24 hours with mature pollen (iii) pollinating flower buds to be opened within 24 hours with pollen from the same flower bud (iv) pollinating flowers at anthesis with stigmatic surface removed with mature pollen grains of the same flower and (v) pollinating flowers at anthesis, the stigmatic surface of which was smeared with germination media (sucrose, boric acid) with mature pollen grains of the same flower. But no seed-set could be obtained adopting the various methods.

2.4. Seed-set

According to Hooker (1894), ginger is a species never setting seed. Attempts by various workers so far, have failed to achieve seed-set in ginger. Factors contributing for this debacle are not properly understood. East (1940) and Fryxell (1957) suspected that the failure to set seed may be due to the incompatibility. Ramachandran (1969) and Ratnambal (1979) remarked that the failure of seed-set in ginger is due to chromosomal abberations. Pillai *et al.* (1978) opined that failure of seed setting in ginger may be due to varying reasons ranging from defects in micro and mega sporogenesis or lack of suitable pollinating agents or the failure of pollen germination on the stigma or due to incompatibility if the ovule is viable.

From the literature reviewed so far, it is inferred that attempts to achieve seed-set in ginger have not succeeded till date and even the exact reasons behind the failure are not properly understood.

2.5. Role of growth regulators on flowering, pollination and seed-set

Growth regulators as an aid to induce flowering and seed-set in ginger is a novel approach and so, no literature is available in this respect. However, Nair *et al.* (1980) have suggested to explore the possibility

of inducing flowering and seed-set in ginger through the use of growth regulators.

Hormonal concept of flowering was proposed by Cajlachjan in 1936. Failure of earlier attempts to control flowering by the external application of growth regulators spread a smoke of suspicion on the regulatory effects of growth substances on flowering. However, observation that some naturally occurring growth substances in the meristematic region of many plants varied considerably as they transit from vegetative to reproductive phase, suggested that the external application of growth regulators during the flower induction period might result in the regulation of flowering. The report that growth substances are present at the various parts of the flower and during the various phases of flower development (Leopold and Kriedemann, 1980) provided positive proof to the concept of hormonal control over flowering. These workers provided experimental evidences to confirm that there is an endogenous control of flowering monitored by growth substances. Leopold and Kriedemann (1980) further indicated that auxins, cytokinins and ethylene exercise regulatory activities in the developmental activities like flower initiation, sex expression, fruit-set and fruit growth.

2.5.(1). Auxins

Auxins are found to perform the dual role of promoting as well as inhibiting flowering. Liverman (1955) proposed that auxins are the controlling entities in flowering process.

Thurlow and Bonner (1947) observed that application of NAA 500 ppm delayed and even prevented flowering in soyabean. Similarly, in the case of lettuce, inhibitory effect of auxin was found in the form of delayed flowering. These initial observations put forward the belief that auxins generally inhibit flowering. However, in pineapple auxin application induced flowering (Clark and Kerns, 1942). Stimulatory effect of auxin on the development of the orchid embryo sac has been observed by Healop-Harrison (1957). He found that auxin introduced to the orchid ovary by pollination, triggers the entire development of the embryo sac. Thus, it was concluded that until and unless the flower is pollinated or supplied with an external source of auxin, the orchid embryo sac failed to develop beyond the single cell stage. The profound positive influence of auxins on the maturation of the onion anther sac was demonstrated by Vasil (1957).

Thereafter, evidences for the positive effects of auxins on flowering had been furnished by various workers.

Auxins were found to induce and promote flowering in certain short-day plants (Ogawa, 1961; Maheshwari and Venkataraman, 1966 and Nitsch, 1968) as well as some long-day plants (Michniewicz and Kamienska 1965). In addition to their role in flower initiation and development, auxins are found to influence the pollen tube growth (Leopold and Kriedemann, 1980).

Takeyosi and Fujii (1961) noticed the production of large amounts of auxin by the flower bud in the petals, pollen and ovary of some flowers at the time of flower opening. Auxin produced in the flower parts is expected to prevent the abscission of the flower.

Leopold and Kriedemann (1980) proposed that growth and differentiation activities which are influenced by endogenous auxins could be altered by exogenous auxin application.

2.5.(ii) Cytokinins

Regulatory activities manifested by cytokinins in the various aspects of flowering have been revealed of late. Positive influence of cytokinins in inducing flowering in photoperiod requiring species was indicated by Maheshwari and Venkataraman (1966) and Michniewicz and Kamienska (1965). Nakayama et al. (1962) portrayed

the enhanced flowering obtained in some species by cytokinin application. Stages of sex expression (Negi and Olmo, 1966), fruit-set (Crane and van Overbeek, 1965) and fruit growth (Weaver and van Overbeek, 1963) were found to alter by cytokinins in some instances. Favourable role of kinins on the maturation of onion anther sac was outlined by Vasil (1957).

2.5.(iii). Ethylene

Influence of ethylene on flowering was practically unknown until Rodriguez (1932) observed that ethylene applications can induce flowering. Burg and Burg (1966 a & b) came forward with the theory that the stimulation of flowering in pineapple by auxin application is actually due to the subsequent ethylene production. Ethylene control of flowering had been reported in the bromeliads by Cathey and Taylor (1970).

An overall assessment of the effect of auxins, cytokinins and ethylene on the flowering behaviour of various crops clearly indicate their profound positive regulatory role.

Materials and Methods

MATERIALS AND METHODS

The present investigation on the 'effect of growth regulators on flowering, pollination and seed-set in ginger' was carried out at the College of Horticulture, Vellanikkara during the period 1981-1983. The crop raised during the planting season of 1981-82 under field conditions was lost due to the incidence of soft rot disease. Studies were repeated in 1982-'83 season both in pot culture and under field conditions.

Details of the materials utilised and methodology followed are furnished hereunder.

Cultivation

The land was tractor ploughed, levelled and beds of 3 x 1 m size and 20 cm height were formed. Channels of 30 cm width were provided around each bed.

Soft rot free ginger rhizomes collected from Horticultural Research Station, Ambalavayal were utilized as planting material. Rhizomes soaked for 30 minutes in a solution containing 0.25 per cent Emisan and 0.0125 per cent Ekalux were shade dried and utilized for planting. The selected seed rhizomes were cut into bits of 15 g. weight having one or two viable, healthy buds. These bits were

planted in beds at a spacing of 25 x 25 cm. Package of practices (Kerala Agricultural University, 1981) were followed.

For pot culture, treated rhizome bits weighing 15 g. were planted in pots of size 28 x 33 cm filled with pot mixture (1:1:1).

Planting was undertaken during the second week of April in pot culture and on 1st June in the main field.

3.1. Flowering behaviour of different varieties under Vellanikkara conditions

In order to assess the nature and extent of flowering of different varieties of ginger, the germplasm collection maintained at the All India Co-ordinated Spices and Cashew Improvement Project, Vellanikkara sub centre was made use of. The collection included 25 varieties viz., Nadia, Maran, Jorhut, Wynad Kumarakulam, Burdwan, Narasapatta, Ernad Chernad, Thodupuzha, Arippa, Juggijan, Vengara, Taiwan, Bajpai, Rajakkad, Wynad Manantody, Valluvanad, Vazhakkulam, Jamaica, Beharica, Wynad Local, Rio-de-Janeiro, Palai, Kuruppampady, Kottayam and Bhola.

Each variety comprised of 144 plants each planted in three plots of size 3 x 1 m each adopting a spacing of 25 cm between plants and rows. The following observations were recorded using the entire population.

(i) Tiller production per plant at the time of flowering.

(ii) Duration in days taken from planting to flowering and

(iii) Number of plants flowered.

3.1.1. Inflorescence characteristics of Rio-de-Janeiro and Maran

The same population of 144 plants utilised for assessing the flowering behaviour was made use of to study the characteristics of inflorescence in respect of the varieties, Rio-de-Janeiro and Maran. The following observations were made.

(i) Number of inflorescence per plant in terms of scape, terminal and total.

(ii) Number of flowers per inflorescence.

(iii) Days taken from inflorescence initiation to completion of flowering.

3.1.1.1. Scape development and flowering sequence in Rio-de-Janeiro and Maran

To study the development of ginger inflorescence, 25 plants each were tagged at random both in Rio-de-Janeiro and Maran. For further studies on floral biology 20 inflorescence and 100 flowers were again selected randomly from the selected plants.

Sequential development of inflorescence (scape) was followed as a function of time. Succession of blooming

was also observed. Photographs showing scapes at different stages of development and blooming were taken.

3.1.2. Abnormal floral structures

Abnormalities in floral structures, if any were observed and recorded.

3.2. Floral biology of Rio-de-Janeiro and Maran

3.2.1. Anthesis

With the objective of understanding the exact time of flower opening, 50 inflorescence were tagged individually and observed at 15 minutes interval from 1230 hours onwards, till the cessation of flower opening process. Time of anthesis in Rio-de-Janeiro and Maran was determined.

3.2.2. Flower retention and anther dehiscence

The inflorescence tagged for determining the time of anthesis were utilised for ascertaining the time of retention of individual flowers and anther dehiscence in the two varieties viz., Rio-de-Janeiro and Maran.

Period of retention of a flower since blooming was determined by observing the flower at two hourly intervals upto 2400 hours and again at 0600 hours.

Anther sacs were examined 15 minutes prior to flower opening and thereafter at every five minutes to arrive at the time of anther dehiscence.

3.2.3. Pollen characteristics

Anther sacs collected from the fully opened flowers just prior to the time of anther dehiscence were allowed to dehisce in water taken in a petridish. The pollen suspension prepared by gentle crushing of such anthers was utilized to study the various aspects of pollen viz., morphology, fertility, production and germination.

3.2.3.1. Pollen production

Pollen production was estimated adopting haemocytometer method. Haemocytometer employed in this study was Neubauer type having improved double ruling.

Flowers possessing mature anthers (each ginger flower has one anther/stamen) about to dehisce were collected. Five anthers/stamens were separated with a dissection needle and taken in a test tube. The anthers were gently crushed and 2.50 ml of water containing 0.25 per cent Calgon (Oberle and Geortzen, 1952) was added. Contents of the test tube were thoroughly stirred to obtain an even dispersion of the pollen grains in the suspension.

A drop of the suspension drawn in a fine pipette was transferred to each of the two counting chambers. The pollen grains in each of the four corner squares of each counting chamber were counted with the help of a hand tally counter and by using the low power objective of the microscope. For each treatment 10 such estimates were made and the total flowers examined per treatment was 50.

The number of pollen grains per flower was calculated as per the following expression.

$$N = \frac{25 \times V\ 10^4}{n}$$

Where

N = Number of pollen grains per flower

X = Mean number of pollen grains counted per corner square

V = Volume of suspension made up with anthers in cc. (In the present investigation V = 2.50cc.).

n = Number of anthers with which the suspension was made up. (Being equal to five in the present experiment).

3.2.3.2. Pollen morphology

A drop of pollen suspension prepared was transferred to acetocarmine kept on a clean slide. After covering with a clean cover slip, the slides were kept as

such for 10 minutes for the pollen grains to get stained and were examined under a microscope. Shape of pollen grains was determined by visual assessment. The diameter and exine thickness of the pollen grain were measured using an ocular micrometer. Mean values of the diameter and exine thickness of 250 normal well shaped and well stained pollen grains selected at random were recorded.

3.2.3.3. Pollen fertility

Fertility of the pollen grains were assessed based on the stainability and shape. Pollen grains normally shaped and stained in acetocarmine were treated as fertile whereas unstained and irregular shaped but stained were regarded as sterile. From the mean of ten microscopic fields fertility percentage was worked out.

3.2.4. Stigma receptivity, style length and ovary length

Stigma receptivity was assessed by the visual observation of the stigmatic surface under a hand lens. Presence of an ooze on the stigmatic surface was considered as the positive evidence for stigma receptivity.

Length of the style and ovary were measured with a Vernier Calipers.

3.2.5. Pollen germination and pollen tube growth

For determining pollen germination, in vitro, 400 pollen grains of Rio-de-Janeiro were counted from 10 microscopic fields and for pollen tube growth 10 pollen tubes were measured 24 hours after anther dehiscence using an ocular micrometer. Germination was expressed in percentage and tube growth in μ.

Photomicrographs were taken to illustrate the pattern of pollen tube growth.

The effect of the following media on pollen germination was assessed.

(i) Flowers kept moistened.
(ii) Pollen dispersed in distilled water.
(iii) Pollen kept in the medium containing 5, 10, 15 and 20 per cent sucrose.
(iv) Pollen kept in three per cent gelatin, eight per cent sucrose and 60 ppm boric acid.
(v) Pollen kept in 15 per cent sucrose, 300 ppm $Ca(NO_3)_2$, 100 ppm $Mg(NO_3)_2$, 200 ppm KNO_3, 100 ppm boric acid and one per cent agar

and

(vi) Pollen dispersed in growth regulator solutions. viz., IAA and GA both at 5, 10, 15, 20 and 25 ppm concentration.

The above treatments were tried using the hanging drop method under the following three conditions.

(i) In a desiccator, the bottom chamber filled with distilled water.

(ii) In BOD incubator set to 26.5°C.

(iii) In a BOD incubator set to 26.5°C and the pollen grains covered with moist cotton.

3.3. Pollination

Hand pollination was carried out with the following variables.

a) Stages of flower

(i) At bud stage ie. one day prior to flower opening.

(ii) Immediately after flower opening.

(iii) Three hours after flower opening.

b) Condition of stigma and style

(i) Stigma intact

(ii) Stigma just decapitated

(iii) Stigma and half the style removed

(iv) Stigma and entire style removed

c) Density of pollination

(i) Smearing once with standard brush

(ii) Smearing twice with standard brush

(iii) Smearing thrice with standard brush

d) Effect of mixed pollen

(i) Mature pollen grains of ginger

(ii) Ginger pollen mixed with Alpinia pollen

(iii) Ginger pollen mixed with Hedychium pollen

(iv) Ginger pollen mixed with Kaempheria pollen

(v) Ginger pollen mixed with Costus pollen

e) Supplementary pollination

Pollination done immediately after flower opening was supplemented with repeated pollinations twice at two hours interval.

Each of the above experiments was tried with 10 flowers and the experiment was repeated five times.

3.4. Success of pollination and seed-set

Retention of flowers was taken as the indication of the success of pollination and initiation of seed-set. The basal portion of faded flowers was examined under a hand lens for any indication of seed-set.

3.5. Growth regulator treatments

The experiment was laid out in Randomised Block Design with three replications on Rio-de-Janeiro variety of ginger. The treatments consisted of three growth

regulators viz., Kinetin, Ethrel and NAA, each at three levels. The levels tried were 5, 10 and 15 ppm in Kinetin, 25, 50 and 100 ppm in Ethrel and 10, 25 and 50 ppm in NAA.

All the three growth regulators at the specified levels were tried with two per cent urea and without urea.

Growth regulators with and without urea were applied as four foliar sprays adopting the following schedule.

(i) First spray at 105 days after planting.
(ii) Second spray at 120 days after planting.
(iii) Third spray at 135 days after planting.
(iv) Fourth and final spray at 150 days after planting.

Effect of growth regulators with and without urea on Rio-de-Janeiro was assessed on flowering behaviour, floral biology, pollination and seed-set adopting the procedures already detailed in Sections 3.1 to 3.4.

3.6. Statistical analysis

The data were analysed statistically using the analysis of variance technique as applied to the Factorial experiment in RBD design as per the methods suggested by Snedecor and Cochran (1967). Transformations were made wherever necessary. Significant results were compared after finding out the critical differences.

Results

RESULTS

Results of the investigation are presented in the following sections. The analysis of variance table is furnished in Appendix I.

4.1. Flowering behaviour of available varieties under Vellanikkara conditions

Among the 25 varieties of ginger maintained under the All India Co-ordinated Cashew and Spices Improvement Project, Vellanikkara Sub-Centre, flowering was observed only in Rio-de-Janeiro and Maran. Flowering behaviour of these two varieties is presented in Table 1.

Table 1 Flowering behaviour of Rio-de-Janeiro and Maran under Vellanikkara conditions

Variety	No. of plants observed	Average No. of tillers/ plant at the time of flowering	Average No. of days taken from planting to flowering	No. of plants flowered	Mean duration of flowering (days)	Peak time of flowering (weeks from planting)
Rio-de-Janeiro	144	26.66	163.67	45.00	23.67	23.00
Maran	144	15.40	136.25	6.00	18.15	19.00

It can be seen from Table 1 that flowering was earlier in Maran in respect of days taken for flowering. Number of tillers per plant at the time of flowering also was less in Maran compared to Rio-de-Janeiro. However, in terms of the plants flowered and the duration of flowering, Rio-de-Janeiro was superior to Maran. An overall supremacy of Rio-de-Janeiro over Maran in respect of all aspects of flowering behaviour excepting earliness was evidenced from the data.

Peak time of flowering coincided with the middle of the flowering period in both the varieties and was 23 and 19 weeks after planting in Rio-de-Janeiro and Maran respectively. Period of peak flowering was the third week of October for Maran and the third week of November for Rio-de-Janeiro planted on first June under field conditions. However, in Rio-de-Janeiro grown in pot culture and planted in the second week of April, peak flowering was noted in the second week of October.

Weather data during the period of plant growth are presented in Appendix II. The rainfall received during the period from planting to flowering worked out to229.30 cm in the case of Maran and238.80 cm in the case of Rio-de-Janeiro. A striking difference in the sunshine requirement for flowering was observed, the values being

480.20 hours for Maran and 675.25 hours for Rio-de-Janeiro. In the case of Rio-de-Janeiro planted during the second week of April and grown in pot culture, more sunshine hours (764.96 hours) were required compared to 675.25 hours for the crop planted in June and grown under field conditions.

4.1.1. Inflorescence characteristics of Rio-de-Janeiro and Maran

Data presented in Table 2 indicated the wide variation in the inflorescence characteristics of Rio-de-Janeiro and Maran.

Table 2 Inflorescence characteristics of Rio-de-Janeiro and Maran.

Variety	Mean number of inflorescence/ plant			Mean number of flowers per inflorescence	Days taken from inflorescence initiation to completion of flowering
	Terminal	Scape	Total		
Rio-de-Janeiro	3.83	14.67	18.50	29.67	48.00
Maran	-	3.00	3.00	15.00	40.00

Both terminal and scape types of inflorescence were observed in Rio-de-Janeiro (Plate I) whereas only scapes could be found in Maran. Terminal inflorescence in Rio-de-Janeiro was to a tune of 20.70 per cent of the total inflorescence produced. As may be evidenced from the data with respect to the total number of inflorescence per plant, Rio-de-Janeiro (18.50) was superior to Maran (3.00). Number of flowers per inflorescence was more in Rio-de-Janeiro (29.67) compared to Maran (19.00). Time taken from the initiation of the inflorescence to the completion of flowering was 48 and 40 days in Rio-de-Janeiro and Maran respectively.

4.1.1.1. Scape development and flowering sequence in Rio-de-Janeiro and Maran

Sequential steps of scape development and flowering in Rio-de-Janeiro are illustrated in Table 3 and Plates I and II.

Identical pattern of scape development was observed in Maran also excepting the fact that it took only 25 days for the full development of the inflorescence and the stalk as against 29 days in Rio-de-Janeiro. To reach the last phase of stage nine, Maran took 40 days as against 48 days in the case of Rio-de-Janeiro.

Table 3 Sequential steps of scape development and flowering in Rio-de-Janeiro as a function of time

Stage of inflore-scence develop-ment	Mean No. of days taken to reach the stage	Description of the stage
1	0	Initiation of the dome shaped structure
2	10	Swelling of the top end of the dome shaped structure
3	17	Inflorescence shapes with closely packed bracts along with the elongation of the stalk of the inflorescence
4	24	Bract development on the inflorescence with further elongation of the stalk
5	26	Bract spaces and further elongation of the stalk
6	29	Full development of the inflorescence and the stalk
7	30	First flower appears on the lower most fertile bract
8	30 to 38	Flowering continues in acropetal succession. flower opening at the rate of one per day
9	39 to 48	Flowering continues in acropetal succession flower opening at the rate of two to three per day

Plate I Scape and terminal types of inflorescence and scapes at different stages of development and flowering. (Coloured sgs)

S - Scape type of inflorescence

T - Terminal type of inflorescence

Plate II Scapes at different stages of development and flowering. (Coloured sgs)

1 Stage 1 of scape development
2 Stage 2 of scape development
3 Stage 3 of scape development
4 Stage 4 of scape development
5 Stage 5 of scape development
6 Stage 6 of scape development
7 Stage 7 of scape development
8 Stage 8 of scape development
9 Stage 9 of scape development


T
S
4
3
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4.2. Floral biology of Rio-de-Janeiro and Maran

4.2.1. Anthesis

Anthesis time varied from 1400 hours to 1545 hours in Rio-de-Janeiro as against 1430 hours to 1600 hours in Maran (Table 4). In Rio-de-Janeiro, initiation and completion of flower opening were earlier compared to Maran. Spread over time of anthesis was shorter in Maran compared to Rio-de-Janeiro. It was also found that the peak time of flower opening was between 1445 to 1500 hours in Rio-de-Janeiro and 1500 to 1515 hours in Maran.

Both in Rio-de-Janeiro and Maran, soil moisture was found to play an important role in flower opening. As the plants were grown under moisture stress conditions, flowers failed to open. However, as the stress was removed by irrigation, the flower opening revived.

4.2.2. Flower retention and anther deniscence

Data pertaining to flower retention and anther deniscence in Rio-de-Janeiro and Maran are portraited in Table 5. Retention time of individual flowers expressed in the table is arbitrary. As observed at 2400 hours, flowers of both Rio-de-Janeiro and Maran were intact but were dried or/and rotten at 0600 hours.

Table 4 Time of anthesis in ginger varieties Rio-de-Janeiro and Maran

Time (hours)	Number of flowers opened (out of 100)	
	Rio-de-Janeiro	Maran
1230	-	-
1245	-	-
1300	-	-
1315	-	-
1330	-	-
1345	-	-
1400	2	-
1415	5	-
1430	30	1
1445	55	3
1500	90	40
1515	95	80
1530	99	95
1545	100	98
1600	-	100
1615	-	-
1630	-	-

Table 5 Flower retention and anther dehiscence in Rio-de-Janeiro and Maran.

Variety	Retention time of individual flowers (in hours)	Time of anther dehiscence (minutes after flower opening)
Rio-de-Janeiro	>10 and <16	10 to 15
Maran	>9½ and <15.5	20 to 25

Anther dehiscence took place in close proximity to the time of flower opening ie. 10 to 15 minutes after flower opening in Rio-de-Janeiro and 20 to 25 minutes after flower opening in Maran.

4.2.3. Pollen characteristics

Number of pollen grains per flower varied from 1,87,500 to 1,91,500 in Rio-de-Janeiro and Maran respectively (Table 6). It is also evidenced from the data that pollen grains of both Rio-de-Janeiro and Maran possessed identical morphological features namely, spherical with a thick exine. As can be seen from Table 6, the mean diameter of pollen grains was 81.47 μ in Rio-de-Janeiro and 79.68 μ in the case of Maran and the

corresponding exine thickness was 9.73 μ and 10.15 μ respectively. Rio-de-Janeiro recorded a pollen fertility percentage of 12.48 (sterility being 87.52 per cent) as against 16.42 (83.58 per cent sterility) in Maran.

Table 6 Pollen characteristics of Rio-de-Janeiro and Maran (Mean values).

Variety	Pollen production (No.of pollen grains per flower)	Shape	Diameter (μ)	Exine thickness (μ)	Pollen fertility (%)	Pollen sterility (%)
Rio-de-Janeiro	1,87,500	Spherical	81.47	9.73	12.48	87.52
Maran	1,91,500	Spherical	79.68	10.15	16.42	83.58

Photomicrographs of the stained fertile pollen (note the thick exine) and unstained sterile pollen are presented in Plate III.

4.2.4. Stigma receptivity, style length and ovary length

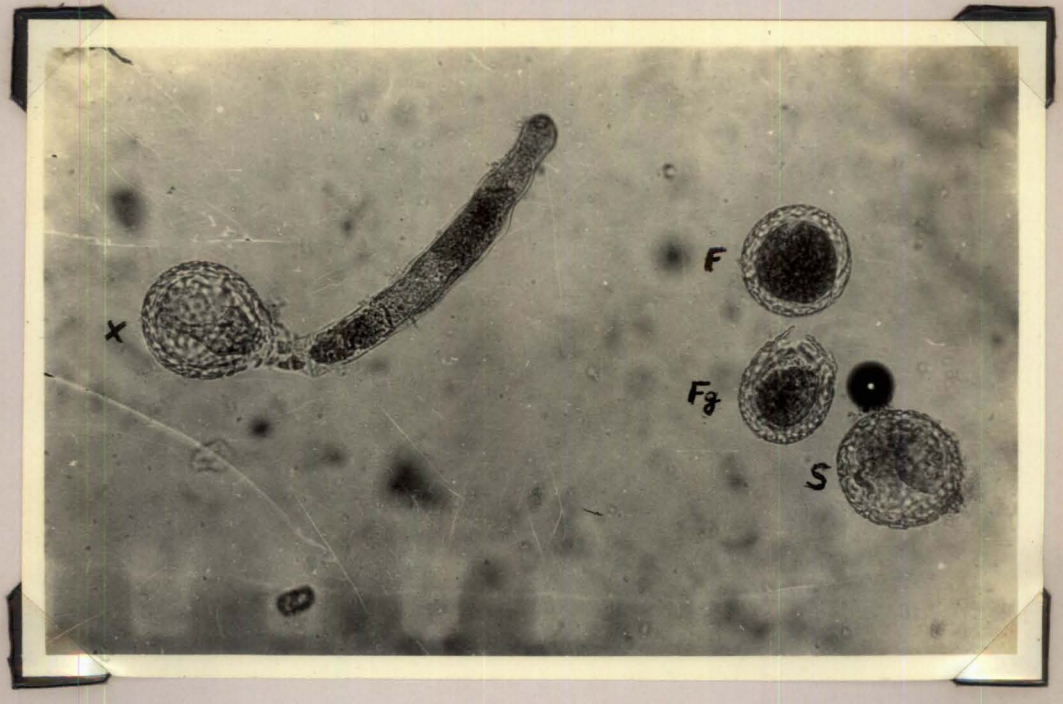
A perusal of the data presented in Table 7 indicate that there was difference between Rio-de-Janeiro and Maran with respect to stigma receptivity. Stigma receptivity assessed by the presence of an ooze on the

Plate III — Fertile and sterile pollen grains of Rio-de-Janeiro and initial and developing stages of pollen germination. (×900)

F - Stained fertile pollen
S - Unstained sterile pollen

Fg - Initial stages of pollen germination - exine breaks

x - Developing stages of pollen germination


X
F
Fz
S

stigmatic surface revealed that stigma remained receptive from one hour to five hours after flower opening.

It is further indicated in Table 7 that the mean style length was 3.72 cm in the case of Rio-de-Janeiro and 3.50 cm in the case of Maran whereas the corresponding values of ovary length were 2.50 mm and 2.60 mm respectively for the two varieties studied. Both Rio-de-Janeiro and Maran possessed very thin styles.

Table 7 Stigma receptivity, style length and ovary length of Rio-de-Janeiro and Maran.

Variety	Stigma receptivity (hours after flower opening)	Mean style length (cm)	Mean ovary length (mm)
Rio-de-Janeiro	1 to 5	3.72	2.50
Maran	1 to 5	3.50	2.60

4.2.5. Pollen germination

Data on the germination percentage of pollen of ginger (var. Rio-de-Janeiro) as influenced by media and conditions are furnished in Table 8. It is evident from the data that among the three conditions tried, pollen grains failed to germinate in desiccator, the bottom chamber

Table 8 Germination percentage of ginger (var. Rio-de-Janeiro) pollen as influenced by media and conditions.

Media	Germination percentage in		
	Desiccator; bottom chamber filled with distilled water	B.O.D. incubator (26.5°C)	With moist cotton covering in B.O.D. incubator (26.5°C)
Flowers kept moistered	0	0	1.00
Pollen dispersed in distilled water	0	0	0.50
Pollen kept in 5% Sucrose solution	0	0	1.50
10% Sucrose solution	0	0	4.00
15% Sucrose solution	0	0	3.50
20% Sucrose solution	0	0	1.00
Pollen kept in 8% sucrose, 3% gelatin and 60 ppm boric acid	0	0	6.20
Pollen kept in 15% sucrose, 300 ppm $Ca(NO_3)_2$, 100 ppm $Mg(NO_3)_2$, 200 ppm KNO_3, 100 ppm boric acid and 1% agar	0	0	1.00
Pollen dispersed in growth regulator solutions			
IAA 5 ppm	0	0	0
IAA 10 ppm	0	0	0
IAA 15 ppm	0	0	0
IAA 20 ppm	0	0	0
IAA 25 ppm	0	0	0
GA 5 ppm	0	0	0
GA 10 ppm	0	0	0
GA 15 ppm	0	0	0
GA 20 ppm	0	0	0
GA 25 ppm	0	0	0

of which was filled with distilled water and B.O.D. incubator (26.5°C). However, positive response was obtained as the pollen grains were kept with a moist cotton covering in B.O.D. incubator (26.5°C). Among the various media tried, growth regulator solutions (both IAA and GA) at 5, 10, 15, 20 and 25 ppm concentrations recorded zero percentage germination under all the three conditions. Media containing sucrose were found to have significant influence on pollen germination as tried with moist cotton covering in B.O.D. incubator (26.5°C). The germination percentage increased from 1.50 per cent to four per cent with an enhancement in sugar concentration from five per cent to ten per cent. However, further increase in sucrose concentration from 10 per cent to 15 per cent and then to 20 per cent recorded a drop in pollen germination percentage from four to 3.5 and then to one. Thus, it appeared that the maximum pollen germination could be obtained by using a media with sucrose concentration in between 5 and 10 per cent.

Incorporation of three per cent gelatin and 60 ppm boric acid to eight per cent sucrose solution helped in getting maximum pollen germination percentage of 6.20 under the moist cotton covering in B.O.D. incubator (26.5°C). However, a combination of 15 per cent sucrose, 300 ppm Ca $(NO_3)_2$, 100 ppm Mg $(NO_3)_2$, 200 ppm KNO_3, 100 ppm boric acid and

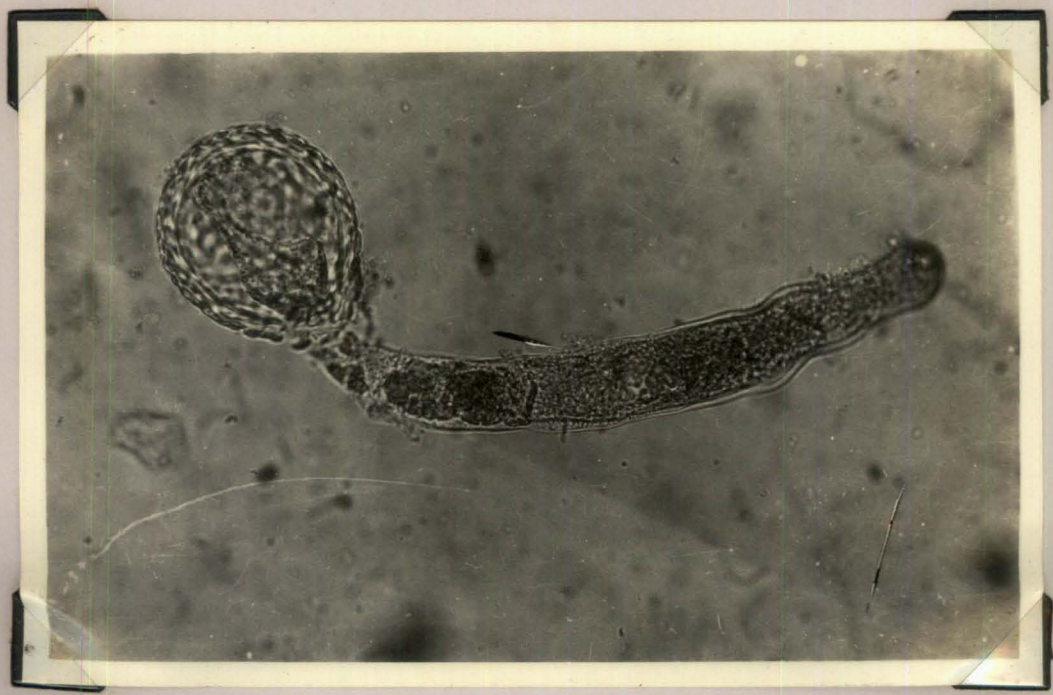
one per cent agar recorded poor pollen germination, the percentage being one. Thus, it is evidenced that the addition of the nitrate salts of Ca, Mg and K and agar exercised a negative effect on pollen germination.

Based on these results, the medium containing eight per cent sucrose, three per cent gelatin and 60 ppm boric acid was selected for further germination studies.

Germination of the pollen grains was first evidenced as bursting of the exine and subsequent growth of the pollen tube (Plate III). An enlarged view of the pollen tube growth showed that the dense contents of the pollen grains got translocated into the pollen tube indicating that pollen germination was an extension of the intine through the broken exine (Plate IV). Pollen tube as observed after 24 hours in the media was 108 μ in length and 16.70 μ in width. At the advanced stages of pollen germination, pollen tube was found to grow in a coiled manner.

4.3. Pollination

Pollination was carried out at the various stages of flowering viz., bud stage, immediately after flower opening and three hours after flower opening, condition of stigma viz., intact, just decapitated, stigma and half



the style removed and stigma and the entire style removed; density of pollen in terms of smearing with standard brush viz., once, twice and thrice; supplementary pollination viz., twice at two hours interval supplementing the initial pollination undertaken immediately after flower opening and using mixed pollen viz., mature ginger pollen, ginger pollen + Alpinia pollen, ginger pollen + Hedychium pollen, ginger pollen + Kaempheria pollen and ginger pollen + Costus pollen.

4.4. Seed-set

No seed-set was obtained in Rio-de-Janeiro and Mysore in any of the pollination combinations tried (Section 4.3).

4.5. Effect of growth regulators on flowering, pollination and seed-set in Rio-de-Janeiro

4.5.1. Flowering characteristics

Flowering characteristics of Rio-de-Janeiro as influenced by growth regulator and urea treatment are presented in Table 9.

4.5.1.1. Days from planting to flowering

Statistical analysis of the data revealed that growth regulators, urea and their interaction had no

significant effect on the number of days taken for flowering. However, certain trends were observed. All the growth regulator treatments with the only exception of NAA 50 ppm required more number of days for flowering compared to the control. Mean number of days to flowering was the maximum for plants treated with NAA 10 ppm (178.83 days) while it was the least for NAA 50 ppm (164.66 days). Eventhough not significant, it appears that urea had a delaying effect on flowering. The observed non significance of urea may be due to the high variability noticed between replicated measurements. Among the treatments included in this study, NAA 50 ppm without urea was the only treatment capable of inducing early flowering in Rio-de-Janeiro. It was also seen that higher the concentration of growth regulators, lesser is the days taken from planting to flowering.

4.5.1.2. Number of tillers per plant at the time of flowering

With respect to the number of tillers per plant at the time of flowering, the effect of growth regulators, urea and interaction were found to be not significant. The trend observed with growth regulator treatments was that all the growth regulators resulted in lesser number of tillers per plant at the time of flowering compared to

control. The effect was more pronounced with Kinetin followed by Ethrel. Invariably in all the cases, lower the concentrations of growth regulator lesser was the number of tillers at the time of flowering. Urea was found to contribute more number of tillers per plant at the time of flowering, the effect being more evidenced with higher concentrations of growth regulators. The interaction effects were more relevant with NAA treatments compared to Kinetin and Ethrel treatments.

4.5.1.3. Duration of flowering

Results of the statistical analysis of data on the duration of flowering showed that neither the effect of growth regulators nor that of urea were statistically significant whereas the effect of their interaction was significant. Though not significant, all the growth regulator treatments excepting 25 and 50 ppm Ethrel exhibited marginal advantage over control Kinetin 15 ppm (24.22 days) followed by NAA 25 ppm (22.33 days) recorded the maximum duration of flowering. Urea was found to enhance the duration of flowering in combination with Kinetin and Ethrel treatments but had a negative effect in combination with NAA. Among the treatments without urea NAA 50 ppm (26.66 days) recorded a marginal increase

on the duration of flowering. In combination with urea Kinetin 15 ppm (24.33 days), Ethrel 100 ppm (22.80 days), Kinetin 10 ppm (21.33 days) and NAA 10 ppm (21 days) recorded longer flowering period compared to control.

4.5.2. Inflorescence characteristics

Data on the effect of growth regulators and urea on the inflorescence characteristics of Rio-de-Janeiro are furnished in Table 10.

4.5.2.1. Total inflorescence per plant

A perusal of the data on the total inflorescence per plant revealed that growth regulators had a significant influence on the total number of inflorescence per plant. All the growth regulator treatments with the exception of 25 and 50 ppm Ethrel recorded significantly more number of inflorescence per plant compared to control. Effect of urea was found to be not significant. However, urea had an aparent negative influence (Plates V and VI). The interaction effect was found to be significant. In the absence of urea NAA 50 ppm (33.67) and Kinetin 15 ppm (30.93) (Plates VII and VIII) recorded more number of inflorescence over control (Plate V). Though statistically not significant, Kinetin 10 ppm without urea (Plate IX)

Plate V Number of inflorescence per plant - Control without urea

Plate VI Number of inflorescence per plant - Control with urea.

Plate VII Number of inflorescence per plant - NAA 50 ppm without urea.

Plate VIII Number of inflorescence per plant - Kinetin 15 ppm without urea.

Plate IX Number of inflorescence per plant - Kinetin 10 ppm without urea.

Plate X Number of inflorescence per plant - Kinetin 15 ppm with urea.

also appears to favour inflorescence production. In combination with urea, Kinetin 15 ppm (25.00) (Plate X), NAA 10 ppm (24.67), Kinetin 10 ppm (22.67), Ethrel 100 ppm (21.67), Kinetin 5 ppm (19.00) and NAA 25 ppm (16.33) were found to be significantly superior to control (Plate VI) with respect to total inflorescence per plant.

4.5.2.2. Number of scapes per plant

Effects of growth regulators, urea and their interaction were significant on the number of scapes per plant. With the exception of all the three concentrations of Ethrel and Kinetin at 5 ppm growth regulator treatments were significantly superior to control in this respect. Urea exhibited a negative influence regarding the number of scapes per plant. In the absence of urea and NAA 50 ppm (28.33) and Kinetin 15 ppm (28.33) produced maximum number of scapes per plant followed by Kinetin 10 ppm (23.33). In combination with urea, number of scapes per plant decreased and the maximum value obtained was with NAA 10 ppm (21.33). Among the treatments with urea, all the concentrations of Kinetin and NAA except NAA 50 ppm and Ethrel 100 ppm were significantly superior to control.

4.5.2.3. Number of terminal inflorescence per plant

Statistical analysis of the data showed that growth regulators and urea had no significant influence

on the number of terminal inflorescence per plant whereas the interaction effect was significant. However, urea seems to exert a positive influence on the production of terminal inflorescence. Among the different treatments, Kinetin 15 ppm with urea (6.00) NAA 50 ppm (5.33) and Kinetin 10 ppm (4.33) without urea recorded more number of terminal inflorescence per plant.

4.5.2.4. Length of inflorescence

Length of the inflorescence was found to vary significantly with growth regulators and urea treatments though their interaction did not exhibit any significant influence. Among the growth regulator treatments NAA 50 ppm (5.68 cm), Kinetin 15 ppm (5.33 cm), NAA 25 ppm (5.13 cm) and Kinetin 10 ppm (5.00 cm) were significantly superior to control. Urea contributed for shorter inflorescence in all treatments except in combination with Kinetin 5 ppm and Ethrel 25 ppm where it resulted in longer inflorescence.

4.5.2.5. Number of flowers per inflorescence

Neither the effect of growth regulators nor that of urea were significant on the number of flowers produced per inflorescence. However, their interaction effect was

significant. A trend that urea contributing for slight reduction in the number of flowers per inflorescence was observed. Among the growth regulator treatments, excepting Kinetin 5 ppm and Ethrel 25 ppm others contributed for more number of flowers per inflorescence. In the absence of urea none of the treatments was found to have significant effect over control. Maximum flower production was noticed with NAA 50 ppm followed by control. In combination with urea, higher concentrations of Kinetin and Ethrel and lower concentrations of NAA were found to be significantly superior to control.

4.5.3. Abnormalities in floral structure

Abnormalities in floral structure encountered because of growth regulator and urea treatments are outlined in Table 11. Abnormalities observed were confined to the androecium. One of the plants treated with Kinetin 5 ppm and 2 per cent urea produced a flower having a single fertile anther lobe instead of the normal two numbers. One flower each obtained from the plant subjected to Kinetin 10 ppm with urea and NAA 25 ppm without urea treatments had two fertile stamens per flower instead of one seen in normal flower. As the occurrence of these abnormalities was an isolated incidence, no generalized effect of growth regulator treatments on the occurrence

of abnormal floral structures could be drawn from the results.

4.5.4. Floral biology of Rio-de-Janeiro

No variation was observed among the growth regulator and urea treatments with respect to the anthesis time, time of anther dehiscence and retention of individual flowers. Invariably, in all cases, identical values were obtained as those of control.

Table 11 Abnormal floral structures in different treatments

Treatment	Abnormal floral part	Abnormality
Kinetin 5 ppm + Urea	Anther lobes	One fertile anther lobe per flower instead of the normal two
Kinetin 10 ppm + Urea	Stamen	Two fertile stamens per flower instead of the one in normal flower
NAA 25 ppm	Stamen	Two fertile stamens per flower instead of the one in normal flower

4.5.5. Pollen characteristics of Rio-de-Janeiro

4.5.5.1. Pollen production and morphological features

Effect of growth regulators and urea on the extent of pollen production in terms of the number of pollen grains per flower and morphological features viz., diameter and exine thickness of pollen grains of Rio-de-Janeiro are given Table 12.

4.5.5.1.1. Extent of pollen production

Data furnished in Table 12 indicated that the number of pollen grains per flower varied significantly with growth regulator treatments. All the growth regulator treatments recorded more number of pollen grains per flower over control. However, significant difference was noticed only with NAA 10 ppm and Kinetin (5, 10 and 15 ppm levels). Though Urea had no significant influence, a positive trend in respect of pollen production had been indicated. Interaction effect was significant. Kinetin 15 ppm (6,45,800), Kinetin 10 ppm (5,88,500), NAA 50 ppm (4,37,500) and NAA 25 ppm (4,34,000) without urea and Kinetin 5 ppm (5,52,800), NAA 25 ppm (5,15,600) and NAA 10 ppm (5,17,400) with urea were found to yield promising results as compared to control.

4.5.5.1.2. Diameter of pollen grains

Statistical analysis of the data relating to diameter of pollen grains indicated that neither the growth regulators nor urea had any significant influence on pollen diameter. However, an apparent favourable effect of urea and a positive effect of NAA treatments at all the three levels (the maximum being with NAA 25 ppm) had been observed. Interaction effect of growth regulators and urea was found to be significant. In the absence of urea, Kinetin 15 ppm was significantly different from control. With urea, none of the treatments were different from control.

4.5.5.1.3. Exine thickness of pollen grains

Significant influence of the growth regulator on the exine thickness of the pollen grains was noticed (Table 12). Kinetin 5 ppm (13.20 µ) and NAA 50 ppm (12.69 µ) recorded significantly thicker exines compared to control (10 µ). Kinetin 15 ppm and NAA 10 ppm were the only cases exhibiting lesser exine thickness than control. However, this effect was not statistically significant. The exine thickness was found to reduce with higher concentrations of Kinetin and Ethrel and lower levels of NAA. Effect of urea and interaction were

not significant. The minimum value of exine thickness was observed with Ethrel 100 ppm in combination with urea (8.09 µ) followed by Kinetin 15 ppm without urea (8.67 µ).

4.5.5.2. Fertility of pollen grains

Data on the effect of growth regulators and urea on pollen fertility and sterility of ginger (var.Rio-de-Janeiro) are outlined in Table 13.

Statistical analysis of the data revealed that growth regulators, urea and their interaction had significant influence on the fertility and sterility of pollen grains. NAA 50 ppm, Kinetin 15 ppm, Kinetin 10 ppm, Ethrel 25 ppm and NAA 25 ppm were significantly superior to control in respect of higher fertility and lower sterility. Urea was found to aid in improving the fertility and reducing the sterility. Interaction effect was also significant. Ethrel 25 ppm (20.05 per cent), Kinetin 15 ppm (19.01 per cent), Kinetin 10 ppm (18.71 per cent) and NAA 50 ppm (18.58 per cent) NAA 25 ppm (15.90 per cent) and Ethrel 50 ppm (15.73 per cent) with urea were found to contribute for increased pollen fertility. A corresponding reduction in sterility was also observed in those treatments. Among the treatments devoid of urea none

of the growth regulators showed significant variation over control.

Growth regulators recorded a significant influence on unstained sterile pollen percentage and irregular shaped sterile pollen percentage. Urea and interaction effects were not significant.

NAA 50 ppm was the only growth regulator treatment which recorded significantly lesser sterility due to unstaining over control.

In the case of pollen sterility due to irregular shape Kinetin 5 ppm recorded significantly lesser sterility percentage over control whereas NAA 50 ppm resulted in significantly more sterility percentage over control.

4.5.6. Length of style and ovary

Style length and ovary length of Rio-de-Janeiro in respect of growth regulator and urea treatments are presented in Table 14.

4.5.6.1. Style length

The effect of growth regulators and urea on the style length of Rio-de-Janeiro was not statistically significant whereas the interaction effect was significant.

Table 14 Length of style and ovary of ginger (var. Rio-de-Janeiro) as influenced by growth regulators and urea

Treatments	Style length (cm)			Ovary length (mm)		
	With urea	Without urea	Growth regulator (mean)	With urea	Without urea	Growth regulator (mean)
Kinetin 5 ppm	3.57	3.63	3.60	2.00	2.50	2.25
Kinetin 10 ppm	3.65	3.70	3.68	2.08	2.67	2.38
Kinetin 15 ppm	3.72	3.80	3.76	2.75	2.83	2.79
Ethrel 25 ppm	3.60	3.50	3.55	2.38	2.00	2.19
Ethrel 50 ppm	3.70	3.60	3.65	2.50	2.08	2.29
Ethrel 100 ppm	4.10	3.70	3.90	2.60	2.17	2.38
NAA 10 ppm	3.67	3.70	3.68	2.50	2.50	2.50
NAA 25 ppm	3.60	3.90	3.75	2.33	2.38	2.36
NAA 50 ppm	3.30	4.00	3.65	2.27	2.17	2.22
Control	3.90	3.50	3.70	2.25	2.50	2.38
Urea (mean)	3.68	3.70	-	2.37	2.38	-
C.D. (growth regulator)		-			-	
C.D. (Urea)		-			-	
C.D. (interaction)		3.189			-	

All the growth regulator treatments with the exceptions of Ethrel 100 ppm, Kinetin 15 ppm and NAA 25 ppm resulted in reduced style length compared to control. Style length was found to reduce with lower concentrations of Kinetin and Ethrel. Ethrel 100 ppm with urea (4.10 cm) followed by NAA 50 ppm without urea (4.00 cm) contributed for maximum style length.

4.5.6.2. Ovary length

Statistical analysis of the data showed that the effects of growth regulators, urea and their interaction were not significant. Among the growth regulators Kinetin 15 ppm and NAA 10 ppm were the only treatments which recorded longer ovaries compared to control. Kinetin 15 ppm without urea (2.83 mm), Kinetin 15 ppm with urea (2.75 mm), Kinetin 10 ppm without urea (2.67 mm) and Ethrel 100 ppm with urea (2.60 mm) were the only cases where the ovary length was found to have marginal increase over the control without urea.

4.5.7. Pollen germination and pollen tube growth

4.5.7.1. Pollen germination

Growth regulators, urea and their interaction were found to exert significant influence on the germination of ginger pollen (var. Rio-de-Janeiro). Higher

concentrations of Kinetin and NAA and the lowest concentration of Ethrel recorded significantly high percentage of pollen germination over control. Urea was found to have a significant and positive influence on pollen germination. In combination with urea Ethrel 25 ppm (11.50 per cent), NAA 50 ppm (11.00 per cent), Kinetin 15 ppm (11.00 per cent), Kinetin 10 ppm (10.50 per cent), NAA 25 ppm (9.40 per cent) and Ethrel 50 ppm (9.00 per cent) with urea were found to enhance the pollen germination significantly over the control. Among the treatments without urea, NAA 50 ppm (8.00 per cent), NAA 25 ppm (7.50 per cent), Kinetin 15 ppm (7.20 per cent) and Kinetin 10 ppm (7.00 per cent) recorded better pollen germination compared to control.

4.5.7.2. Pollen tube growth

It may be seen from Table 15 that growth regulators had a significant regulatory role on pollen tube growth. With the exceptions of NAA 50 ppm (161 µ) and NAA 25 ppm (140 µ), growth regulator treatments contributed for poor pollen tube growth over control. Though statistically not significant, urea was found to have an apparently favourable influence on pollen tube growth. The maximum value of pollen tube growth was obtained in

Table 15 Effect of growth regulators and urea on pollen germination and pollen tube growth

Treatments	Pollen germination (%)			Pollen tube growth (μ) 24 hrs after anther dehiscence		
	With urea	Without urea	Growth regulator (mean)	With urea	Without urea	Growth regulator (mean)
Kinetin 5 ppm	7.50 (15.89)	6.60 (14.89)	7.05 (15.39)	56.00	52.00	54.00
Kinetin 10 ppm	10.50 (18.88)	7.00 (15.34)	8.75 (17.11)	82.00	64.00	73.00
Kinetin 15 ppm	11.00 (19.37)	7.20 (15.56)	9.10 (17.46)	92.00	76.00	84.00
Ethrel 25 ppm	11.50 (19.80)	6.20 (14.42)	8.85 (17.11)	60.00	40.00	50.00
Ethrel 50 ppm	9.00 (17.43)	5.50 (13.56)	7.25 (15.50)	68.00	56.00	62.00
Ethrel 100 ppm	7.00 (15.33)	4.70 (12.52)	5.85 (13.92)	70.67	68.00	69.33
NAA 10 ppm	8.00 (16.41)	6.20 (14.40)	7.10 (15.41)	118.00	100.00	109.00
NAA 25 ppm	9.40 (17.85)	7.50 (15.89)	8.45 (16.87)	148.00	132.00	140.00
NAA 50 ppm	11.00 (19.37)	8.00 (16.43)	9.50 (17.90)	162.00	160.00	161.00
Control	7.06 (15.39)	6.20 (14.42)	6.63 (14.91)	116.00	108.00	112.00
Urea (mean)	9.19 (17.57)	6.51 (14.74)	-	97.27	85.60	-
C.D.(growth regulator)		(0.791)		36.716		
C.D. (Urea)		(0.354)		-		
C.D. (Interaction)		(1.119)		-		

Values in parentheais indicate the angular transformed ones

the case of NAA 50 ppm with urea (162 μ) followed by NAA 50 ppm without urea (160 μ). A trend was observed that higher the concentration of NAA better would be the pollen tube growth.

4.5.8. Pollination

All the plants subjected to growth regulator treatments with and without urea were pollinated in all possible combinations. All the variables outlined in section 4.3 were tried.

4.5.9. Seed-set

In none of the combinations involving growth regulators, with and without urea and pollination procedure variables, seed was obtained. In all the cases, flowers were found to dry or/and rot by the next day morning.

Discussion

DISCUSSION

Ginger and its products have commanded considerable commercial significance in the international trade. The ever increasing demand and its suitability for varied preparations have putforth 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. Research work on crop improvement programmes in ginger is scanty but for the introduction and selection of high yielding varieties. Considerable variability has been observed among the varieties available under cultivation. However, attempts to manipulate the genotype variability through introduction and selection have failed to cope with the demand for special ginger types. Thus, it appears that the advantage of genetic variability is yet to be tapped and exploited for the benefit of cultivators. Evolving superior types through breeding appears to be a way out in this respect.

Shy and irregular flowering, poor pollen germination and the failure to set seeds are the main barriers for breeding in ginger. Lack of systematic

studies on the floral structure and floral biology of ginger also poses problems in the crop improvement programmes in ginger. A thorough understanding of the flowering behaviour and floral biology of the available ginger varieties was thus reckoned as the theme of the present investigation. The effect of growth regulators and urea on the flowering behaviour, floral biology, floral structures, pollination and seed-set also was evaluated. The results of the study are discussed in the following sections.

5.1. Flowering behaviour of available varieties under Vellanikkara conditions

It was observed that flowering is restricted to two varieties viz., Rio-de-Janeiro and Maran out of the 25 varieties. It is thus apparent that varieties vary widely in respect of flowering. Marked differences between Rio-de-Janeiro and Maran in all aspects of flowering also provided positive evidences for the varietal influence on the flowering behaviour of ginger.

Earlier flowering noticed in Maran may be attributed to its lesser photo-periodic requirement compared to Rio-de-Janeiro. The observation that Maran had lesser number of tillers at the time of flowering compared to Rio-de-Janeiro may be due to an inherent

character in tiller production. The report by Nybe (1978) that Maran produces lesser number of tillers than Rio-de-Janeiro also supports this view. Longer duration of flowering in Rio-de-Janeiro may be attributed to its advantage over Maran in respect of number of inflorescence per plant and number of flowers per inflorescence.

5.1.1. Inflorescence characteristics of Rio-de-Janeiro and Maran

Earlier workers have reported that inflorescence in ginger develops mostly as scapes originating from the rhizome and occasionally as terminal spike at the tip of the tiller. Results of the present investigation showed that terminal inflorescence was restricted to Rio-de-Janeiro. Varietal influence on the occurrence of terminal inflorescence is thus indicated. It seems that the hormone/factor governing flowering in ginger is present in the underground stem. Translocation of this hormone/factor to the tiller tip may be responsible for the occurrence of terminal inflorescence. Varietal influence on the translocation of this hormone/factor may be the reason for the presence or absence of terminal inflorescence in ginger.

During the present investigation, it was revealed that the percentage of terminal inflorescence in Rio-de-Janeiro was 20.70 per cent as against two per cent reported by Jayachandran et al. (1979). Climatic and seasonal factors may be held responsible for this variation.

Presence of more number of inflorescence and more flowers per inflorescence in Rio-de-Janeiro compared to Maran, is an observation of significance to breeders. Similarly, the duration of flowering being more extended in Rio-de-Janeiro, it is possible to undertake sizeable number of pollinations, that too repeatedly after assessing the success.

5.1.1.1. Scape development

Sequential steps of scape development, revealed that swelling on the top end of the dome shaped structure marked the visual initiation of the inflorescence development. In the absence of such a swelling, the dome shaped structure might have remained in vegetative phase. Presence of the flowering response/hormone, availability of photoperiodic requirement and soil moisture are the factors likely to decide the phase of the dome shaped structure.

For the full development of the inflorescence and stalk in Rio-de-Janeiro, it took 29 days as against the earlier reports ie. 20 to 25 days (Jayachandran *et al.*, 1979). Similarly blooming in an inflorescence was over within 9 to 18 days, whereas Jayachandran *et al.* (1979) had reported a blooming period of 23 to 28 days.

Lesser time taken by Maran over Rio-de-Janeiro, for the full development of the inflorescence may be due to the lesser number of bracts and flowers in Maran. Varietal factors also might be a cause for this phenomenon.

Acropetal succession of flower opening as observed in this study was in agreement with the report by Jayachandran *et al.* (1979). The observed blooming pattern of single flower per day initially and two to three flowers per day in the later stages is a new information.

5.2. Floral biology

5.2.1. Anthesis

Soil moisture was found to have a marked influence on the anthesis of ginger in general. Flowers failed to open under moisture stress conditions. Comparatively early flower opening in Rio-de-Janeiro thus appears to be owing to its inherently high moisture content which might

have contributed the initial moisture required for blooming.

Anthesis time in Rio-de-Janeiro observed during the present study, almost coincided with the earlier reports (Pillai et al., 1978 and Jayachandran et al., 1979) within the limitations of the climatic variations. Anthesis time in ginger is restricted to a period of 2.30 to 2.45 hours that too with 35 to 40 per cent flowers opening within 15 minutes during the peak period of flower opening.

5.2.2. Flower retention and anther dehiscence

Purseglove et al. (1981) have reported that ginger flowers are fragile and short lived. Results of the present study were in confirmity with this. Drying or/and rotting of flowers was found to take place in between 2400 hours and 0600 hours. Further studies appear necessary to locate the exact time of flower fragility.

No mention is available in the literature on the exact time of anther dehiscence except for a statement that it coincided with flower opening. However, it was observed that anther dehiscence does not exactly coincide with flower opening and it takes place 10 to 15 minutes (Rio-de-Janeiro) and 20 to 25 minutes (Maran)

after flower opening. Thus, it appears that the collection of pollen grains from fully opened flowers is possible.

5.2.3. Pollen characteristics

It was observed during the present study that pollen grains are present in plenty in ginger flowers, the count per flower varying from 1,87,500 in Rio-de-Janeiro to 1,91,500 in Maran. The data reveal that availability of pollen grains is not a limiting factor in ginger breeding.

With respect to the morphological features of pollen grains, the results obtained in this study were in agreement with the report by Pillai et al. (1978). Presence of the thick exine accounting to 23.88 per cent of the diameter in Rio-de-Janeiro and 25.46 per cent in Maran is an observation of considerable significance.

Pollen fertility assessed by the stainability with acetocarmine was as low as 12.48 per cent in Rio-de-Janeiro and 16.42 per cent in Maran. These values are far lesser than the reported values of 35 per cent by Pillai et al. (1978) and 24 per cent by Jayachandran et al. (1979). Poor fertility/high sterility of pollen grains thus seems to be a matter of concern to the breeders.

5.2.4. Stigma receptivity, style length and ovary length

Stigma receptivity of ginger has not been properly understood. Results of the present study indicated that stigma remained receptive (as judged from the presence of an ooze) from one hour to five hours after flower opening. Accordingly it is suggested that the best time for pollination in ginger is within one to five hours after flower opening.

Long but thin style observed in Rio-de-Janeiro and Maran may be a cause of hindrance to successful pollination.

Short and thick style and long ovaries may be helpful in achieving pollination success in ginger.

5.2.5. Pollen germination

Poor pollen viability had been suspected to be one of the factors responsible for pollination failures in ginger. Specific temperature as well as humidity requirement for pollen germination may be evidenced from the results of the studies on pollen germination. Fulfilment of either of these conditions failed to give positive response.

Growth regulators viz., IAA and GA were found to have no positive effect on pollen germination. Though sugar was found to have an influence on pollen viability, the effect does not appear to be of osmotic type.

Among the various media tried, the one with eight per cent sucrose, three per cent gelatin and 60 ppm boric acid recommended by Pillai et al. (1978) was found to be the best. The best condition identified for pollen germination was keeping with moist cotton covering in BOD incubator (26.5°C). Even under the best condition and the best medium identified during the present investigation, the maximum pollen germination obtained was as low as 6.20 per cent as against the reported figures of 14.5 per cent by Pillai et al. (1978).

Initiation of pollen germination was marked by exine bursting and the extension of the intine as the pollen tube. Coiling of the pollen tube observed during the advanced stages of pollen germination, seems to be a contributing factor for the pollination failure. Prior to coiling, the pollen tube attained a length of 108 μ. Since the style length was 3.72 cm, the possibility of the pollen tube reaching the ovary prior to coiling is remote. Moreover, once the coiling is started, pollen tube

penetration through the thin stigma is likely to be more difficult. Thus, it is clear that poor pollen germination and defective pollen tube growth are certain practical problems encountered in the pollination studies of ginger.

5.3. Pollination

An evaluation of the variables employed in the pollination studies based on the results discussed so far is furnished below.

Among the stages of flower utilised for pollination, bud stage and the stage immediately after flower opening were unsatisfactory as stigma was not receptive at these stages. The third stage, viz., three hours after flower opening was well within the period of stigma receptivity.

Removal of the stigma and partial and complete removal of the style also failed to give successful pollination. If the inhibitory substances present at the stigmatic surface or stylar neck were the reasons for the failure, success could have been obtained through the removal of stigma and style. Similarly, if the unconducive nature of the thin style for pollen tube penetration, was the factor for pollination failure,

success could have been obtained under complete removal of stigma and the entire style. But the cut end of the style had only less surface area to hold the pollen. If this point is viewed in the light of poor pollen germination, the pollination set back appears to be justifiable.

In pollination studies, adequate pollen was ensured through increased density of pollen and supplimentary pollinations. But, the accommodative area of the stigmatic surface and the cut end of the style was limited. Poor pollen germination coupled with poor pollen accommodative power at the receiving end might have been contributory factors for negative results.

Pollination with mixed pollen has not been successful in inducing the necessary stimulus required for successful pollination.

5.4. Seed-set

Fertilisation failure owing to poor pollen viability and defective pollen tube growth resulted in seed-set failure in ginger in the present investigation.

5.5. Effect of growth regulators on flowering, pollination and seed-set in Rio-de-Janeiro.

5.5.1. Flowering characteristics

5.5.1.1. Days from planting to flowering

Among the growth regulators tried NAA 50 ppm was found to induce early flowering. Positive influence of auxins in inducing flowering had been reported by Maheshwari and Venkataraman (1966) and Michniewicz and Kamienska (1965).

NAA treatments have shown the trend of inducing earlier flowering with increasing concentrations from 10 ppm to 50 ppm. Thus it seems that higher concentrations of NAA, above 50 ppm might be helpful in inducing still earlier flowering.

The observation that urea delayed the flowering may be explained from its effects on promoting vegetative growth (Section 4.5.1.2).

5.5.1.2. Number of tillers per plant at the time of flowering

Growth regulators exhibited a negative influence whereas urea had a positive effect on the number of tillers produced per plant at the time of flowering. These observations help in judging the effect of growth regulators and urea on plant growth and flowering.

5.5.1.3. Duration of flowering

Kinetin (15 ppm) was found to contribute for extended flowering period in Rio-de-Janeiro. This observation is supported by the inference that Kinetin 15 ppm produced more number of inflorescence per plant (Section 4.5.2.1.) and more number of flowers per inflorescence (Section 4.5.2.5).

5.5.2. Inflorescence characteristics

5.5.2.1.Total inflorescence per plant

Positive effects of NAA and Kinetin on inflorescence production were evidenced during the present investigation. Evidences of favourable effects of auxins on flowering had been reported by Clark and Kerns (1942) in pineapple. Since Ethrel treatments recorded lesser number of inflorescence per plant, it appears that the mechanism of promoting the inflorescence production by NAA in ginger is not identical to the one in pineapple. The favourable influence of Kinetin on inflorescence production is in conformity with the reports by Maheshwari and Venkataraman (1966) and Nakayama et al. (1962).

Negative influence of urea on inflorescence production may be due to its positive effect on tiller production and its tendency to retain the plant in vegetative phase.

5.5.2.2. Number of scapes per plant

Results and trends being in identical line as with Section 5.5.2.1. same explanations hold good here also.

5.5.2.3. Number of terminal inflorescence per plant

Kinetin 10 and 15 ppm and NAA 50 ppm resulted in the production of terminal inflorescence. Positive influence of urea was also evidenced in this respect.

Exogenously applied Kinetin and NAA might have moved from the leaves to the apical lateral buds through the phloem, transforming the vegetative bud to floral bud and thereby producing a terminal inflorescence. Exogenous application of growth regulators might have either triggered the activity or substituted the role of the naturally occurring flowering hormone/response.

5.5.2.4. Length of inflorescence

Favourable effects of NAA (50 and 25 ppm) and Kinetin (15 and 10 ppm) and the negative influence of urea could be explained on the same lines as discussed in Section 5.5.2.1.

5.5.2.5. Number of flowers per inflorescence

NAA 50 ppm without urea could be singled out as the only treatment capable of producing more number of flowers per inflorescence compared to control. In view of the favourable influence of Ethrel (25 and 50 ppm), it appears that the positive effect of NAA on the number of flowers per inflorescence may be due to the ethylene mediated mechanism as in pineapple.

5.5.3. Abnormalities in floral structures

Occurrence of abnormal floral structures being isolated cases, it is too early to make any inference on the role of growth regulators and urea in this respect.

5.5.4. Floral biology of Rio-de-Janeiro

The observations that growth regulators and urea treatments had no significant influence on the time of anthesis, anther dehiscence and retention of individual flowers point out that the floral biology of ginger is not regulated by growth regulators but by varietal and climatic factors.

5.5.5. Pollen characteristics

5.5.5.1.Pollen production and morphological features

5.5.5.1.1. Extent of pollen production

The observed favourable effect of growth regulators and urea may be attributed to the stimulatory effect of growth regulators at the gene level, manifested as the activation of specific gene resulting in the synthesis of specific mRNA and enzymes and the contributory role of urea and growth regulators to the cell content. Among the growth regulators, NAA and Kinetin were found to favour the pollen production. The role of auxin as a constituent of pollen (Takeyoshi and Fujii, 1961) and the positive effect of auxins and cytokinins on the maturation of onion anther sac (Vasil, 1957) may be quoted in support of the present observations. Stimulatory effect of cytokinins in cell division by increasing the synthesis of DNA and m RNA and that of auxin by increasing the ribosomal RNA might have resulted in enhanced pollen production.

5.5.5.1.2. Diameter of pollen grains

The apparent positive influence of NAA treatments and urea on the diameter of pollen grains could be explained on the same lines as in Section 5.5.5.1.1.Moreover, auxin induced cell elongation as a result of the micro

fibril synthesis and its incorporation into the core of the cellwall contributing to an increase in the dry weight of the wall may be another possible reason for the increase in pollen diameter. Effects of auxins on the synthesis of m RNA, RNA directed protein synthesis and the synthesis of cellulose synthetase also might have contributed to the increased pollen diameter.

5.5.5.1.3. Exine thickness of pollen grains

Exine thickness was found to have a marginal reduction with higher levels of Kinetin and Ethrel and lower levels of NAA. Effect of auxins on cellwall thickening has been discussed in Section 5.5.5.1.2. The exine of the pollen appears to be a deposit of microfibrils on the inner face of the cellwall. This consideration, if held valid explains the thicker exines as a result of higher NAA concentrations. Ethylene is found to promote the cellwall permeability by its cellwall dissolution effect. Owing to this effect of ethylene on pollen walls, reduced exine thickness resulted. Urea by its effect in enhancing the turgour and stress on the cellwall also reduces the exine thickness. Thus, the reduced exine thickness as a result of Ethrel with urea treatment appears to be justifiable.

5.5.5.2. Fertility of pollen grains

Favourable influence of growth regulators and urea on pollen fertility may be due to their regulatory role on the synthesis of specific nucleic acids, enzymes and cell constituents, governing the pollen fertility.

5.5.6. Style length and ovary length

Short style and long ovary are desirable for success in pollination. Results of the present study showed that growth regulator and urea treatments were not capable enough in manipulating the style and ovary to the advantage of breeding. Since no substantial ovary development could be achieved by the growth regulator and urea treatments, the possibility of obtaining a parthenocarpic fruit development appears to be remote. However, the higher concentrations of Kinetin (with and without urea) and Ethrel (with urea) have indicated some promising results in this respect and hence are worth persuing.

5.5.7. Pollen germination and pollen tube growth

Pollen germination was achieved with growth regulator treatments in general with the only exception of the higher concentration of Ethrel. Higher concentrations of

NAA and Kinetin favoured pollen germination. Urea also contributed for the same. A comparison of these results with the one on the effect of growth regulators and urea on the fertility of pollen grains showed identical pattern. Thus, it appears that the acetocarmine test of pollen fertility in ginger is a true indication of the pollen viability in respect of Rio-de-Janeiro variety. The explanations offered on the influence of growth regulators and urea on pollen fertility are thus valid here too.

Enhanced pollen tube growth was achieved with NAA 50 ppm and 25 ppm. Leopold and Kriedemann (1980) have furnished the evidence for the influence of auxins on pollen tube growth. An appraisal of the trend indicates that still higher concentrations of NAA may be worth trying to achieve better pollen tube growth.

5.5.8. Pollination

Failure in pollination studies inspite of growth regulator application indicates the necessity for a more systematic study based on the results of the present investigation.

5.5.9. Seed-set

It was not possible to overcome the long prevailing problem of failure in seed-set in ginger by

the application of growth regulators with and without urea. However it was possible to throw much light on the factors contributing to the hindrances in seed-set. Toxic substances at the stigmatic surface was found to be not a cause for pollination failures. The problems of poor pollen viability and pollen tube growth were tackled to an extent by growth regulator application. In an attempt to overcome the incompatibility problems, mixed pollen was employed for pollination. The failure to achieve seed-set, even with mixed pollen indicates that incompatibility may not be the factor causing seed-set failure. Effect of growth regulators and urea on the structural and functional aspects of ginger flower was indicated during the present investigation. These positive responses can be exploited for better results. The impact of growth regulators with and without urea on mega and micro sporogenesis which was not taken up in the present investigation is a field for future studies.

Summary

SUMMARY

Flowering characteristics of the available varieties of ginger and the effect of growth regulators on the flowering characteristics, pollination and seed-set in Rio-de-Janeiro variety of ginger were studied at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara during 1981-'83.

Main findings of the study are summarised hereunder.

Among the 25 varieties available at Vellanikkara, flowering was restricted to two varieties viz., Rio-de-Janeiro and Maran. Flowering was early in Maran whereas, in respect of the number of plants flowered, duration of flowering, number of inflorescence per plant and number of flowers per inflorescence, Rio-de-Janeiro was superior. Both scape and terminal inflorescence were observed in Rio-de-Janeiro whereas it was restricted to scapes only in Maran. Sequential steps of scape development and flowering were outlined. From the emergence of the scape to the completion of flowering, it took 45 days in Rio-de-Janeiro as against 40 days in Maran. Flowering time varied

from 1400 hours to 1545 hours (the peak being between 1445 to 1500 hours) in Rio-de-Janeiro and 1430 to 1600 hours (the peak being between 1500 to 1515 hours) in Maran. Fragile and short lived nature (<16 hours) of ginger flowers was noticed. It was observed that anther dehiscence takes place 10 to 15 minutes (Rio-de-Janeiro) and 20 to 25 minutes (Maran) after flower opening. Plenty of pollen grains, the count per flower being 1,87,000 in Rio-de-Janeiro and 1,91,500 in Maran, are present in ginger flowers. Pollen grains of both Rio-de-Janeiro and Maran were spherical with thick exines - the thickness being 9.73 µ in Rio-de-Janeiro and 10.15 µ in Maran. Pollen fertility, assessed by the stainability with acetocarmine, was as low as 12.48 per cent in Rio-de-Janeiro and 16.42 per cent in Maran. In both the varieties, stigma remained receptive from one hour to five hours after flower opening. Long but thin style was noticed both in Rio-de-Janeiro and Maran.Style length varied from 3.72 cm (Rio-de-Janeiro) to 3.50 cm (Maran). Ovary length was found to be 2.50 mm in Rio-de-Janeiro and 2.60 mm in Maran.

Studies with the pollen grains of Rio-de-Janeiro indicated the specific requirements of temperature and humidity for pollen germination. IAA and GA had no positive effect on pollen germination. Among the various

media tried, the one with eight per cent sucrose, three per cent gelatin and 60 ppm boric acid was found to be the best. The best condition identified for pollen germination was keeping with moist cotton covering in BOD incubator (26.5°C). The maximum pollen germination obtained was 6.20 per cent. Coiling of the pollen tube was observed during the advanced stages ie. at a length of 108 u. The possibility of the pollen tube reaching the ovary through the thin style of 3.7? cm length, prior to coiling appears to be remote.

Pollination was carried out in Rio-de-Janeiro and Maran employing the variables like stage of flower (bud stage, immediately after flower opening and three hours after flower opening), condition of stigma (partial and complete removal of style), density of pollen (smearing once, twice and thrice with standard brush), mixed pollen (ginger pollen mixed with either Alpinia, Hedychium, Kaempheria or Costus pollen) and supplementary pollination (repeated pollination twice at two hours interval). No seed-set was obtained in any of these cases.

Growth regulators and urea were found to have no significant influence on the flowering characteristics of Rio-de-Janeiro. However, positive influence of NAA 50 ppm on inducing early flowering and Kinetin 15 ppm

on the duration of flowering was indicated. Urea had an apparently negative effect on the flowering characteristics.

Positive effects of NAA and Kinetin on inflorescence production were observed. Production of terminal inflorescence was favoured by Kinetin 10 ppm and 15 ppm and NAA 50 ppm. Urea seems to favour the production of terminal inflorescence. Favourable effects of urea on the length of the inflorescence were indicated. NAA 50 ppm without urea produced apparently more number of flowers per inflorescence over control. Effects of growth regulators and urea were not significant in this respect. A trend that urea contributing for slight reduction in the number of flowers per inflorescence was observed.

Abnormal floral structures occurred as isolated cases and so the effect of growth regulators and urea was not inferred.

Growth regulator and urea treatments had no influence on the floral biology of ginger.

NAA 10 ppm and all the three levels of Kinetin produced more number of pollen grains per flower. Urea

also exhibited a positive trend in this respect. An apparent positive influence of NAA and urea treatments on the pollen diameter was noticed. Marginal reduction of exine thickness was achieved with higher levels of Kinetin and Ethrel and lower levels of NAA. Ethrel 100 ppm in combination with urea recorded the minimum exine thickness. Growth regulators, urea and their interaction had significant influence on the fertility of pollen grains. Among the growth regulator treatments, NAA 50 ppm, Kinetin 15 ppm and 10 ppm recorded significant increment in pollen fertility over control. Urea was found to aid in enhancing the pollen fertility and reducing the pollen sterility. Ethrel 25 ppm in combination with urea recorded the maximum pollen fertility.

A marginal reduction in style length was achieved with the lower concentrations of Kinetin and Ethrel. Kinetin 15 ppm and NAA 10 ppm resulted in longer ovaries. Maximum ovary length was achieved with Kinetin 15 ppm without urea.

Among the growth regulator treatments, higher concentrations of NAA and Kinetin and the lowest concentration of Ethrel recorded significantly higher percentage of pollen germination over control. Urea exerted a favourable effect on pollen germination.

Maximum pollen germination was achieved with ethrel 25 ppm with urea. Effect of growth regulators and urea on the fertility of pollen grains and pollen germination followed an identical trend.

With the exceptions of NAA 25 ppm and 50 ppm the growth regulator treatments contributed for poor pollen tube growth. Urea appears to favour the pollen tube growth. The maximum value of pollen tube growth was obtained in the case of NAA 50 ppm with urea (162 μ).

All the plants of Rio-de-Janeiro subjected to growth regulator treatments with and without urea were pollinated in all possible combinations. Variables like the stage of flower, condition of stigma, density of pollen, mixed pollen and supplementary pollination were tried. In none of combinations, seed-set was achieved. Incompatibility does not appear to be a reason for the failure in achieving seed-set in ginger.

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* Originals not seen

APPENDIX - I

Analysis of variance - Mean sum of square values

Character	Growth regulator (df = 9)	Urea (df = 1)	Growth regulator x Urea (df = 9)	Error (df= 38)
Days from planting to flowering	87.276^{NS}	120.417^{NS}	7.898^{NS}	48.697
Tillers per plant at the time of flowering	149.659^{NS}	493.066^{NS}	42.919^{NS}	133.056
Duration of flowering	16.717^{NS}	21.444^{NS}	35.348**	9.482
Total inflorescence per plant	321.389**	189.038^{NS}	137.815*	55.805
Number of scape per plant	222.039**	370.017*	133.239*	52.361
Number of terminal inflorescence per plant	6.854^{NS}	2.017^{NS}	10.350*	4.208
Length of inflorescence	2.134**	3.495*	0.724^{NS}	5.819
Number of flowers per inflorescence	32.085^{NS}	58.945^{NS}	49.174**	16.560
Pollen production (No.of pollen grains/flower)	568.293**	5.382^{NS}	721.253**	191.674
Diameter of pollen grains	65.820^{NS}	113.493^{NS}	173.913**	48.826
Exine thickness of pollen grains	10.285**	0.410^{NS}	6.308^{NS}	3.175
Fertile pollen percentage	19.809**	144.709**	8.680**	2.469
Sterile pollen percentage (unstained)	149.450**	102.757^{NS}	26.788^{NS}	26.898
Sterile pollen percentage (irregularly shaped)	32.852**	0.65^{NS}	20.601^{NS}	10.904
Sterile pollen percentage (total)	20.309**	104.017**	5.197**	1.035
Style length	0.056^{NS}	0.008^{NS}	0.155**	0.037
Ovary length	0.181^{NS}	2.407^{NS}	1.964^{NS}	9.594
Pollen germination percentage	10.149**	120.134**	2.884**	0.459
Pollen tube growth	8531.711**	2041.667^{NS}	72.333^{NS}	983.309

NS - Not Significant

* - Significant at 5% level

** - Significant at 1% level

APPENDIX - II

Weather data during the period of plant growth - April to December, 1983.

Month	Temperature (°C)		Relative humidity			Total rainfall mm	Rainy days	Total sunshine hours
	Minimum	Maximum	0730 hours	1430 hours	Mean			
April	25.80	36.20	-	-	66.00	-	-	270.90
May	25.50	35.10	-	-	69.00	37.40	3.00	240.50
June	24.50	31.90	90.00	69.00	79.00	387.20	19.00	113.80
July	23.70	29.70	94.00	79.00	87.00	580.60	28.00	89.50
August	29.10	23.80	93.50	80.40	87.00	754.70	26.00	61.30
September	29.50	23.40	93.00	75.00	84.00	494.16	24.00	108.00
October	31.20	23.10	90.00	64.00	77.00	149.80	6.00	215.80
November	31.80	22.30	84.00	58.00	71.00	60.20	3.00	244.90
December	31.20	23.90	71.00	55.00	63.00	24.40	3.00	215.60

Source : Meterological Observatory, Vellanikkara.

EFFECT OF GROWTH REGULATORS ON FLOWERING, POLLINATION AND SEED-SET IN GINGER (*Zingiber officinale*, Rosc.)

By

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ABSTRACT OF A THESIS

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ABSTRACT

Flowering behaviour and floral biology of Rio-de-Janeiro and Maran and the effect of growth regulators viz., Kinetin (5, 10 and 15 ppm), Ethrel (25, 50 and 100 ppm) and NAA (10, 25 and 50 ppm) in combination with two per cent urea and without urea on flowering, pollination and seed-set in Rio-de-Janeiro were studied at the College of Horticulture, Kerala Agricultural University, Vellanikkara during 1981-'83.

The objective of the investigation was to assess the available varieties of ginger for flowering behaviour and to evaluate the effect of growth regulators with and without urea on the flowering behaviour, floral biology, floral structure, pollination and seed-set in Rio-de-Janeiro. The possibility of overcoming the problems of shy and irregular flowering, poor pollen germination and the failure to set seeds also was explored during the investigation.

Among the 25 varieties studied, flowering was observed only in two varieties viz., Rio-de-Janeiro and Maran. Considerable variation was noticed between Rio-de-Janeiro and Maran with respect to flowering behaviour, extent of flowering, types of inflorescence, time taken for scape development, anthesis, anther dehiscence, pollen production, pollen fertility, style length and ovary length. Long but thin style was noticed both in Rio-de-Janeiro and Maran.

Irrespective of the variety, flowers were found to rot/ and dry within 16 hours after flower opening.

The maximum pollen germination (6.20 per cent) was obtained in the medium containing eight per cent sucrose, three per cent gelatin and 60 ppm boric acid under moist cotton covering in BOD incubator (26.5°C). Coiling of the pollen tube during the advanced stages of growth was noticed.

Pollination carried out in Rio-de-Janeiro and Maran employing the variables like stage of flower (bud stage, immediately after flower opening and three hours after flower opening), condition of stigma (partial and complete removal of style), density of pollen (smearing once, twice and thrice with standard brush), mixed pollen (ginger pollen with either Alpinia, Hedychium, Kaempheria or Costus pollen) and supplementary pollination (repeated pollination twice at two hours interval) failed to record any positive evidence of seed-set.

Effect of growth regulators and urea on flowering behaviour, inflorescence characteristics and floral structures of ginger was evidenced during the study. Favourable influence of NAA 50 ppm on inducing early flowering, Kinetin 15 ppm on the duration of flowering, NAA and Kinetin on inflorescence production, NAA 50 ppm without urea on the number of flowers per inflorescence, NAA 10 ppm and all the three levels of Kinetin on pollen production, NAA on pollen

diameter, higher levels of Kinetin and Ethrel and lower levels of NAA on exine thinning, Ethrel 25 ppm in combination with urea, Kinetin 15 ppm with urea, NAA 50 ppm and Kinetin 10 ppm on pollen fertility, lower levels of Kinetin and Ethrel on reducing the style length, Kinetin 15 ppm and NAA 10 ppm on ovary length, Ethrel 25 ppm with urea and higher levels of NAA and Kinetin on pollen germination and NAA 50 ppm on pollen tube growth was revealed during the study.

Floral biology of Rio-de-Janeiro was not influenced by growth regulator and urea treatments.

Abnormal floral structures occurred as isolated cases and therefore the role of growth regulators and urea in this respect was not clear from the results.

Pollination carried out in ginger (var.Rio-de-Janeiro) plants, subjected to growth regulator treatments with and without urea, employing the variables like stage of flower, condition of stigma, density of pollen, mixed pollen and supplementary pollination failed to result in seed-set.