EXPLOITATION OF INDUCED VARIABILITY FOR CROP IMPROVEMENT IN GINGER (*Zingiber officinale* Rosc.)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

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VELLANIKKARA, THRISSUR-680656
KERALA, INDIA

2003

DECLARATION

I hereby declare that this thesis entitled **"Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)"** is a record of research work done independently by **Mr. G. Shankar** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Acknowledgment

"All men like to think, they can do it alone, but a real man knows there's no substitute for support, encouragement or crew" - Allen

First and foremost, I wish to place on record my deep sense of gratitude and I feel myself short of words to express the boundless love, affection, constant encouragement and inspiration showered by my parents with out which I could never complete this endeavour successfully.

I express my deepest sense of respect, gratitude and indebtedness to Dr.Alice Kurian, Associate Professor, Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University and chairperson of my advisory committee for her meticulous guidance, constant encouragement, ever willing help, constructive criticism, continuous support, creative ideas, well timed advice, painstaking scrutiny of the manuscript, extreme patience, motherly affection, good under standing, guiding spirit. Gifted I am, to relish a few from her ocean of knowledge, experience, perseverance and perfection. I considered myself fortunate in having the privilege of being guided by her.

I wish to extent my sincere gratitude with due respect and whole hearted thankfulness to Dr. E.V. Nybe, Associate Professor and Head, Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, for his scholarly suggestions, critical scrutiny of the manuscript and unbound support rendered throughout my investigation.

I extend my profound sense of gratitude to Dr. P. A. Valsala, Associate Professor, Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, for her unwavering encouragement, unflagging

perseverance, well-timed and price less support, eminent suggestions, critical assessment and invigorating discussions through out my study.

I am very much obliged to Dr. Luckins C. Babu,, Associate Dean, College of Forestry, Vellanikkara, for his timely suggestions and help rendered which made the successful completion of this thesis possible.

With deep respect and esteem regards, my heartful thanks are due to Dr. P. A. Nazeem, Associate Professor, Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, for providing ample freedom of work, valuable and timely suggestions and expert counseling of this manuscript.

It gives me great privilege to express heartful thanks to teaching and non-teaching staffs of Plantation Crops and Spices Department for their timely suggestions and encouragements provided throughout the course of my study.

I duly acknowledge the full heart, personal sacrifices, incessant encouragement, moral support and timely persuasions by my friends Rajasekar, Chandraseggar, VIswanathan, Suresh, Sundararasu, Hagi Khan and Vezhavendhan not only in my research and throughout my career.

On a personal note, I would like to pour out my heartful love, but mere words can't express my soulful gratitude to my beloved friends "Jyothi, R, and Teena Joy and their family members" for their unbridled help and stupendous sacrifice for me during the course and the affection shown by them gave me enough mental strength and joy to go through all tedious circumstances in Kerala.

I specially acknowledge Karthikeyan, Venkatesh, Ramesh and Ponnaiyan for their boundless love and affection, rendered help in my career.

My sincere thanks to all my batch friends Mini, Sujatha, Binu, Gana, Arul, Biju, Boopathi, Muthu, Manimala and Rajesh for their pleasant friendship and company that made my stay in Kerala quite happy and comfortable.

I thankfully acknowledge the co-operation and help rendered by my seniors Arunachalam, Karthi, Lakshmikanthan, Vineel, Kali, Makesh, Sajithiranath, Karups and Dinesh.

A special word of thanks to my friends Murugan, Naga, Kamalakanna, Sambha, Chandrahasan, Gopi, Bhavani, Mani, Jinna, Mahadev, Pradeep and Subba for their joyful company and lively inspirations received during the period of study.

The sincere help, support and encouragement of my dear friend Santhosh and Prince in computer work is deeply acknowledged.

I am gratefully acknowledge KAU for the award of research fellowship.

I am forever beholden to all of my family members with special thanks to my brothers (Venkatesh, Sathish, Ravi, Ramkumar), Sisters (Vennila, Manju, Devi) and baby (Swarnalatha) for their boundless affection, moral support and inspiring encouragement which becomes the summum bonum for me to lift up to this stage.

A word of apology to those I have not mentioned in person and a note of thanks to one and all that worked for the successful completion of the endeavour.

Above all, I bow my head before GOD ALMIGHTY without whose blessings I would not have completed this venture successfully.

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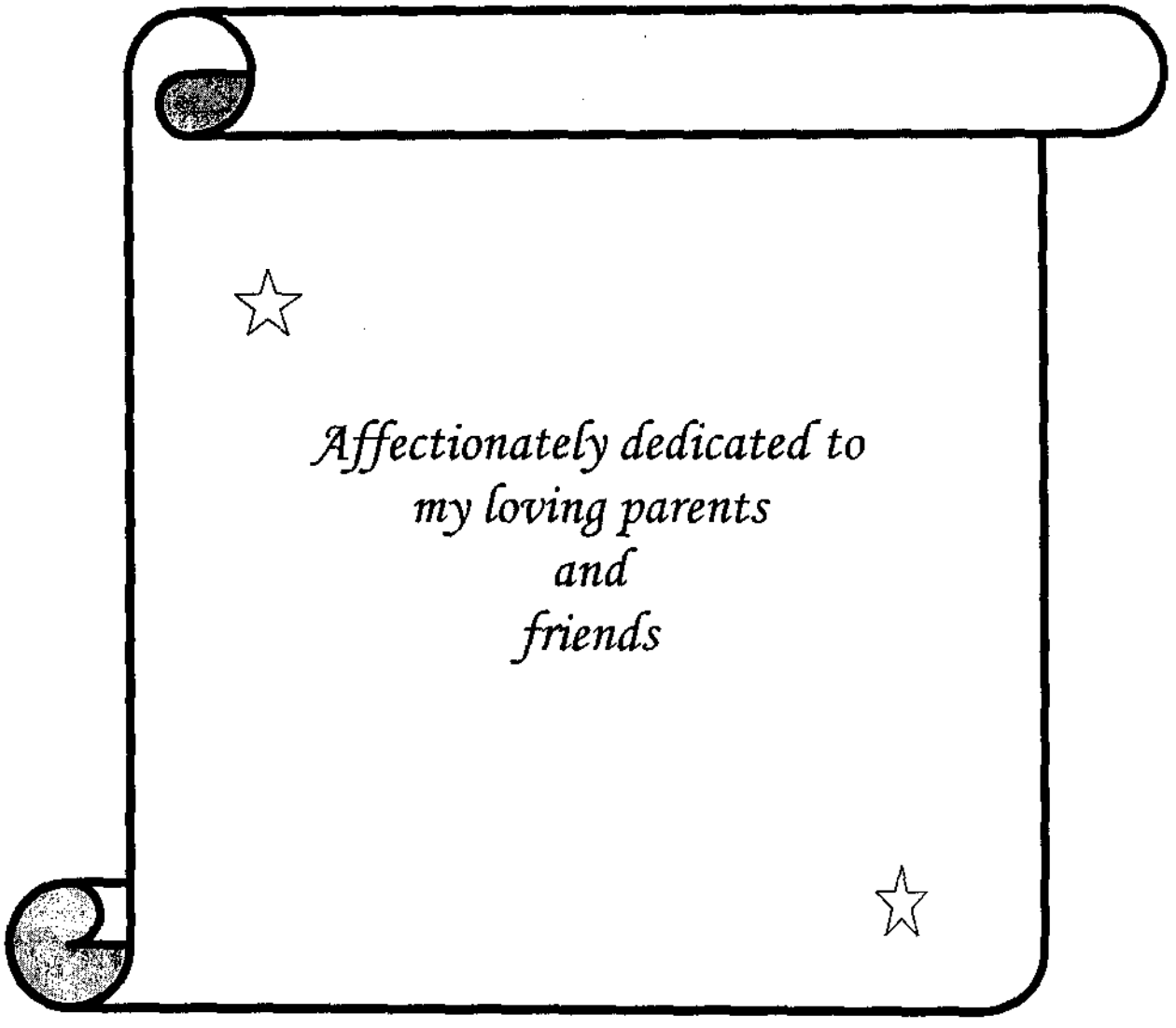

Affectionately dedicated to
my loving parents
and
friends

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µm	- micrometer
2, 4 – D	- 2, 4, Dichlorophenoxy Acetic Acid
BA	- Benzyl Adenine
CH	- Casien Hydrolysate
CW	- Coconut Water
DAP	- Days After Pollination
GA_3	- Gibberellic Acid
ha	- hectare
HCl	- Hydrochloric Acid
HNO_3	- Nitric Acid
IAA	- Indole – 3 – Acetic Acid
IBA	- Indole Butyric Acid
kg	- kilogram
l	- litre
mg	- milligram
mm	- millimetre
MS	- Murashige and Skoog's (1962) medium
NA	- Not available/ Not applicable
NAA	- α Naphthalene Acetic Acid
PEG	- Polyethylene glycol
SH	- Schenk and Hildebrandt (1972) medium
t	- tonnes
V/V	- Volume in volume


INTRODUCTION

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), is one of the oldest and renowned commercial spices esteemed for its aroma, flavour and pungency. It is much valued as a spice, medicine as well as vegetable since very ancient days (Sasikumar, 1996). According to the ayurvedic system of medicine, ginger is considered to be carminative, stimulant and administered in dyspepsia and flatulent colic. It is also prescribed as an adjunct to many tonics and stimulating remedies. The folk use of this ancient spice for gastrointestinal problems has been given scientific approval. Today, it is used mainly to prevent the symptoms of travel sickness (Langner, 1998). It is used as anti-inflammatory, anti-emetic and to treat rheumatic conditions. It also has aphrodisiac values, besides its use in tinctures and as a flavourant. In western medicine, it is used to prevent motion sickness, nausea, dizziness as well as for post operative vomiting and vomiting during pregnancy (Grant, 2000).

Ginger has been considered indispensable in the culinary art for flavouring of foods. A number of alcoholic beverages such as brandy, wine, beer and ale are prepared in foreign countries. Ginger oil also finds use in perfumery, pharmaceuticals and has industrial use (Pruthi, 1998). In the present day, the use of ginger in different forms is increasing which has resulted in a hike in demand world over.

In the global scenario, India still continues to be the largest producer, consumer and exporter of ginger and its products. In India, ginger occupies 83, 940 ha with a production of 3, 06, 960 tonnes of which 6, 580 tonnes valued at Rs. 22.95 crores were exported during 2000 – 2001 (Johny and Ravindran, 2002). Ginger registered 4.05 per cent growth in export quantity and 10.15 per cent in export value.

Although ginger is grown in almost all parts of India, Kerala makes significant contribution through production of world renowned 'Cochin' and 'Calicut' ginger and contributes 33 per cent of the Indian production. In Kerala, ginger is cultivated in an area of 14, 568 ha with a production and productivity of 49, 946 tonnes and 3, 428 kg ha^{-1} respectively (FIB, 2001).

Inspite of the fact that ginger is an important and ancient spice, no major break through in boosting the production and increasing exports has been possible. The ginger production in India is handicapped by the occurrence of devastating diseases like soft rot and bacterial wilt, higher cost of production and non availability of better genotypes. In the export front, high fibre content and dull colour of dried rhizomes are factors limiting international competitiveness. In order to put the ginger industry on sound footings, it is therefore imperative to develop high yielding disease resistant varieties, with low fibre, high volatile oil and oleoresin contents. The recent issue of globalization demands cost effectiveness and product diversification so as make Indian ginger competitive in the international trade.

The genetic variability in ginger is locked up due to exclusive vegetative propagation. Conventional generative breeding cannot be employed mainly due to lack of seed set in nature. Hence, most of the crop improvement programmes of this species are confined to evaluation and selection of naturally occurring clonal variations. The existing population of ginger shows narrow genetic variability for morphological, economic and qualitative traits. The available gene pool is not so far fully exploited for crop improvement.

Conventional *in vivo* methods like artificial cross pollination on stigma and on style cut at various heights, bud pollination, chemically aided pollination, mixed pollination and mentor pollination tried to induce seed set, have not given any positive response so far.

Creation of variability is imperative to step up yield potential, improve quality attributes like low fibre content and high flavour components and resistance to diseases like soft rot and bacterial wilt. The deeply seated ovary, which cannot be reached by the germinating pollen, the main hindrance for obtaining seed set in ginger could be overcome by *in vitro* placental pollination and fertilization (Valsala, 1994). The germination of *in vitro* produced seeds continues to be highly erratic and the conditions for successful regeneration are yet to be standardized.

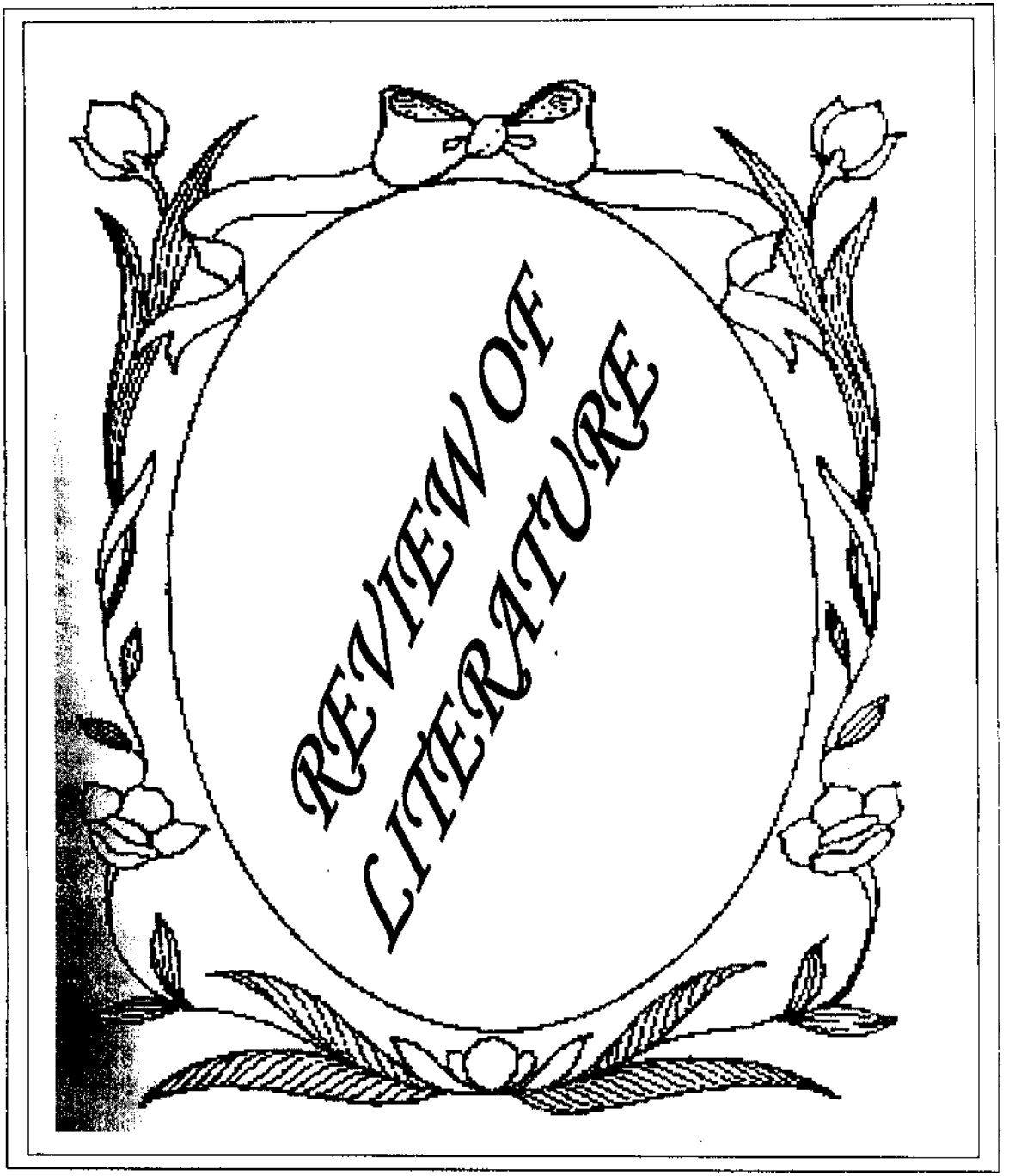
Induction of variability in commercial cultivars of ginger through colchiploidy attempted at the Department of Plantation Crops and Spices during 1994 – 1996 generated two autotetraploids and five promising variants (Sheeba, 1996). Detailed variability analysis of these variants is warranted to isolate commercially viable types. The high pollen fertility observed in the autotetraploids could be used with advantage, in heterozygous breeding. Generation of progenies with altered ploidy level is possible by crossing autotetraploids and diploids, which can be exploited for targeted characters. The success of the present study will open new vistas in crop improvement of ginger.

Therefore, the present investigations were undertaken with the following objectives:

To evaluate the variability in colchicine induced variants of ginger for morphological characters, anatomical features, floral characters, economic attributes, qualitative traits and incidence of pest and disease in order to isolate commercially acceptable types.

To attempt *in vivo* and *in vitro* crossing between autotetraploids and diploids.

To standardize the conditions for successful germination of *in vitro* produced ginger seeds to create genetic variability and to isolate hybrids with targeted characters.


REVIEW OF
LITERATURE

2. REVIEW OF LITERATURE

The accumulated literature on various aspects relevant to the subject matter of the present investigation "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)"is reviewed here under.

2.1 ORIGIN AND DISTRIBUTION

Ginger is not seen in wild state, hence the country of origin is not known with certainity. The sanskrit name 'Singabera' gave rise to the greek name 'Zingiberi' and to the late latin 'Zingiber'. It might have originated in South East Asia, especially in India or China, and later introduced to countries like Japan, Sierraleone, Nigeria, Australia and West Indies. It was the Spanyards who introduced ginger to Jamaica which is now world famous for the quality (Purseglove, 1978).

2.2 TAXONOMY

Ginger, (*Zingiber officinale* Rosc.) is a monocot belonging to the family Zingiberaceae of the order Zingiberales. The family Zingiberaceae consists of two sub families (ie) aromatic Zingiberoidae and non aromatic Costoideae. The sub family Zingiberoideae include important genera like *Aframomum, Amomum, Curcuma, Elettaria, Hedychium, Kaempferia and Zingiber.* The principal genus of Costoideae is *Costus* (Purseglove, 1978).

The genus *Zingiber* consist of about 80 to 90 species of perennial rhizomatous herbs distributed through out South East Asia and extending to Queensland and Japan (Purseglove *et al.*, 1981).

Z. mioga, a condimental vegetable, rarely sets seed as reported by Adaniya (1991). Sirat (1994) used *Z. ottensii* rhizome as a poultice in postnatal treatments and as an appetizer.

Theilade and Mood (1999) characterized *Z. viridiflavum* and *Z. chlorobracteatum* by their slender fusiform inflorescence with yellow-green bracts while *Z. flagelliforme* displays a peculiar reproduction habit in its tapering leafy

shoots which bend over to trail on the ground where plantlets produced in the leaf axis take root. *Z. velutinum* is a tall plant with large ovate inflorescence and bright red, densely hairy bracts and *Z. phillippsii* is morphologically characterised by its purple stems and glabrous leaves. *Z. collinsii*, a new species from Vietnam placed in section *Zingiber*, have the inflorescence on a radical, short erect shape (Theilade and Mood, 1999). *Z. matutumense* has a close similarity to *Z. spectabile* in the structure of inflorescence, but with distinct vegetative characters and a different coloration of inflorescence and flowers as reported by Mood and Theilade (2001).

2.3 CYTOGENETICS

The chromosome number of 2n = 22 for ginger (*Zingiber officinale* Rosc.) has been confirmed by many cytogeneticists (Morinaga *et al.*, 1926; Chakravarthi, 1948 and Sathiabhama, 1988), eventhough Takahashi (1931) reported chromosome number of 2n = 24 for the species. Chakravarthi (1948) concluded in view of the bivalent association in diploid species of *Z. cassumnar* and *Z. zerumbet*, that *Z. mioga* is to be considered as a pentaploid. Pillai *et al.* (1978) observed that meiosis is highly irregular in ginger with only 46.6 per cent of PMCs showing bivalents and the rest showing univalents, trivalents and quadrivalents. Ratnambal and Nair (1982) concluded that the structural chromosome aberrations had a significant influence in lowering the fertility in cultivars of *Z. officinale*. Adaniya (1991) investigated variation in the number of chromosomes of self pollinated progenies of *Z. mioga* and reported that the chromosome number varied from 2n = 46 to 2n = 60. Autotetraploids (2n=44) were produced in ginger by colchicine treatment (Ramachandran and Nair, 1992 and Sheeba, 1996).

The karyotype study of nine cultivars of ginger by Sathiabhama (1988) showed that there is considerable difference in the morphology of their chromosome, such as length, centromere position, total chromatin length and total chromosome volume. The classification of the kayotype showed that *Z. officinale* belongs to the primitive species of angiosperms. Irregular meiosis consisting of univalents, trivalents, quadrivalents, pentavalents and hexavalents besides bivalents during microsporogenesis was also observed.

2.4 CROP IMPROVEMENT IN GINGER

Crop improvement work related to ginger was taken up with widened objectives such as to develop high yielding varieties with wide adaptation, with high oil, oleoresin and low fibre, besides being resistant to major diseases and pests.

2.4.1 Variability studies

Ginger is a vegetatively propagated crop and the agro-ecological conditions have a greater role to play in the various morphological, yield and quality attributes. The numbers of clones in ginger available are limited and each centre of production produces a distinctive type and this may be due to soil, climatic and cultural conditions (Purseglove, 1978). The same variety grown under different conditions show marked difference in various attributes. Very little variability has been reported among the genotypes grown in the same area, but with good amount of variability among the varieties grown in different states. Sreekumar *et al.* (1980) reported that improved ginger cultivars such as Suprabha, Suruchi and Suravi performs well for each of a number of yield and quality related traits. Varada, Mahima and Rejatha are released varieties from Kerala. Varada exhibits wide adaptability while Mahima is notable for the high rhizome yield and Rejatha for the volatile oil content (Naidu *et al.*, 2000 and Sarma and Sasikumar, 2002).

2.4.1.1 *Vegetative and economic attributes*

Nybe (1978) investigated morphological variations in twenty five types of ginger and found height of plant, number of leaves per tiller, number of roots per plant was maximum in Valluvanad and number of primary fingers per plant and secondary fingers per plant was more in Wynad Local and Bajpai respectively. Mohanty *et al.* (1981) studied twenty eight cultivars of ginger for variations in number of tillers, number of leaves, plant height, leaf width, weight of straw, number of adventious roots, number of root tubers, total number of rhizome fingers, girth of secondary fingers and rhizome yield. The varietal differences for all the characters studied were significant. Ravindran *et al.* (1994) evaluated accessions for various morphological, yield and quality characters and revealed considerable variability for

most of these traits. Sujatha *et al.* (1994) reported plant height to be highest in cultivar Culcutta, more leaves and tillers in PGS-10 and maximum fresh yield in Kuruppampady and dry yield in PGS-667. Himgiri was found to perform well for tillers per plant, leaves per plant, leaf length and breadth, rhizome length and breadth and yield and the performance was consistent under both rainfed and irrigated conditions (Korla, 1999).

Nybe *et al.* (1980) revealed that morphological characters and economic characters such as length and girth of primary and secondary fingers were found positively correlated with yield. Weight of mother rhizome and internodal distance of rhizome were the most variable characters. The yield was positively and significantly correlated with tillers per clump, internodal distance of rhizome and plant height and was negatively correlated with fibre content as reported by Chandra and Govind (1999).

2.4.1.2 Flowering

2.4.1.2.1 Flowering behaviour

Ginger is a shy flowering crop, when raised from seed bits of around 15 g and maintained as an annual crop (Hooker, 1894 and Holttum, 1950). Pillai *et al.* (1978) reported that, of the 35 germplasm collections maintained in CPCRI, Kasaragod all but, six flowered and the flowering started in the last week of October and lasted till early December, the peak being in November. Nybe (1978) reported 0.5 to 12 per cent flowering in various ginger types under vellanikkara conditions. Flowering was not observed in any of the tetraploid ginger plant by Ratnambal and Nair (1982). But Ramachandran (1982) reported that induced tetraploids of ginger flowered during the second year of planting. Sheeba (1996) observed flowering in autotetraploid ginger derived from the variety Rio-de-Janeiro during August in the second year of planting and from Himachal Pradesh in subsequent years.

Valsala (1994) reported that biennial plants flowered as early as first July and and it extented to last week of October where as plants maintained as annuals flowered only by middle of September and the season extented to third week of November. The seven cultivars studied registered cent per cent flowering when they were maintained as biennials and in annuals, percentage of flowering was

only 25 per cent. Soo *et al.* (2000) observed that flowering occured between 18 and 25 August irregularly, at 16.00 – 17.00 h.

2.4.1.2.2 Inflorescence

Inflorescence in ginger is a bracteate spike or raceme directly springing from the rhizome, rarely emerging terminally from the aerial shoot. The inflorescence has a slender scape of 10 to 20 cm and a cylindrical cone like spike 4 to 7 cm length and 1.50 to 2.50 cm in diameter with appressed ovate or elliptic green bracts. Each bract subtends a single flower with a lateral or obliquely posterior bracteole. Occasionally in a bract more than one flower is seen. The bracts are spirally arranged (Nybe, 1978; Pillai *et al.*, 1978 and Jayachandran *et al.*, 1979).

2.4.1.2.3 Floral morphology

The flowers are small as compared to those of other related genera and are borne in the axil of a bract. They are trimerous, zygomorphic, bisexual and epigynous. The calyx is tubular or bell shaped dividing above into three short teeth and split on one side. The corolla is tubular below with three yellowish lobes of which the dorsal lobe is curved over the anther. Androecium consists of six stamens of which the outer three are reduced to staminodes and are seen at the base of the corolla tube. The inner lateral stamens are united 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 stamen has short, broad filament and two prominent anther lobes. The style is long and slender and passes through the groove formed by the anther lobes and ends in a capitate stigma. Stigma has a circular aperture surrounded by stiff hairs and it protrudes above the anthers (Nybe, 1978; Jayachandran *et al.*, 1979 and Soo *et al.*, 2000). Ovary is inferior, trilocular with several ovules per ovary (Pillai *et al.*, 1978). The ovary measured a mean length of 2.71 mm and diameter of 2.59 mm and with a mean ovule number of 24.43. The ovules measured a mean length of 539.13 μm and breadth of 324.41 μm at the middle (Valsala, 1994).

Homeosis has played an important role in the evolution of the flowers of the Zingiberales in which in some members, the outer androecial whorl are replaced by a

lip and in some members, the inner androecial whorl are replaced by petaloid staminodes (Kirchoff, 1991).

Das (1999) reported ginger flowers as hermaphrodite with pin and thrum type incompatibility and dehisced pollen grains did not reach the stigma head.

2.4.1.2.4 Floral biology

Ginger inflorescences take approximately 29 to 32 days from the spike initiation to first blooming. In a spike, one or two flowers opened daily and blooming of flowers in a spike was completed with in a period of 9 to 22 days. The blooming of individual flowers takes place in an acropetal succession and the flower falls on the next day of blooming (Usha, 1984 and Valsala, 1994).

Valsala (1994) observed that anthesis started by 3.00 p.m and continued upto 4.00 p.m. In contrast Das (1999) found that anthesis under green house occurred around 13.00 – 14.00 h and under field conditions around 9.00 – 10.00 h.

According to Pillai *et al.* (1978) and Jayachandran *et al.* (1979), anther dehiscence almost coincided with the flower opening. In contrast, Usha (1984) and Valsala (1994) observed anther dehiscence 10 min to 2 h after opening. Time of stigma receptivity remains still unravelled owing to the failure of hand pollination to set seeds.

2.4.1.2.5 Pollen morphology

The pollen grains of ginger flowers are round, ovoid to ellipsoidal, highly heterogeneous in size and filling and are limited by a very thick exine. In some species of *Zingiber*, the pollen grains are spherical with cerebroid sculpturing. All species have a coherent exine and an intine consisting of a thick, radially channelled outer layer and a thin, finely granular inner layer. (Theilade *et al.*, 1993). In *Z. spectabile*, the mature pollen exhibited an extremely thin and discontinous exine (Theilade and Theilade, 1996). Exine is striate or tuberculate to areolate in *Zingiber officinale* (Mangaly and Nayar, 1990). A well defined pore is absent in pollen grains. The anther lobes are filled with plenty of pollen grains (Pillai *et al.*, 1978; Jayachandran *et al.*, 1979 and Usha, 1984).

2.4.1.2.6 Pollen fertility and germination

Pollen fertility in ginger reported by various workers ranged from 0.9 to 40 per cent (Pillai *et al.*, 1978; Usha, 1984; Sathiabhama, 1988; Valsala, 1994; Bindu, 1997 and Adaniya, 1998).

Pillai *et al.*(1978) obtained 14.50 per cent pollen germination in a medium with 8 per cent sucrose, 3 per cent gelatin and 60 ppm boric acid in moist chamber operated at 26.5^{0}C. With the same medium, Usha (1984) obtained only 6 per cent pollen germination. Sathiabhama (1988) reported 17.81 per cent pollen germination in the same medium except for the reduction of gelatin to one per cent. Maximum pollen tube length observed was 108 µm and coiling of pollen tube was also noticed. Valsala (1994) got only 7.51 per cent pollen germination in ME_3 medium. The pollen tube growth was significantly higher in the ME_3 medium and maximum tube length obtained was 1042.17 µm. Bindu (1997) got a pollen germination ranging from 2.80 to 6.13 per cent and mean pollen tube length ranging from 51.9 to 72.16 µm in ME_3 medium.

Sheeba (1996) reported that high pollen fertility (64 %) was recorded for the autotetraploid derived from Rio-de-Janeiro. Shirai (2001) reported that induced tetraploid strains had higher pollen fertility and germination (27.4 to 74.2 % and 4.8 to 12.9 % respectively) than the diploid counterparts (0.3 to 6.2 % and 0.0 to 0.1 %) respectively.

2.4.1.2.7 Stigma receptivity, style length, ovule size and ovary size

Stigma receptivity of ginger could not be correctly ascertained owing to the failure of seed set on hand pollination. Observations under hand lens by Jayachandran *et al.* (1979) indicated that stigma receptivity coincided with anther dehiscence. Valsala (1994) reported variations in style length (3.0 to 4.2 cm), ovary length (2.0 to 3.0 mm) and diameter (2.0 to 3.0 mm), ovule length (469.87 to 617.62 µm) and ovule breadth (303.26 to 373.13 µm) of different ginger cultivars.

2.4.1.3 Quality attributes

Nybe *et al.* (1980) reported that Rio-de-Janeiro was the best in oleoresin recovery where as Karakal recorded maximum oil content. However, the yield of aromatic oil and oleoresin when worked out on per hectare basis were found

maximum in Maran. They also observed minimum fibre content in China and maximum in Kuruppampady closely followed by Maran. Sreekumar *et al.* (1980) evaluated 30 ginger cultivars and the results indicated that cultivars Thingpuri, Rio-de-Janeiro and China were suitable for fresh ginger production and Tura, Nadia and Maran suitable for dry ginger. Maran was found suitable for the extraction of oleoresin and volatile oil content, but it had high fibre content (Saikia and Shadeque, 1992). Considerable variations were observed by Shamina *et al.* (1997) for total free amino acids, protein and total phenols. Nizam and Jayachandran (1997) observed maximum non-volatile ether extract in Kuruppampady. Korla (1999) observed that though SG 61 had high volatile oil and oleoresin content, it had high crude fibre content and poor yield. More fibre content in Khasi local and less in Nadia was reported by Chandra and Govind (1999). Gingerol content, antioxidant activity and pungency intensity were higher in tetraploid types than in diploid types as investigated by Nakasone *et al.* (1999). Singh *et al.* (2000) observed highest rhizome yield and oil content in Thinladium and Nadia respectively.

2.4.1.4 Incidence of pests and diseases

Pests and diseases are important production constraints. The common pathogens associated were *Ralstonia* sp, *Pythium* spp and *Fusarium oxysporum*. *Pratylenchus coffeae* increased the severity of infection by these pathogens along with *Fusarium* as reported by Rajan *et al.* (2002).

None of the accession screened by Ravindran *et al.* (1994) showed tolerance or resistance to leaf spot disease, rhizome rot, bacterial wilt. Maximum variation within cultivated ginger occurs in northern India. This is suggested to be due to geographical spread from its centre of origin in South East Asia accompanied by genetic differentiation into locally adapted populations caused by mutations.

All the twenty five types in ginger studied were susceptible to soft rot, leaf spot and shoot borer incidence and among them, Maran was relatively tolerant to soft rot whereas Rio-de-Janeiro was the most susceptible type as reported by Nybe (1978). Ali *et al.* (1994) observed SG-600 to be most resistant cultivar against *Pythium* spp and heavy losses occurred in local cultivars.

Setty *et al.* (1995) observed that though none of the cultivars were resistant against leaf spot and Narasapatom and Tura were moderately resistant with a disease

index of less than 5 per cent. Singh and Edison (2000) reported that accessions SG-554 and RGS-5 were resistant to Phyllosticta leaf spot disease under Chhattisgarh conditions.

Pruthi (1998) reported *Conogethes punctiferalis*, as a serious pest that damages by boring central shoots of plants, which turn yellow and finally dry the crop.

Dohroo (2001) found *Pythium, Fusarium and Verticillium* to be associated with storage rot of ginger and these diseases reached its maximium intensity in April at 18.5 ^{0}C and 67.5 per cent relative humidity.

2.4.2 Correlation studies

Correlation studies have shown significantly positive relation of rhizome weight with plant height, number of tillers and leaf number (Sreekumar *et al.*, 1980). Nybe *et al.* (1980) reported morphological characters such as length and girth of primary and secondary fingers were found positively correlated with yield. They further reported that rhizome yield was found to vary significantly among the types, maximum being in Nadia followed by Bajpai, Maran and Naraspattom. Saikia and Shadeque (1992) observed leaves per clump, tillers per clump and shoot height to be positively correlated with yield. Significant genotype difference for pseudostem length, rhizome length and breadth and yield per plant were observed by Korla and Tiwari (1999). Das *et al.* (2000) reported that linear component was significant for tiller number, plant height and yield while the non-linear component was significant for plant height. Correlation studies revealed that rhizome yield per plant was positively and significantly correlated with plant height, number of leaves per plant, number of tillers per plant, leaf length and width as reported by Singh (2001).

Shading the ginger plants reduced stomatal density as investigated by Hui (1998). Path coefficient analysis for rhizome yield and its related traits revealed very high positive direct effects of stomatal number as reported by Das (1999) in ginger.

Higher rhizome yield was positively associated with greater chlorophyll-a and carbohydrate and lower polyphenol levels in the leaf as observed by Rai *et al.* (1999).

Significant positive association of oil content with rhizome yield per plant and oleoresin content was observed by Singh *et al.* (2000).

2.4.3 Selection

The improvement work was confined mainly to collection of germplasm from different area, their comparative yield evaluation and selection. Hybridisation is not feasible since majority of cultivars do not flower and none of them set seeds in nature. Mohanty *et al.* (1981) reported highest rhizome yield in Rio-de-janeiro and China followed by Vingra selection. Yadav (1999) reported high heritability coupled with high genetic advance for plant height, leaf length, tillers per plant, number of primary and secondary rhizomes, weight of primary rhizome and rhizome yield per plant indicating that desirable improvement in these traits can be brought about through selection.

Good quality ginger contains low fibre content and sufficient amount of protein and fat. The varieties having high fibre content have low market value. Nybe *et al.* (1980) evaluated twenty five cultivars and reported that Karakkal contained highest essential oil content with minimum driage, Thodupuzha with maximum driage, Rio-de-Janiero with maximum oleoresin and China and Nadia with minimum fibre. Sreekumar *et al.* (1980) recorded dry ginger recovery in range of 17.7 per cent in China to 28.0 per cent in Tura, fibre content ranged from 3.5 per cent in Tura to 6.0 per cent in Jorhat and highest oleoresin in Rio-de-Janeiro (10.8 %).

2.4.4 Mutation breeding

Mutation breeding appears to have a special advantage in adding specific characteristics in vegetatively propagated crops like ginger, as a large number of genetically identical individuals can be obtained once the useful mutants are obtained through physical and chemical mutagens. Suravi is an induced mutant from Rudrapur Local as reported by Edison *et al.* (1991). Jayachandran and Mohanakumaran (1992) irradiated cultivar Rio-de-Janeiro with 0.5 – 1.5 krad gamma rays and reported that increasing the doses of gamma ray reduced the vigour of the mutants but the incidence of chlorophyll mutants increased. Gridharam and Balakrishanan (1992) also reported no stimulatory effect on the vegetative characters subsequent to irradiation with gamma rays. The doses of radiation tried did not help to overcome absence of seed setting in ginger, since none of the plants produced viable pollen.

2.4.5 Polyploidy breeding

In ginger, flowering is sparse and one reason suggested for failure of seed setting is chromosomal sterility. Polyploidy breeding therefore offers some hope to explore the possibilities of inducing variability. Ramachandran (1982) reported that tetraploids were more vigorous than diploids and flowered during the second year of induction of colchicine. On the contrary, Ratnambal and Nair (1982) reported that majority of tetraploids were stunted with dark green leaves, increased number of epidermal and stomatal cells per unit area and flowering was not observed in any of the tetraploid plants. Tetraploids produced higher yield with larger rhizomes but the oil content was lowered by 0.5 per cent as reported by Ramachandran and Nair (1992). Sheeba (1996) observed the autotetraploids to have larger epidermal cells and stomata with reduced number per mm^2 of leaf and with increased pollen fertility. She also recorded number of stomata per mm^2 in range of 16 to 46 with stomatal length (133.28 µm to 199.92 µm) and stomatal width (63.30 µm to 133.28 µm) in ginger and the epidermal size was found to be increased by 50 – 60 per cent in autotetraploids compared to diploids. Oil cells are present in the leaf, shoot apex and root apex and are more or less spherical, containing stored volatile oil. The number of oil cells are higher in apical and nodal regions than in the internodal region and the size of oil cells is dependent on their number (Remashree *et al.*, 1999).

2.4.6 *In vitro* hybridisation

In vitro crossing between selected culivars showed that Rio-de-Janeiro as female parent can be crossed with Kuruppampady, SG-66, Nadia and as male parent with Kuruppampady, Nadia, SG-66 and Bajpai. Selfing studies showed that the cultivars Rio-de-Janeiro, Kuruppampady, Bajpai, SG-66 and Nadia can be selfed by *in vitro* pollination and fertilization techniques (Bindu, 1997).

2.5 POLLINATION AND SEED SET UNDER *IN VIVO* CONDITION

Ginger is reported to be a species never setting seeds (Hooker, 1894). Pillai *et al.* (1978) reported that flower structure of ginger manifest an adaptation suitable for entomophily. Hand pollination using large quantities of pollen grains in Rio-de-Janeiro was not effective in achieving seed set (Jayachandran *et al.*, 1979 and

Usha, 1984). Pollination after removal of stigma and style was also not effective in obtaining seed set.

Sathiabhama (1988) tried many techniques such as bud pollination, artificial sibbing, artificial cross pollination between cultivars, chemically aided pollination, mixed pollination and mentor pollination but failed to get seed set in ginger. Adaniya (2001) concluded that the optimum *in vitro* pollen germination of the tetraploid ginger was at 17-20 ^{0}C under 100 per cent relative humidity.

2.6 REASONS FOR LACK OF SEED SET IN GINGER

Several reasons were put forward for lack of seed set in ginger. One school of thought was that failure of seed set is due to self-incompatibility existing in stigma and style (Pillai *et al.*, 1978). Usha (1984) observed that incompatibility may not be the factor for failure of seed set, as it failed to set seed following bud pollination or after removing the stigma and style. Das (1999) reported that lack of seed set despite selfing and cross-pollination might be due to non-homology of bivalents, with irregular segregation of genomic complements leading to sterile gamete formation.

Chromosomal aberrations during meiosis and defects in micro and megasporogenesis, which lead to defective gametes and sterility, are reasons suggested by Pillai *et al.* (1978). Even though high sterility exists in ginger, some amount of pollen germination occurs. Since anther lobes of ginger contain a lot of pollen grain, low pollen fertility may not be the reason for lack of seed set in ginger.

Pollen pistil interaction under *in vivo* conditions using fluorescence microscopy was studied by Sathiabhama (1988). It was observed that pollen tube growth was only 95 µm where as the style length was upto 39000 µm and the possibility of pollen tube reaching the ovule at 39000 µm is quite remote and suggested that in sufficient pollen tube growth is the main reason for lack of seed set. She further opined that presence of spiny stigma prevent germination of pollen grains either by injuring the pollen grains or by preventing the adherence of pollen grains in stigmatic surface. The pollen tube growth was very slow and as it advanced, gets coiled. Besides, the opened flowers are retained in the plant for less than 12 h after anthesis and by the time the pollen germinate and grow, abscission layer is formed in the stylar region and flowers are shed.

2.7 *IN VITRO* POLLINATION AND FERTILIZATION

The technique of *in vitro* pollination and fertilization is an effective tool for getting seed set in species where pre fertilization barriers block seed set. Some of the barriers to fertilization are (a) inability of pollen to germinate on foreign stigma, (b) failure of the pollen tube to reach the ovule due to excessive length of the style, or slow growth of the pollen tube, which fails to reach the stigma before the ovary abscises, (c) bursting of the pollen tube in the style. The most important application of the technique is for the production of rare hybrids (Bhojwani and Razdan, 1983)

2.7.1 Technique of *in vitro* pollination and fertilization

The pre-requisites and procedures to be adopted for the successful *in vitro* pollination has been described by Kanta and Maheshwari (1963).

The technique consists of

(1) A detailed study of floral biology of the crop.

(2) Pollen germination and pollen tube growth.

(3) Standardisation of a surface sterilization technique for flowers and flower buds without affecting viability of pollen grains and receptivity of gynoecium.

(4) Identification of a suitable medium which support, the development of the ovule or ovary into mature seed or fruit.

(5) Standardisation of appropriate pollination technique so that fertilization and growth of ovule takes place.

(6) Identification of suitable culture conditions which will promote growth of ovule to viable seeds.

(7) Histological examination of ovule or ovary at various stages to know the fertilization and development of embryo and endosperm.

(8) Standardisation of culture conditions for germination of seeds.

2.7.2 *In vitro* pollination methods

Different methods were suggested for *in vitro* pollination viz., stigmatic, stylar, intra-ovarian, placental, modified placental and ovular or test tube fertilization (Bhojwani and Razdan, 1983). Valsala (1994) reported successful development of ovule after placental pollination in ginger. In placental pollination, the placenta bearing ovules were exposed by completely peeling and removing the ovary wall and

pollination was done on the whole unit and alternatively they were cut into two pieces also (Rangaswamy and Shivanna, 1971a). In modified placental pollination, ovary wall of one locule was peeled away and pollination was done on the exposed ovules (Rangaswamy and Shivanna, 1971b). Valsala (1994), Renjith (1999) and Bhurke (2002) reported seed development through placental pollination in ginger, turmeric and kacholam respectively.

2.8 *IN VITRO* FERTILIZATION AND SEED SET

Valsala (1994) reported seed set in ginger for the first time by *in vitro* pollination when ovule cultured in half MS + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} with sucrose 3 per cent. Organic supplements coconut water (15 % v/v) and casein hydrolysate (200 mg l^{-1}) enhanced the development.

Renjith (1999) obtained seed set in turmeric by *in vitro* pollination. The medium of half MS + BAP 1 mg l^{-1} + kinetin 1 mg l^{-1} + NAA 0.5 mg l^{-1} with sucrose 3 per cent was found to be the best for ovary and ovule development. Organic supplements coconut water (15 % v/v) and casein hydrolysate (200 mg l^{-1}) enhanced the development.

Bhurke (2002) achieved seed set in kacholam by *in vitro* pollination in the medium of half MS + BAP 0.5 mg l^{-1} + NAA 3.0 mg l^{-1} with sucrose 3 per cent. Organic supplements did not favour ovule development.

2.9 FACTORS AFFECTING SEED SET *IN VITRO*

2.9.1 Age of flower buds

Generally, flower buds on the day of anthesis or one or two days after anthesis respond to *in vitro* pollination. The seed set was reported to be higher when the ovules were excised 1-2 days after anthesis than on the day of anthesis (Kanta *et al.*, 1962; Kanta and Maheshwari, 1963 and Rangaswamy and Shivanna, 1967). On the contrary, in *Lilium*, while making interspecific crosses, seed set was observed when the flowers were pollinated three days prior to anthesis and success rate was reduced when pollination was done two days prior to anthesis (Tuyl *et al.*, 1991). In the case of ginger, the flower buds have to be collected on the day of anthesis or on the next day of anthesis as reported by Valsala (1994).

2.9.2 Surface sterilization

An effective surface sterilization technique is an important pre requisite for successful *in vitro* culture. Various surface sterilization techniques without affecting pollen viability and receptivity of the ovules have been tried by many workers.

Valsala (1994) perfected a three-step surface sterilization procedure in ginger. The flower buds on the day of anthesis, prior to flower opening were surface sterilized by dipping in 500 mg l^{-1} streptocycline for 1 h followed by wiping with 70 per cent alcohol and rinsing with 0.1 per cent mercuric chloride for 3 minutes.

Renjith (1999) utilized similar procedure for sterilization of turmeric flowers but with a lower concentration of streptocycline (350 mg l^{-1})

Bhurke (2002) reported that fytolon (2500 mg l^{-1}) showed complete suppression of bacteria in kacholam.

2.9.3 Nature of the explant

Nature of the explant has major influence on the success of *in vitro* pollination. The size of the gynoecium or floral appendages may influence seed set following *in vitro* pollination. The factors like the physical condition of the ovary or ovules, the extent and the type of dissection done to expose the ovules, the parts of the pistil being removed or retained constitute the nature of explant.

Zenketeler (1984) and Razdan (1993) reported that ovaries large in size and containing many ovules are the best experimental material for *in vitro* pollination. Most of the initial successful attempts of *in vitro* pollination are reported in members of families Solanceae (*Nicotiana tabacum, N. alata, N. rustica, Petunia hybrida),* Papaveraceae (*Papaver somniferum, Argemone mexicana, Eschscholtzia californica* and Caryophyllaceae *(Melandrium album, M. rubrum, Dianthus caryophyllus, Agrostemma githago)* where in all the above cases, the placentae are covered with several hundred ovules. Abundant supply of viable pollen and abundant growth of pollen tubes on ovules and placentae are other important factors for successful *in vitro* pollination.

Castano and Proft (2000) noted that success has been obtained mainly with the species of the families Brassicaceae, Caryophyllaceae, Liliaceae, Papaveraceae, Primulaceae and Solanaceae. The direct *in vitro* pollination of ovules allows to overcome some pre and post zygotic barriers of incompatibility resulting in

subsequent development of embryos in 57 species representing 14 families. The best results were obtained in species in which the ovaries are large and contain many ovules.

2.9.4 The culture medium

An appropriate culture media combination is necessary for the successful development of *in vitro* fertilized ovule into mature seed.

In the initial years of *in vitro* pollination and fertilization technique, the Nitsch medium identified for ovary development was used (Kanta and Maheswari, 1963 and Usha, 1965).

Modifed Nitsch's medium in some angiosperms was suggested by Kanta and Maheshwari (1963) while Steward and Hsu (1978) developed a medium for intraspecific and interspecific hybrids from young fertilized ovules of cotton.

Tuyl *et al* (1991) reported that MS medium was also useful for the production of interspecific hybrids of *Gossypium, Nicotiana* and *Lilium* following *in vitro* pollination.

Valsala (1994) tried MS medium at full and half strength, SH medium and Nitsch's medium and found half MS, SH and Nitsch's medium suitable for ovary development of ginger after *in vitro* pollination.

Renjith (1999) found half MS superior to full MS for the ovary establishment in turmeric after *in vitro* pollination.

Castano and Proft (2000) identified both MS and Gamborg's B_5 medium for the culture of isolated ovules of chichory after *in vitro* ovular pollination.

Bhurke (2002) reported that cultured ovary of kacholam developed in MS medium at half and full strength supplemented with hormones but half MS medium was superior for ovule development. There was no ovary development without hormones.

2.9.5 Sucrose concentration

The sucrose concentration influences the development of ovary, ovule and germination of seeds after *in vitro* pollination.

Generally sucrose has been used at a concentration of 4-5 per cent (Kanta and Maheshwari, 1963 and Usha, 1965)

For the swelling of capsules of *Lilium* hybrids, MS medium supplemented with 7 per cent sucrose gave best results (Tuyl *et al.*, 1991)

Valsala (1994) found 6-8 per cent sucrose optimum for the development of ovaries and ovules of ginger while Renjith (1999) found 3 per cent sucrose superior to 6 per cent in turmeric.

Castano and Proft (2000) used MS medium and Gamborg's B_5 medium with 4 and 6 percent sucrose.

In *Lilium*, a high concentration of 10 per cent sucrose gave successful results when cultured in Brewbaker and Kwack's agar plate medium (Chi, 2000)

Bhurke (2002) observed that 3 per cent sucrose was the best for ovule swelling of kacholam.

2.9.6 Effect of growth regulators and supplements

Casein hydrolysate at 500 ppm was found to enhance ovule development in some angiosperms (Kanta and Maheshwari, 1963). Usha (1965) observed seed set in *Antirrhinum majus* through *in vitro* pollination on addtion of 25 per cent coconut water to Nitsch medium. In *Lilium*, auxin is essential at the initial stages of ovule development (Tuyl *et al.*, 1991).

Valsala (1994) cultured pollinated gynoeciums of ginger in half MS with various growth regulators and reported maximum seed development in half MS + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} supplemented with coconut water 15 percent (v/v). The auxins and cytokinins alone, induced ovule development but combinations proved to be better. The combinations of NAA 0.5 mg l^{-1} with varying concentrations of BAP from 2 to 10 mg l^{-1} showed positive effect. The effect of BAP could be replaced by kinetin (2 and 5 mg l^{-1}) or 2 iP (2.5 mg l^{-1}). The effect of NAA (0.5 to 2 mg l^{-1}) could be replaced by 2,4-D (0.5 to 1 mg l^{-1}) or IAA (0.05 to 0.2 mg l^{-1}). GA did not favour ovary and ovule development. Supplements like coconut water (10 to 12 per cent v/v) and casein hydrolysate (200 to 500 mg l^{-1}) enhanced ovule development. Similarly, inflorescence extract of ginger (0.3 to 3 %) promoted ovule development.

Renjith (1999) observed that *in vitro* pollinated gynoeciums of turmeric recorded maximum ovule swelling when cultured in half MS with NAA 0.5 mg l^{-1} and BAP and kinetin both at 1 mg l^{-1} and 3 per cent sucrose. Organic supplements like

coconut water 15 per cent v/v and casein hydrolysate 200 mg l^{-1} enhanced ovule development.

Castano and Proft (2000) cultured ovules from *in vitro* pollinated flowers of chicory on various media combinations. The ovules developed into seedlings in three combinations viz.,

(i) MS + IAA 4 mg l^{-1} + kinetin 0.5 mg l^{-1} + GA_3 5 mg l^{-1} with 4 percent sucrose and supplemented with glycine 9.5 mg l^{-1} + casein hydrolysate 500 mg l^{-1}.

(ii) B_5 + IAA 4 mg l^{-1} + kinetin 0.5 mg l^{-1} + GA_3 5 mg l^{-1} with 4 percent sucrose and supplemented with glycine 7.5 mg l^{-1} + casein hydrolysate 500 mg l^{-1}.

(iii) MS + IAA 0.5 mg l^{-1} + kinetin 0.5 mg l^{-1} + GA_3 5 mg l^{-1} with 4 percent sucrose and supplemented with glycine 7.5 mg l^{-1} + casein hydrolysate 500 mg l^{-1}.

Bhurke (2002) reported the following four combinations favourable for ovule development of kacholam. Maximum ovule swelling was observed in cultures with hormone combinations viz.

(i) Half MS + 2,4-D 0.2 mg l^{-1}

(ii) Half MS + BA 1.0 mg l^{-1} + kinetin 1.0 mg l^{-1}

(iii) Half MS + BA 0.5 mg l^{-1} + NAA 3.0 mg l^{-1}

(iv) Half MS + BA 1.0 mg l^{-1} + kinetin 3.0 mg l^{-1} + 2, 4-D 0.2 mg l^{-1}

2.9.7 Culture conditions

There is hardly any data on the precise effect of light on the response of *in vitro* pollinated ovules. Cultures were usually stored in darkness or near darkness (Rangaswamy and Shivanna, 1967).

In *Petunia diffusa,* day light (10 –12 ft candle at 25 ± 2^0c) was found optimum for *in vitro* seed development (Rangaswamy and Shivanna, 1967).

Tuyl *et al.* (1991) incubated cultures of *Lilium* in a climate room of light intensity 12 W m^{-2}, photoperiod 16 h and temperature 24 ^{0}c for the production of interspecific hybrids. The cultures were incubated at 26 ± 2^0C in an air-conditioned culture room under diffused light as reported by Valsala (1994) in ginger, Vijayasree (2001) in turmeric and Bhurke (2002) in kacholam.

2.9.8 Genotype

Gengenbach (1977) and Bajaj (1979) reported evidence of genotypic variation in the response of *in vitro* pollinated ovaries of maize.

2.10 EMBRYO AND ENDOSPERM DEVELOPMENT FOLLOWING *IN VITRO* FERTILIZATION

In *Papaver rhoeas*, the embryos after intra-ovarian pollination grew faster than in nature. The cotyledons differentiated within 14 days after pollination and by the third week the plumule and the root tip were well differentiated and one month old seeds had fully developed embryo (Kanta, 1960).

Kanta and Maheshwari (1963) reported that in test-tube fertilization of *Eschscholzia californica*, the embryo showed normal development during its early growth. However the endosperm was poorly developed and degenerated before becoming cellular. In test-tube fertilization of *Nicotiana rustica* and *N.tabaccum* fertilized ovules enlarged considerably but 25 per cent of them aborted showing only an irrergular embryonal mass and a degenerating endosperm.

In the test-tube fertilization of *Papaver somniferum*, the embryo developed concomitantly with the endosperm. Normally developing seeds from three days old culture showed a two celled pro-embryo. Dissections from nine days old seed showed a globular pro-embryo. In fifteen days old seed a differentiated embryo and twenty two days old seed a fully formed normal dicotyledonous embryo and cellular endosperm (Kanta and Maheshwari, 1963).

Rangaswamy and Shivanna (1967) reported that fertilization took place within 24 h after placental pollination of petunia. Mature seeds bearing dicotyledonous adult embryos were formed 24 days after selfing.

Histological examination of placental pollinated ovules four DAP of ginger showed eight celled pro-embryo and sections of developed ovules 40 DAP showed deeply stained embryo along with starch and oil grains as reported by Valsala (1994). Similar studies by Bhurke (2002) in kacholam also revealed distinct embryonic tissue which increased in size in the subsequent stages of the developing ovules, thus supporting the success of fertilization.

2.11 GERMINATION STUDIES

2.11.1 Embryo rescue technology

In crop improvement programme, sometimes it may become necessary to make crosses between two genera or species or varieties, which cannot be conveniently hybridised using conventional methods, due to several crossing barriers. These crossing barriers may be pre-fertilization or post-fertilization barriers (Dinesh *et al.*, 1999).

Pre-fertilization barriers can be overcome by excision methods or *in vitro* pollination and fertilization technique where as post fertilization barriers can be overcome by Embryo Rescue Technology (Bhojwani and Razdan, 1983).

Distant hybridization is the most commonly used system for the transfer of specific characters viz., resistance to biotic and abiotic stress, yield increment and good quality attributes. In such distant crosses, often the embryo aborts at an early stage of development, so that no mature seed can be obtained. Currently embryo rescue holds great promise not only for effective wide crosses, but also for obtaining plants form inherently weak embryos, obtaining haploid plants as well as for shortening the breeding cycle as reported by Sharma *et al.* (1996).

Embryo rescue technology is adopted in situations where the hybrid embryo fails to attain maturity due to embryo-endosperm incompatibility or poor development of the endosperm. The cultured embryos can directly develop into young seedlings. When embryos cannot be easily excised, whole ovule can be cultured and similarly when ovule is too small to be removed, whole ovary can be cultured. Thus embryo rescue technique is used for embryo culture, ovule culture and ovary culture since in each case the objective is to rescue the embryo. This method can be utilized for the production of rare hybrids, which are otherwise impossible in nature due to embryo abortion. Inomata (1996) described methods of overcoming interspecific and intergeneric barriers in *Brassica* and its wild relatives, through embryo, ovule and ovary culture. The method is successfully utilized for interspecific hybridisation in crops like tomato and brinjal (Bletsos *et al.*, 1998).

The technique helps to maintain the integrity of the hybrid genome in a developmentally arrested or an abortive embryo and its potential to resume normal growth may be realized if it supplied with favourable environmental conditions (Bridgen, 1994).

2.11.1.1 Embryo culture

Embryo culture can be defined as the *in vitro* culture zygote, pro-embryo or mature embryo (Bhojwani and Razdan, 1983).

Since the turn of the century, when Hanning (1904) first cultured embryos of the cruciferous genera (*Raphanus* and *Cochleria*), *in vitro* zygotic embryo culture technique have been developed with significant applications in plant breeding and horticulture, as well as in basic studies on embryo physiology and biochemistry. Many embryos do not survive *in vivo* or become dormant for long periods of time and therefore successful *in vitro* embryo culture methods have become quite important.

2.11.1.1.1 Technique for dicot embryo culture

The technique eventually consists of excision and transfer of the embryo from the abortive seed to a culture medium, which will support the development of the same to full maturity (Bhojwani and Razdan, 1983). The excision of mature embryos can be done with relative ease by splitting open the seed. Seeds with a hard coat are dissected after soaking them in water (Raghavan, 1977). Smaller embryos require careful dissection with the aid of a microspatula and transferred to the nutrient medium. The presence of oil prevents drying up of the embryo.

Raghavan and Torrey (1963) adopted the following procedure for isolating torpedo shaped younger embryos from the ovules of *Capsella bursa- pastoris*. The embryo is confined to one longitudinal half of the ovule and are clearly visible through the chalaza either because of the transparent vesicle of their suspensor. The ovule from which the embryo has to be excised was placed in the depression of a new slide with a drop of medium. With the help of a sharp mounted blade, the ovule was split longitudinally to isolate the half containing the embryo. By carefully tearing apart the ovular tissues the entire embryo could be removed.

2.11.1.1.2 Technique for monocot embryo culture

In monocots, a well studied plant for embryo culture is barley (*Hordeum vulgare*). Norstog (1965) has described the following procedure for the isolation of immature barley embryos of smaller than 0.2 mm size. In barley, the region of the ovule that contains the embryo is beak like. The beak was excised and transferred to a

drop of sterile paraffin oil. Tissues of the beak were carefully teased apart to release the embryo. The isolated embryo was lifted out in the oil film using a microspatula and transferred to the nutrient medium. The presence of oil prevents drying up of the embryo.

2.11.1.1.3 Embryo-nurse endosperm transplant

The nutritional requirements of younger embryos are complicated, as they require more morphological differentiation. The technique of embryo-nurse endosperm transplant is suggested in such situations.

Zeibur and Brink (1951) showed that *in vitro* growth of excised immature embryos (300 – 1100 μm long) of barley was considerably promoted by surrounding the embryo with the endosperm of another seed of the same species.

A modified endosperm transplant technique for younger embryo culture has been described by Lautour *et al.* (1978) and Williams and Lautour (1980). They inserted an excised hybrid embryo into a cellular endosperm dissected out from a normally developing ovule of one of the parents or a third species and cultured the nurse endosperm with the transplanted embryo on an artificial medium. Using this technique many interspecific hybrids could be produced in *Trifolium* genus which could not be reared by growing embryos directly on the medium.

2.11.1.2 Types of embryo culture

According to Bhojwani and Razdan (1983), there are two types of embryo culture (immature embryo culture and mature embryo culture). Immature embryo culture is used mainly to grow immature embryos originating from unripe or hybrid seeds which fail to germinate. Excising such embryos is arduous and generally a complex nutrient medium is required to raise them to produce plants. The chances of success in this type of culture depend largely on the developmental stages of the excised embryo. In case of mature embryo culture, mature embryos are excised from ripe seeds and cultured mainly to avoid inhibition in the seed for germination. This type of culture is relatively easy as the embryos required a simple nutrient medium containing mineral salts, sugar and agar for growth and development.

2.11.1.3 Role of suspensor in embryo culture

The suspensor is an ephemeral structure found at the radicular end of the pro-embryo and attains maximum development by the time embryo reaches the globular stage. Studies suggest the active involvement of the suspensor in the development of young embryos (Bhojwani and Razdan, 1983).

Many painstaking studies have shown that in cultures, the presence of a suspensor is critical, particularly for the survival of young embryos (Cionini *et al.*, 1976). The role of suspensor can be replaced by hormones like gibberellin and kinetin as reported by Cionini *et al.* (1976). The growth promoting activity of the suspensor is maximal at the early heart stage of the embryo. Moreover Monnier (1978) has pointed out that the suspensor must be intact for the survival of early shaped embryos. If the embryo is excised from the suspensor at an early stage, the wound results in embryo abortion. Therefore, for the successful culture of younger embryos, suspensor may be required.

2.11.1.4 Precocious germination

Precocious germination is the phenomenon of seedling formation without complex normal embryogenic development. Excised immature plant embryo on nutrient medium sometimes tend to bypass the stage of dormancy and cease to undergo the linear embryogenic mode of development and develop into weak seedlings (Bhojwani and Razdan, 1983).

Kent and Brink (1947) reported that precocious germination of excised immature barley embryos can be prevented by incorporating casein hydrolysate in the culture medium. Norstog (1972) have shown that exogenous factors like reduced oxygen tension, elevated temperature and high light intensity prevented precocious germination. Studies conducted further showed that presence of some inhibitors like ABA might be preventing precocious germination in normal embryo development (Norstog, 1972).

2.11.1.5 Culture requirements

2.11.1.5.1 Media requirements for developing embryo

The selection of the right culture medium that would support progressive and orderly development of embryos excised at different stages of development is the most important aspect of embryo culture. Hanning (1904) reported that mineral salt - sucrose solution was sufficient to culture mature embryos (2mm long) of crucifers.

Raghavan (1966) recognized two phases of embryo development with respect to nutrition (a) the heterotrophic phase – during this early phase, the embryo was dependent upon the endosperm and the surrounding maternal tissues, (b) the autotrophic phase – during this phase, the embryo was capable of synthesing substances required for its growth. The media required changes in the two phases and even with in the two phases. The exogenous requirement of the cultured embryos becomes irrespectively simpler with the age of the embryo.

2.11.1.5.2 Growth regulators

An auxin or cytokinin, or both, are required for the growth of embryo and for callusing of embryos.

Raghavan and Torrey (1964) reported that for cultivating globular embryos of *Capsella* on mineral salt-vitamin medium with only 2 per cent sucrose, it was necessary to supplement it with IAA, kinetin and adenine sulphate.

The plantlets were obtained from zygotic embryos of *Taxus brevifolia* when cultured on half strength Gamborg's B_5 medium supplemented with BA 10 μm (Chee, 1995) while the plantlets were obtained from 40 days old embryos of *Juniperus cedrus* when cultured on Quoirin and Le Poivere half strength medium supplemented with BA 5 μm (Harry *et al.*, 1995).

2.11.1.5.3 Carbohydrate and osmotic pressure of culture medium

A suitable source of carbon energy is generally required for the activation of excised mature and immature embryos.

Sucrose is the best form and commonly used carbohydrate for embryo culture (Beek *et al.*, 1944). Sucrose is also essential in the medium to maintain suitable

osmolarity, which is extremely important for immature embryos. Eight to twelve per cent was required for the culture of pro-embryos (Norstog, 1961 and Monnier, 1978).

The stimulatory effect of high concentrations (12 – 18 %) of sucrose on *in vitro* development of excised globular embryos (smaller than 80 µm) of C*apsella* can be obtained by combinations of IAA (0.1 mg l^{-1}), kinetin (0.001 mg l^{-1}) and adenine sulphate (0.001 mg l^{-1}) added to the basal medium with only 2 per cent sucrose (Raghavan and Torrey, 1964).

Pancholi *et al.* (1995) reported that immature embryos excised from *Musa velutine* seeds when cultured on half strength MS medium with 2 per cent sucrose resulted in plantlet formation.

2.11.1.5.4 Mineral salts

Monnier (1978) observed that though MS medium supported maximum growth of embryos of C*apsella bursa-pastoris*, the survival frequency of the embryos were very low, where as in the Knop's medium which was least toxic, the growth of the embryo was very poor. So he developed Monnier's medium which was a modification of MS medium, containing high concentrations of K^+ and Ca^{2+} and a reduced level of NH_4^+ ions supported both growth and survival of embryos.

2.11.1.5.5 Amino acids and vitamins

The addition of amino acids, singly or in combination to the culture medium may stimulate embryo growth.

Hanning (1904) reported that asparagine was very effective in enhancing embryo growth of crucifers.

Casein hydrolysate (CH) an amino acid complex was used widely as an additive to the embryo culture. Kent and Brink (1947) observed that CH inhibited precocious germination and promoted pre-germinal embryo development in *Hordeum vulgare.* According to them, the optimum level of CH for *Hordeum vulgare* was 500 mg l^{-1} while CH was 0.1 g l^{-1} for *Cucumis melo* embryos to form plants (Beharav and Coheu, 1995).

2.11.1.5.6 Natural plant extracts

Beek *et al.* (1942) observed that normal seedlings from *Datura* embryos as small as 150 – 200 µm long and as young as 10 DAP could be developed by incorporating coconut water in the culture medium containing mineral salts, vitamins and amino acids. Later, it has enabled the successful culture of young embryos of a number of species.

2.11.1.5.7 pH of the medium

Pancholi *et al.* (1995) reported that the optimum pH for the growth and morphogenesis of immature embryos of *Musa velutina* under *in vitro* conditions was 5.8 at 23 to 28 ^{0}C which resulted in plantlet formation.

2.11.1.5.8 Culture maintenance

Embryos of most plants grew well at temperature between 25 and 30 ^{0}C (Narayanaswamy and Norstog, 1964). According to them, light was not critical for embryo growth. However, in barley, light was known to suppress precocious germination of immature embryos (Norstog, 1972)

Beharav and Coheu (1995) reported that the hybrid embryo of the cross between *Cucumis melo* and *Cucumis metuliferus* was grown best at 25 ^{0}C with a photoperiod of 16 h. The zygotic embryos of *Taxus brevifolia* produced organs like shoot and root under 16 h photoperiod (Chee, 1995).

2.11.1.6 Routes of hybrid plant production through embryo culture

2.11.1.6.1 Direct organogenesis

For the culture of immature embryos of *Actinidia*, the best medium was MS basal containing GA_3 0.01 – 0.05 + mannitol 8 per cent as suggested by Kin *et al.* (1995).

Lekshmy (1989) cultured embryos of mature hybrid seeds of banana from fully ripe bunch. Seeds were surface sterilized with one per cent silver nitrate for 10 min before extraction and excised embryos developed to plantlets when cultured on modified Knudson's medium. Sudhadevi *et al.* (1993) reported multiple shoots from hybrid embryo between Kew X Ripley Queen varieties of pineapple on MS medium

containing NAA 3 mg l^{-1} and BAP 2 mg l^{-1}. Elongation of shoots was observed on MS with BAP 4 mg l^{-1} and IBA 1 mg l^{-1} and rooting by giving a pulse treatment with IBA 2 mg l^{-1}. Muniswamy and Sreenath (1996) reported that zygotic embryos of *Coffea bengalensis* were matured on MS medium with 1 mg l^{-1} ABA + BAP or kinetin by 30 days. They germinated into plantlets when cultured on half strength MS with BAP 0.1 mg l^{-1}. Buyukalaca *et al.*(1997) reported that embryos of pistachio seeds were germinated on solid, growth regulator-free MS medium and half strength MS without sucrose and liquid growth regulator-free half strength MS medium without sucrose. Asif *et al.* (2001) observed that germination was more in *in vitro* zygotic embryo culture of wild banana compared to green house grown seeds. Burun and Poyrazoglu (2002) observed the highest plantlet development from barley embryos in Randolph and Cox medium. But Qing *et al.* (2002) reported that the best medium for culturing young embryos of apricot was modified SH medium. Ondrej *et al.* (2002) observed better development of Cucumis embryos was achieved on medium with lower concentration of GA_3 but high GA_3 concentration positively stimulated embryogenesis of zygote and pro-embryo culture.

2.11.1.6.2 Indirect organogenesis

Wang (1987) obtained callus induction and plant regeneration from mature embryos of maize. Callus was obtained on MS medium with 1– 2 mg l^{-1} 2,4-D and plant regeneration was obtained on MS medium without hormones. Roy and De (1989) reported tissue culture and plant regeneration from immature embryo explants of *Calotropis gigantea.* Callus was obtained from immature embryos when cultured on MS medium with NAA 0.1 mg l^{-1} and BAP 0.1 to 0.5 mg l^{-1}. Rooting was reduced when shoots were transferred to auxin supplemented Bonner's solution or half strength MS basal salt solutions.

2.11.1.6.3 Somatic embryogenesis

Somatic embryogenesis is most common route of plantlet production from embryos. Chuang and Chang (1987) observed somatic embryogenesis and plant regeneration in callus culture derived from mature zygotic embryos of *Dysosma pleiantha.* Callus was obtained in MS medium supplemented with 2,4-D 1 mg l^{-1}.

Embryoids were formed on media containing 2,4-D 0.1 to 0.5 mg l^{-1} and they germinated on MS or B_5 with BAP 1 mg l^{-1} and $GA_3$1 mg l^{-1}. Guerra and Handro (1988) reported somatic embryogenesis and plantlet generation in embryo cultures of *Eutrpe edulis*. Direct embryogenesis was obtained on Linsmaeir and Skoog medium containing 2,4-D 50 mg l^{-1}. Plantlets were obtained when somatic embryos were transferred to medium containing NAA and 2iP or without growth regulators.

2.11.1.2 Ovule culture

The technique of *in vitro* pollination consists of culture of ovules or ovaries after pollination, for the development of seeds. Even though *in vitro* pollination would help to induce seed set, the later development of the seeds may be limited by various unknown factors. For successful *in vitro* culture of the gynoecium, the knowledge of culture of ovules or ovaries would be helpful to manipulate the conditions for the development. This is of help in the crosses where in embryo rescue technique fails to support embryo development beyond heart or torpedo-shaped stages.

The first attempt to isolate ovules and culture them under aseptic conditions was made by White (1932) in *Antirrhinum majus* . However the technique was developed and perfected at the Department of Botany, University of Delhi. The first successful culture of ovules was reported by Maheshwari (1958) in *Papaver rhoeas*. The ovules were cultured on Nitsch medium containing vitamins, kinetin (0.4 mg l^{-1}) and IAA (5 mg l^{-1}). Raising mature seeds by culturing ovules of *Allium cepa* containing globular or older embryos is comparatively easier as reported by Guha and Johri (1966). Spiegel-Roy *et al.* (1985) obtained normal embryos and seedlings by culturing abortive ovules and seeds of rape on Nitsch medium with IAA 10^{-5} M and GA_3 10^{-6} M. Kristiansen and Vainstein (1995) obtained viable hybrids using ovule culture in genus *Alstroemeria*. The ovule culture will allow novel crosses and will offer new possibilities for the introduction of desirable genes into tulip cultivars as reported by Custers *et al.* (1995). Rhee *et al.* (1997) studied embryo development in different cross cultivars of *Brassica* sp and *Raphanus sativus* using ovule culture. Adventitious embryos induced from the papaya ovules were transferred into a hormone free MS medium to regenerate plantlets as reported by Tokumoto *et al.* (2000). Ishikawa *et al.* (2001) efficiently produced interspecific hybrids by

culturing immature ovules with placenta 7-14 days after pollination on 2 g l^{-1} Gelrite-solidified MS medium containing 3 percent sucrose. Ovule culture 60 days after pollination was recommended for producing interspecific hybrids in large scale crosses of lily as studied by Jin *et al.* (2001). Michishita *et al.* (2001) obtained hybrid seedlings from *Rhododendron kaempferia* ovules by exposing ovules of 4 months after crossing to 50 mg l^{-1} GA_3. Notsuka *et al.* (2001) observed the rate of plant recovery *in vitro* ranged from 0.6 to 24.9 % in F_1 progenies produced through in ovulo embryo culture. The best medium was modified SH medium with 4 per cent sugar with 4 per cent sorbitol for culturing apricot ovules as reported by Qing *et al.* (2002).

2.11.1.3 Ovary culture

The technique of ovary culture was developed by Nitsch (1951) who grew detached ovaries of *Phaseolus vulgaris* and *Lycopersicon esculentum* on synthetic medium. The fruit developed *in vitro* were smaller than the natural ones but they contained viable seeds.

Guha and Johri (1966) cultured ovaries of *Allium cepa* in Nitsch medium with 5 per cent sucrose. Removal of perianth prior to culture retarded the ovary growth markedly.

Bajaj and Collins (1968) cultured pollinated flowers of the cultivated strawberry on White's medium and observed ripening was hastened by GA as well as high light intensity.

Nomura *et al.* (1994) produced interspecific hybrids between *Allium chinense* and *A. fistulosum* by culturing ovaries 5 DAP on MS medium containing 5 per cent sucrose at 25 ^{0}C. Gong *et al.* (1995) observed the development of excised ovaries from the cross *Brassica pekinensis* X *Sinapsis alba* when cultured in presence of IAA 1.5 –2.0 mg l^{-1}.

Campion *et al.* (1995) reported the presence of 2,4-D or NAA, together with BA in the medium is required to induce an acceptable yield of gynogenic embryos from the unfertilized ovary in onion. Interspecific hybrids were obtained with the introduction of valuable traits from wild species of Cyclamen into *C.persicum* through ovary culture as reported by Ewald (1996). Ovary culture is a novel method for direct

organogenesis in onion resulting in the formation of multiple shoot structures when cultured in medium containing 2 mg l^{-1} Thidiazuron (TDZ) as reported by Luthar and Bohanec (1999). Creij *et al.* (2000) observed the addition of 9 per cent sucrose to medium for ovary slice culture, started at 3 or 5 weeks after pollination significantly improved the germination percentage of ovules in interspecific *Tulipa gesneriana* crosses. Jin *et al.* (2001) produced hybrid plantlets from lily species through ovary slice culture when cultured on a hormone-free MS medium supplemented with 6 per cent sucrose.

2.11.2 Germination of *in vivo* produced seeds

Cardamom seeds gave highest germination (39.33 %) after acid scarification for 10 min and soaking in GA for 24 h combined with sowing in the open site (Raja, 1993). Germination of cardamom seeds improved significantly after acid treatment (nitric acid 25 % for 10 min) and continuous water washing for 24 days giving 85 per cent germination as observed by Chaudhury and Chandel (1995). Korikanthimath and Mulge (1998) found acid treatment (nitric acid 20 % for 10 min), GA_3 100 mg l^{-1} for 12 h and Planofix (NAA 75 mg l^{-1} for 12 h) increased germination of cardomom seeds. Higher doses of growth regulators decreased germination.

Spiegel-Roy *et al.* (1985) reported Nitsch's medium with IAA 10^{-5} M and GA_3 10^{-6} M as the best for germination of seeds of seedless grape cultivars.

Deemir and Gunay (1996) observed the increased germination percentage in seeds of cucumber after priming with 3 per cent KNO_3 for 5 days.

Acacia nilotica seeds scarified with H_2 SO_4 followed by washing and drying and then treated with KNO3 at 2 per cent for 24 h showed higher germination percentage as reported by Palani *et al* (1996). Treatment with KCl, KH_2PO_4 for 24 h had no germination effect.

Cantliffe and Abebe (1996) reported priming the tomato seeds with KNO_3 or PEG 8000 solutions for 6-8 days consistently improved germination at 35 ^{0}C.

Khandelwal and Sen (1996) observed one year old seeds of *Eragrostis* soaked in 50 ppm KNO_3 for 24 h showed higher germination.

Toria seeds soaked in 0.25%, 0.5%, 1% and 2% of KCl, KH_2PO_4 or Na_2HPO_4 for 10 h showed significantly higher germination as reported by Paul and Sarma (1996).

Renjith (1999) reported the turmeric seeds produced under *in vivo* germinated under *in vitro* on moist filter paper.

Bhattacharya and Khurpe (2001) reported soaking of papaya seeds for 24 h in 200 ppm GA increased germination to 12-79 per cent. They observed maximum germination of 95.5 per cent after 7-8 days when naked embryos of papaya seeds were cultured in MS medium supplemented with TDZ (1.0 μM l^{-1}) and maintained in light at 30 ^{0}C.

Roy and Banerjee (2001) observed seed germination of *Geodorum densifolium* was very high (96 %) in all the basal media, with Knudson'C and half MS being slightly more productive than Vacin and Went.

2.11.3 Germination of *in vitro* produced seeds

The *in vitro* raised seeds may often require special pre treatments, media and culture conditions for germination. *In vitro* seeds of *Papaver rhoeas* soaked in tap water for 24 h germinated in modified white's medium (Kanta, 1960).

Usha (1965) observed seeds of *Antirrhinum majus* produced by *in vitro* pollination germinated in Nitsch medium with 4 per cent sucrose.

In vitro produced seeds of *Petunia* germinated on moist filter paper and on nutrient agar medium on which it was developed. The germination was more on moist filter paper (Rangaswamy and Shivanna, 1967).

In vitro produced seeds of *Nicotiana tabacum* varied in their time taken for germination and sometimes the seeds dried out and passed to dormancy (Balatkova *et al.*, 1976)

The karyopses of maize produced *in vitro* dried at room temperature for several days and then kept for germinate on moist filter paper at 28 ^{0}C germinated within 7 days after incubation (Gengenbach, 1977).

Valsala (1994) reported ginger seed incubated in a medium of half MS with 2,4-D 8.0 mg l^{-1} for two months and the seed was then transferred to a plant growth regulator combination of BAP 9.0 mg l^{-1} and 2,4-D 0.1 mg l^{-1} in half MS showed germination.

2.12 MICROPROPAGATION OF GINGER

The growth and performance of micro propagated ginger was comparable with that of plants derived from pieces of rhizome ("seed") in field trials as reported by Smith and Hamill (1996). In the first generation *ex vitro*, micro propagated plants had significantly reduced rhizome yields with smaller knobs and more roots, than "seed" – derived plants. Micro propagated plants had a greater shoot: root ratio compared with "seed" – derived plants. Shoots from micro propagated plants were also significantly smaller, with a greater number of shoots per plant.

2.12.1 Enhanced release of axillary bud

The first report of clonal propagation of ginger under *in vitro* conditions through enhanced release of axillary bud was given by Hosoki and Sagawa (1977). Buds from rhizome in storage were cultured in a medium consisting of MS major elements, Ringe-Nitsch minor elements and vitamins, 2.0 per cent sucrose and BAP 1 mg l^{-1}. De Lange *et al.* (1987) developed a method for elimination of nematodes from ginger by tissue culture. Shoot buds that were successfully encapsulated in 4 per cent sodium alginate gel germinated *in vitro* to form roots and shoots. These plantlets showed no symptoms of ginger yellows disease caused by *Fusarium oxysporum* f.sp. *Zingiberi* and the pathogen was not observed on this plant tissue during a standard *in vitro* diagnostic test (Sharma *et al.*, 1994). Dogra *et al* (1994) achieved *in vitro* propagation of ginger using rhizome buds and these buds produced multiple shoots when cultured aseptically on MS medium supplemented with 2.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. The greatest number of roots was formed on medium supplemented with 1 mg l^{-1} NAA. Plants were successfully established in the field and were morphologically similar. Sharma and Singh (1997) reported high frequency *in vitro* multiplication of disease-free clones of ginger by culturing small, active buds on MS medium supplemented with 2 mg l^{-1} kinetin and 20 g l^{-1} sucrose. An average of 7.7 shoots per bud was obtained after 4 weeks of culture. These *in vitro* derived plants performed well under field conditions, were morphologically identical to the mother plants and were free of ginger yellows (*Fusarium oxysporum* f.sp. *Zingiberi*). Well-developed rhizomes obtained from tissue cultured plants did not rot for up to six months, thus indicating

that the method is also effective against storage rot caused by *Fusarium oxysporum* f.sp.*Zingiberi*.

Babu *et al.* (1992) reported plantlet formation from immature inflorescence (1 to 10 days old flower buds) in modified MS medium supplemented with BA 10 mg l^{-1} and 2,4-D 0.2 mg l^{-1}. These plantlets were easily established in soil and it is concluded that this method could be useful in limiting the spread of rhizome rot and bacterial wilt through vegetative propagation.

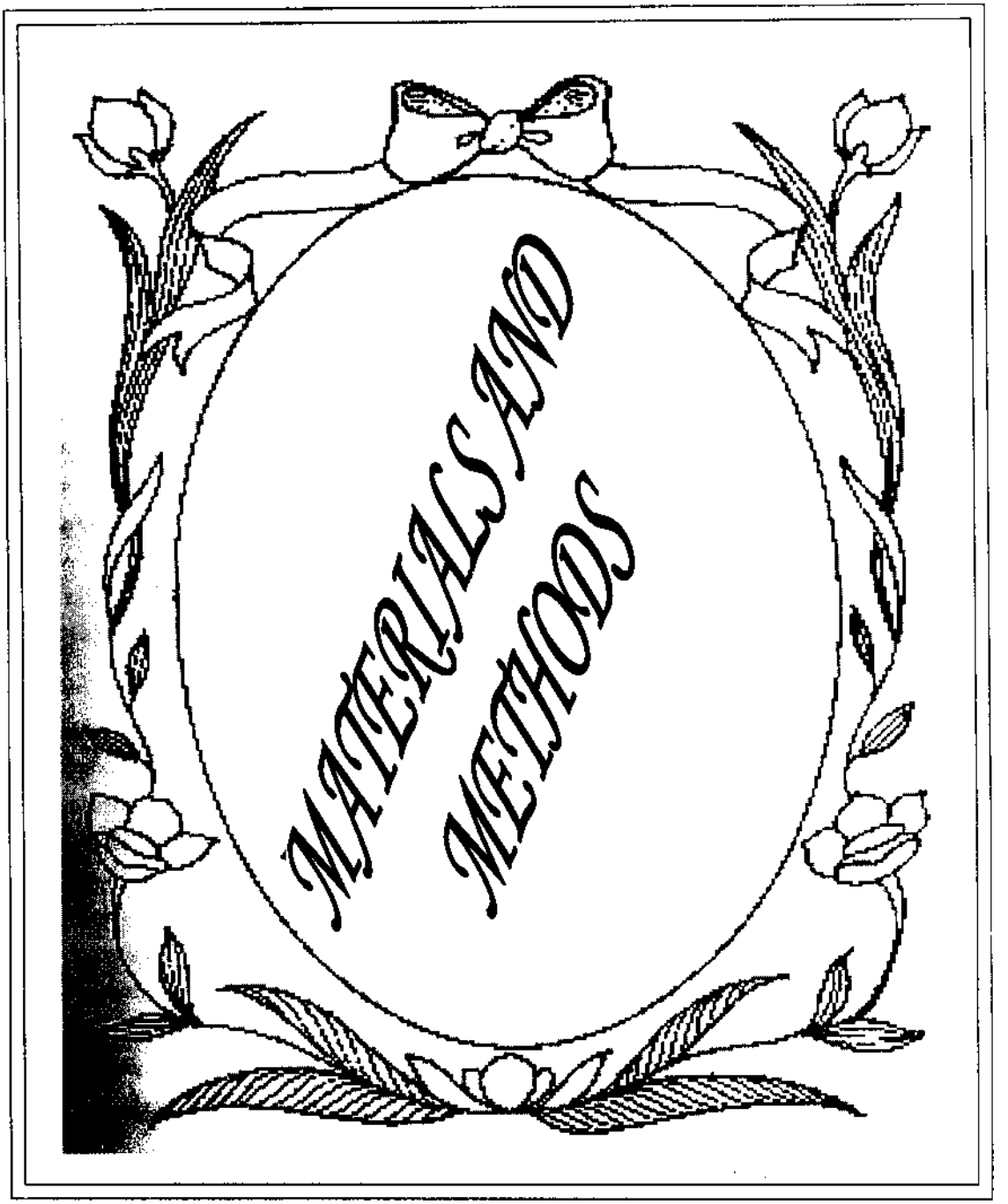
Illg and Faria (1995) reported micro propagation of *Zingiber spectabile* (ornamental ginger). Multiple buds were obtained from axillary buds on MS medium with IAA 10 μM. Shoots were obtained on half MS with BA 10 μM and roots were obtained on half MS with 5 μM IAA or NAA.

2.12.2 Callus mediated organogenesis

Ilahi and Jabeen (1987) have successfully produced plantlets through callus mediated organogenesis. They reported that half strength of MS inorganic was more favourable for microprogation of ginger than full strength. Choi (1991) reported callus mediated organogenesis in ginger from explants of pseudostem containing one leaf blade. Callusing was best on medium containing NAA 0.5 ppm while shoot and root formation were best on medium containing NAA 0.1 to 1 ppm and BA 1 ppm. Rout and Das (1997) also reported efficient plant regeneration in *Z. officinale* achieved using callus derived from shoot primordia grown on MS media. Organogenesis was best on media supplemented with BA (5 mg l^{-1}), IAA (1 mg l^{-1}), adenine sulphate (100 mg l^{-1}) and 3 per cent sucrose. Shoots were rooted on half MS supplemented with IBA or IAA (1 mg l^{-1}) and 2 per cent sucrose. Palai *et al.* (2000) reported plant regeneration from callus culture on MS basal medium supplemented with BAP 5 mg l^{-1}, 100 mg l^{-1} adenine sulphate, IAA 1.5 mg l^{-1} and 3 per cent sucrose. Regenerated shoots were rooted on half strength basal medium MS medium supplemented with IAA 0.25-0.5 mg l^{-1} with in 7-8 days of culture. These *in vitro* grown plantlets were successfully established in soil.

2.12.3 Somatic embryogenesis

In monocots, immature inflorescences, immature embryos, or mature seeds are the choice explants for initiating embryogenic cultures (Bhaskaran and Smith, 1990). Somatic embryogenesis was reported in ginger by Kackar *et al.* (1993). The leaf segments in MS medium with sucrose 8 per cent and Dicamba 2.7 μM produced embryoids. IAA and NAA were not effective in producing embryogenic cultures. Plant regeneration was achieved when embryogenic cultures were transferred to MS medium containing BAP 8.9 μM. Histological studies revealed various stages of somatic embryogenesis characteristic of monocot system. The *in vitro* raised plants were successfully established in soil.


MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigations on "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices and at the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during March 2001 to January 2003.

The details regarding the experimental material and methodology adopted for conducting various aspects of the study are presented in this chapter.

3.1 EXPERIMENTAL MATERIAL

Seven variants evolved through colchicine treatment along with three check varieties maintained at the Department of Plantation Crops and Spices formed the experimental material as indicated in Table 1 (Plates 1a and 1b).

Table 1. Details of experimental material selected for the study

Experimental material	Treatments	Designated as
Z – O – 78	Autotetraploid from Himachal Pradesh evolved through 0.25 per cent colchicine treatment by injection method.	V_1
Z – O – 86	Autotetraploid from Rio-de-Janeiro evolved through 0.1 per cent colchicine treatment by hole method.	V_2
Z – O – 89	Variant from Himachal Pradesh evolved through 0.1 per cent colchicine treatment by hole method.	V_3
Z – O – 90	Variant from Himachal Pradesh evolved through 0.1 per cent colchicine treatment by hole method.	V_4
Z – O – 92	Variant from Himachal Pradesh evolved through 0.25 per cent colchicine treatment by hole method.	V_5
Z – O – 95	Variant from Himachal Pradesh evolved through 0.4 per cent colchicine treatment by hole method.	V_6
Z – O – 97	Variant from Maran evolved through 0.25 per cent colchicine treatment by hole method.	V_7
Rio-de-Janeiro	-	V_8
Maran	-	V_9
Himachal Pradesh	-	V_{10}

3.2 VARIABILITY STUDIES

Experimental design	:	RBD
Treatments	:	Ten
Replications	:	Three

3.3 MANAGEMENT OF CROP

The crop was raised in two consecutive seasons (May 2001 to January 2002 and May 2002 to January 2003). The field was prepared well and raised beds of 3 m x 1m size were taken giving 50 cm inter channel. The selected cultivars were planted at random. Seed bits of 15-20 g weight were planted at a spacing of 25 cm x 25 cm on the raised beds, accommodating 48 plants per bed. The crop was maintained following the cultural, manurial and other plant protection operations as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 1996). Five potted plants of each cultivar were also maintained for getting sufficient number of flowers for *in vitro* studies.

3.4 MORPHOLOGICAL CHARACTERS

The cultivars were examined for the variation in salient morphological characters. Four sample plants were selected randomly from each replication for recording the various morphological observations six months after planting.

The morphological characters include

Plant height in cm

Pseudostem height in cm

Pseudostem thickness in mm

Pseudostem circumference in cm

Pubescence of pseudostem

Number of tillers per plant

Number of leaves per tiller

Number of leaves per plant

Leaf length and breadth in cm

Leaf area in cm^2

Leaf area index

Leaf thickness in mm

Leaf shape

Leaf colour (upper and lower surface)

Hairiness on leaf (upper and lower surface)

Stipules

Number of roots

Root length in cm

Plant height was measured from the ground level to the top of the longest leaf. Pseudostem height was measured from the ground level to the growing point. Pseudostem thickness and leaf thickness were measured using vernier caliper. Leaf length from the base to the tip of fourth leaf from the top of the main tiller and leaf breadth at the centre was measured.

Leaf area was calculated using the equation A = -24 + 3.312 L (Sheeba, 1996). Leaf Area Index (LAI) was calculated using the formula suggested by Watson (1952).

$$LAI = \frac{\text{Total leaf area of plant}}{\text{Land area occupied by plant}}$$

3.5 ANATOMICAL FEATURES

Observations on anatomical features were taken from the fourth leaf from the top of four plants from each replication. Photomicrographs were also taken.

3.5.1 Cuticle thickness

Thin transverse sections were prepared as suggested by Loequin and Langerson (1978). The sections were mounted on a slide with glycerol. The measurements on cuticle thickness were made at 10 X magnification using calibrated ocular micrometer. Measurements were taken from different points of each section and mean was worked out.

3.5.2 Stomatal count and size

The stomatal count and size were recorded following the method described by Jambhale and Nerker (1980). The leaves were collected after exposure to sunlight for two to four hours, preferably at noon. Lower epidermis of leaves were stripped off and placed on a clean glass slide. A drop of two per cent silver nitrate solution was added on the strip and kept for one minute. The strip was then washed thoroughly with distilled water and fixed in a few drops of hyposolution (25 g sodium thiosulphate and 0.10 g potassium metabisulphite dissolved in 200 ml distilled water) for a few minutes. After washing in distilled water, it was mounted in glycerin and observed under microscope. Number of stomata per mm^2 of leaf was counted. The length and width of stomata were measured from six cells selected at random using a calibrated ocular micrometer and expressed in µm.

3.5.3 Epidermal cell count and size

Similar to stomatal observations, epidermal cell size was measured and the number of cells per mm^2 was also counted.

3.6 FLORAL CHARACTERS

3.6.1 Flowering behaviour

The flowering behaviour and the percentage of flowering for selected cultivars were examined.

3.6.2 Inflorescence development and floral biology

The plants were regularly observed and the stage at which the inflorescence primordium was 4 to 5 cm long was taken as the stage of visual initiation of flowering. The duration from initiation of inflorescence to blooming and the period from first flower opening to last flower opening were recorded daily by monitoring anthesis. Observations on inflorescence size, number of inflorescence per plant, number of flowers per inflorescence and number of flowers per plant were recorded. The time of anthesis and anther dehiscence were also observed.

3.6.3 Morphology of flowers

The floral characters of different cultivars were examined. The flower length and style length of flowers emerging from lower, middle and upper portions of the inflorescence were measured in cm. The length and diameter of the ovary were measured using a vernier caliper and expressed in mm. The number of ovules per ovary was counted. The length and breadth of ovules were recorded in µm using a calibrated ocular micrometer.

3.7 POLLEN STUDIES

3.7.1 Pollen fertility and viability studies

The fertility and viability of pollen grains from various cultivars at early, mid and late flowering season were recorded from flowers at lower, middle and upper portions of the inflorescence.

3.7.1.1 Estimation of pollen fertility

Pollen grains scooped out from flower buds at the time of anthesis were stained using 1 per cent acetocarmine and viewed at 100 X magnification. All the pollen grains that were well stained were counted as fertile and unstained as sterile. The observations were recorded from ten microscopic fields. The fertility per cent was calculated using the formula

$$\frac{\text{Number of well stained pollen grains in a field}}{\text{Total number of pollen grains in a field}} \times 100$$

3.7.1.2 Measurement of pollen size

The heterogenity in pollen size in a microscopic field was recorded by noting the diameter of five larger and five smaller pollen grains from various cultivars at different flowering season using a calibrated ocular micrometer and expressed in µm and the fertility level was determined by staining with acetocarmine method. Photomicrographs were also taken.

3.8 POLLEN VIABILITY STUDIES

The pollen grains were collected at anthesis and were incubated in a moisture chamber along with a drop of ME_3 medium. The composition of the ME_3 medium is given in Table 2. The observations were taken 24 h after incubation. The total number of pollen grains as well as the number of germinated grains were counted from 10 microscopic fields and the mean germination percentage was worked out. Mean and maximum pollen tube growth attained was also measured using a calibrated ocular micrometer. Photomicrographs of the germinated pollen grains were also taken.

Table 2. Composition of modified ME_3 medium (Leduc *et al.*, 1990)

Constituents	Concentration (mg l^{-1})
Macronutrients	
$MgSO_4.7H_2O$	370.00
KNO_3	950.00
H_2PO_4K	85.00
$CaCl_2$	880.00
NH_4NO_3	412.50
KCl	175.00
Na_2EDTA	7.45
$FeSO_4.7H_2O$	5.55
Micronutrients	
H_3BO_3	50.00
$MnSO_4.H_2O$	16.80
$ZnSO_4.7H_2O$	10.50
KI	0.83
$Na_2MO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Vitamins	
Thiamine	1.00
Pyrodoxine	1.00
PEG – 4000	120000.00
pH	5.8

3.9 ECONOMIC ATTRIBUTES

3.9.1 Rhizome characters

The number of primary, secondary and tertiary fingers in a clump was recorded from the randomly selected four plants from each replication.

The rhizomes originating from the seed material were taken as primary fingers and rhizomes originating from the primary finger were considered as secondary fingers and the rhizomes from secondary finger considered as tertiary fingers.

Length, breadth, girth, weight, number of nodes and internodal length of primary, secondary and tertiary fingers were measured and mean worked out.

3.9.2 Fresh rhizome yield

The rhizomes were harvested eight months after planting by uprooting individual clumps. The fresh rhizome yield was expressed as yield per plant (g) and yield per hectare (tonnes).

3.9.3 Dry rhizome yield

Dry rhizome yield per hectare was calculated for each cultivar using the corresponding dry recovery percentage and was expressed as g per plant and tonnes per hectare.

3.10 QUALITY ATTRIBUTES

The variability in the selected cultivars for the following quality parameters was examined. Three replications were maintained for each cultivar.

3.10.1 Colour of rhizome flesh and powder

The colour of both rhizome flesh and powder was recorded.

3.10.2 Dry recovery percentage

The per cent recovery of dry rhizome to fresh rhizome was estimated by sun drying 500 g of fresh rhizome immediately after harvest until a constant weight was

obtained. For quick drying, the rhizomes were chopped into pieces of 2-3 mm thickness.

3.10.3 Estimation of crude fibre

The crude fibre content was estimated as per AOAC (1980) and expressed as percentage.

3.10.4 Estimation of volatile oil

Volatile oil was estimated by water distillation adopting Clevenger trap method as per AOAC (1980) and expressed as percentage. Twenty five g of powdered sample from each cultivar was used for analysis. The oil production per hectare was calculated from the oil recovery percentage and the dry rhizome yield per hectare. The quality of the oil from cultivars was assessed by sensory evaluation and scoring technique based on colour and aroma of oil.

3.10.5 Estimation of oleoresin

The oleoresin content was estimated using the soxhlet extraction apparatus as per AOAC (1980) with solvents acetone and ethyl acetate. Five g of powdered sample was refluxed with 250 ml of solvent. The solvent was removed through evaporation from the extract until a constant weight was reached. The oleoresin content was expressed in percentage. The oleoresin production per hectare was calculated from the oleoresin recovery percentage and the dry rhizome yield per hectare. The quality of oleoresin from different cultivars was assessed by sensory evaluation and scoring technique based on colour and aroma of oleoresin. The duration for extraction of oleoresin with the two selected solvents was also recorded.

3.11 INCIDENCE OF PESTS AND DISEASES

3.11.1 Incidence of shoot borer

Incidence of shoot borer (*Conogethes punct*iferalis) on the crop was recorded as number of infected tillers per clump and number of infected plants and both were expressed in percentage.

3.11.2 Incidence of rhizome rot or soft rot

The incidence of rhizome rot caused by *Pythium aphanidermatum* was recorded four times (July, August, September, and October) during the growth period. The mean percentage of infection was worked out from the number of plants infected out of the total number of plant population in each plot for each cultivar.

3.11.3 Incidence of bacterial wilt

The incidence of bacterial wilt caused by *Ralstonia solanacearum* was recorded four times (July, August, September, and October) during the growth period. The percentage of infection was worked out from the number of plants infected out of the total number of plant population in each plot. Ooze test from rhizomes at harvest was also done and scored as "+++" maximum intensity, "++" moderate intensity, "+" light intensity and "-" nil.

3.11.4 Incidence of leaf spot

The incidence of leaf spot caused by *Phyllosticta zingiberi* was scored into five intensities based on the number of spots on the leaf (i.e.) very light (1 spot), light (2 to 5 spots), medium (6 to 15 spots), severe (16 to 40 spots) and very severe (more than 40 spots) (Nybe, 1978). They were given numericals for statistical analysis (very light-0, light-1, medium-2, severe-3 and very severe-4). From each observation plant, ten leaves were randomly selected for taking observation.

3.12 CONTROLLED *IN VIVO* POLLINATION

Controlled *in vivo* pollination was carried out in the cultivars at bud stage (1 day prior to flower opening), immediately after flower opening and 3 h after flower opening adopting the following techniques.

3.12.1 *In vivo* stylar pollination

It was done by cutting the styles at different length and by applying pollen grains along with pollen germinating medium (ME_3) at the cut end at the receptive stage.

3.12.2 *In vivo* stigmatic pollination

Stigmatic pollination was tried after removing the floral appendages like corolla, anther and stigma and smearing pollen grains along with ME_3 medium.

3.12.3 *In vivo* intra ovarian pollination

Various techniques were adopted for *in vivo* intra ovarian pollination. In all the following cases, the style and the corolla tube were removed just above the ovary and pollination was done in the following ways.

(i) Pollen grains were applied on the top of the cut surface.

(ii) A slit was made on the top of the ovary by inserting a surgical blade and pollen grains were applied in the slit.

(iii) A pollen suspension was prepared in the pollen germinating medium and this was injected into the ovary with the help of a syringe.

In all the above techniques, pollination was carried out using the pollen from the same flower (artificial self pollination), pollen from separate flowers of the same variety (artificial sibling), pollen from flowers of the other selected varieties (artificial cross pollination) and mixed pollen from other genera like *Kaempferia* and *Costus* which also belong to the family Zingiberaceae. In addition, *in vivo* pollination was also carried out in *Zingiber zerumbet* using pollen from *Z. officinale* (Plate 2).

3.13 IN VITRO POLLINATION

3.13.1 Collection of explant

Flower buds were collected on the day of anthesis at 12 noon prior to flower opening. The flower buds ready to dehisce protruding from the bract were scooped out with the help of forceps and needle without injuring the ovary. The calyx surrounding the base of the corolla tube was removed, wiped with 70 per cent alcohol and the flower buds were quickly transferred into conical flask containing sterile water.

Himachal Pradesh
(Diploid)

Z – O – 78
(Autotetraploid)

Plate 1a. Plants of Himachal Pradesh and its corresponding autotetraploid

Rio-de-Janeiro
(Diploid)

Z – O – 86
(Autotetraploid)

Plate 1b. Plants of Rio-de-Janeiro and its corresponding autotetraploid

Zingiber officinale

Zingiber zerumbet

Plate 2. *Zingiber* species

3.13.2 Transfer area and aseptic manipulations

All the aseptic manipulations such as surface sterilization of flower buds, pollination and inoculation of ovules, subsequent sub culturing and various *in vitro* seed germination studies including embryo rescue were carried out under the hood of a clean laminar air flow chamber. The working table of the laminar air flow chamber was first wiped with absolute alcohol and then exposed to ultra violet light for 20 to 30 minutes. The petridishes as well as the inoculation aids were first steam sterilized and then flame sterilized before use.

3.13.3 Surface sterilization

Flower buds were surface sterilized prior to anthesis ie 3.00 p.m. The flowers were immersed in distilled water and cleaned carefully to remove soil particles adhering to the base of the ovary. The flowers were then cut into two parts i.e. upper part with the anther lobe and the lower part with the ovary along with a small portion of the style. The prepared androecium and gynoecium parts were first wiped with 70 per cent alcohol followed by 0.05 per cent streptocycline dip for 2 h. They were continuously agitated manually to avoid bacterial interference. Separation of the anther and ovary portions was done to ensure that the gentle rinsing of anther portion does not allow loss of pollen grains during washing. This also facilitated vigorous shaking and rinsing of the ovary portion to clean the basal part of the ovary where more contamination from soil could be found adhering. Then they were washed and treated with 0.1 per cent mercuric chloride for 3 minutes, rinsed thrice with sterile distilled water to remove traces of sterilant from the surface and then dried on sterile flamed filter paper.

3.14 PREPARATION OF MEDIA

The chemicals used for preparing various media were of analytical grade obtained from British Drug House (BDH), Sisco Research Laboratories (SRL), Merck or Sigma.

Borosilicate glassware of corning/borosil brand were used for *in vitro* studies. They were cleaned initially using boiled tap water for half an hour. On cooling, they were washed with detergent solution rinsed with potassium dichromate solution

in sulphuric acid, then washed free of detergent using tap water and finally rinsed with distilled water. Washed glassware were dried in hot air oven at 60 ^{0}C and were stored away from dust and contaminants.

Standard procedures were adopted (Gamborg and Shyluk, 1981) for the preparation of media. The pH of the medium was adjusted to 5.7. Semisolid media was prepared by adding good quality agar (0.375 %). In case of liquid media, strips of Whatman No. 1. filter paper were used to support the explants. Sterilization of medium was done by subjecting the media to a temperature of 121^{0}C at a pressure of 1.06 kg cm^2 for 20 minutes in an autoclave (Dodds and Robert, 1982). After sterilization, the media were allowed to cool to room temperature and stored in cool, dry place.

3.15 *IN VITRO* POLLINATION METHODS IN GINGER

Placental pollination, modified placental pollination and ovular or test tube fertilization were tried as reported by Valsala (1994) for the present study. The ovule development was scored as "+++" good swelling, "++" moderate swelling, "+" low swelling and "-" no swelling. In placental pollination, placenta bearing the ovules was exposed by completely peeling the ovary wall and pollination was done on it with pollen grains suspended in ME_3 medium. Modified placental pollination was done by peeling away the ovary wall of one locule and pollinating on the exposed ovules. In ovular pollination, the seperated ovules were put in a cavity slide containing pollen suspension in pollen germinating medium for 24 h. After pollination, it was transferred to inoculation medium. *In vitro* selfing as well as crossing between cultivars were done adopting this method. The number of ovules developed per culture and the percentage of cultures showing ovule development at different flowering season were recorded.

3.16 POLLEN PISTIL INTERACTION AFTER *IN VIVO* AND *IN VITRO* POLLINATION

Fluorescence microscopic technique (Kho and Baer, 1968 and Kho *et al.*, 1980) was employed to study pollen germination and tube growth after *in vivo* and *in vitro* pollination. The pollinated pistils were fixed in FAA (formalin 10 ml,

acetic acid 10 ml and ethyl acetate 8 ml) at intervals of 3, 6, 9, 12, 24, 36, 48 h after pollination. After 24 h of fixation, the materials were transferred to glass vials containing 1N NaOH for 10 h at room temperature in order to soften the tissue. The materials were then washed carefully with distilled water and stained with 0.1 per cent aniline blue in 0.1 N K_2HPO_4 for 18 h. After adequate staining, the pistils were mounted in 80 per cent glycerol on a microscopic slide and viewed through fluorescence microscope and photomicrographs were taken.

3.17 BASAL MEDIA FOR CULTURE ESTABLISHMENT

Murashige and Skoog's (MS) medium (1962) at half strength, Schenk and Hildebrandt (SH) medium (1972) and Nitsch medium (1951) were used as basal media in the present study. The composition of these media is given in Table 3. The basal media alone and also supplemented with different levels of different growth regulators along with different organic media supplements served as treatments. The pollinated ovaries and ovules after *in vitro* placental pollination were cultured in the media. The swelling of ovary was measured using ocular micrometer whereas ovules were scored at 20 DAP as "+++" good, "++" moderate and "+" low and the cultures showing ovule development were expressed as percentage.

3.18 STANDARDISATION OF MEDIA SUPPLEMENTS

3.18.1 Influence of auxins and cytokinins on ovule development

The effects of auxins and cytokinins alone and in combination were studied.

3.18.1.1 Influence of auxin

The basal medium with 3 per cent sucrose and 200 mg l^{-1} CH was supplemented with NAA (0.5, 1.0) mg l^{-1}, IAA (0.5) mg l^{-1} and 2,4 – D (0.5) mg l^{-1}.

3.18.1.2 Influence of cytokinin

The basal medium with 3 per cent sucrose and 200 mg l^{-1} CH was supplemented with BAP (2.5 mg l^{-1}) and kinetin (2.5 mg l^{-1}).

3.18.1.3 Influence of auxin and cytokinin combinations

The treatment details are described in Table 4.

Table 3. Composition of different culture media used for culture establishment and seed germination

Constituents	Concentration (mg l^{-1})		
	MS medium	***SH medium***	***Modified Nitsch medium***
Macronutrients			
KNO_3	1900.00	2500.0	1250.00
NH_4NO_3	1650.00	-	-
H_2PO_4K	170.00	-	1250.00
$MgSO_4.7H_2O$	370.00	400.00	-
$CaCl_2.2H_2O$	440.00	200.00	-
$Category(NO_3)_2.4H_2O$	440.00	200.00	5000
NH_4SO_4	-	-	-
Micronutrients			
H_2SO_4 Sp. Gr. 1.83	-	-	0.5 ml
H_3BO_3	6.200	5.0	500.00
$MnSO_4.4H_2O$	22.300	-	3000.00
$ZnSO_4.7H_2O$	8.600	1.0	500.00
$Na_2MO_4.2H_2O$	0.250	0.10	25.00
$CuSO_4.5H_2O$	0.025	1.00	25.00
$CoCl_2.6H_2O$	0.025	20.00	-
KI	0.830	10.00	-
Na_2EDTA	33.600	15.00	-
$MnSO_4.H_2O$	-	10.00	-
$FeSO_4.7H_2O$	27.800	15.00	-
$FeC_6O_5H_7.5H_2O$	-	-	10
Vitamins Thiamine, HCl	0.10	5.00	-
Pyridoxine, HCl	0.50	0.50	15
Nicotinic acid	0.05	5.00	-
Niacin	-	-	75
Others			
Glycine	2.00	-	450
Myo-inositol	100.00	1000.00	-
Sucrose	30000.00	30000.00	30000.00
Calcium pantothenate	-	-	15
pH	5.8	5.8	5.8

Table 4. Auxin and cytokinin combinations for ovule development of ginger after *in vitro* placental pollination

Growth regulator concentrations in mg l^{-1}		
NAA 0.5 + BAP 2.5	IAA 0.1 + BAP 2.5	2,4 – D 0.1 + BAP 2.5
NAA 0.5 + BAP 5.0	IAA 0.2 + BAP 2.5	2,4 – D 0.2 + BAP 2.5
NAA 0.5 + BAP 7.5	IAA 0.25 + BAP 2.5	2,4 – D 0.5 + BAP 2.5
NAA 0.5 + BAP 10.0	IAA 0.25 + BAP 5.0	2,4 – D 0.5 + BAP 5.0
NAA 0.5 + BAP 15.0	IAA 0.25 + BAP 7.5	2,4 – D 0.5 + BAP 7.5
NAA 0.5 + BAP 20.0	IAA 0.25 + BAP 10.0	2,4 – D 0.5 + BAP 10.0
NAA 1.0 + BAP 2.5	IAA 0.25 + BAP 15.0	2,4 – D 0.5 + BAP 15.0
NAA 1.5 + BAP 2.5	IAA 0.25 + BAP 20.0	2,4 – D 0.5 + BAP 20.0
NAA 2.0 + BAP 2.5	IAA 0.5 + BAP 2.5	2,4 – D 0.75 + BAP 2.5
NAA 2.5 + BAP 5.0	IAA 0.5 + BAP 5.0	2,4 – D 1.0 + BAP 2.5
NAA 2.5 + BAP 10.0	IAA 0.5 + BAP 10.0	Dicamba (0.50, 0.75, 1.00) + BAP 2.5 Dicamba 2.5 + BAP 0.5

3.18.2 Influence of GA on ovule development

The effect of different levels of GA_3 alone or in combination with different levels of auxin and cytokinin was studied in basal medium of half MS along with 3 per cent sucrose and 200 mg l^{-1} CH. The treatment details are given in Table 5.

Table 5. Gibberellin tried for ovule development of ginger after *in vitro* placental pollination

Sl. No.	Growth regulator treatments in mg l^{-1}
1.	GA_3 (1.0, 2.5, 5.0, 7.5, 10.0)
2.	GA_3 5.0 + NAA 0.5
3.	GA_3 5.0 + BAP 2.5
4.	GA_3 1.0 + NAA 0.5 + BAP 2.5

3.18.3 Influence of tryptophan on ovule development

The effect of tryptophan alone at different levels (0.25, 0.50, 0.75, 1.00 mg l^{-1}) or in combination with cytokinin (BAP 2.5 mg l^{-1}) was studied in half MS basal medium with 3 per cent sucrose + 200 mg l^{-1} CH.

3.18.4 Influence of carbon source on ovule development

The influence of different levels of sucrose (3.0, 6.0, 9.0, 12.0 %) on ovary and ovule development was studied. The medium of half MS + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} + CW 15 per cent v/v was supplemented with different concentrations of sucrose.

3.18.5 Influence of organic media supplements on ovule development

The influence of organic media supplements such as coconut water (CW) (5 to 25 v/v), casein hydrolysate (CH) (200 to 1000 mg l^{-1}) and L-glutamine (LG) (25 to 500 mg l^{-1}) on ovule development was studied. The basal medium used was half MS + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} + sucrose 3 per cent. The liquid endosperm collected from tender coconut was used to study the influence of coconut water.

3.18.6 Influence of vitamin supplements on ovule development

The effect of doubling the vitamin stock of MS medium was studied. Four hormone combinations as shown below were tried. The basal medium was half MS along with 3 per cent sucrose and CH 200 mg l^{-1}.

(i) BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1}

(ii) BAP 2.5 mg l^{-1} + 2, 4 -D 0.5 mg l^{-1}

(iii) BAP 2.5 mg l^{-1} + Dicamba 0.5 mg l^{-1}

(iv) BAP 2.5 mg l^{-1} + IAA 0.5 mg l^{-1}

3.18.7 Influence of physical conditions on ovule development

In this experiment, the physical forms of the medium such as solid, liquid and semisolid were incubated under both light and dark conditions. The basal medium used was half MS + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} + sucrose 3 per cent + CW 15 per cent.

In all the above treatments studied, placental pollination as detailed in 3.15 was done.

3.19 CULTURE CONDITIONS

The cultures were incubated at 26 ± 2^{0}C in an air-conditioned culture room under diffused light. Humidity in the culture room varied between 60 to 80 per cent according to the climate, which prevailed at the time.

3.20 POST POLLINATION CHANGES

Observations were recorded at intervals of 20 days commencing from the 20th DAP till 80 days for the change in development of ovules and the size was measured using an ocular micrometer.

3.21 SEED VIABILITY TEST

The viability of the seeds was tested from 40 to 80 DAP with tetrazolium stain (2,3,5 triphenoxy tetrazolium chloride). The seeds were first soaked in distilled water for 24 h. Then they were cut into two halves longitudinally under a dissection microscope, immersed in 0.1 per cent tetrazolium solution of pH 6.5 to 7.0 and kept in dark at 38^{0}C until the solution was completely evaporated out. The sections were observed under stereo microscope and the embryo stained red were taken as viable.

3.22 *IN VITRO* SEED GERMINATION STUDIES

The *in vitro* produced seeds after placental pollination were subjected to different germination treatments as detailed below and the germination percentage was noted.

3.22.1 Embryo rescue studies

3.22.1.1 Embryo culture

The seeds obtained on 20, 40, 60 and 80 DAP were subjected to embryo rescue studies. Eighty days old seeds were soaked in sterile water for 24 h to soften the seed coat. The technique of embryo rescue was done under the hood of a clean laminar air flow chamber using a dissection microscope.

The technique followed in this study was transfer of embryo along with endosperm. A perfect cut was given to the seed at micropylar end without injuring the embryo. Then with the help of forceps, a slight pressure was applied at the chalazal end so as to squeeze out the embryo along with the endosperm. The rescued embryo with a small bit of endosperm was incubated in half MS medium, Knudson C medium and Tomato embryo culture medium containing combinations of NAA (0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 mg l^{-1}), IAA (0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 mg l^{-1}) and 2,4-D (0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 mg l^{-1}). The composition of Knudson C and Tomato embryo culture media is given in Tables 6 and 7.

3.22.1.2 Ovule culture

The pollinated ovules in half MS + BAP (2.5 mgl^{-1}) + NAA (0.5 mgl^{-1}) at different stages of development (5,10,15,20,25,50,80 DAP) were cultured in various media viz., half MS, MS, Knudson C, SH, B_5, Taira and Larter's modified Norstog, Tomato embryo culture alone and also with different concentrations of various growth regulators. The composition of B_5, Taira and Larter's modified Norstog media is given in Tables 8 and 9. The various growth regulator combinations tried are listed in Table 10.

3.22.2 Primary treatments

The seeds obtained on 80 DAP were subjected to various primary treatments like imbibition in water, keeping on moist filter paper, moist sterile sand, moist sterile sand with vermiculite (1:1) and basal medium such as full strength MS, half strength MS, Knudson C, SH, B_5, Tomato embryo culture medium and Taira and Larter's modified Norstog medium (solid, liquid and semi solid).

3.22.3 Influence of physical conditions of medium on seed germination

The seeds obtained on 80 DAP were incubated in half MS and full MS (solid, semi solid and liquid) media alone and also with different combinations of growth regulators. The treatment combinations tried are furnished in Table 11.

Table 6. Composition of Knudson C medium used for seed germination

Constituents	Concentration (mg l^{-1})
Ca $(NO_3)_2$.4H2O	1000.00
$FeSO_4.H_2O$	25.00
KH_2PO_4	250.00
$MgSO_4$. $7H_2O$	250.00
$(NH_4)_2SO_4$	250.00
$MnSO_4$	7.50
Sucrose	20000.00
Agar	9000.00
pH	5.8

Table 7. Composition of Tomato embryo culture medium used for seed germination

Constituents	Concentration (mg l^{-1})
NH_4NO_3	1601.00
KNO_3	2022.00
$NaH_2PO_4.H_2O$	276.02
$CaCl_2$. $2H_2O$	441.07
$MgSO_4$. $7H_2O$	739.50
H_3BO_3	9.28
$MnSO_4$. H_2O	16.90
$ZnSO_4$. $7H_2O$	11.50
$CuSO_4$. $7H_2O$	0.375
Na_2MoO_4. $2H_2O$	0.242
$CoCl_2$. $6H_2O$	0.238
KI	0.830
$FeSO_4$. $7H_2O$	27.80
Na_2EDTA	37.22
Sucrose	60000.00
Inositol	20.00
Nicotinic acid	0.50
Pyridoxine	0.50
Thiamine	1.00
Glycine	0.50
pH	5.8

Table 8. Composition of B_5 medium used for seed germination

Constituents	Concentration (mg l^{-1})
Macronutrients	
KNO_3	2500.00
NH_4NO_3	-
H_2PO_4K	-
$MgSO_4.7H_2O$	122.09
$CaCl_2.2H_2O$	113.24
$Ca(NO_3)_2.4H_2O$	-
NH_4SO_4	134.00
Micronutrients	
H_2SO_4 Sp. Gr. 1.83	-
H_3BO_3	3.0
$MnSO_4.4H_2O$	10.00
$ZnSO_4.7H_2O$	2.00
$Na_2MO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.75
Na_2EDTA	37.3
$MnSO_4.H_2O$	10.00
$FeSO_4.7H_2O$	27.80
$FeC_6O_5H_7.5H_2O$	10.00
Vitamins	
Thiamine, HCl	1.00
Pyridoxine, HCl	1.00
Nicotinic acid	-
Niacin	-
Others	
Glycine	-
Myo-inositol	100
Sucrose	30000.00
Calcium pantothenate	-
pH	5.8

Table 9. Composition of Taira and Larter's modified Norstog medium used for seed germination

Constituents	Concentration (mg l^{-1})
KH_2PO_4	900.00
KCl	750.00
$MgSO_4.H_2O$	750.00
$CaCl_2.2H_2O$	750.00
$MnSO_4.H_2O$	3.00
H_3BO_3	0.50
$ZnSO_4.7H_2O$	0.50
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.020
$NaMoO_4.2H_2O$	0.030
Fe EDTA	25.00
Meso-inositol	100.00
Thiamine HCl	1.00
Pyrodoxine HCl	1.00
Nicotinic acid	1.00
Casein hydrolysate	2500.00
Maleic acid *	500.00
Agar	10000.00
Sucrose	51300.00
pH (before autoclaving)	5.5

- Maleic acid dissolved in 150 ml water, pH adjusted to 5.5 with NaOH before adding to the medium.

Table 10. Growth regulator treatments tried for germination of ovules

Sl.No.	Growth regulator treatments in mg l^{-1}
1.	IAA (0.05, 0.1, 0.3, 0.5, 1.0)
2.	NAA (1.0, 1.5, 2.0, 2.5, 5.0)
3.	2, 4 – D (0.1, 0.3, 0.5, 1.0, 2.0)
4.	Dicamba (0.25, 0.5, 1, 2.5, 5.0)
5.	BAP (5, 7.5, 10)
6.	Kinetin (5, 7.5, 10)
7.	GA_3 (1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0)
8.	Ethylene (1.0, 3.0, 5.0)
9.	IAA (0.05, 0.1, 0.3, 0.5, 1.0, 2.0) + BAP (0.5, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0)
10.	NAA (0.1, 1.0, 1.5, 2.0, 2.5, 5.0) + BAP (1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0)
11.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + BAP (0.1, 2.5, 5.0, 7.5, 9.0, 10.0, 15.0, 20.0)
12.	IAA (0.05, 0.1, 0.2, 0.3, 0.5) + Kinetin (0.5, 2.0, 3.0, 5.0)
13.	NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5.0) + Kinetin (0.5, 2.0, 3.0, 5.0)
14.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + Kinetin (0.5, 2.0, 3.0, 5.0)
15.	IAA (0.05, 0.1, 0.2, 0.3, 0.5) + GA_3 (2.0, 5.0, 9.0) + Tomato juice (5 %)
16.	NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5.0) + GA_3 (2.0, 5.0, 9.0) + Tomato juice (5 %)
17.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + GA_3 (2.0, 5.0, 9.0) + Tomato juice (5 %)
18.	NAA (0.1, 1.0, 1.5, 2.0, 2.5, 5.0) + BAP (1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0) + Tomato juice (5 %)
19.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA3 (1, 2.5, 5) + Tomato juice (5 %)

Table 11. Growth regulators tried along with physical conditions of media for germination

Sl. No.	Growth regulator treatments in mg l^{-1}
1.	Half MS + GA_3 (0.5, 5, 25, 50, 100)
2.	Half MS + GA_3 (1.0, 5.0, 10.0) + Ethylene (0.25, 0.50, 0.75, 1.00)
3.	Half MS + 2,4 – D (0.1, 0.3, 0.5) + BAP (2.5, 5.0, 10.0) + GA_3 (1.0, 2.0, 5.0)
4.	Half MS + 2,4 – D (0.1, 0.25, 0.5) + BAP (3.0, 6.0, 9.0)

3.22.4 Pulse treatment with growth regulators

The seeds obtained on 80 DAP were treated for half an hour with different concentrations of auxins, cytokinins, gibberellin and ethylene.

The various growth regulators tried at different concentrations as initial pulse treatment are given below

3.22.5.1 Auxins

IAA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250 mg l^{-1})

NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250 mg l^{-1})

2,4-D (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250 mg l^{-1})

IBA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250 mgl^{-1})

Dicamba (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250 mgl^{-1})

3.22.5.2 Cytokinins

BAP (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500 mg l^{-1})

Kinetin (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500 mg l^{-1})

3.22.5.3 Gibberellin and Ethylene

Both the growth regulators were subjected to filter sterilization and then used.

GA_3 (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000 mg l^{-1})

Ethylene (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000 mg l^{-1})

The seeds after treatment were transferred to half MS medium alone and also with different concentrations of different growth regulators as given in Table 12.

Table 12. Growth regulator combinations tried in the medium after soaking the seeds in growth regulators for germination

Sl.No.	Growth regulator treatments mg l^{-1}
1.	IAA (0.1, 0.5) + BAP (5, 10)
2.	IAA 0.5 + GA_3 (1, 2.5, 5)
3.	IAA 0.5 + Ethylene (1, 3, 5)
4.	NAA (1, 2.5) + BAP (5, 10)
5.	NAA 2.5 + GA_3 (1, 2.5, 5)
6.	NAA 2.5 + Ethylene (1, 3, 5)
7.	2, 4 – D (0.25, 0.5) + BAP (7.5, 10)
8.	2, 4 – D 0.5 + GA_3 (1, 2.5, 5)
9.	2, 4 – D 0.5 + Ethylene (1, 3, 5)
10.	BAP (5, 7.5, 10) + GA_3 (1, 2.5, 5)
11.	BAP (5, 7.5, 10) + Ethylene (1, 3, 5)
12.	GA_3 (1, 2.5, 5) + Ethylene (1, 3, 5)
13.	IAA (0.1, 0.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)
14.	IAA (0.1, 0.5) + BAP (5, 10) + Ethylene (1, 3, 5)
15.	NAA (1, 2.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)
16.	NAA (1, 2.5) + BAP (5, 10) + Ethylene (1, 3, 5)
17.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA_3 (1, 2.5, 5)
18.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + Ethylene (1, 3, 5)

3.22.5 Mechanical scarification

The seeds obtained on 80 DAP were pounded with sterile sand and on a piece of marble until the seed coat ruptured.

3.22.6 Chemical scarification

Eighty days old seeds were scarified with HCl (10, 25, 50 %), HNO_3 (10, 25, 50 %), H_2SO_4 (10, 25, 50 %) and salicyclic acid (0.001, 0.01, 0.1, 0.5 %) for one to ten minutes.

3.22.7 Stratification

The seeds obtained on 80 DAP were kept at 2 ^{0}C and at 4 ^{0}C for 3 to 24 h.

3.22.8 Washing the seeds in running water and sowing

Eighty days old seeds were washed at time intervals of 3, 6, 9 and 12 h.

3.22.9 Hydration – dehydration – rehydration

The seeds obtained on 80 DAP were initially hydrated for 12 h, then dehydrated for 3, 6, 9 and 12 h and again rehydrated for another 12 h.

The seeds after treatment as detailed from 3.22.5 to 3.22.9 were washed with sterile water and then transferred to half MS medium alone and also with GA_3 (0.5, 5.0, 25.0, 50.0, 100.0 mg l^{-1}) and GA_3 (1.0, 5.0, 10.0 mg l^{-1}) + ethylene (0.25, 0.50, 0.75, 1.00 mg l^{-1}) and BAP (2.5, 5.0, 10.0 mg l^{-1}) + 2,4 – D (0.1, 0.3, 0.5 mg l^{-1}) + GA_3 (1.0, 2.0, 5.0 mg l^{-1}).

3.22.10 Treatment with solvents

Eighty days old seeds were treated with acetone (1, 5, 10, 20 %), methanol (1, 5, 10, 20 %) and ethanol (1, 5, 10, 20 %) for 1 to 10 minutes.

3.22.11 Treatment with NaCl and NaOH

The seeds obtained on 80 DAP were treated with NaCl (0.1, 0.25, 0.5, 1 %) and NaOH (1, 5, 10, 15, 20 %) for 10 minutes.

3.22.12 Influence of HCN

Eighty days old seeds were treated with HCN (2, 4, 6, 8, 10 mg l^{-1}) for 10 minutes.

3.22.13 Treatment with thiourea

Eighty days old seeds were treated with thiourea (0.1, 0.25, 0.5, 0.75, 1.0 %) for 10 minutes.

3.22.14 Treatment with enzyme

The seeds obtained on 80 DAP were treated with pectinase (1, 5, 10, 15, 20 %) for 10 minutes.

3.22.15 Manual nicking of seeds

Eighty days old seeds were soaked in sterile water for 1 day and both soaked seeds as well as unsoaked seeds were used for manual pricking by pins.

3.22.16 Influence of cut at chalazal and micropylar end

Eighty days old seeds were given cut at chalazal, micropylar and at both ends.

The seeds after treatment as detailed from 3.22.10 to 3.22.16 were washed with sterile water and then transferred to half MS, full MS, Knudson C, Tomato embryo culture media alone and also with BAP (6.0 mg l^{-1}) + 2,4 – D (0.5 mg l^{-1}) + GA_3 (5.0 mg l^{-1}).

3.22.17 Influence of priming of seeds

Eighty days old seeds soaked in water for one day, unsoaked seeds, seeds cut at micropylar, chalazal and at both ends, seeds pricked with sterile pin were primed with chemicals at a stipulated time as given in Table 13.

Table 13. Seed priming chemicals for germination of ginger seed

Seed priming chemicals with concentration	Treatment duration
PEG 4000 (3, 6, 9, 12, 15) %	5 days
PEG 6000 (3, 6, 9, 12, 15) %	5 days
Mannitol (3, 6, 9, 12, 15) %	5 days
KCl (0.25, 0.5, 1.0, 2.0) %	6 h
KH_2PO_4 (0.25, 0.5, 1.0, 2.0) %	6 h
Na_2HPO_4 (0.25, 0.5, 1.0, 2.0) %	6 h
$CaCO_3$ (0.25, 0.5, 1.0, 2.0) %	6 h
KNO_3 (25, 50, 100, 250, 500, 750, 1000) ppm	8 h
KNO_3 (1, 3, 5, 7, 10) %	5 days

The seeds after treatment were washed with sterile water and then transferred to half MS, full MS, Knudson C, Tomato embryo culture medium + 3 per cent sucrose alone and also with growth regulators as listed in Table 14.

Table 14 Growth regulator treatments tried for culturing primed seeds for germination

Sl. No.	Growth regulator treatments mg l^{-1}
1.	IAA (0.1, 0.5, 1)
2.	NAA (1, 2.5, 5)
3.	2, 4 – D (0.25, 0.5, 1)
4.	Dicamba (0.25, 0.5, 1)
5.	BAP (5, 7.5, 10)
6.	Kinetin (5, 7.5, 10)
7.	GA_3 (1, 2.5, 5)
8.	Ethylene (1, 3, 5)
9.	IAA (0.1, 0.5) + BAP (5, 10)
10.	IAA (0.1, 0.5) + Kinetin (5, 10)
11.	IAA 0.5 + GA_3 (1, 2.5, 5)
12.	IAA 0.5 + Ethylene (1, 3, 5)
13.	NAA (1, 2.5) + BAP (5, 10)
14.	NAA (1, 2.5) + Kinetin (5, 10)
15.	NAA 2.5 + GA_3 (1, 2.5, 5)
16.	NAA 2.5 + Ethylene (1, 3, 5)
17.	2, 4 – D (0.25, 0.5) + BAP (7.5, 10)
18.	2, 4 – D (0.25, 0.5) + Kinetin (7.5, 10)
19.	2, 4 – D 0.5 + GA_3 (1, 2.5, 5)
20.	2, 4 – D 0.5 + Ethylene (1, 3, 5)
21.	BAP (5, 7.5, 10) + GA_3 (1, 2.5, 5)
22.	BAP (5, 7.5, 10) + Ethylene (1, 3, 5)

Table 14 Growth regulator treatments tried for culturing primed seeds for germination (Continuation)

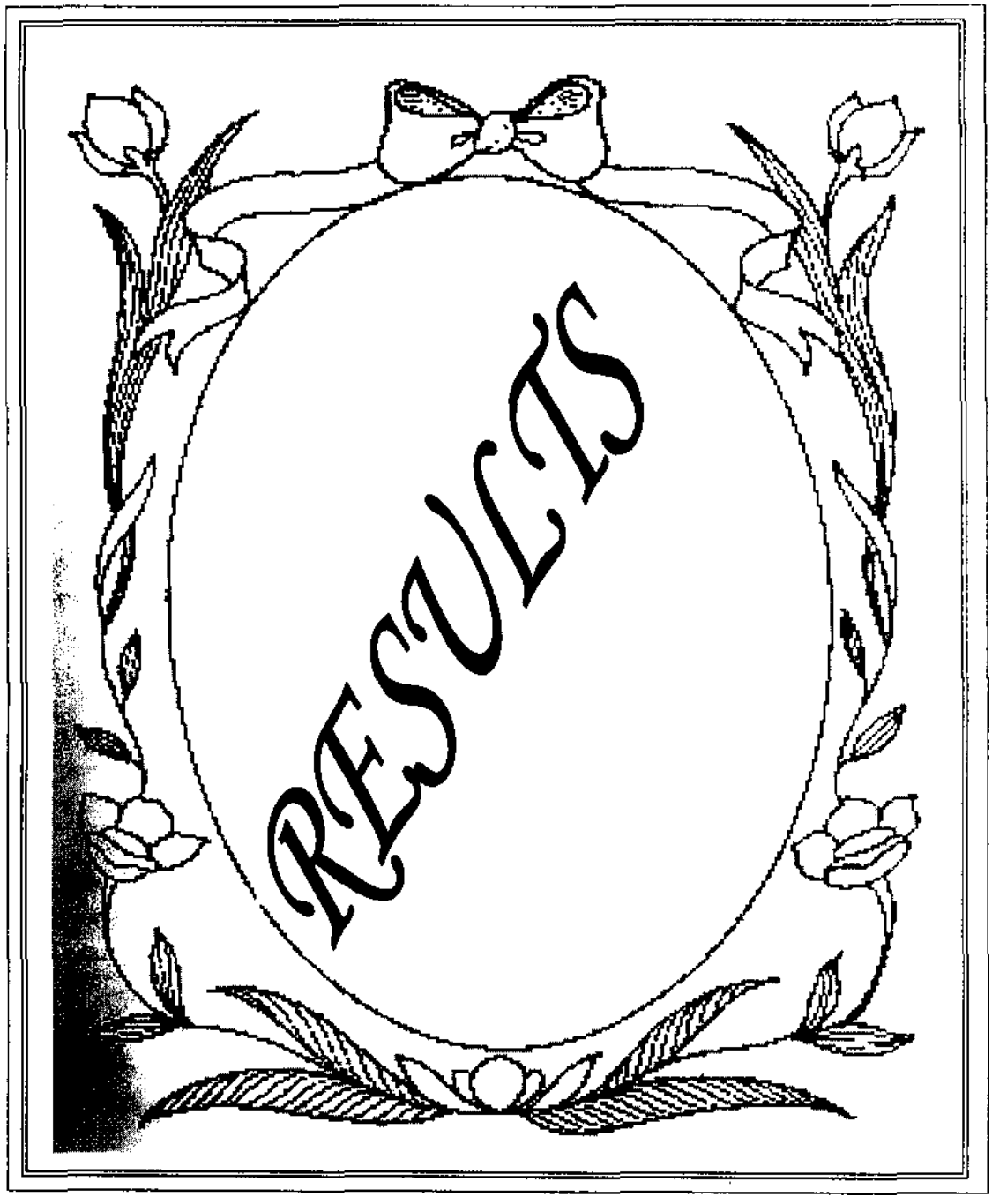
Sl. No.	Growth regulator treatments mg l^{-1}
23.	Kinetin (5, 7.5, 10) + GA_3 (1, 2.5, 5)
24.	Kinetin (5, 7.5, 10) + Ethylene (1, 3, 5)
25.	GA_3 (1, 2.5, 5) + Ethylene (1, 3, 5)
26.	IAA (0.1, 0.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)
27.	IAA (0.1, 0.5) + BAP (5, 10) + Ethylene (1, 3, 5)
28.	IAA (0.1, 0.5) + Kinetin (5, 10) + GA_3 (1, 2.5, 5)
29.	IAA (0.1, 0.5) + Kinetin (5, 10) + Ethylene (1, 3, 5)
30.	NAA (1, 2.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)
31.	NAA (1, 2.5) + BAP (5, 10) + Ethylene (1, 3, 5)
32.	NAA (1, 2.5) + Kinetin (5, 10) + GA_3 (1, 2.5, 5)
33.	NAA (1, 2.5) + Kinetin (5, 10) + Ethylene (1, 3, 5)
34.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA_3 (1, 2.5, 5)
35.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + Ethylene (1, 3, 5)
36.	2, 4 – D (0.25, 0.5, 1) + Kinetin (7.5, 10) + GA_3 (1, 2.5, 5)
37.	2, 4 – D (0.25, 0.5, 1) + Kinetin (7.5, 10) + Ethylene (1, 3, 5)
38.	IAA (0.1, 0.5) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)
39.	IAA (0.1, 0.5) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)
40.	NAA (1, 2.5) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)
41.	NAA (1, 2.5) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)
42.	2, 4 – D (0.25, 0.5, 1) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)
43.	2, 4 – D (0.25, 0.5, 1) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5,5)

3.23 *IN VIVO SEED* GERMINATION STUDIES

The treatments as detailed from 3.22.4 to 3.22.17 were done and the seeds were sown in sterile moist sand and moist filter paper.

3.24 STATISTICAL ANALYSIS

The statistical analysis adopted was for Randomised Block Design (RBD) with the treatments arranged in a factorial set up. MSTATC package was used to analyse the data using the standard statistical procedure as per Panse and Sukhatme (1978).


RESULTS

4. RESULTS

The results of the investigations on "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)" are described in this chapter under the following headings:

1. Evaluation of induced variants for variability in morphological characters, anatomical features, floral characters, economic attributes, quality attributes and incidence of pests and diseases.
2. Response to *in vivo* pollination.
3. Response to *in vitro* pollination.
4. Pollen pistil interaction.
5. Standardization of media and supplements for culture establishment.
6. Attempting controlled selfing and crossing among the selected cultivars.
7. Post pollination changes.
8. Seed viability test using tetrazolium staining.
9. Germination studies.

4.1 EVALUATION STUDIES

4.1.1 Morphological characters

The variability in selected cultivars was examined for salient morphological characters (Figure 1). The cultivars showed significant variation for all morphological characters listed in (Table 15) but the year wise variation with respect to plant height and number of leaves per plant and variation among treatments within year with respect to plant height and number of roots was not significant.

With respect to plant height, the cultivars V_9 and V_1 registered the maximum value (80.27 cm and 89.23 cm) whereas V_4 the minimum value (56.57 cm and 58.34 cm) during first and second year respectively. Pooled data also indicated the superiority of V_1 (79.92 cm) and inferiority of V_4 (57.43 cm) with respect to plant height. Maximum number of tillers was observed in V_2 (10.25 and 6.33) while minimum in V_6 (3.17) and V_4 (3.17) at the first and second year respectively. Considering the pooled data also, V_2 recorded the maximum value (8.29) and V_4 the minimum value (3.33). In respect of number of leaves per tiller, V_9 (15.47) and

Table 15. Variability in morphological characters of ginger cultivars

Sl. No.	Cultivar	Plant height (cm)			Number of tillers per plant			Number of leaves per tiller			Number of leaves per plant			Number of roots			Root length (cm)		
		I year	II year	Pooled	I year	II year	Pooled	I year	II Year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled
1.	Z-O-78 (V_1)	70.60	89.23	79.92	5.25	4.67	4.96	13.68	12.43	13.06	72.00	59.08	65.54	44.33	42.58	43.46	12.58	12.03	12.31
2.	Z-O-86 (V_2)	77.22	77.08	77.15	10.25	6.33	8.29	11.04	11.65	11.35	107.58	74.50	91.04	67.00	61.50	64.25	13.36	13.16	13.26
3.	Z-O-89(V_3)	75.83	75.93	75.88	3.33	4.33	3.83	14.60	10.86	12.73	49.00	47.50	48.25	49.50	43.08	46.29	11.73	11.92	11.82
4.	Z-O-90 (V_4)	56.51	58.34	57.43	3.5	3.17	3.33	8.23	11.25	9.74	28.50	35.50	32.00	48.50	41.67	45.08	11.86	11.42	11.64
5.	Z-O-92 (V_5)	63.15	74.66	68.91	3.5	4.00	3.75	9.90	11.34	10.62	33.08	46.08	39.58	41.58	35.17	38.38	12.03	10.14	11.08
6.	Z-O-95 (V_6)	66.82	74.81	70.82	3.17	4.08	3.63	10.45	12.12	11.29	33.83	49.33	41.58	41.83	36.17	39.00	12.19	9.48	10.83
7.	Z-O-97 (V_7)	58.42	65.20	61.81	3.58	4.50	4.04	11.43	13.25	12.34	39.75	59.83	49.79	62.08	54.67	58.38	14.33	12.13	13.23
8.	Rio-de-Janeiro (V_8)	74.60	63.76	69.18	5.75	4.25	5.00	14.40	12.08	13.24	81.08	51.83	66.46	69.75	62.42	66.08	12.77	11.68	12.22
9.	Maran (V_9)	80.27	68.13	74.20	3.92	3.42	3.67	15.47	14.13	14.80	59 92	48.25	54.08	60.17	57.50	58.83	14.01	12.53	13.27
10.	Himachal Pradesh(V_{10})	75.51	83.48	79.50	6.67	3.33	5.00	11.44	16.15	13.80	74.07	54.08	64.08	41.50	42.08	41.79	12.23	11.86	12 04
Mean		69.89	73.06	71.48	4.89	4.21	4 55	12.07	12.53	12.30	57.88	52.60	55.24	52.63	47.68	50.15	12.71	11.63	12.17
*			12.81			1.16			2.15			15.86			3.05			0.80	
**			NS			0 52			0.96			NS			1.36			0 36	
***			NS			1.64			3.04			22.42			NS			1.13	

* CD (5 %) for treatment means
** CD (5%) for year means
*** CD (5%) for (treatment X year) interaction means.
NS Non Significant

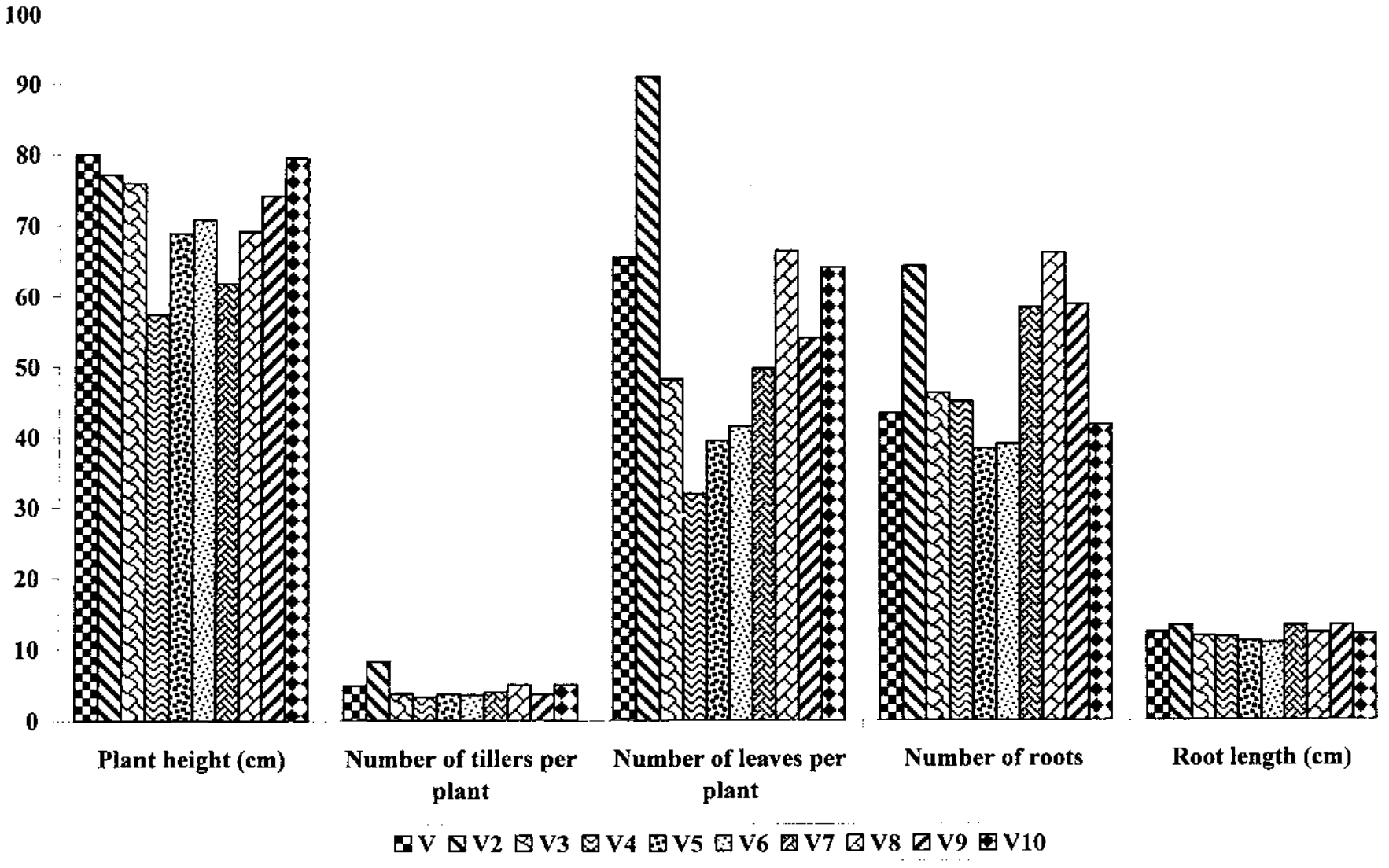


Fig. 1 Variability in morphological characters of ginger cultivars

V_{10} (16.16) recorded the maximum number while V_2 the minimum number (8.23 and 11.25) at the respective first and second year. In pooled analysis also, V_9 was the superior cultivar with maximum number of leaves per tiller (14.80) whereas V_4 had minimum number (9.74). In production of maximum number of leaves per plant, V_2 ranked top (107.58 and 74.5) at the respective first and second year with pooled maximum value of 91.04 whereas V_4 had minimum number (28.5 at first year and 35.5 at second year) with pooled lowest value (32.00). V_8 produced the maximum number of roots (69.75 at first year, 62.42 at second year and 66.08 for pooled data) while the minimum was observed in V_{10} (41.5 at first year), V_5 (35.17 at second year) and V_5 (38.38 for pooled data). It was observed that V_7 and V_2 had the longest root (14.33 cm and 13.16 cm respectively) whereas V_3 and V_6 produced the shortest root (11.73 cm and 9.5 cm) during the respective first and second year. The pooled analysis indicated that V_9 had maximum value (13.27 cm) and V_6 the minimum value (10.83 cm).

4.1.2 Pseudostem characters

Significant cultivar difference was observed with respect to pseudostem height and thickness whereas circumference was not significant (Table 16). V_1 recorded the maximum height (80.66 cm) whereas V_{10} recorded maximum thickness (9.98 mm) and circumference (3.35 cm). Hairiness of pseudostem was absent in all the cultivars selected for the study.

4.1.3 Leaf characters

The selected cultivars showed significant variation in various leaf characters except shape, stipules, hairyness, leaf area and leaf area index (Table 17). Colour of leaf in the different cultivars varied from light green to dark green in the upper and lower surface. Leaf shape was linear lanceolate. Hairyness on both surfaces of leaf and stipules were absent in all the cultivars. V_9 had the maximum leaf length (24.53 cm) and V_2 the minimum length (22.34 cm). Maximum leaf breadth was observed in V_3 (3.08 cm) and the minimum in V_6 (1.92 cm). V_{10} recorded maximum leaf thickness (0.221 mm) and minimum in V_9 (0.178 mm). With respect to leaf area

Table 16. Variability in pseudostem characters of ginger cultivars

Sl. No.	Cultivar	Pseudostem height (cm)	Pseudostem thickness (mm)	Pseudostem circumference (cm)
1.	Z-O-78 (V_1)	80.66	8.53	3.07
2.	Z-O-86 (V_2)	74.92	9.10	3.03
3.	Z-O-89 (V_3)	73.83	8.82	3.01
4.	Z-O-90 (V_4)	54.06	7.89	2.97
5.	Z-O-92 (V_5)	67.47	8.63	3.02
6.	Z-O-95 (V_6)	69.29	9.27	2.98
7.	Z-O-97 (V_7)	62.84	8.55	3.02
8.	Rio-de-Janeiro (V_8)	59.74	8.54	2.97
9.	Maran (V_9)	62.80	7.17	3.00
10.	Himachal Pradesh (V_{10})	78.38	9.98	3.35
Mean		68.40	8.65	3.04
CD (5 %)		13.33	0.35	NS

Table 17. Variability in leaf characters of ginger cultivars

Sl. No.	Cultivar	Leaf colour		Leaf length (cm)	Leaf breadth (cm)	Leaf thickness (mm)	Leaf area (cm^2)	Leaf Area Index
		upper surface	lower surface					
1.	Z-O-78 (V_1)	Dark green	Green	23.34	2.16	0.205	3150.30	5.04
2.	Z-O-86 (V_2)	Dark green	Green	22.34	2.23	0.199	3725.33	5.96
3.	Z-O-89 (V_3)	Medium green	Medium green	24.27	3.08	0.200	2682.84	4.29
4.	Z-O-90 (V_4)	Dark green	Green	24.11	2.46	0.201	1980.03	3.17
5.	Z-O-92 (V_5)	Dark green	Green	23.62	2.07	0.197	2500.02	4.00
6.	Z-O-95 (V_6)	Medium green	Green	23.54	1.92	0.210	2662.25	4.26
7.	Z-O-97 (V_7)	Green	Green	22.57	2.06	0.179	3036.47	4.86
8.	Rio-de-Janeiro (V_8)	Green	Green	24.39	2.52	0.207	2941.97	4.71
9.	Maran (V_9)	Green	Medium green	24.53	2.50	0.178	2762.79	4.42
10.	Himachal Pradesh (V_{10})	Green	Light green	24.49	2.74	0.221	3087.47	4.94
Mean		-	-	23.72	2.37	0.200	2852.95	4.57
CD (5 %)		-	-	0.27	0.14	0.008	NS	NS

per plant and leaf area index, V_2 recorded maximum values (3725.33 cm^2 and 5.96) respectively whereas V_4 was inferior (1980.03 cm^2 and 3.17 respectively).

4.1.4 Anatomical features

The study revealed significant variation in cuticle, stomatal and epidermal cell characters among the selected cultivars (Table 18).

4.1.4.1 Cuticle thickness

Cuticle thickness of the ginger cultivars ranged from 16.36 µm (V5) to 24.54 µm (V_1) (Plates 3a and 3b). The variants except autotetraploids (V_1 and V_2), recorded less cuticle thickness than the check varieties (V_8, V_9 and V_{10}). The autotetraploids V_1 and V_2 had cuticle thickness of 24.54 µm and 21.81 µm respectively which was more than the corresponding diploids (V_{10} and V_8).

4.1.4.2 Stomatal count and size

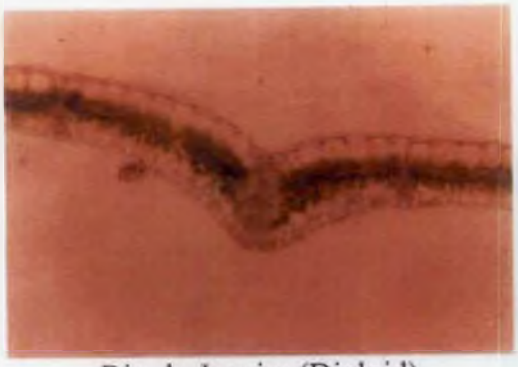
Compared to check varieties (V_8, V_9 and V_{10}), all the selected variants recorded lesser stomatal number with increased size (Plates 4a and 4b). In the check varieties, the stomatal number ranged from 42.08 (V_9) to 46.33 (V_8) with length (147.24 µm) and width (81.81 µm to 84.53 µm) whereas the variants recorded a range of (17.33 in V_7 to 28.00 in V_6) for stomatal number, (171.78 µm in V_5 to 212.68 µm in V_2) for length and (95.43 µm inV_6 to 133.61µm V_7) for width.

4.1.4.3 Epidermal cell number and area

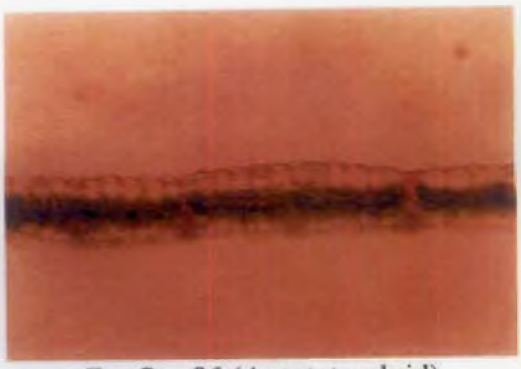
The autotetraploids recorded increased epidermal cell area (51.57 mm^2 in V_1 and 53.99 mm^2 inV_2) with less number of cells per mm^2 (27.75 in V_1 and 26.50 in V_2). The other variants and the diploids showed a range of (28.38 mm^2 in V_9) to (30.60 mm^2 in V_8) for cell area and (46.75 in V_8 to 50.42 in V_9) for number of cells.

4.1.5 Floral characters

Among the ten cultivars studied, flowering was observed only in V_1 (Z – O – 78), V_2 (Z – O – 86), V_8 (Rio-de-Janeiro), V_9 (Maran) and V_{10} (Himachal

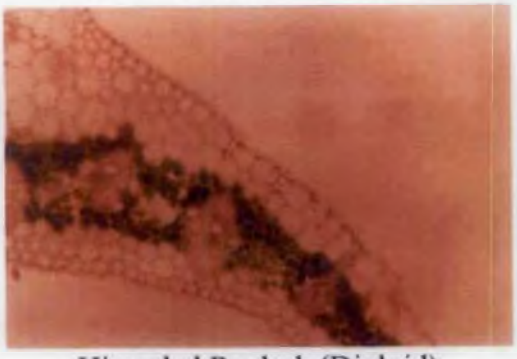


Rio-de-Janeiro (Diploid)

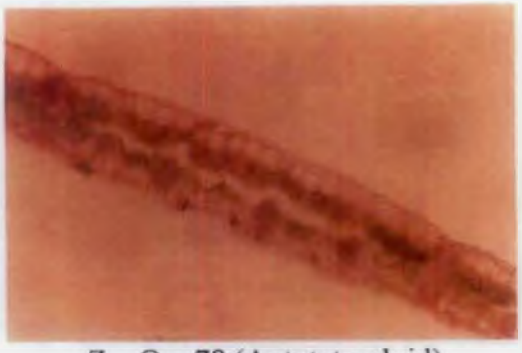


Z – O – 86 (Autotetraploid)

Plate 3a. Cuticle of Rio-de-Janeiro and its corresponding autotetraploid (40 X)



Himachal Pradesh (Diploid)



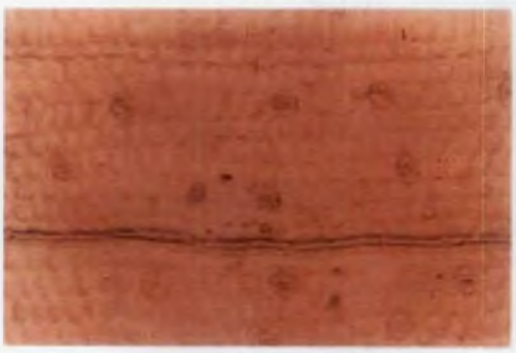
Z – O – 78 (Autotetraploid)

Plate 3b. Cuticle of Himachal Pradesh and its corresponding autotetraploid (40 X)

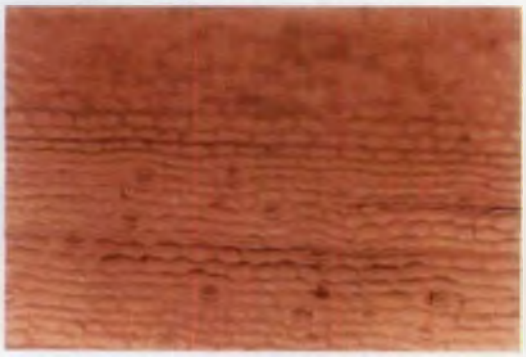
Rio-de-Janeiro (Diploid)

Z – O – 86 (Autotetraploid)

Plate 4a. Stomata of Rio-de-Janeiro and its corresponding autotetraploid (100 X)



Himachal Pradesh (Diploid)



Z – O – 78 (Autotetraploid)

Plate 4b. Stomata of Himachal Pradesh and its corresponding autotetraploid (10 X)

Table 18. Variability in anatomical features of ginger cultivars

Sl. No	Cultivar	Cuticle thickness (μm)	Stomatal number per mm^2	Stomatal length (μm)	Stomatal width (μm)	Number of epidermal cells per mm^2	Epidermal cell area (mm^2)
1.	Z-O-78 (V_1)	24.54	21.42	188.14	122.70	27.75	51.57
2.	Z-O-86 (V_2)	21.81	18.08	212.68	130.88	26.50	53.99
3.	Z-O-89 (V_3)	19.09	24.00	179.96	114.52	48.00	29.83
4.	Z-O-90 (V_4)	17.72	17.83	196.32	130.88	49.92	28.67
5.	Z-O-92 (V_5)	16.36	26.50	171.78	106.34	47.58	30.08
6.	Z-O-95 (V_6)	17.72	28.00	177.23	95.43	49.33	29.01
7.	Z-O-97 (V_7)	19.09	17.33	199.05	133.61	49.00	29.21
8.	Rio-de-Janeiro (V_8)	20.45	46.33	147.24	81.80	46.75	30.60
9.	Maran (V_9)	20.45	42.08	147.24	81.80	50.42	28.38
10.	Himachal Pradesh(V_{10})	23.18	45.33	147.24	84.53	49.67	28.81
Mean		20.04	28.69	176.69	108.25	44.49	34.01
CD (5 %)		3.62	1.18	3.82	4.60	1.55	1.06

Pradesh) (Plate 5). The cultivars V_3 (Z – O – 89), V_4 (Z – O – 90), V_5 (Z – O – 92), V_6 (Z – O – 95) and V_7 (Z – O – 97) failed to flower.

4.1.5.1 Flowering behaviour

The crop duration from planting to initiation of flowering in the five cultivars ranged from 133.6 (V_9) to 164.8 (V_{10}) days with a mean of 151.8 days (Table 19). V_9 was the earliest flowering cultivar (133.6 days) whereas V_{10} was the latest flowering cultivar (164.8 days). The duration of flowering was maximum in V_8 (54 days) followed by V_1 (40 days) and was minimum in V_2 (25 days) with a mean of 37.6 days. The percentage of flowering (number of plants flowered) ranged from 34.72 per cent in V_2 to 88.53 per cent in V_8 with a mean of 57.78 per cent.

4.1.5.2 Inflorescence development and floral biology

The duration from initiation of floral primordium to blooming did not show much variability between cultivars and it ranged from 28.33 (V_1) to 30.33 (V_8). The spike bears appressed ovate or elliptic green bracts with spiral arrangement. Each bract subtended a single flower or cluster of two or three flowers with a lateral or obliquely posterior bracteole. In a spike one or two flowers opened daily and blooming of flowers in a spike was completed within a period of 13.66 days (V_2) to 22.33 days (V_8).

The inflorescence of ginger is a bracteate spike directly springing from the rhizome and rarely emerging terminally from the aerial shoot (Plate 6). The floral primordium recorded a mean length of 4.53 cm at the time of visual initiation and attained a mean length of 18.00 cm within a period of one month (Table 20). By this time, the scape and cone shaped spike were well developed and length of scape varied from 10.06 cm (V_9) to 15.4 cm (V_{10}) and length of cone from 4.03 cm (V_8) to 6.4 cm (V_1). The maximum total length of the inflorescence was observed in V_{10} (20.73 cm) and minimum in V_9 (15.09 cm).

The number of inflorescence springing from the rhizome ranged from 2.67 in (V_9 and V_{10}) to 4.67 in V_8 whereas the number of inflorescence emerging terminally from the aerial shoot ranged from 0.33 in V_9 to 1.00 in V_8. The maximum total number of inflorescence per plant was observed in V_8 (5.67) whereas minimum in

Table 19. Variability in flowering behaviour of ginger cultivars

Sl. No.	Cultivar	Crop duration for initiation of flowering (days)	Date of first flowering	Date of last flowering	Flowering duration (days)	Percentage of flowering
1.	Z-O-78 (V_1)	141.4	25 – 09 – 2002	03 – 11 – 2002	40	49.64
2.	Z-O-86 (V_2)	156.6	12 – 10 – 2002	05 – 11 – 2002	25	34.72
3.	Rio-de-Janeiro (V_8)	161.6	08 – 10 – 2002	30 – 11 – 2002	54	88.53
4.	Maran (V_9)	133.6	13 – 09 – 2002	19 – 10 – 2002	37	51.90
5.	Himachal Pradesh (V_{10})	164.8	20 – 10 – 2002	20 – 11 – 2002	32	64.12
Mean		151.8	-	-	37.6	57.78

V_9 (3.00). The number of flowers contained per spike ranged from 16.33 in V_2 to 20.66 in V_8 .The flowers produced per plant was maximum in V_8 (117.14) and minimum in V_9 (58.98).

The time of anthesis did not show much variability between cultivars and it commenced by 3.00 p.m and continued upto 4.00 p.m (Plate 7). Anther dehiscence did not occur simultaneously with flower opening and was observed between 4.30 p.m and 5.00 p.m. The interval between flower opening and anther dehiscence ranged from 0.5 h to 2 h (Table 20).

The peculiar character observed in cultivar V_3 was that though it showed inflorescence development, no flowers were produced. In the succeeding season it failed to produce even inflorescence (Plate 8).

4.1.5.3 Morphology of flowers

The flowers are trimerous, zygomorphic, bisexual and epigynous. The calyx is tubular, dividing above into short teeths and split on one side. The corolla is tubular below (2.0 to 2.5 cm long) with three yellowish lobes, of which the dorsal one is curved over the anther. Androecium consists of 6 stamens of which the outer three are reduced to staminodes and are seen at the base of the corolla tube. The inner lateral stamens are united to form a showy deep purple coloured labellum. The posterior stamen of the inner whorl is the only fertile stamen and is enclosed in the labellum. Stamen has short broad filament and two prominent anther lobes. The style passes through the groove formed by the anther lobes and ends in a capitate stigma. The stigma has a circular apical aperture surrounded by stiff hairs and it protrudes above the anthers. Cultivar difference was observed with respect to length of flowers emerging from different portions of the inflorescence (Table 21). V_1 recorded highest mean flower length of 3.69 cm (Plate 9). The mean flower length observed was 3.72 cm at lower, 3.62 cm at middle and 3.43 cm at upper portion of inflorescence. The flowers are characterized by a long style which recorded a mean length of 3.38 cm in the cultivars studied. V_1 recorded the maximum style length of 3.47 cm (Plate 10). The style length showed variations depending on the different position of flowers in an inflorescence and it ranged from 3.32 cm (V_8) to 3.72 cm (V_9) emerging at lower position, 3.32 cm (V_9) to 3.54 cm (V_8) at middle position and 3.14 cm (V_2) to

Plate 5. Ginger cultivar Maran in flowering

Plate 6. Inflorescence of *Zingiber officinale*

Plate 7. Opened flower on the day of anthesis – (best suitable for *in vitro* placental pollination)

Plate 8. Inflorescence with no flower in variant Z – O – 89

A. Rio-de-Janeiro
B. Z – O – 86
C. Maran
D. Z – O – 78
E. Himachal Pradesh

Plate 9. Flower length of ginger cultivars

(Diploid) (Autotetraploid)

Plate 10. Flower of diploid and autotetraploid ginger with androecium and gynoecium

Table 20. Variability in inflorescence development and floral biology of ginger cultivars

Sl. No.	Cultivar	Duration from initiation to blooming (days)	Blooming period of an inflorescence	Length of floral primordium (cm)				Number of inflorescence per plant emerging from			Number of flowers per scape	Number of flowers per plant	Time of anthesis (p.m)	Anther dehiscence (p.m.)
				At visual initiation	30 days after			Shoot tip	rhizome	Total				
					Scape length	Cone length	Total length							
1.	Z-O-78 (V_1)	28.33	15.66	4.83	13.98	6.40	20.38	0.67	4.00	4.67	18.66	87.14	3.15 – 4.00	5.00
2.	Z-O-86 (V_2)	29.33	13.66	5.00	11.42	4.68	16.10	0.67	3.67	4.33	16.33	70.71	3.00 – 4.00	5.00
3.	Rio-de-Janeiro (V_8)	30.33	22.33	4.66	13.66	4.03	17.69	1.00	4.67	5.67	20.66	117.14	3.00 – 3.30	5.00
4.	Maran (V_9)	29.67	15.33	4.00	10.06	5.03	15.09	0.33	2.67	3.00	19.66	58.98	3.00 – 3.15	4.30
5.	Himachal Pradesh (V_{10})	29.67	15.66	4.16	15.4	5.33	20.73	0.67	2.67	3.33	19.66	65.47	3.00 – 3.30	4.45
Mean		29.47	16.53	4.53	12.91	5.09	18.00	0.67	3.54	4.21	48.99	79.94	-	-

Table 21. Variability in floral morphology of ginger cultivars

Sl. No	Cultivar	Flower length (cm) at different positions of inflorescence				Style length (cm) at different positions of inflorescence				Ovary length (mm)	Ovary diameter (mm)	Number of ovules per ovary	Ovule length (μm)	Ovule breadth (μm)
		lower	middle	upper	Mean	lower	middle	upper	Mean					
1.	Z-O-78 (V_1)	3.82	3.66	3.6	3.69	3.58	3.42	3.4	3.47	2.18	2.14	18.8	566.06	363.19
2.	Z-O-86 (V_2)	3.8	3.56	3.36	3.57	3.54	3.36	3.14	3.35	1.96	1.92	21.2	549.70	346.83
3.	Rio-de-Janeiro (V_8)	3.5	3.78	3.3	3.53	3.32	3.54	3.26	3.37	2.04	2.02	23.2	520.25	327.2
4.	Maran (V_9)	3.88	3.5	3.44	3.61	3.72	3.32	3.18	3.41	3.00	3.02	23.8	575.87	337.01
5.	Himachal Pradesh (V_{10})	3.58	3.6	3.46	3.55	3.36	3.4	3.2	3.32	2.06	2.04	20.8	533.26	320.66
Mean		3.72	3.62	3.43	3.59	3.50	3.41	3.24	3.38	2.25	2.23	21.56	549.02	338.98

3.4 cm (V_1) at upper portion. The ovary measured a mean length of 2.25 mm and diameter of 2.23 mm. The ovary of V_9 was the largest followed by that of V_1. The number of ovules in an ovary ranged from 18.8 (V_1) to 23.8 (V_9) with a mean length of 21.56. Microscopic measurement of size of ovule showed a mean length of 549.02 µm and breadth of 338.98 µm at the middle with maximum length in V_9 (575.87 µm) and maximum breadth in V_1 (363.19 µm).

4.1.5.4 Pollen morphology

Ginger flowers carry plenty of pollen grains in single bilobed anther. The pollen grains were round, highly heterogeneous in size and filling and were limited by a thick exine. A well defined pore is absent in the pollen grains.

4.1.5.5 Influence of season on size of pollen grains of ginger cultivars

The estimation of diameter of pollen grains of ginger cultivars showed that they are heterogeneous in size and the diameter ranged from 38.18 µm in V_9 to 103.62 µm in V_2 (Table 22). The diameter of larger pollen grains ranged from 109.07 µm in V_2 during early season to 70.89 µm in V_9 during late season and they were deeply stained. The smaller pollen grains showed a range of 54.53 µm in V_1 to 32.72 µm in V_{10} during mid season. In all the seasons, V_2 produced the biggest pollen grains with a mean diameter of 103.62 µm whereas V_9 produced the smallest pollen grains (38.18 µm).

4.1.5.6 Influence of season on pollen fertility and viability of ginger cultivars

The pollen fertility studied during different periods of flowering season in five ginger cultivars showed that the fertility was high (46.01 %) during the mid period of the flowering season compared to late (36.62 %) and early period (21.38 %) (Table 23). During the mid period of the season, fertility percentage in ginger cultivars ranged from 28.11 per cent (V_8) to 68.38 per cent (V_1) while it was only 17.31 per cent (V_{10}) to 63.50 per cent (V_2) during the late period of the season. During the early period, it ranged from 11.72 per cent (V_8) to 38.92 per cent (V_2).

The viability of pollen grains of ginger cultivars showed difference depending on the season of flowering and it was generally high in early (8.36 %) and mid season

Table 22. Influence of season on pollen size of ginger cultivars

Sl. No.	Cultivar	Diameter of larger pollen grains (μm)				Diameter of smaller pollen grains (μm)			
		Early season	Mid season	Late season	Mean	Early season	Mid season	Late season	Mean
1.	Z-O-78 (V_1)	89.98	89.98	95.43	91.80	49.08	54.53	51.81	51.81
2.	Z-O-86 (V_2)	109.07	100.89	100.89	103.62	43.63	46.35	43.63	44.54
3.	Rio-de-Janeiro (V_8)	106.34	98.16	89.98	98.16	43.63	46.35	35.45	41.81
4.	Maran (V_9)	84.53	84.53	70.89	79.98	65.45	35.45	43.63	38.18
5.	Himachal Pradesh (V_{10})	87.25	89.98	84.52	87.23	40.90	32.72	43.63	39.08
Mean		95.43	92.71	88.33	92.16	42.54	43.08	43.63	43.08

Table 23. Influence of season on pollen fertility and viability of ginger cultivars

Sl. No.	Cultivar	Pollen fertility (%)				Pollen viability (%)			
		Early season	Mid season	Late season	Mean	Early season	Mid season	Late season	Mean
1.	Z-O-78 (V_1)	27.05	68.38	54.44	49.96	11.81	10.60	9.56	10.66
2.	Z-O-86 (V_2)	38.92	38.32	63.50	56.91	13.70	12.80	11.98	12.83
3.	Rio-de-Janeiro (V_8)	11.72	28.11	22.61	20.81	6.56	5.94	4.72	5.74
4.	Maran (V_9)	17.08	32.60	25.23	24.97	4.93	5.68	3.68	4.76
5.	Himachal Pradesh (V_{10})	12.12	32.62	17.31	20.68	4.78	3.85	2.68	3.80
Mean		21.38	46.01	36.62	34.67	8.36	7.77	6.52	7.56

(7.77 %) compared to late season (6.52 %) of flowering (Table 23). During the early season, the viability percentage ranged from 4.78 per cent (V_{10}) to 13.70 per cent (V_2) and in mid season, it ranged from 3.85 per cent (V_{10}) to 12.8 per cent (V_2) among cultivars. During late season, it ranged from 2.68 per cent (V_{10}) to 11.98 per cent (V_2).

4.1.5.7 Influence of position of flowers in the inflorescence on pollen fertility and viability of ginger cultivars

The flowers emerging from the lower, middle and upper portions of the inflorescence recorded fertility values of 36.03 per cent, 31.24 per cent and 36.74 per cent respectively (Table 24).

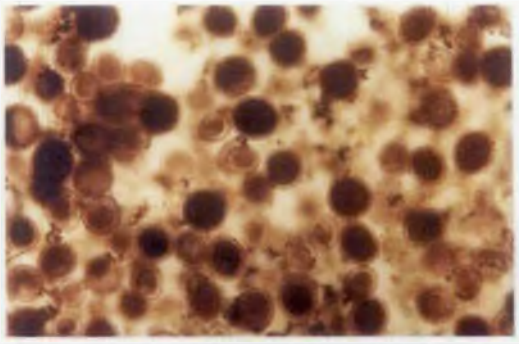
The data presented in Table 24 indicated that the germination percentage of pollen grains irrespective of the cultivars was maximum (9.18 %) from lower portion of the inflorescence followed by middle (7.48 %) and upper (6.02 %) portion.

4.1.5.8 Influence of season and position of flowers in the inflorescence on pollen tube length of ginger cultivars

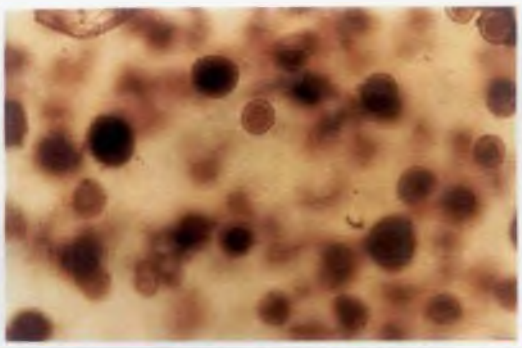
The results showed that the tube length of germinated pollen grains varied greatly depending on the season, position of flowers in the inflorescence and cultivars (Table 25). The mean tube length of the germinated pollen grains collected during early, mid and late seasons were 155.38 µm, 240.68 µm and 184.96 µm respectively. The mean pollen tube length was maximum during mid season but the cultivars V_2 and V_8 recorded maximum tube length during late season. Pollen tube length showed variation depending on the position of flowers on the inflorescence and cultivars. Maximum tube length was recorded for flowers from middle portion of inflorescence (226.05 µm) followed by lower portion of inflorescence (220.06 µm).

4.1.5.9 Influence of cultivars on pollen size, fertility, viability and tube length

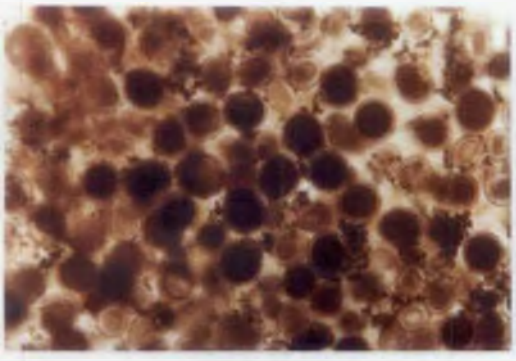
Cultivar difference existed with respect to season and position of flowers in the inflorescence on pollen size, fertility, viability and tube length (Tables 22, 23, 24 and 25). Among the cultivars, V_2 recorded the maximum diameter of pollen grain (103.62 µm) while V_9 was with minimum diameter (38.18 µm). Irrespective of the season and position of flowers, V_2 recorded highest mean pollen fertility (56.91 %) and viability (12.83 %) followed by V_1 (49.96 % and 10.66 %) and it was low in V_{10}



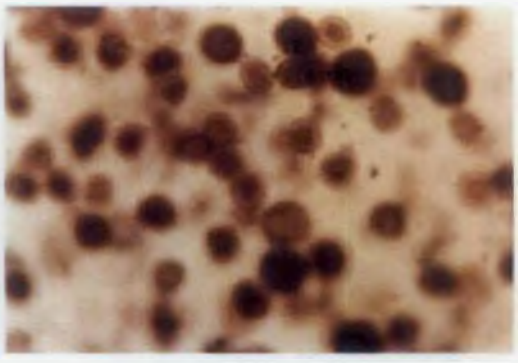
Z – O – 78 (Autotetraploid)



Himachal Pradesh (Diploid)

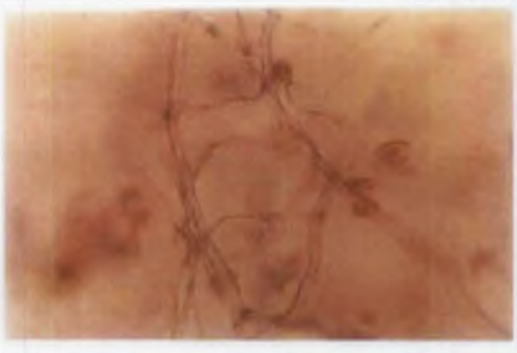


Z – O – 86 (Autotetraploid)

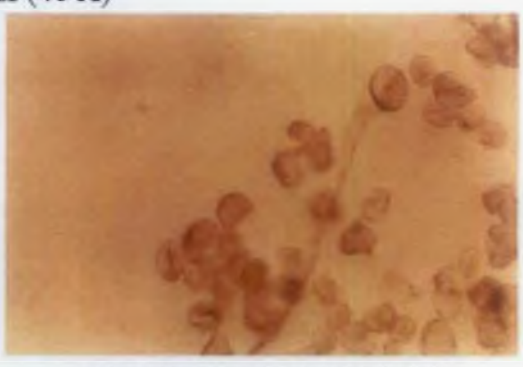


Rio-de-Janeiro (Diploid)

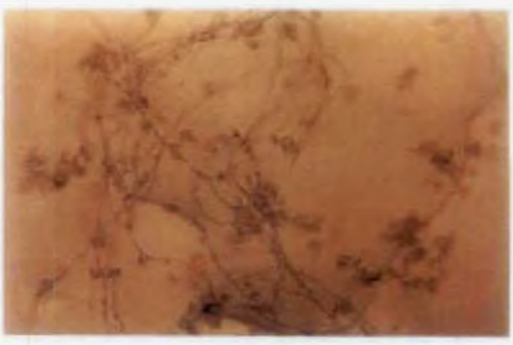
Plate 11.Stained pollen grains of diploids and its corresponding autotetraploids (40 X)



Z – O – 78 (Autotetraploid)



Himachal Pradesh (Diploid)



Z – O – 86 (Autotetraploid)

Rio-de-Janeiro (Diploid)

Plate 12. Pollen germination and tube growth in ME_3 medium (40 X)

Table 24. Influence of position of flowers in the inflorescence on pollen fertility and viability of ginger cultivars

Sl. No.	Cultivar	Pollen fertility (%)				Pollen viability (%)			
		Position of flowers in the inflorescence				Position of flowers in the inflorescence			
		lower	middle	upper	Mean	lower	middle	upper	Mean
1.	Z-O-78 (V_1)	59.31	49.09	41.47	49.96	12.48	10.50	9.01	10.66
2.	Z-O-86 (V_2)	57.15	45.34	68.24	56.91	14.41	12.83	11.24	12.83
3.	Rio-de-Janeiro (V_8)	19.5	16.37	26.47	20.81	7.35	5.58	4.28	5.74
4.	Maran (V_9)	20.97	24.82	29.12	24.97	6.28	4.74	3.27	4.76
5.	Himachal Pradesh (V_{10})	23.12	20.56	18.38	20.68	5.37	3.74	2.30	3.80
Mean		36.03	31.24	36.74	34.67	9.18	7.48	6.02	7.56

Table 25. Influence of season and position of flowers in the inflorescence on pollen tube length of ginger cultivars

Sl. No.	Cultivar	Season (μm)				Position of flowers in the inflorescence (μm)				Maximum pollen tube length (μm)
		Early	Mid	Late	Mean	Lower	Middle	Upper	Mean	
1.	Z-O-78 (V_1)	423.53	448.26	175.59	349.13	355.55	446.43	245.39	349.13	1177.92
2.	Z-O-86 (V_2)	209.40	459.17	486.38	384.98	484.25	402.45	268.24	384.98	1390.60
3.	Rio-de-Janeiro (V_8)	43.62	94.21	208.32	115.38	142.74	116.70	86.71	115.38	981.60
4.	Maran (V_9)	43.62	92.70	22.90	53.08	52.35	75.25	31.63	53.08	736.20
5.	Himachal Pradesh (V_{10})	56.71	109.03	31.62	65.80	65.43	89.43	42.53	65.80	458.08
Mean		155.38	240.68	184.96	193.68	220.06	226.05	134.90	193.68	948.88

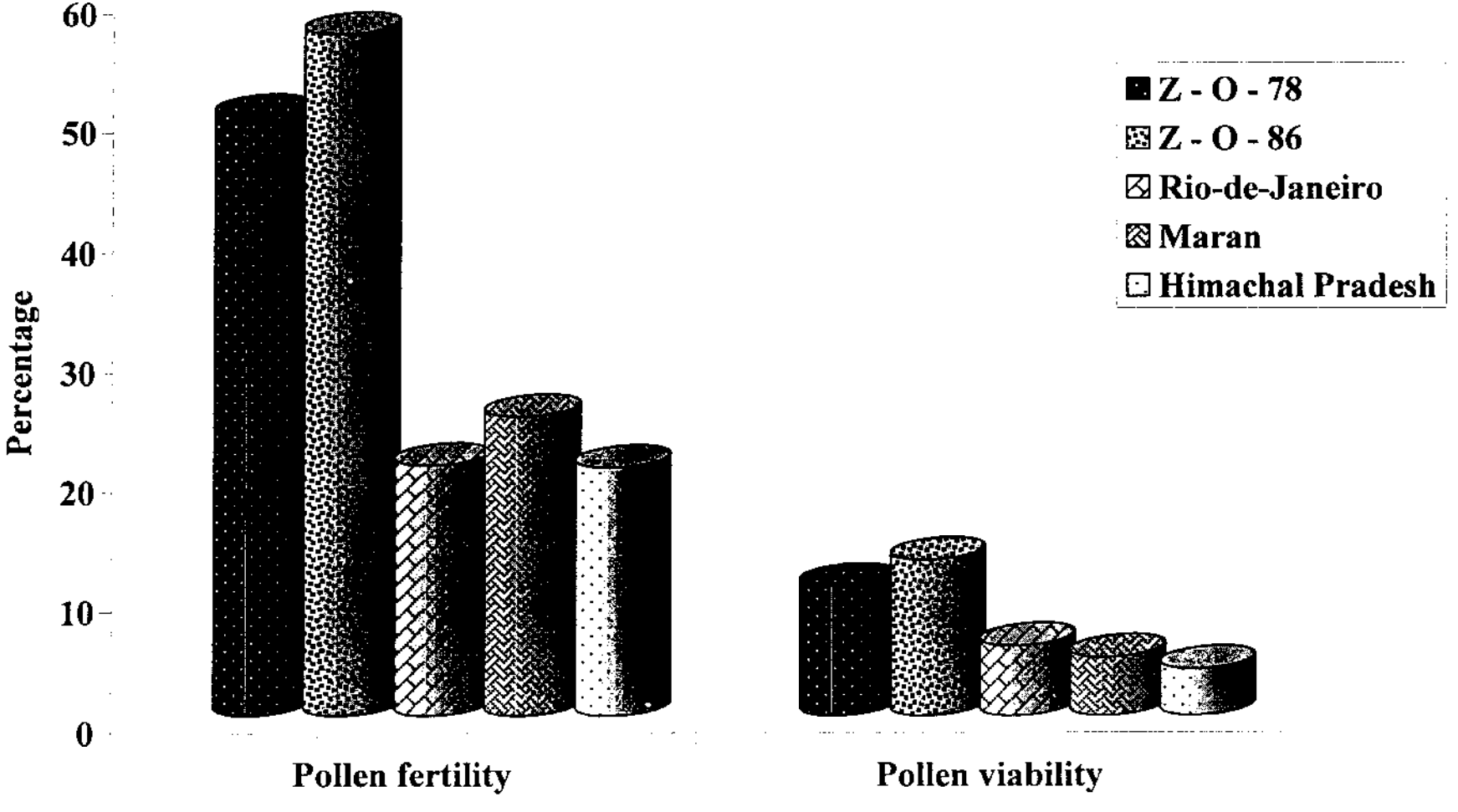


Fig. 2 Influence of cultivars on pollen fertility and viability

(20.68 % and 3.80 %) respectively (Plate 11 and Figure 2). The autotetraploid V_1 and V_2 showed increased pollen tube length. The maximum mean tube length (384.98 µm) and also the highest tube length (1390.6 µm) were recorded by V_2 whereas V_9 recorded the minimum (53.08 µm) mean tube length (Plate 12).

4.1.6 Economic attributes

4.1.6.1 Rhizome characters

The variability in rhizome characters viz., number of fingers, length, breadth, girth, weight, number of nodes and internodal distance of primary, secondary and tertiary fingers are presented in Tables 26, 27 and 28 (Plate 13a and 13b).

4.1.6.1.1 Primary finger

The treatments showed significant variation for all the primary finger characters but the year wise variation except for length, breadth, internodal distance and variations among treatments within year except for breadth and girth were not significant. The pooled data revealed V_1 with maximum number of fingers (14.27) and V_6 with minimum (4.58). During first and second year, V_4 had maximum length (4.32 cm and 3.93 cm) while V_9 and V_2 had minimum length (3.1 and 2.67 cm respectively). Maximum breadth was observed in V_3 (3.28 cm) and V_4 (3.12 cm) whereas minimum in V_1 (1.86 cm) and V_2 (1.31 cm) during the respective first and second year. V_{10} had the highest value (7.69 cm) for girth while V_7 had the lowest value (5.59 cm) in case of pooled data. The pooled data for weight, revealed that V_6 registered maximum weight (13.18 g) while V_1 had minimum weight (8.60 g). Number of nodes and internodal distance were maximum in V_{10} (6.09) and V_7 (0.79 cm) respectively while the same was minimum in V_4 (5.38) and V_2 (0.38 cm) respectively.

4.1.6.1.2 Secondary finger

The treatments showed significant variation for all the secondary finger characters except length and breadth. Interaction was absent in case of length and breadth and so pooled analysis was not done and interpretation of results was done based on individual years data. Year wise variation was non significant for number, length and breadth while variation between the treatments within year was significant

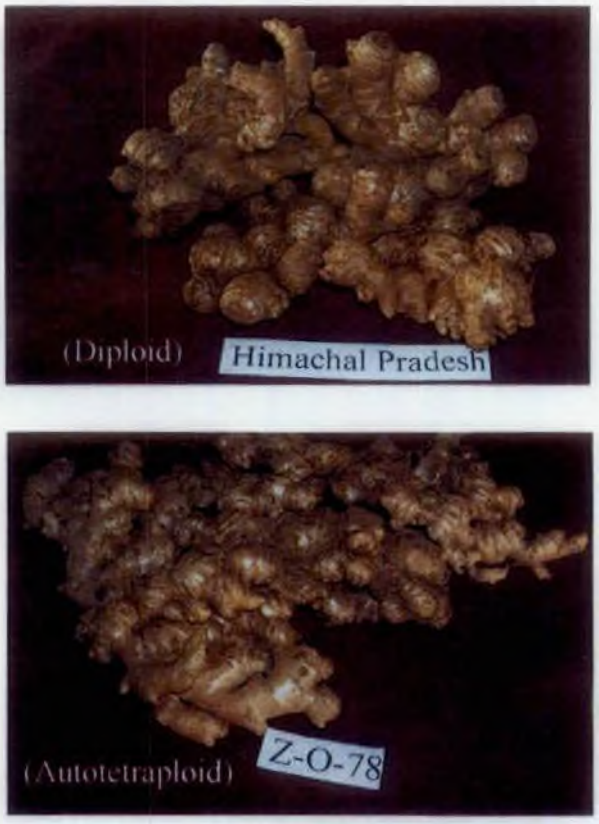


Plate 13a. Rhizomes of Himachal Pradesh and its corresponding autotetraploid

Plate 13b. Rhizomes of Rio-de-Janeiro and its corresponding autotetraploid

Table 26. Variability in primary finger characters of ginger cultivars

Sl. No	Cultivar	Number			Length (cm)			Breadth (cm)			Girth (cm)			Weight (g)			Number of nodes			Internodal distance (cm)		
		I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled
1.	Z-O-78 (V_1)	14.24	14.30	14.27	3.35	2.72	3.03	1.86	2.44	2.15	7.23	7.43	7.33	8.17	9.03	8.60	5.75	5.67	5.71	0.44	0.40	0.42
2.	Z-O-86 (V_2)	11.37	9.77	10.57	4.03	2.67	3.35	2.81	1.31	1.75	6.30	5.63	5.97	11.42	10.02	10.72	5.67	5.64	5.65	0.38	0.38	0.38
3.	Z-O-89(V_3)	9.57	8.49	9.03	3.20	3.17	3.18	3.28	2.12	2.70	7.40	7.17	7.28	12.20	13.30	12.75	5.75	5.75	5.75	0.57	0.58	0.57
4.	Z-O-90 (V_4)	5.64	4.54	5.09	4.07	3.94	4.00	3.11	3.11	3.11	7.30	7.00	7.15	12.70	12.90	12.80	5.42	5.33	5.37	0.56	0.53	0.55
5.	Z-O-92 (V_5)	14.47	11.58	13.02	3.95	3.47	3.71	2.08	2.35	2.22	7.19	7.31	7.25	12.40	11.60	12.00	5.50	5.45	5.48	0.58	0.55	0.56
6	Z-O-95 (V_6)	4.77	4.38	4.58	4.09	3.55	3.82	2.37	2.16	2.26	7.52	7.62	7.57	14.01	12.35	13.18	5.58	5.55	5.57	0.57	0.58	0.57
7	Z-O-97 (V_7)	14.00	13.76	13.88	4.32	3.93	4.13	2.79	2.88	2.84	5.41	5.77	5.59	11.52	12.21	11.86	5.75	5.67	5.71	0.82	0.75	0.79
8.	Rio-de-Janeiro (V_8)	6.53	7.57	7.05	4.00	3.59	3.80	2.17	2.47	2.32	6.35	6.10	6.23	11.08	11.18	11.13	5.42	5.52	5.47	0.76	0.72	0.74
9.	Maran (V_9)	6.66	6.74	6.70	3.10	3.30	3.20	2.06	1.75	1.91	6.70	6.81	6.76	10.47	10.55	10.51	5.33	5.43	5.38	0.53	0.52	0.53
10.	Himachal Pradesh(V_{10})	7.90	9.20	8.55	3.32	3.34	3.33	2.73	2.75	2.74	7.67	7.71	7.69	12.62	12.38	12.50	6.17	6.01	6.09	0.58	0.57	0.58
	Mean	9.52	9.03	9.28	3.74	3.37	3.56	2.46	2.33	2.40	6.91	6.86	6.88	11.66	11.55	11.61	5.63	5.60	5.62	0.58	0.56	0.57
	*	1.37			0.49			0.53			0.26			1.99			0.37			0.04		
	**	NS			0.22			0.23			NS			NS			NS			0.02		
	***	NS			NS			0.76			0.37			NS			NS			NS		

* CD (5 %) for treatment means
** CD (5%) for year means
*** CD (5%) for (treatment X year) interaction means
NS Non Significant

Table 27. Variability in secondary finger characters of ginger cultivars

Sl. No.	Cultivar	Number			Length (cm)			Breadth (cm)			Girth (cm)			Weight (g)			Number of nodes			Internodal distance (cm)		
		I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled
1.	Z-O-78 (V_1)	27.39	27.70	27.55	3.45	3.31	3.38	1.93	1.82	1.88	6.22	6.19	6.20	9.73	9.87	9.80	5.50	4.98	5.24	0.32	0.31	0.32
2.	Z-O-86 (V_2)	23.20	20.62	21.91	4.81	3.51	4.16	2.19	1.18	1.69	5.70	5.94	5.82	11.42	12.43	11.83	6.33	5.09	5.71	0.39	0.39	0.39
3.	Z-O-89(V_3)	11.60	12.31	11.95	4.26	3.39	3.82	2.75	1.73	2.24	5.65	5.75	5.70	13.17	13.53	13.35	5.25	5.12	5.18	0.77	0.76	0.76
4.	Z-O-90 (V_4)	12.37	13.02	12.70	4.19	3.32	3.75	2.81	1.81	2.31	5.75	6.02	5.89	13.07	13.37	13.22	5.08	5.08	5.08	0.79	0.71	0.75
5.	Z-O-92 (V_5)	21.73	20.39	21.06	3.72	2.56	3.14	2.33	1.56	1.95	6.02	5.99	6.01	12.17	11.57	11.87	5.67	5.40	5.54	0.58	0.56	0.57
6.	Z-O-95 (V_6)	14.70	12.84	13.77	4.02	2.67	3.34	2.30	1.59	1.94	5.77	5.64	5.71	13.60	12.77	13.18	5.33	4.95	5.14	0.51	0.50	0.51
7.	Z-O-97 (V_7)	35.55	37.37	36.46	4.49	2.81	3.65	2.57	1.18	1.88	3.47	3.48	3.48	11.50	12.10	11.8	6.58	4.95	5.77	0.62	0.59	0.61
8.	Rio-de-Janeiro (V_8)	21.62	20.11	20.86	4.81	3.84	4.33	2.20	1.99	2.09	6.12	5.70	5.91	10.55	10.55	10.55	6.08	5.02	5.55	0.82	0.78	0.80
9.	Maran (V_9)	21.25	19.22	20.23	4.17	3.11	3.64	2.45	1.53	1.99	5.14	5.18	5.16	9.93	10.24	10..09	6.17	4.81	5.49	0.62	0.60	0.61
10.	Himachal Pradesh(V_{10})	17.36	15.73	16.55	3.75	3.27	3.51	2.37	1.86	2.12	6.47	6.34	6.41	12.20	12.83	12.52	5.25	5.25	5.25	0.64	0.60	0.62
Mean		20.68	19.93	20.30	4.17	3.18	3.67	2.39	1.63	2.01	5.63	5.62	5.63	11.73	11.93	11.83	5.73	5.07	5.40	0.61	0.58	0.59
*		1.68			Intraction is absent			Intraction is absent			0.29			2.51			0.66			0.04		
**		NS									0.13			1.12			0.30			0.02		
***		NS									NS			3.55			0.94			NS		

* CD (5 %) for treatment means
** CD (5%) for year means
*** CD (5%) for (treatment X year) interaction means
NS Non Significant

only for weight and number of nodes. V_7 registered maximum number of fingers (36.46) while V_3 had minimum number (11.95) in case pooled data. In relation to pooled analysis of data for girth showed that maximum and minimum girth was observed in V_{10} (6.41 cm) and V_7 (3.48 cm) respectively. V_6 (13.60 g) at first year and V_3 at second year had maximum weight while it was minimum in V_1 (9.73 g and 9.87 g) at both years. V_7 (6.58) and V_5 (5.40) produced the maximum number of nodes (5.77) whereas V_4 the minimum number of nodes (5.08) respectively. Considering pooled data for internodal distance, it was highest in V_8 (0.80 cm) and lowest in V_1 (0.32 cm).

4.1.6.1.3 Tertiary finger

The treatments showed significant variation for all tertiary finger characters except number and weight of tertiary fingers. Since interaction was absent, pooled analysis was not done and conclusion was drawn from the results of the individual year for number and weight of tertiary fingers. Year wise variation and variation among treatments within year was significant only in case of number of nodes. In relation to pooled analysis, V_6 had maximum length (4.67 cm) while V_9 (2.97 cm) had minimum length. Maximum and minimum breadth was observed in V_4 (2.79 cm) and V_5 (1.04 cm) respectively. V_7 registered maximum number of nodes (4.82) and internodal distance (0.44 cm). The same was minimum in V_1 (3.91) and V_2 (0.25 cm) respectively.

4.1.6.2 Fresh rhizome yield

Yield potential of the selected cultivars was recorded as g per plant and tonnes per hectare (Figure 3). The fresh rhizome yield exhibited significant variation among cultivars and among year of cultivation (Table 29). Variation between treatments within year was also significant. V_8 registered maximum yield per plant (277.84 g) with maximum yield per hectare (25.37 t) during the first year whereas V_1 recorded maximum (168.18 g per plant and 15.37 t ha^{-1}) during the second year. Pooled analysis of the yield per plant and per hectare showed significant variation among the cultivars and indicated V_8 as superior cultivar (175.35 g per plant and 16.02 t ha^{-1}) which was on par with V_{10} (156.73 g per plant and 14.32 t ha^{-1}), V_3 (150.89 g per

Table 28. Variability in tertiary finger characters in different ginger cultivars

Sl. No	Cultivar	Number			Length (cm)			Breadth (cm)			Girth (cm)			Weight (g)			Number of nodes			Internodal distance (cm)		
		I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled
1.	Z-O-78 (V_1)	19.92	17.66	18.79	3.33	3.32	3.33	1.37	1.39	1.38	4.60	4.70	4.65	8.23	8.33	8.28	4.00	3.82	3.91	0.32	0.26	0.29
2.	Z-O-86 (V_2)	16.52	15.77	16.15	4.09	4.24	4.17	1.69	1.87	1.78	4.00	3.91	3.95	7.28	7.94	7.61	3.92	5.28	4.60	0.24	0.26	0.25
3.	Z-O-89(V_3)	11.03	11.93	11.48	3.51	3.62	3.57	2.48	2.34	2.41	4.85	4.81	4.83	9.00	7.63	8.32	3.83	4.69	4.26	0.44	0.42	0.43
4.	Z-O-90 (V_4)	9.10	8.14	8.62	4.19	4.54	4.36	2.78	2.81	2.79	4.07	4.13	4.10	10.90	11.20	11.05	3.83	5.22	4.53	0.41	0.35	0.38
5.	Z-O-92 (V_5)	20.37	18.88	19.60	2.96	3.18	3.07	0.95	1.12	1.04	4.51	4.50	4.51	7.36	7.87	7.61	4.50	4.79	4.64	0.32	0.34	0.33
6.	Z-O-95 (V_6)	9.73	12.77	11.25	4.63	4.70	4.67	2.16	2.23	2.20	4.84	4.81	4.23	10.00	11.00	10.50	4.75	3.73	4.24	0.30	0.31	0.30
7.	Z-O-97 (V_7)	25.34	20.89	22.78	3.71	3.79	3.75	2.32	2.54	2.43	3.60	3.73	3.66	10.23	12.30	11.27	4.92	4.73	4.82	0.44	0.42	0.43
8.	Rio-de-Janeiro (V_8)	15.65	14.79	15.22	4.09	4.18	4.13	1.72	1.95	1.84	3.85	3.83	3.84	6.25	6.73	6.49	4.67	4.18	4.42	0.40	0.41	0.40
9.	Maran (V_9)	15.66	12.75	14.20	2.90	3.04	2.97	2.00	2.18	2.09	3.65	3.54	3.59	9.47	9.20	9.34	4.92	4.42	4.67	0.31	0.32	0.32
10.	Himachal Pradesh(V_{10})	12.48	12.86	12.67	4.63	4.68	4.65	1.70	1.70	1.70	5.00	4.85	4.93	10.17	11.40	10.78	4.75	4.34	4.55	0.38	0.39	0.38
Mean		15.58	14.64	15.11	3.81	3.93	3.87	1.92	2.01	1.97	4.30	4.28	4.29	8.89	9.36	9.13	4.41	4.52	4.46	0.36	0.35	0.35
*		Interaction is absent			0.83			0.42			0.19			Interaction is absent			0.69			0.05		
**					NS			NS			NS						0.31			NS		
***					NS			NS			NS						0.98			NS		

* CD (5 %) for treatment means
** CD (5%) for year means
*** CD (5%) for (treatment X year) interaction means
NS Non Significant

Table 29. Variability in economic attributes of ginger cultivars

Sl. No.	Cultivar	Fresh rhizome yield g plant^{-1}			Fresh rhizome yield t ha^{-1}			Dry rhizome yield t ha^{-1}		
		I year	II year	Pooled	I year	II year	Pooled	I year	II year	Pooled
1.	Z-O-78 (V_1)	73.57	168.18	120.88	6.72	15.37	11.05	1.52	3.47	2.49
2.	Z-O-86 (V_2)	163.34	125.00	144.17	13.93	11.42	12.68	2.69	2.20	2.45
3.	Z-O-89 (V_3)	221.95	79.83	150.89	20.28	7.29	13.79	4.06	1.45	2.76
4.	Z-O-90 (V_4)	50.00	50.00	50.00	4.57	4.57	4.57	0.82	0.82	0.82
5.	Z-O-92 (V_5)	82.78	95.24	89.01	7.57	8.70	8.13	1.39	1.59	1.49
6.	Z-O-95 (V_6)	69.45	111.11	90.28	6.34	10.15	8.25	1.23	2.01	1.62
7.	Z-O-97 (V_7)	80.45	90.94	85.70	7.35	8.31	7.83	1.55	1.71	1.63
8.	Rio-de-Janeiro (V_8)	277.84	72.87	175.35	25.37	6.66	16.02	4.44	1.20	2.82
9.	Maran (V_9)	138.89	87.45	113.17	12.69	7.99	10.34	2.67	1.73	2.20
10.	Himachal Pradesh(V_{10})	157.02	156.44	156.73	14.35	14.30	14.32	3.08	3.07	3.08
Mean		131.53	103.71	117.62	11.92	9.48	10.70	2.35	1.93	2.14
CD for treatment means		72.82			6.65			1.27		
CD for year means		32.57			2.97			0.57		
CD for (treatment X year) interaction means		102.99			9.40			1.80		

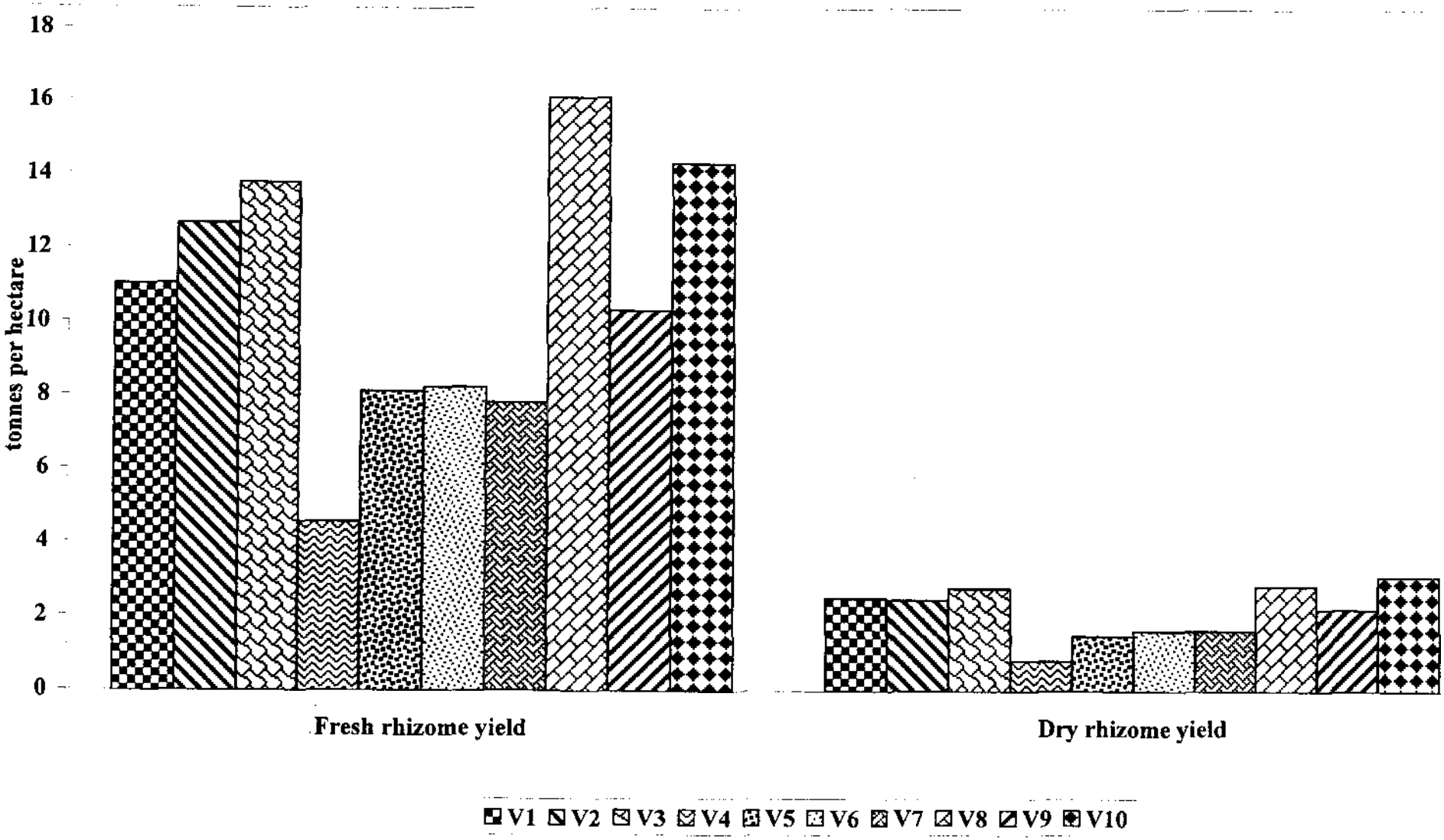


Fig. 3 Variability in economic attributes of ginger cultivars

plant and 13.79 t ha^{-1}), V_2 (144.17 g per plant and 12.68 t ha^{-1}), V_1 (120.88 g per plant and 11.05 t ha^{-1}) and V_9 (113.17 g per plant and 10.34 t ha^{-1}) while V_4 as inferior cultivar (50 g per plant and 4.57 t ha^{-1}).

4.1.6.3 Dry rhizome yield

The cultivars selected for the study showed significant variation with regard to dry rhizome yield (t ha^{-1}) during the two years (Table 29). The dry rhizome yield exhibited significant variation among treatments within year as well as between years of cultivation. The maximum dry rhizome yield was recorded in V_8 (4.44 t ha^{-1}) and V_1 (3.47 t ha^{-1}) during first and second year respectively whereas minimum in V_4 (0.82 t ha^{-1}) in both the years. The pooled analysis indicated V_{10} as superior cultivar with maximum dry rhizome yield (3.08 t ha^{-1}) and showing stable performance over the years (Figure 3). The cultivars V_8 (2.82 t ha^{-1}), V_3 (2.76 t ha^{-1}), V_1 (2.49 t ha^{-1}), V_2 (2.45 t ha^{-1}), and V_9 (2.2 t ha^{-1}) were on par with superior cultivar V_{10}. V_4 remained inferior (0.82 t ha^{-1}) in pooled analysis also.

4.1.7 Quality attributes

4.1.7.1 Colour of rhizome flesh and powder

The cultivars showed variation in both flesh colour as well as powder colour of rhizome (Table 30). Both flesh and powder colour varied from yellow to bright yellow.

4.1.7.2 Dry recovery percentage

The cultivars selected for the study exhibited significant variation with respect to dry recovery percentage (Table 30). Driage ranged from 17.33 per cent in V_8 to 22.56 per cent in V_1. The autoteraploids V_1 and V_2 recorded enhanced driage when compared to corresponding diploids (Figure 4). The other variants showed less driage compared to the check cultivars.

4.1.7.3 Estimation of crude fibre

Significant variation was observed among the selected cultivars with regard to fibre content (Table 30). The autotetraploids V_1 and V_2 recorded low fibre content

Table 30. Variability in quality attributes of ginger cultivars

Sl. No.	Cultivar	Rhizome flesh colour	Rhizome powder colour	Driage (%)	Fibre content (%)
1.	Z-O-78 (V_1)	Yellow	Bright yellow	22.56	3.39
2.	Z-O-86 (V_2)	Bright yellow	Bright yellow	19.30	2.70
3.	Z-O-89(V_3)	Yellow	Yellow	19.67	3.32
4.	Z-O-90 (V_4)	Yellow	Bright yellow	18.00	3.47
5.	Z-O-92 (V_5)	Yellow	Yellow	18.30	4.98
6.	Z-O-95 (V_6)	Yellow	Yellow	19.76	3.83
7.	Z-O-97 (V_7)	Yellow	Yellow	20.72	4.17
8.	Rio-de-Janeiro (V_8)	Yellow	Bright yellow	17.93	4.82
9.	Maran (V_9)	Bright yellow	Yellow	21.47	4.18
10.	Himachal Pradesh (V_{10})	Yellow	Bright yellow	21.43	4.51
Mean		-	-	19.91	3.94
CD (5 %)		-	-	2.48	0.27

when compared to corresponding diploids. Autotetraploid derived from Rio-de-Janeiro (V_2) recorded the lowest value (2.70 %) whereas V_5 the highest value (4.98 %) (Figure 4).

4.1.7.4 Estimation of volatile oil

With respect to volatile oil content (%) and oil yield (kg ha^{-1}) during the second year, the cultivars exhibited variation (Table 31). Highest oil content was observed in V_2 (2.07 %) and the lowest in V_7 (1.20 %) (Figure 4). With respect to oil yield per hectare, V_8 registered the maximum value of 76.02 kg ha^{-1} followed by autotetraploid V_2 (55.50 kg ha^{-1}) while V_4 was found to be an inferior cultivar (10.38 kg ha^{-1}). The colour of oil varied from light yellow to dark yellow. Sensory evaluation indicated that V_8 had good sensory score ("++++") and the least preferred was V_5 and V_6 ("+").

4.1.7.5 Estimation of oleoresin

The oleoresin extracted using acetone and ethyl acetate exhibited variation among the cultivars in both content as well as yield (kg ha^{-1}) during the second year (Tables 32, 33 and Figure 4).

With respect to oleoresin extracted with acetone, V_2 recorded maximum content (9.16 %) and minimum in V_3 (4.47 %). V_8 gave the maximum yield per hectare (280.15 kg ha^{-1}) followed by V_2 (246.28 kg ha^{-1}) whereas V_4 was found to be an inferior cultivar (41.95 kg ha^{-1}). The duration of extraction of oleoresin using acetone ranged from 65 to 85 minutes.

With respect to oleoresin extracted with ethyl acetate, the maximum content was observed in V_2 (7.74 %) and minimum in V_5 (1.26 %). V_8 produced the maximum yield per hectare (288.66 kg ha^{-1}) followed by V_2 (207.97 kg ha^{-1}).V_4 remained inferior with an yield of 14.23 kg ha^{-1}. The duration of extraction of oleoresin using acetone varied from 60 to 75 minutes.

When the extraction efficiency of the two solvents was compared it was found that the content was more with acetone (5.91 %) compared to ethyl acetate (3.86 %).

The colour of oleoresin extracted using acetone and ethyl acetate varied from pale brown to dark brown. Sensory evaluation of oleoresin indicated that V_8 had the most pleasing aroma with acetone and ethyl acetate as solvents.

Table 31. Variability in volatile oil attributes of ginger cultivars

Sl. No.	Cultivar	Oil content (%)	Oil yield (kg ha^{-1})	Oil colour	Sensory evaluation
1.	Z-O-78 (V_1)	1.27	19.02	Dark yellow	++
2.	Z-O-86 (V_2)	2.07	55.50	Dark yellow	++
3.	Z-O-89(V_3)	1.27	49.91	Dark yellow	+++
4.	Z-O-90 (V_4)	1.27	10.38	Light yellow	++
5.	Z-O-92 (V_5)	1.27	17.65	Light yellow	+
6.	Z-O-95 (V_6)	1.60	19.73	Light yellow	+
7.	Z-O-97 (V_7)	1.20	18.63	Dark yellow	++
8.	Rio-de-Janeiro (V_8)	1.73	76.02	Light yellow	++++
9.	Maran (V_9)	1.27	33.76	Light yellow	+++
10.	Himachal Pradesh (V_{10})	1.60	49.17	Light yellow	++
Mean		1.45	34.98	-	-
CD (5 %)		0.17	18.72	-	-

Table 32. Variability in oleoresin attributes extracted with acetone of ginger cultivars

Sl.No.	Cultivar	Oleoresin content (%)	Oleoresin yield (kg ha^{-1})	Oleoresin colour	Sensory evaluation
1.	Z-O-78 (V_1)	7.40	114.20	Dark brown	++
2.	Z-O-86 (V_2)	9.15	246.28	Dark brown	++
3.	Z-O-89(V_3)	4.46	182.62	Dark brown	++++
4.	Z-O-90 (V_4)	5.09	41.95	Pale brown	+
5.	Z-O-92 (V_5)	5.53	76.93	Pale brown	+
6.	Z-O-95 (V_6)	5.17	63.11	Pale brown	+
7.	Z-O-97 (V_7)	5.41	83.49	Pale brown	++
8.	Rio-de-Janeiro (V_8)	6.27	280.15	Dark brown	++++
9.	Maran (V_9)	5.58	152.50	Dark brown	++
10.	Himachal Pradesh (V_{10})	4.99	153.93	Dark brown	+++
Mean		5.91	139.52	-	-
CD (5 %)		1.05	77.21	-	-

Table 33. Variability in oleoresin attributes extracted with ethyl acetate of ginger cultivars

Sl.No.	Cultivar	Oleoresin (%)	Oleoresin yield (kg ha^{-1})	Oleoresin colour	Sensory evaluation
1.	Z-O-78 (V_1)	5.75	87.86	Dark brown	++
2.	Z-O-86 (V_2)	7.74	207.97	Dark brown	++
3.	Z-O-89 (V_3)	1.69	68.85	Dark brown	++++
4.	Z-O-90 (V_4)	1.73	14.23	Pale brown	+
5.	Z-O-92 (V_5)	1.26	17.44	Pale brown	+
6.	Z-O-95 (V_6)	1.99	24.92	Pale brown	++
7.	Z-O-97 (V_7)	3.52	55.62	Pale brown	+
8.	Rio-de-Janeiro (V_8)	6.46	288.66	Dark brown	++++
9.	Maran (V_9)	4.04	106.97	Dark brown	+++
10.	Himachal Pradesh (V_{10})	4.36	134.50	Dark brown	++
Mean		3.86	100.70	-	-
CD (5 %)		0.50	66.52	-	-

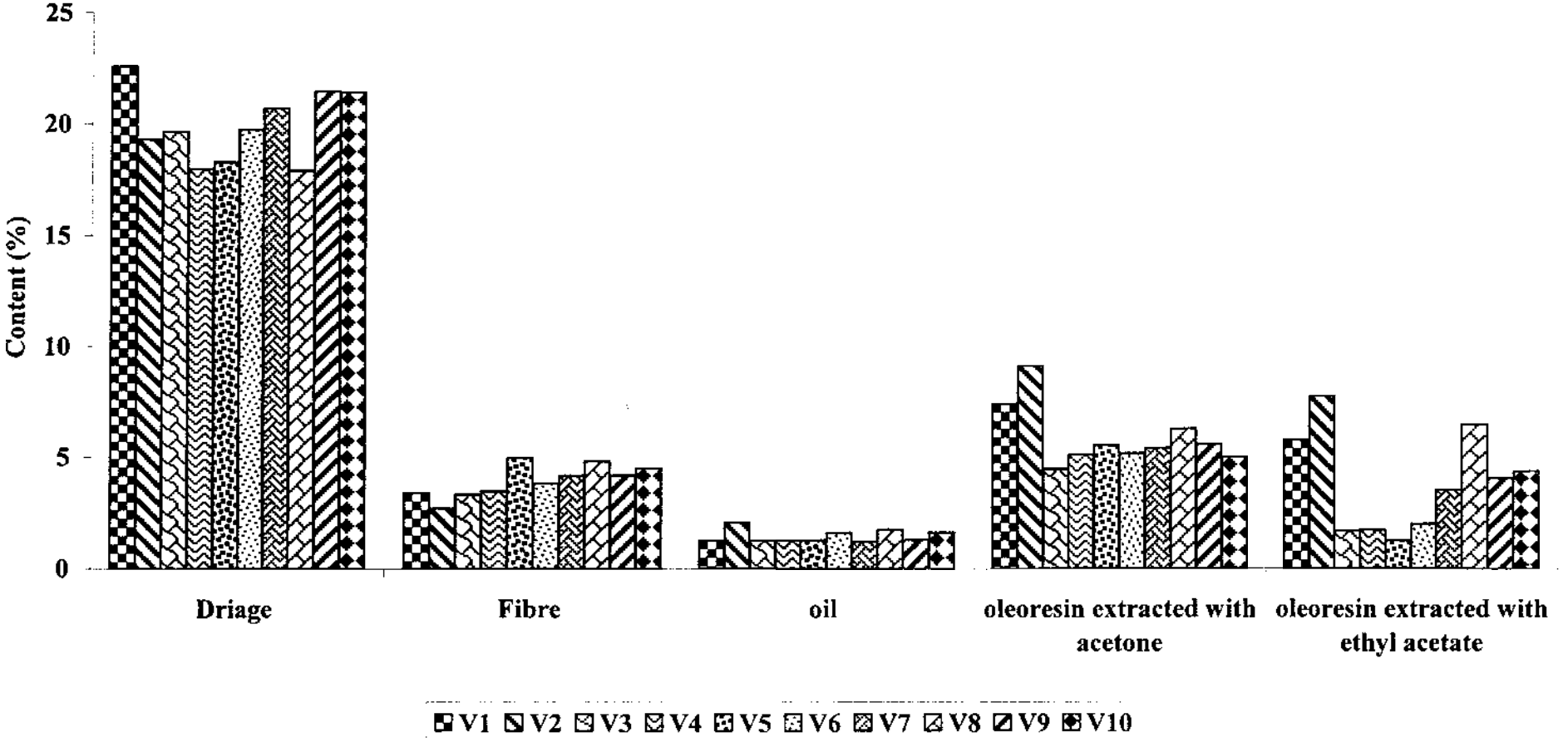


Fig. 4. variability in quality attributes of ginger cultivars

4.1.8 Incidence of pests and diseases

Data on incidence of shoot borer, soft rot, bacterial wilt and leaf spot are presented in Table 34.

4.1.8.1 Incidence of shoot borer

Significant variation was observed among cultivars for the percentage of tillers attacked and plants attacked by shoot borer. V_8 showed the least percentage of attacked tillers (19.99 %) whereas V_7 had the least percentage of attacked plants (29.37 %). V_4 showed the maximum incidence of both shoot borer attacked tillers (50.82 %) and attacked plants (67.32 %). The autotetraploid V_2 derived from V_8 also showed less incidence similar to V_8.

4.1.8.2 Incidence of soft rot

The cultivars showed significant difference in incidence of soft rot disease. Among the ten cultivars studied, V_{10} showed the least susceptibility (20.28 %) whereas V_4 showed maximum susceptibility (53.24 %).

4.1.8.3 Incidence of bacterial wilt

The cultivars differed significantly for the incidence of bacterial wilt disease. V_{10} was found to be the least susceptible cultivar (10.14 %), which was confirmed by ooze test also. V_9 was found to be the most susceptible cultivar (35.83 %). It was further observed that V_{10} was found to be on par with V_1 (an autotetraploid from V_{10}) while V_9 with V_2 (an autotetraploid from V_8). Ooze test carried out from rhizomes at harvest showed maximum intensity of wilt disease in (V_1, V_2, V_7, V_8), moderate in (V_3, V_6, V_9), light in (V_4, V_5) and nil in V_{10}.

4.1.8.4 Incidence of leaf spot

With regard to leaf spot disease, no significant difference was observed among the cultivars. Cultivars were scored for the disease intensity and the disease was scored as very light (0.68) to medium (2.00). V_1 and V_6 were found to be the least susceptible cultivars, which is scored as very light (0.68) while V_8 showed the maximum incidence, which is scored as medium (2.00).

Table 34. Variability in incidence of pests and diseases in ginger cultivars

Sl. No.	Cultivar	Shoot borer incidence (%)		Soft rot incidence (%)	Bacterial wilt intensity		Leaf spot intensity	
		tillers attacked	plants attacked		Percentage of incidence	Scoring by ooze test in rhizomes	Grade	Category
1.	Z-O-78 (V_1)	30.29	46.55	30.55	15.72	+++	0.67	Very light
2.	Z-O-86 (V_2)	23.80	35.62	47.92	27.08	+++	1.33	Light
3.	Z-O-89(V_3)	41.85	62.26	24.31	13.19	++	1.33	Light
4.	Z-O-90 (V_4)	50.82	67.32	53.24	17.59	+	1.67	Light
5.	Z-O-92 (V_5)	38.89	52.69	46.67	15.83	+	1.00	Light
6.	Z-O-95 (V_6)	32.96	33.83	43.21	21.57	++	0.68	Very light
7.	Z-O-97 (V_7)	20.15	29.36	25.00	22.50	+++	1.00	Light
8.	Rio-de-Janeiro (V_8)	19.99	40.15	31.39	22.64	+++	2.00	Medium
9.	Maran (V_9)	28.06	42.53	25.00	35.83	++	1.33	Light
10.	Himachal Pradesh (V_{10})	34.61	57.97	20.28	10.14	-	1.67	Light
Mean		32.14	46.83	34.74	20.21	-	1.27	-
CD (5 %)		5.87	9.09	15.88	11.07	-	NS	-

4.2 INVESTIGATIONS ON CONTROLLED *IN VIVO* POLLINATION IN GINGER

In order to overcome the barriers in seed set in nature, various *in vivo* pollination techniques such as stylar, stigmatic, intraovarian were carried out in five cultivars viz., Z – O – 78, Z – O – 86, Rio-de-Janeiro, Maran and Himachal Pradesh at three stages such as 1 day prior to flower opening, immediately after flower opening and 3 h after flower opening. Even the stigma and style were removed at different heights in these varieties and artificial (self, sibbling and cross) pollination and mixed pollen from other genera like *Kaempferia* and *Costus* were employed. Crossing of *Zingiber zerumbet* with *Zingiber officinale* was also tried. None of these pollination techniques succeeded in inducing fruit set in ginger.

4.3 INVESTIGATIONS ON *IN VITRO* POLLINATION IN GINGER

Ovules were found to develop in the three methods tried; placental pollination, modified placental pollination and ovular or test tube fertilization (Table 35). The ovule swelling was good and development was maximum in placental pollination (17.86) followed by ovular or test tube fertilization (15.02). It was minimum in modified placental pollination technique (6.14). The percentage of cultures with maximum developed ovules was found in placental pollination (90 %) followed by ovular or test tube fertilization (85 %) and modified placental pollination (75 %).

Table 35. Different methods of *in vitro* pollination in ginger

Sl. No.	Methods of pollination	Ovule swelling	Percentage of cultures with well developed ovules	No. of ovules developed per culture
1.	Placental pollination	+++	90	17.86
2.	Modified placental pollination	++	75	6.14
3.	Ovular or test tube fertilization	+	85	15.02

Average of 20 observations 20 DAP
Scoring: " +++" good; "++" moderate; "+" low
Medium: Half MS +sucrose 3% + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) +CW 15% v/v

4.4 POLLEN PISTIL INTERACTION AFTER *IN VITRO* AND *IN VIVO* POLLINATION

Gynoeciums after *in vitro* placental pollination and *in vivo* stigmatic, stylar as well as intra ovarian pollination were examined for pollen germination and fertilization using fluorescence microscopy.

The pollen pistil interaction studies after *in vitro* placental pollination involving diploid (Rio-de-Janeiro) X autotetraploid (Z – 0 – 78) and autotetraploid (Z – 0 – 78) X diploid (Rio-de-Janeiro) showed that pollen grains germinate readily on the ovules under *in vitro* condition. It was observed that pollen germination starts within3 h of pollination and attained a length which was sufficient to cover the total length of the ovule and to effect to fertilization by 24 h (Plate 14a).

The pollen pistil interaction studies in the aforesaid crosses after *in vivo* stylar, stigmatic and intra ovarian pollination showed that pollen grains failed to germinate under *in vivo* condition (Plate 14b).

4.5 STANDARDIZATION OF MEDIA AND SUPPLEMENTS FOR CULTURE ESTABLISHMENT

4.5.1 Basal medium for culture establishment

In order to get initial *in vitro* culture establishment, surface sterilized ovaries on the day of anthesis and placental pollinated ovules were incubated in three different basal media viz., half MS, SH and Nitsch with and without supplements. The results are presented in Table 36. There was no ovary development in the simple basal medium with 3 per cent sucrose. The ovaries developed in all the three media when they were supplemented with BAP 2.5 mg l^{-1}, NAA 0.5 mg l^{-1}, sucrose 3 per cent and coconut water 15 per cent v/v. The mean diameter of the ovary was only 2.6 mm on the day of pollination. It developed upto 5.1 mm in the artificial media within 20 DAP. The half MS with supplements recorded the maximum ovary development of 7.9 mm followed by SH (5.9 mm) and Nitsch (5.7 mm).

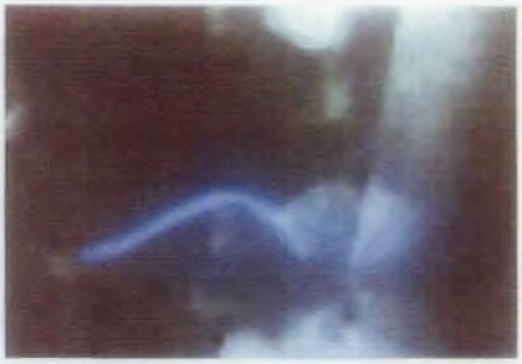
The maximum percentage of cultures subsequent to placental pollination with developed ovules (90 %) was found in half MS with supplements followed by SH

(3 hours after *in vitro* placental pollination)

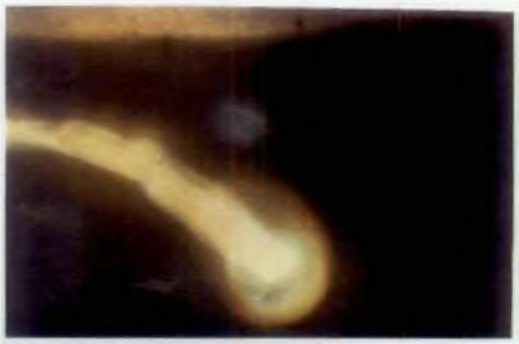
(24 hours after *in vitro* placental pollination)

Autotetraploid (Z - O - 78) X Diploid (Rio-de Janeiro)

(3 hours after *in vitro* placental pollination)



(12 hours after *in vitro* placental pollination)



(24 hours after *in vitro* placental pollination)

Lengthy pollen tube observed 36 hours after *in vitro* placental pollination

Diploid (Rio-de Janeiro) X Autotetraploid (Z - O - 78)

Plate 14a. Pollen pistil interaction after *in vitro* placental pollination (400 X)

Diploid (Rio-de Janeiro) X Autotetraploid (Z - O - 78)

Plate 14b. Pollen pistil interaction after *in vivo* intra ovarian pollination (400 X)

Table 36. Basal medium for culture establishment of ginger ovary and ovule

Sl. No.	Treatments	Diameter of ovary (mm)			Ovule swelling	Percentage of cultures showing ovule development
		Maximum	Minimum	Mean		
1.	Half MS + sucrose 3 %	3.3	2.7	2.8	++	40
2.	Half MS + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v	7.9	3.2	5.1	+++	90
3.	SH + sucrose 3 %	3.1	2.6	2.8	+	25
4.	SH + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v	5.9	2.9	4.2	++	50
5.	Nitsch + sucrose 3 %	3.1	2.2	2.7	+	15
6.	Nitsch + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v	5.7	2.7	3.7	+	40
Initial size of ovary		**3.1**	**1.98**	**2.5**	**NA**	**NA**

(50 %) and Nitsch (40 %) with supplements. The scoring for swelling of ovules for the different basal media with and without supplements also showed that half MS with supplements favoured maximum swelling (Table 36).

4.5.2 Influence of growth regulators on ovule development in ginger after *in vitro* placental pollination.

4.5.2.1 Influence of auxins

The data presented in Table 37 showed that auxins induced ovule development in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1}. NAA 0.5 to 1.0 mg l^{-1} induced moderate ovule swelling in 100 per cent of cultures. 2,4 – D 0.5 mg l^{-1} and IAA 0.5 mg l^{-1} resulted in low ovule swelling in 72.22 and 66.66 per cent of cultures respectively.

4.5.2.2 Influence of cytokinins

The data on the influence of cytokinins on ovule development (Table 38) revealed that both BAP and kinetin favoured ovule development individually in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1}. BAP 2.5 mg l^{-1} favoured moderate ovule swelling in 83.33 per cent of cultures while kinetin 2.5 mg l^{-1} was found to favour low ovule swelling in 72.22 per cent cultures.

4.5.2.3 Influence of auxins and cytokinins

4.5.2.3.1 Influence of NAA and BAP

The auxin, NAA at concentrations of 0.5 to 2.5 mg l^{-1} along with cytokinin, BAP at 2.5 to 20.0 mg l^{-1} in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1} supported ovule development in varying degrees after *in vitro* placental pollination in the cross Rio-de-Janeiro X Z – O – 78. NAA 0.5 mg l^{-1} + BAP 2.5 mg l^{-1} favoured good ovule swelling with maximum percentage of cultures showing ovule devlopment (94.44 %). NAA 0.5 mg l^{-1} + BAP 5.0 mg l^{-1} and NAA 1.0 mg l^{-1} + BAP 2.5 mg l^{-1} resulted in moderate ovule swelling while all other combinations including control showed low ovule swelling. The percentage of cultures showing ovule development ranged from 66.66 per cent to 94.44 per cent in hormonal combinations whereas in control, it was only 27.77 per cent (Table 39).

Table 37. Influence of auxins on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	NAA 0.5	++	100.00
2.	NAA 1.0	++	100.00
3.	IAA 0.5	+	66.66
4.	2,4 – D 0.5	+	72.22
5.	Control (Basal Medium)	+	16.66

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Rio-de-Janeiro X Z – 0 – 78

Table 38. Influence of cytokinins on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	BAP 2.5	++	83.33
2.	Kinetin 2.5	+	72.22
3.	Control (Basal Medium)	+	11.11

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Rio-de-Janeiro X Z – 0 – 78

Table 39. Influence of NAA and BAP on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	NAA 0.5 + BAP 2.5	+++	94.44
2.	NAA 0.5 + BAP 5.0	++	88.88
3.	NAA 0.5 + BAP 7.5	+	88.88
4.	NAA 0.5 + BAP 10.0	+	83.33
5.	NAA 0.5 + BAP 15.0	+	88.88
6.	NAA 0.5 + BAP 20.0	+	77.77
7.	NAA 1.0 + BAP 2.5	++	72.22
8.	NAA 1.5 + BAP 2.5	+	66.66
9.	NAA 2.0 + BAP 2.5	+	66.66
10.	NAA 2.5 + BAP 5.0	+	66.66
11.	NAA 2.5 + BAP 10.0	+	72.22
12.	Control (Basal Medium)	+	27.77

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Rio-de-Janeiro X Z – 0 – 78

4.5.2.3.2 Influence of IAA and BAP

The combinations of IAA (0.10 to 0.25) mg l^{-1} along with BAP (2.5 to 10.0) mg l^{-1} in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1} supported ovule development after placental pollination in the cross Maran X Z – O – 78. IAA (0.25 to 0.50) mg l^{-1} with BAP (5 to 20) mg l^{-1} did not favour ovule development. The percentage of cultures showing response ranged from 27.77 per cent to 94.44 per cent in hormonal combinations whereas in control, it was only 16.66 per cent (Table 40).

Table 40. Influence of IAA and BAP on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	IAA 0.1 + BAP 2.5	+	94.44
2.	IAA 0.2 + BAP 2.5	+	77.77
3.	IAA 0.25 + BAP 2.5	+	72.22
4.	IAA 0.25 + BAP 5.0	+	44.44
5.	IAA 0.25 + BAP 7.5	+	27.77
6.	IAA 0.25 + BAP 10.0	+	27.77
7.	IAA 0.25 + BAP 15.0	-	-
8.	IAA 0.25 + BAP 20.0	-	-
9.	IAA 0.5 + BAP 2.5	+	72.22
10.	IAA 0.5 + BAP 5.0	-	-
11.	IAA 0.5 + BAP 10.0	-	-
12.	Control (Basal Medium)	+	16.66

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Maran X Z – 0 – 78

4.5.2.3.3 Influence of 2,4 – D and BAP

The 2,4 – D (0.1 to 1.0 mg l^{-1}) along with BAP (2.5 to 20.0 mg l^{-1}) in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1} supported ovule development after placental pollination in the cross between Maran X Z – O – 86. 2,4 – D (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) showed moderate ovule swelling while all other combinations were similar to control and showed low ovule swelling. The percentage of cultures showing response ranged from 16.66 to 88.88 in hormonal combinations whereas in control, it was only 22.23 per cent (Table 41).

Table 41. Influence of 2,4-D and BAP on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	2,4 – D 0.1 + BAP 2.5	+	27.77
2.	2,4 – D 0.2 + BAP 2.5	+	16.66
3.	2,4 – D 0.5 + BAP 2.5	++	88.88
4.	2,4 – D 0.5 + BAP 5.0	+	77.77
5.	2,4 – D 0.5 + BAP 7.5	+	66.66
6.	2,4 – D 0.5 + BAP 10.0	+	77.77
7.	2,4 – D 0.5 + BAP 15.0	+	83.33
8.	2,4 – D 0.5 + BAP 20.0	+	77.77
9.	2,4 – D 0.75 + BAP 2.5	+	44.44
10.	2,4 – D 1.0 + BAP 2.5	+	38.88
11.	Control (Basal Medium)	+	22.23

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Maran X Z – 0 – 86

4.5.2.3.4 Influence of dicamba and BAP

The auxin, dicamba (0.5 to 10.0) mg l^{-1} along with cytokinin, BAP (2.5 mg l^{-1}) in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1} supported ovule development after placental pollination in the cross between Himachal Pradesh X Z – O – 86. Dicamba (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) favoured moderate ovule swelling while Dicamba (0.75 and 1.00 mg l^{-1}) + BAP (2.5 mg l^{-1}) showed low ovule swelling. Dicamba (2.5 mg l^{-1}) + BAP (0.5 mg l^{-1}) did not favour ovule development. The mean percentage of cultures showing response was 27.77 to 38.88 and in control, it was only 11.11 per cent (Table 42).

Table 42. Influence of dicamba and BAP on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	Dicamba 0.50 + BAP 2.5	++	38.88
2.	Dicamba 0.75 + BAP 2.5	+	33.33
3.	Dicamba 1.00 + BAP 2.5	+	27.77
4.	Dicamba 2.50+ BAP 0.5	-	-
5.	Control (Basal Medium)	+	11.11

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Himachal Pradesh X Z – 0 – 86

4.5.2.4 Influence of GA_3

The data on the influence of GA_3 alone and in combination with NAA and BAP on ovule development are presented in Table 43. GA_3 (1 to 10 mg l^{-1}) and GA_3 (5 mg l^{-1}) + NAA (0.5 mg l^{-1}) or BAP (2.5 mg l^{-1}) in the basal medium of half MS with 3 per cent sucrose level and CH 200 mg l^{-1} did not support ovule development after placental pollination in the cross between Himachal Pradesh X Z – O – 86. But GA_3 (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) induced slightly more ovule swelling (27.77 %) than control (16.66 %).

Table 43. Influence of GA_3 on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	GA_3 1.0	-	-
2.	GA_3 2.5	-	-
3.	GA_3 5.0	-	-
4.	GA_3 7.5	-	-
5.	GA_3 10.0	-	-
6.	GA_3 5.0 + NAA 0.5	-	-
7.	GA_3 5.0 + BAP 2.5	-	-
8.	GA_3 1.0 + NAA 0.5 + BAP 2.5	+	27.77
9.	Control (Basal Medium)	+	16.66

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Himachal Pradesh X Z – 0 – 86

4.5.2.5 Influence of tryptophan

The IAA precursor tryptophan (0.25 to 1.00 mg l^{-1}) alone and with BAP (2.5 mg l^{-1}) in half MS + 3 per cent sucrose + CH 200 mg l^{-1} did not favour ovule development after placental pollination in the cross between Himachal Pradesh X Z – O – 86 (Table 44).

Table 44. Influence of tryptophan on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	Tryptophan 0.25	-	-
2.	Tryptophan 0.5	-	-
3.	Tryptophan 0.75	-	-
4.	Tryptophan 1.00	-	-
5.	Tryptophan 0.25 + BAP 2.5	-	-
6.	Tryptophan 0.5 + BAP 2.5	-	-
7.	Tryptophan 0.75 + BAP 2.5	-	-
8.	Tryptophan 1.00 + BAP 2.5	-	-
9.	Control (Basal Medium)	+	27.77

Average of 18 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Himachal Pradesh X Z – 0 – 86

4.5.3 Influence of carbon source on ovule development

Maximum level of scoring for ovule swelling was observed in 3.0 and 6.0 per cent levels of sucrose with maximum percentage of cultures showing ovule development Ovule swelling and percentage of cultures showing ovule development was decreased at higher concentration of sucrose (Table 45).

Table 45. Influence of sucrose on ovary and ovule development of ginger cultures after *in vitro* placental pollination.

Sl. No	Treatments	Diameter of ovary (mm)			Ovule swelling	Percentage of cultures showing ovule development
		Maximum	Minimum	Mean		
1.	Sucrose 3 %	6.4	3.3	4.7	+++	85
2.	Sucrose 6%	6.4	3.1	4.9	+++	85
3.	Sucrose 9%	3.8	2.7	3.4	++	75
4.	Sucrose 12%	3.1	2.3	2.6	+	55

Average of 20 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low
Medium : Half MS + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v

4.5.4 Influence of organic media supplements on ovule development

The results indicated that the organic media supplements viz., coconut water (CW), casein hydrolysate (CH) and L-glutamine (LG) favoured ovule swelling (Table 46). CW (15 to 25 % v/v) and CH (200 to 500 mg l^{-1}) indicated high ovule swelling whereas CW (5 to 10 % v/v) and CH (100, 750 and 1000 mg l^{-1}) showed moderate ovule swelling. CW (20 and 25 % v/v) and CH (200 mg l^{-1}) had maximum percentage of cultures showing ovule development (95 %). LG (25 to 500 mg l^{-1}) showed moderate ovule swelling and 65 to 75 percentage of cultures showed ovule development and it was comparable to control.

Table 46. Influence of organic supplements on ovule development of ginger after *in vitro* placental pollination

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	Coconut water 5 % v/v	++	70.00
2.	Coconut water 10 % v/v	++	80.00
3.	Coconut water 15 % v/v	+++	85.00
4.	Coconut water 20 % v/v	+++	95.00
5.	Coconut water 25 % v/v	+++	95.00
6.	Casein hydrolysate 200 mg l^{-1}	+++	95.00
7.	Casein hydrolysate 500 mg l^{-1}	+++	85.00
8.	Casein hydrolysate 750 mg l^{-1}	++	75.00
9.	Casein hydrolysate 1000 mg l^{-1}	++	65.00
10.	L – glutamine 25 mg l^{-1}	++	65.00
11.	L – glutamine 50 mg l^{-1}	++	70.00
12.	L – glutamine 100 mg l^{-1}	++	70.00
13.	L – glutamine 250 mg l^{-1}	++	75.00
14.	L – glutamine 500 mg l^{-1}	++	75.00
15.	Control (Basal Medium)	++	70.00

Average of 20 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium : Half MS + sucrose 3 %+ NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1})
Parental combination : Z – 0 – 78 X Maran

4.5.5 Influence of vitamin supplementation on ovule development

The effect of doubling the vitamin stock of MS medium on ovule development was studied (Table 47). The vitamin supplementation was found to enhance high ovule development in the medium half MS + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH (200 mg l^{-1}) with 3 per cent sucrose thus achieving the maximum percentage of cultures showing ovule development (95 %). The ovule development percentage in half MS + 2, 4 – D (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) with 3 per cent sucrose was 80 per cent with moderate ovule swelling while that in half MS + IAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) with 3 per cent sucrose and half MS + dicamba (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) with 3 per cent sucrose was only 65 per cent and 40 per cent respectively with low ovule swelling.

Table 47. Influence of vitamin supplementation on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Growth regulators in mg l^{-1}	Ovule swelling	Percentage of cultures showing ovule development
1.	BAP 2.5 + NAA 0.5	+++	95.00
2.	BAP 2.5 + 2, 4 – D 0.5	++	80.00
3.	BAP 2.5 + Dicamba 0.5	+	65.00
4.	BAP 2.5 + IAA 0.5	+	40.00

Average of 20 observations, 20 DAP
Scoring: " +++" good; "++" moderate; "+" low; "-" nil
Medium: Half MS with doubled vitamin stock + sucrose 3 %+ CH (200 mg l^{-1})
Parental combination : Z – 0 – 78 X Rio-de-Janeiro

4.5.6 Influence of physical conditions in ovule development

The solid, liquid and semisolid form of culture medium supported ovule development following placental pollination (Table 48). The results showed that semisolid medium and solid medium favoured high ovule swelling compared to liquid medium when cultures were kept in dark conditions. Both semisolid and solid medium had maximum percentage of cultures showing ovule development (95 %) and in liquid medium, it was only 85 per cent.

Subjecting the cultures to both light and dark condition (Table 48) showed that continuous dark was better favouring high ovule swelling and maximum percentage of cultures showing ovule development, irrespective of solid, semisolid and liquid medium. In case of continuous light as treatment, it favoured moderate ovule swelling in semisolid medium and light ovule swelling in both solid and liquid medium.

Table 48. Influence of physical conditions on ovule development of ginger after *in vitro* placental pollination.

Sl. No.	Treatments	Solid medium		Liquid medium		Semi solid medium	
		Ovule swelling	Percentage of cultures showing ovule development	Ovule swelling	Percentage of cultures showing ovule development	Ovule swelling	Percentage of cultures showing ovule development
1.	Kept in light conditions	+	85.00	+	80.00	++	85.00
2.	Kept in dark conditions	+++	95.00	++	85.00	+++	95.00

Average of 20 observations 20 DAP
Scoring: " +++" good; "++" moderate; "+" low
Medium–Half MS+sucrose 3 %+ NAA (0.5 mg l^{-1})+ BAP (2.5 mg l^{-1})+ CW15 % v/v

4.6 CONTROLLED SELFING AND CROSSING AMONG SELECTED CULTIVARS

4.6.1 Influence of selfing in ginger cultivars

The data on influence of selfing of ginger cultivars on ovule development are presented in Table 49. All the five cultivars viz., Z – O – 78, Z – O – 86, Rio-de-Janeiro, Maran and Himachal Pradesh could be selfed adopting *in vitro* placental pollination and produced well developed ovules. The percentage of cultures showing response ranged from 50.00 to 83.33 per cent in early, 66.66 to 100.00 per cent in mid and 44.44 to 94.44 per cent in late season. The number of ovules developed per culture ranged between 11.67 in late season to 16.93 in mid season. It was found that mid season favoured more percentage of cultures (87.77 %) with maximum number of well developed ovules per culture (15.82).

The maximum percentage of culture showing response was observed in Z – O – 78 and Rio-de-Janeiro (83.33 %) in early, Z – O – 86 in mid (100 %) as well as in late season (94.44 %). The maximum number of ovules developed per culture was recorded in Rio-de-Janeiro (15.79) in early, Z – O – 86 (16.93 and 15.67) in both mid and late season respectively and minimum in Maran (12.17) in early, Himachal Pradesh (14.63 and 11.67) in both mid and late season respectively. When the mean performance of the cultivars for the three seasons was compared and it was found that the selfing Z – O – 86 resulted in maximum percentage of cultures with well developed ovules and the same was minimum in Himachal Pradesh. The number of ovules developed per culture ranged from 13.42 to 16.01. The crosses involving Himachal Pradesh either as female or pollen parent recorded low percentage of cultures with well developed ovules. The mean percentage of cultures showing response in the three seasons was 77.77 per cent and the mean number of ovules developed per culture was 14.68.

Table 49. Influence of selfing ginger cultivars on ovule development after *in vitro* placental pollination

Sl. No.	Cultivar	Early season		Mid season		Late season		Mean of cultivars	
		Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture
1.	Z – 0 – 78	83.33	14.46	94.44	15.82	88.88	14.32	88.88	14.87
2.	Z – 0 – 86	77.77	15.43	100.00	16.93	94.44	15.67	90.74	16.01
3.	Rio-de-Janeiro	83.33	15.79	94.44	16.05	77.77	14.38	85.18	15.41
4.	Maran	61.11	12.17	83.33	15.69	66.66	13.32	70.37	13.73
5.	Himachal Pradesh	50.00	13.96	66.66	14.63	44.44	11.67	53.70	13.42
Mean of season		71.11	14.36	87.77	15.82	74.44	13.87	77.77	14.68

Average of 18 observations, 20 DAP

Medium : Half MS + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v

4.6.2 Influence of crossing between ginger cultivars

The data on influence of crossing between ginger cultivars on ovule development are presented in Table 50. The autotetraploids [(Z – O – 78) and (Z – O – 86)] as female parent could be crossed with diploids (Rio-de-Janeiro, Maran and Himachal Pradesh) and the percentage of cultures showing response ranged from 66.66 to 88.88 per cent in early season, 83.33 to 100.00 per cent in mid season and 77.77 to 88.88 per cent in late season. The number of ovules developed per culture ranged from 14.03 to 15.83 in early, 14.63 to 16.86 in mid and 13.83 to 15.08 in late season in the above crosses. The autotetraploids [(Z – O – 78) and (Z – O – 86)] as pollen parent could be crossed with diploids (Rio-de-Janeiro, Maran and Himachal Pradesh) and the percentage of cultures showing response ranged from 77.77 to 100.00 per cent in early, 83.33 to 100.00 per cent in mid and 66.66 to 100.00 per cent in late season. The number of ovules developed per culture ranged from 12.86 to 15.64 in early, 14.29 to 16.83 in mid and 12.99 to 15.09 in late season. It was found that mid season favoured more percentage of cultures (92.58 %) with maximum number of well developed ovules per culture (15.52).

The maximum percentage of cultures showing response was observed in cross Rio-de-Janeiro X Z – O – 78 (100 %) and Maran X Z – O – 78 (100 %) in early, Z – O –78 X Rio-de-Janeiro, Rio-de-Janeiro X Z – O – 78, Rio-de-Janeiro X Z – O – 86 and Maran X Z – O – 78 in mid and Rio-de-Janeiro X Z – O – 86 and Maran X Z – O – 78 in late season. The maximum number of ovules observed in Z – O –78 X Rio-de-Janeiro (15.83) in early, Z – O –86 X Rio-de-Janeiro (16.86) in mid and Rio-de- Janeiro X Z – O –78 (15.09) in late season. When the mean performance of the cultivars for the three seasons was compared, it was found that the cross Maran X Z – O – 78 resulted in maximum percentage of cultures with well developed ovules and the same was minimum in the cross Z – O – 78 X Himachal Pradesh and Himachal Pradesh X Z – O – 86. The number of ovules developed per culture ranged from 13.54 to 15.85. The crosses involving Himachal Pradesh either as female or pollen parent recorded low percentage of cultures with well developed ovules. The mean percentage of cultures showing response in the three seasons was 87.98 per cent and the mean number of ovules developed per culture was 14.75.

Table 50. Influence of crossing ginger cultivars on ovule development after *in vitro* placental pollination

Sl. No.	Cultivar	Early season		Mid season		Late season		Mean of cultivars	
		Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture	Cultures with well developed ovules (%)	Number of ovules developed per culture
1.	Z – 0 – 78 X Rio-de-Janeiro	88.88	15.83	100.00	15.96	83.33	14.92	90.74	15.57
2.	Z – 0 – 78 X Maran	83.33	14.92	88.88	15.84	88.88	14.63	87.03	15.13
3.	Z – 0 – 78 X Himachal Pradesh	66.66	14.36	83.33	14.63	77.77	13.83	75.92	14.27
4.	Z – 0 – 86 X Rio-de-Janeiro	83.33	15.83	94.44	16.86	88.88	14.32	88.88	15.67
5.	Z – 0 – 86 X Maran	77.77	14.99	94.44	15.29	77.77	15.08	83.33	15.12
6.	Z – 0 – 86 X Himachal Pradesh	66.66	14.03	88.88	15.05	77.77	14.29	77.77	14.46
7.	Rio-de-Janeiro X Z – 0 – 78	100.00	15.64	100.00	16.83	94.44	15.09	98.15	15.85
8.	Rio-de-Janeiro X Z – 0 – 86	94.44	14.26	100.00	15.89	100.00	14.43	98.15	14.86
9.	Maran X Z – 0 – 78	100.00	14.29	100.00	15.84	100.00	14.16	100.00	14.76
10.	Maran X Z – 0 – 86	88.88	13.33	88.88	14.29	94.44	12.99	90.73	13.54
11.	Himachal Pradesh X Z – 0 – 78	88.88	12.86	88.88	14.76	83.33	13.73	87.03	13.78
12.	Himachal Pradesh X Z – 0 – 86	77.77	12.93	83.33	14.98	66.66	14.03	75.92	13.98
Mean of season		84.72	14.44	92.58	15.52	86.66	14.29	87.98	14.75

Average of 18 observations, 20 DAP

Medium : Half MS + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CW 15 % v/v

4.7 POST POLLINATION CHANGES

4.7.1 Seed set and development after *in vitro* placental pollination

The seed set per culture and seed size after placental pollination differed markedly based on solid media and semisolid media tried (Table 51 and Figure 5). The seed set per culture and number of well developed ovules per culture at 80 DAP in case of semisolid medium were 66.66 per cent and 7.82 respectively while it was only 50 per cent and 5.93 respectively in solid medium.

The seed development measured in terms of the length and breadth of the ovule at intervals of 20 days commencing from 20 DAP to 80 DAP are presented in Table 51. The ovules on the day of anthesis measured a length of 0.55 mm and breadth of 0.34 mm. There was substantial increase in the size of the seeds at 20 DAP as they recorded a mean length of 1.84 mm and breadth of 0.92 mm in semisolid media while it was only 1.79 mm length and 0.88 mm breadth in solid medium. The length and breadth showed linear increase with advance in age irrespective of the medium upto 80 DAP. Seeds from semisolid medium registered a length of 2.54 mm and breadth of 1.63 mm at 80 DAP but it was only 2.30 mm length and 1.45 mm breadth in case of solid medium.

Seeds developed after placental pollination were creamy white during the initial stage of development which changed to purple red colour within a period of 30 to 35 DAP and to black within a period of 55 – 80 DAP.

Microscopic examination of the longitudinal sections of seeds at full maturity revealed two seed coats in arillate seeds, outer one being thick and inner one thin. The seed coat encloses a cavity, which is typical of monocots. In the cavity, endosperm with embedded embryo is seen.

4.8 SEED VIABILITY TEST USING TETRAZOLIUM STAINING

The longitudinal sections of seeds at 40 DAP and 80 DAP were soaked in tetrazolium and observed under stereo microscope. It was observed that the embryos were stained deeply red compared to the surrounding tissues. The embryos of the seeds of 40 DAP took more intense red colour than the seeds of 80 DAP.

Table 51. Seed set and seed development in ginger after *in vitro* placental pollination

Sl. No.	Treatments	Seed set per culture (%)	No. of ovules well developed per culture (80 DAP)	Initial size of ovule on the day of the anthesis		20 DAP		40 DAP		60 DAP		80 DAP	
				Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)
				0.55	.0.34								
1.	Solid medium	57.14	7.2	-	-	1.79	0.88	2.12	1.19	2.27	1.38	2.30	1.45
2.	Semi solid medium	71.42	8.9	-	-	1.84	0.92	2.39	1.24	2.48	1.57	2.54	1.63
Mean		64.28	8.05			1.82	0.9	2.26	1.22	2.38	1.48	2.42	1.54

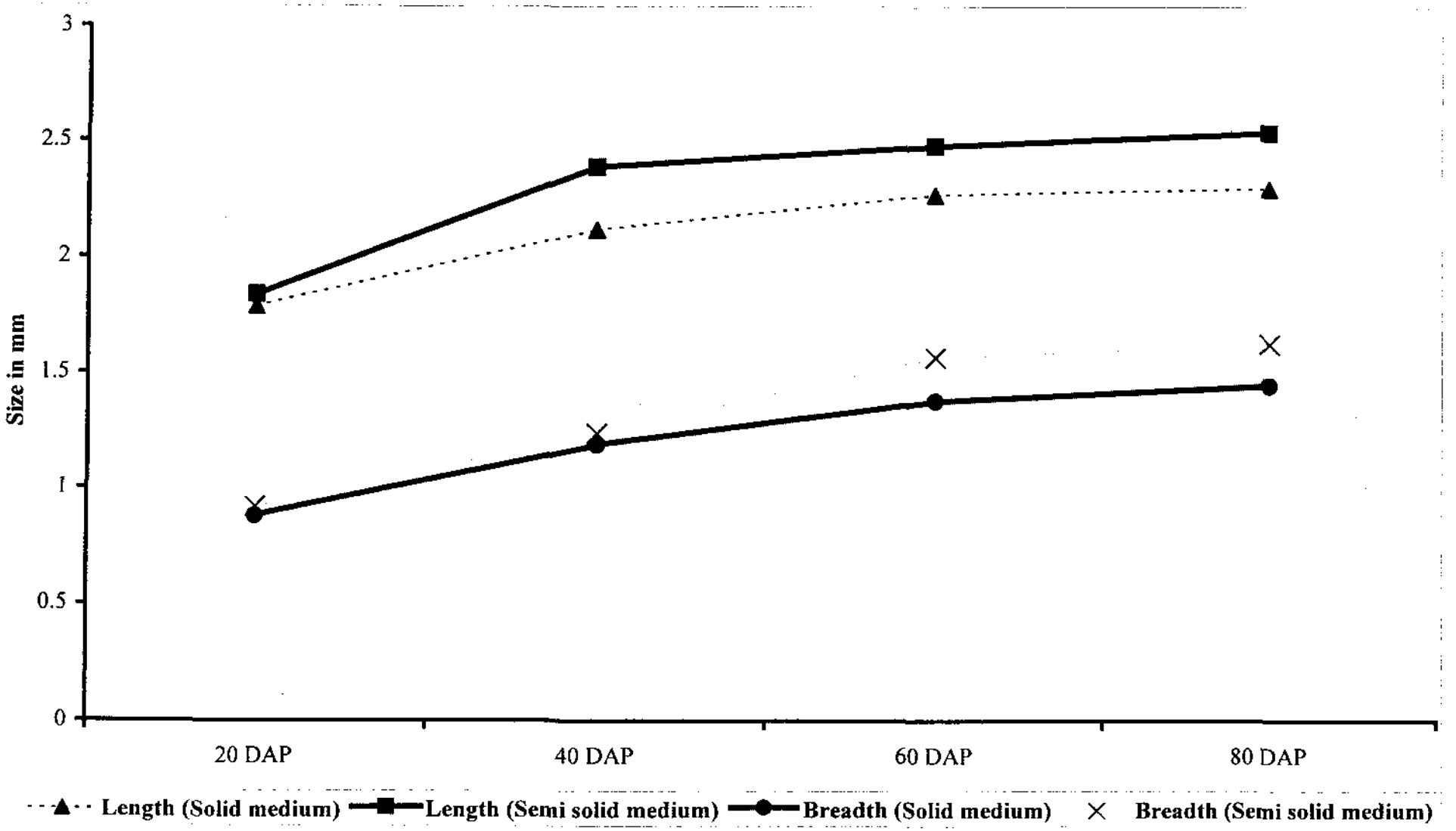


Fig. 5 Seed development in ginger after in vitro placental pollination

4.9 GERMINATION STUDIES OF *IN VITRO* PRODUCED SEEDS

4.9.1 Investigations on embryo rescue studies

4.9.1.1 Embryo culture

Embryos along with endosperm were isolated from seeds of 20, 40, 60 and 80 DAP and were cultured in basal medium of half MS, Knudson C, Taira and Larter's modified Norstog and Tomato embryo culture medium containing various auxins and cytokinins in different combinations. The various treatments had no favourable effect on germination of embryo (Table 52).

Table 52. Effect of media constituents on germination of embryo

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	NAA (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2) mg l^{-1}	Nil
2.	IAA (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2) mg l^{-1}	,,
3.	2, 4 - D (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2) mg l^{-1}	,,
4.	Basal medium alone	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Half MS + 3% sucrose + BAP (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 10, 15, 20) mg l^{-1}
Knudson C + 3% sucrose + BAP (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 10, 15, 20) mg l^{-1}
Taira and Larter's modified Norstog + BAP (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 10, 15, 20) mg l^{-1}
Tomato embryo culture+ 3% sucrose + BAP (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 10, 15, 20) mg l^{-1}
Explant – Embryos along with endosperm 20, 40, 60 and 80 DAP

4.9.1.2 Ovule culture

The pollinated ovules in half MS + BAP (2.5 mg l^{-1}) + NAA (0.5 mg l^{-1}) at different stages of development (5, 10, 15, 20, 25, 50, 80 DAP) were cultured in various medium (half MS, MS, Knudson C, SH, B_5, Taira and Larter's modified Norstog and Tomato embryo culture) alone and also with different concentrations of various growth regulators as furnished in Table 53. The treatments did not induce seed germination.

Table 53. Effect of media constituents on germination of ovule

Sl. No.	Growth regulators in mg l^{-1}	Germination (%)
1.	IAA (0.05, 0.1, 0.3, 0.5, 1.0)	Nil
2.	NAA (1.0, 1.5, 2.0, 2.5, 5.0)	,,
3.	2, 4 – D (0.1, 0.3, 0.5, 1.0, 2.0)	,,
4.	Dicamba (0.25, 0.5, 1, 2.5, 5.0)	,,
5.	BAP (5, 7.5, 10)	,,
6.	Kinetin (5, 7.5, 10)	,,
7.	GA_3 (1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0)	,,
8.	Ethylene (1.0, 3.0, 5.0)	,,
9.	IAA (0.05, 0.1, 0.3, 0.5, 1.0, 2.0) + BAP (0.5, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0)	,,
10.	NAA (0.1, 1.0, 1.5, 2.0, 2.5, 5.0) + BAP (1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0)	,,
11.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + BAP (0.1, 2.5, 5.0, 7.5, 9.0, 10.0, 15.0, 20.0)	,,
12.	IAA (0.05, 0.1, 0.2, 0.3, 0.5) + Kinetin (0.5, 2.0, 3.0, 5.0)	,,
13.	NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5.0) + Kinetin (0.5, 2.0, 3.0, 5.0)	,,
14.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + Kinetin (0.5, 2.0, 3.0, 5.0)	,,
15.	IAA (0.05, 0.1, 0.2, 0.3, 0.5) + GA_3 (2.0, 5.0, 9.0)*	,,
16.	NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5.0) + GA_3 (2.0, 5.0, 9.0)*	,,
17.	2, 4 - D (0.1, 0.3, 0.5, 1.0, 2.0) + GA_3 (2.0, 5.0, 9.0)*	,,
18.	NAA (0.1, 1.0, 1.5, 2.0, 2.5, 5.0) + BAP (1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0)*	,,
19.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA3 (1, 2.5, 5)*	,,
20.	Basal medium alone	,,

Average of 10 observations - 15, 25 and 30 days after culturing

* Addition of tomato juice (5%)

Media – Above treatment + Medium (Half MS, Full MS, Knudson C, SH, B_5, Tiara and Larter's modified Norstog and Tomato embryo culture medium) + 3% sucrose + 15 % coconut water (v/v)

Explant – 5, 10, 15, 20, 25, 50, 80 days old seeds after *In vitro* placental pollination

4.9.2 Effect of primary treatments on seed germination

The eighty days old seeds failed to germinate even after subjecting to various primary treatments like keeping on moist filter paper, moist sterile sand, moist sand with vermiculite (1:1), imbibition in water and basal medium such as MS, half MS, Knudson C, SH, B_5, Taira and Larter's modified Norstog medium and Tomato embryo culture medium as solid, liquid and semisolid forms (Table 54). ***In vitro*** **produced seeds at 80 DAP imbibed in water for one day when cultured in half MS + 2, 4 – D (0.5) mg l^{-1} + BAP (6.0) mg l^{-1} + GA_3 (5.0) mg l^{-1} with 3 per cent sucrose induced germination with radicle emergence after 2 weeks of inoculation.**

Table 54. Effect of primary treatments on germination of ginger seeds produced *in vitro*

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	Keeping on moist filter paper	Nil
2.	Keeping on moist sterile sand	,,
3.	Keeping on moist sand with vermiculite (1:1)	,,
4.	Basal medium MS, half MS, Knudson C, SH, B_5, Taira and Larter's modified Norstog and Tomato embryo culture (Solid, Semisolid, Liquid)	,,
5.	***In vitro*** **produced seed imbibed in water for one day and cultured in half MS + 2, 4 – D (0.5) mg l^{-1} + BAP (6.0) mg l^{-1} + GA_3 (5.0) mg l^{-1} with 3 per cent sucrose**	**10.00**

Average of 10 observations - 15, 25 and 30 days after culturing
Explant – *In vitro* produced seeds (80 days) after placental pollination

4.9.3 Influence of physical conditions of medium on seed germination.

The 80 DAP seeds were subjected to half MS and MS (solid, liquid and semisolid) medium with different concentrations of various growth regulators as given in Table 55. **After two to three weeks of inoculation, solid medium with half**

MS + 2, 4 – D (0.25) mg l^{-1} + BAP (9.0) mg l^{-1} with 3 per cent sucrose showed favourable influence on seed germination with emergence of radicle.

4.9.4 Pulse treatment with growth regulators

The various growth regulator treatments as listed in Table 56 for half an hour followed by inoculation in various media constituents did not show positive influence on seed germination.

4.9.5 Mechanical scarification

Mechanical scarification by pounding the seeds with sterile sand till the rupturing of the seed coat followed by culturing in half MS with various combinations of growth regulators as listed in Table 57 did not favour germination of ginger seeds.

4.9.6 Chemical scarification

Four acids (hydrochloric acid, sulphuric acid, nitric acid and salicyclic acid) at varying concentrations and duration as furnished in Table 58 were tried to enhance germination and the treatments failed to exhibit positive influence.

4.9.7 Stratification

Subjecting the seeds to low temperature treatment at 2 ^{0}C and 4 ^{0}C for varying duration from 3 h to 24 h revealed that stratification had no favourable influence on seed germination (Table 59).

4.9.8 Washing the seeds in running water and sowing

Washing the seeds in running water for varying duration from 3 h to 12 h followed by sowing failed to induce seed germination (Table 60).

4.9.9 Hydration – dehydration – rehydration treatment

Hydration – dehydration – rehydration treatment for varying duration as given in Table 61 failed to induce seed germination.

After imposing the treatments as detailed from 4.9.5 to 4.9.9, the seeds were then cultured in half MS alone and also with GA_3 (1.0, 5.0, 10.0) mg l^{-1} and ethylene

(0.25, 0.50, 0.75, 1.00) mg l^{-1} and also with 2, 4 – D (0.1, 0.3, 0.5) mg l^{-1}, BAP (2.5, 5.0, 10.0) mg l^{-1}, GA_3 (1.0, 2.0, 5.0) mg l^{-1} and GA_3 alone (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1} failed to show positive result of germination.

4.9.10 Treatment with solvents

The various solvent treatment at different concentration as given in Table 62 did not induce germination.

4.9.11 Treatment with NaCl, NaOH, HCN, thiourea and pectinase enzyme

Treating the seeds with NaCl, NaOH, HCN, thiourea and pectinase enzyme (Table 63) failed to exhibit any favourable effect on germination of seeds.

4.9.12 Influence of manual pricking of seeds

Soaking the seeds in water for one day followed by manual pricking by pins and culturing in medium did not show positive effect on germination.

4.9.13 Influence of cut at chalazal and micropylar end

The seeds after cutting at chalazal and micropylar and at both ends did not favour germination.

After imposing the treatments as detailed from 4.9.10 to 4.9.13, the seeds were then cultured in half MS, MS, Knudson C, Tomato embryo culture alone and also with combinations of 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1}. All the treatments failed to induce germination of seed.

4.9.14 Priming of seeds

Water soaked seeds for one day, unsoaked seeds, seeds cut at micropylar and chalazal and at both ends, pierced seeds with sterile pin were primed with different *seed priming chemicals at different concentrations and cultured in half MS and* Knudson C alone and also with different growth regulator concentrations as furnished in Table 64.

Only pierced seeds were imbibed in 1, 3, 5, 7, 10 per cent KNO_3 for 5 days and washed with sterile water and incubated in different combinations of growth

Table 55. Effect of physical conditions and media constituents on germination of ginger seeds produced *in vitro*

Sl. No.	Growth regulators in mg l^{-1}	Physical condition of medium	Germination (%)
1.	Half MS	Solid	Nil
2.	Full MS	Solid	,,
3.	Half MS + GA_3 (0.5, 5, 25, 50, 100) mg l^{-1}	Solid	,,
4.	Half MS + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00) mg l^{-1}	Solid	,,
5.	Half MS + 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} + BAP (2.5, 5.0, 10.0) mg l^{-1} + GA_3 (1.0, 2.0, 5.0) mg l^{-1}	Solid	,,
6.	Half MS	Semi solid	,,
7.	Full MS	Semi solid	,,
8.	Half MS + GA_3 (0.5, 5, 25, 50, 100) mg l^{-1}	Semi solid	,,
9.	Half MS + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00)mg l^{-1}	Semi solid	,,
10.	Half MS + 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} + BAP (2.5, 5.0, 10.0) mg l^{-1} + GA_3 (1.0, 2.0, 5.0) mg l^{-1}	Semi solid	,,
11.	Half MS	Liquid	,,
12.	Full MS	Liquid	,,
13.	Half MS + GA_3 (0.5, 5, 25, 50, 100) mg l^{-1}	Liquid	,,
14.	Half MS + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00)mg l^{-1}	Liquid	,,
15.	Half MS + 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} + BAP (2.5, 5.0, 10.0) mg l^{-1} + GA_3 (1.0, 2.0, 5.0) mg l^{-1}	Liquid	,,
16.	Half MS + 2,4 – D (0.1, 0.5) mg l^{-1} + BAP (3.0, 6.0) mg l^{-1}	Solid	,,
17.	Half MS + 2,4 – D (0.1, 0.5) mg l^{-1} + BAP (3.0, 6.0) mg l^{-1}	Semi solid	,,
18.	Half MS + 2,4 – D (0.1, 0.5) mg l^{-1} + BAP (3.0, 6.0) mg l^{-1}	Liquid	,,
19.	**Half MS + 2,4 – D (0.25) mg l^{-1} + BAP (9.0) mg l^{-1}**	**Solid**	**30.00**
20.	Half MS + 2,4 – D (0.25) mg l^{-1} + BAP (9.0) mg l^{-1}	Semi solid	Nil
21.	Half MS + 2,4 – D (0.25) mg l^{-1} + BAP (9.0) mg l^{-1}	Liquid	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Above combinations with 3 % sucrose
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 56. Effect of pulse treatment and media constituents on germination of ginger seeds produced *in vitro*.

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	IAA (0.1, 0.5) + BAP (5, 10)	Nil
2.	IAA 0.5 + GA_3 (1, 2.5, 5)	,,
3.	IAA 0.5 + Ethylene (1, 3, 5)	,,
4.	NAA (1, 2.5) + BAP (5, 10)	,,
5.	NAA 2.5 + GA_3 (1, 2.5, 5)	,,
6.	NAA 2.5 + Ethylene (1, 3, 5)	,,
7.	2, 4 – D (0.25, 0.5) + BAP (7.5, 10)	,,
8.	2, 4 – D 0.5 + GA_3 (1, 2.5, 5)	,,
9.	2, 4 – D 0.5 + Ethylene (1, 3, 5)	,,
10.	BAP (5, 7.5, 10) + GA_3 (1, 2.5, 5)	,,
11.	BAP (5, 7.5, 10) + Ethylene (1, 3, 5)	,,
12.	GA_3 (1, 2.5, 5) + Ethylene (1, 3, 5)	,,
13.	IAA (0.1, 0.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)	,,
14.	IAA (0.1, 0.5) + BAP (5, 10) + Ethylene (1, 3, 5)	,,
15.	NAA (1, 2.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)	,,
16.	NAA (1, 2.5) + BAP (5, 10) + Ethylene (1, 3, 5)	,,
17.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA_3 (1, 2.5, 5)	,,
18.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + Ethylene (1, 3, 5)	,,
19.	Basal medium alone	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Above treatment + Medium (Half MS and Full MS) + 3% sucrose
Explant – *In vitro* seeds (80DAP) after pulse treatment for half an hour as given below
IAA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250) mgl^{-1}
NAA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250) mgl^{-1}
2,4-D (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250) mgl^{-1}
IBA (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250) mgl^{-1}
Dicamba (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250) mgl^{-1}
BAP (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500) mgl^{-1}
Kinetin (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500) mgl^{-1}
GA_3 (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000) mgl^{-1}
Ethylene (0.1, 0.5, 1.0, 2.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000) mgl^{-1}

Table 57. Effect of mechanical scarification and media constituents on germination of ginger seeds produced *in vitro*

Sl. No	Growth regulators in mg l^{-1}	Germination (%)
1.	Half MS	Nil
2.	Half MS + GA_3 (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1}	,,
3.	Half MS + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00) mg l^{-1}	,,
4.	Half MS + 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} + BAP (2.5, 5.0, 10.0) mg l^{-1} + GA_3 (1.0, 2.0, 5.0) mg l^{-1}	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Above combinations with 3 % sucrose
Explant – *In vitro* seeds (80 DAP) after mechanical scarification

Table 58. Effect of chemical scarification and media constituents on germination of ginger seeds produced *in vitro*

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	HCl (10 %, 25%, 50%) for 1 minute	Nil
2.	HCl (10 %, 25%, 50%) for 3 minutes	,,
3.	HCl (10 %, 25%, 50%) for 5 minutes	,,
4.	HCl (10 %, 25%, 50%) for 7 minutes	,,
5.	HCl (10 %, 25%, 50%) for 10 minutes	,,
6.	HNO_3 (10 %, 25%, 50%) for 1 minute	,,
7.	HNO_3 (10 %, 25%, 50%) for 3 minutes	,,
8.	HNO_3 (10 %, 25%, 50%) for 5 minutes	,,
9.	HNO_3 (10 %, 25%, 50%) for 7 minutes	,,
10.	HNO_3 (10 %, 25%, 50%) for 10 minutes	,,
11.	H_2SO_4 (10 %, 25%, 50%) for 1 minute	,,
12.	H_2SO_4 (10 %, 25%, 50%) for 3 minutes	,,
13.	H_2SO_4 (10 %, 25%, 50%) for 5 minutes	,,
14.	H_2SO_4 (10 %, 25%, 50%) for 7 minutes	,,
15.	H_2SO_4 (10 %, 25%, 50%) for 10 minutes	,,
16.	SA (0.001%, 0.01%, 0.1%, 0.5%) for 1 minute	,,
17.	SA (0.001%, 0.01%, 0.1%, 0.5%) for 3 minutes	,,
18.	SA (0.001%, 0.01%, 0.1%, 0.5%) for 5 minutes	,,
19.	SA (0.001%, 0.01%, 0.1%, 0.5%) for 7 minutes	,,
20.	SA (0.001%, 0.01%, 0.1%, 0.5%) for 10 minutes	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Half MS + 3% sucrose
Half MS + 3% sucrose +GA_3 (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1}
Half MS + 3% sucrose + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00)mg l^{-1}
Half MS + 3% sucrose + 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} + BAP (2.5, 5.0, 10.0) mg l^{-1}
+ GA_3 (1.0, 2.0, 5.0) mg l^{-1}
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 59. Effect of stratification and media constituents on germination of ginger seeds produced *in vitro*

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	Incubating at 2 ^{0}C and 4 ^{0}C for 3 h	Nil
2.	Incubating at 2 ^{0}C and 4 ^{0}C for 6 h	,,
3.	Incubating at 2 ^{0}C and 4 ^{0}C for 9 h	,,
4.	Incubating at 2 ^{0}C and 4 ^{0}C for 12 h	,,
5.	Incubating at 2 ^{0}C and 4 ^{0}C for 15 h	,,
6.	Incubating at 2 ^{0}C and 4 ^{0}C for 18 h	,,
7.	Incubating at 2 ^{0}C and 4 ^{0}C for 21 h	,,
8.	Incubating at 2 ^{0}C and 4 ^{0}C for 24 h	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Half MS + 3% sucrose
Half MS + 3% sucrose + GA_3 (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1}
Half MS + 3% sucrose + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00)mg l^{-1}
Half MS + 3% sucrose+2,4 – D (0.1, 0.3, 0.5) mg l^{-1}+BAP (2.5, 5.0, 10.0) mg l^{-1}+GA_3 (1.0, 2.0, 5.0)mg l^{-1}
Explant – *In vitro* seeds (80 DAP) placental pollination

Table 60. Effect of washing in running water and media constituents on germination of ginger seeds produced *in vitro*

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	Washing the seeds in running water for 3 h	Nil
2.	Washing the seeds in running water for 6 h	,,
3.	Washing the seeds in running water for 9 h	,,
4.	Washing the seeds in running water for 12 h	,,

Average of 12 observations - 15, 25 and 30 days after culturing
Media – Half MS + 3% sucrose
Half MS + 3% sucrose +GA3 (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1}
Half MS + 3% sucrose + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00) mg l^{-1}
Half MS + 3% sucrose+2,4 – D (0.1, 0.3, 0.5) mg l^{-1}+BAP (2.5, 5.0, 10.0) mg l^{-1}+GA_3 (1.0, 2.0, 5.0)mg l^{-1}
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 61. Effect of hydration – dehydration and rehydration and media constituents on germination of ginger seeds produced *in vitro*

Sl. No	Growth regulators in mg l^{-1}	Germination (%)
1.	Hydration for 12 h, dehydration for 3 h and rehydration for 12 h	Nil
2.	Hydration for 12 h, dehydration for 6 h and rehydration for 12 h	,,
3.	Hydration for 12 h, dehydration for 9 h and rehydration for 12 h	,,
4.	Hydration for 12 h, dehydration for 12 h and rehydration for 12 h	,,

Average of 12 observations - 15, 25 and 30 days after culturing
Media – Half MS + 3% sucrose
Half MS + 3% sucrose +GA_3 (0.5, 5.0, 25.0, 50.0, 100.0) mg l^{-1}
Half MS + 3% sucrose + GA_3 (1.0, 5.0, 10.0) mg l^{-1} + Ethylene (0.25, 0.50, 0.75, 1.00)mg l^{-1}
Half MS + 3% sucrose+2,4 – D (0.1, 0.3, 0.5) mg l^{-1}+BAP (2.5, 5.0, 10.0) mg l^{-1}+GA_3 (1.0, 2.0, 5.0)mg l^{-1}
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 62. Effect of solvents and media constituents on germination of ginger seeds produced *in vitro*

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	Acetone (1, 5, 10, 20 %) for 1 minute	Nil
2.	Acetone (1, 5, 10, 20 %) for 5 minutes	,,
3.	Acetone (1, 5, 10, 20 %) for 10 minutes	,,
4.	Methanol (1, 5, 10, 20 %) for 1 minute	,,
5.	Methanol (1, 5, 10, 20 %) for 5 minutes	,,
6.	Methanol (1, 5, 10, 20 %) for 10 minutes	,,
7.	Ethanol (1, 5, 10, 20 %) for 1 minute	,,
8.	Ethanol (1, 5, 10, 20 %) for 5 minutes	,,
9.	Ethanol (1, 5, 10, 20 %) for 10 minutes	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – (Half MS, Full MS, Knudson C, Tomato embryo culture medium) + 3% sucrose alone and supplemented with 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} +BAP (2.5, 5.0, 10.0) mg l^{-1}+GA_3 (1.0, 2.0, 5.0) mg l^{-1}
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 63. Effect of NaCl, NaOH, HCN, thiourea, pectinase enzyme and media constituents on germination of ginger seeds produced *in vitro*.

Sl.No.	Growth regulators in mg l^{-1}	Germination (%)
1.	NaCl (0.1, 0.25, 0.5, 1) % for 10 minutes	Nil
2.	NaOH (1, 5, 10, 15, 20) % for 10 minutes	,,
3.	HCN (2, 4, 6, 8, 10) mg l^{-1} for 10 minutes	,,
4.	Thiourea (0.1, 0.25, 0.5, 0.75, 1) % for 10 minutes	,,
5.	Pectinase (1, 5, 10, 15, 20) % for 10 minutes	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – (Half MS, Full MS, Knudson C, Tomato embryo culture medium) + 3% sucrose alone and supplemented with 2,4 – D (0.1, 0.3, 0.5) mg l^{-1} +BAP (2.5, 5.0, 10.0) mg l^{-1}+GA_3 (1.0, 2.0, 5.0) mg l^{-1}
Explant – *In vitro* seeds (80 DAP) after placental pollination

Table 64. Effect of seed priming and media constituents on germination of ginger seeds produced *in vitro*

Sl. No.	Growth regulators in mg l^{-1}	Germination (%)
1.	IAA (0.1, 0.5, 1)	Nil
2.	NAA (1, 2.5, 5)	,,
3.	2, 4 – D (0.25, 0.5, 1)	,,
4.	Dicamba (0.25, 0.5, 1)	,,
5.	BAP (5, 7.5, 10)	,,
6.	Kinetin (5, 7.5, 10)	,,
7.	GA_3 (1, 2.5, 5)	,,
8.	Ethylene (1, 3, 5)	,,
9.	IAA (0.1, 0.5) + BAP (5, 10)	,,
10.	IAA (0.1, 0.5) + Kinetin (5, 10)	,,
11.	IAA 0.5 + GA_3 (1, 2.5, 5)	,,
12.	IAA 0.5 + Ethylene (1, 3, 5)	,,
13.	NAA (1, 2.5) + BAP (5, 10)	,,
14.	NAA (1, 2.5) + Kinetin (5, 10)	,,
15.	NAA 2.5 + GA_3 (1, 2.5, 5)	,,
16.	NAA 2.5 + Ethylene (1, 3, 5)	,,
17.	2, 4 – D (0.25, 0.5) + BAP (7.5, 10)	,,
18.	2, 4 – D (0.25, 0.5) + Kinetin (7.5, 10)	,,
19.	2, 4 – D 0.5 + GA_3 (1, 2.5, 5)	,,
20.	2, 4 – D 0.5 + Ethylene (1, 3, 5)	,,
21.	BAP (5, 7.5, 10) + GA_3 (1, 2.5, 5)	,,
22.	BAP (5, 7.5, 10) + Ethylene (1, 3, 5)	,,
23.	Kinetin (5, 7.5, 10) + GA_3 (1, 2.5, 5)	,,
24.	Kinetin (5, 7.5, 10) + Ethylene (1, 3, 5)	,,
25.	GA_3 (1, 2.5, 5) + Ethylene (1, 3, 5)	,,

Table 64. Effect of seed priming and media constituents on germination of ginger seeds produced *in vitro* (continuation)

Sl. No.	Growth regulators in mg l^{-1}	Germination (%)
26.	IAA (0.1, 0.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)	,,
27.	IAA (0.1, 0.5) + BAP (5, 10) + Ethylene (1, 3, 5)	,,
28.	IAA (0.1, 0.5) + Kinetin (5, 10) + GA_3 (1, 2.5, 5)	,,
29.	IAA (0.1, 0.5) + Kinetin (5, 10) + Ethylene (1, 3, 5)	,,
30.	NAA (1, 2.5) + BAP (5, 10) + GA_3 (1, 2.5, 5)	,,
31.	NAA (1, 2.5) + BAP (5, 10) + Ethylene (1, 3, 5)	,,
32.	NAA (1, 2.5) + Kinetin (5, 10) + GA_3 (1, 2.5, 5)	,,
33.	NAA (1, 2.5) + Kinetin (5, 10) + Ethylene (1, 3, 5)	,,
34.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + GA_3 (1, 2.5, 5)	,,
35.	2, 4 – D (0.25, 0.5, 1) + BAP (7.5, 10) + Ethylene (1, 3, 5)	,,
36.	2, 4 – D (0.25, 0.5, 1) + Kinetin (7.5, 10) + GA_3 (1, 2.5, 5)	,,
37.	2, 4 – D (0.25, 0.5, 1) + Kinetin (7.5, 10) + Ethylene (1, 3, 5)	,,
38.	IAA (0.1, 0.5) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)	,,
39.	IAA (0.1, 0.5) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)	,,
40.	NAA (1, 2.5) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)	,,
41.	NAA (1, 2.5) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)	,,
42.	2, 4 – D (0.25, 0.5, 1) + BAP (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5, 5)	,,
43.	2, 4 – D (0.25, 0.5, 1) + Kinetin (3, 6, 10) + GA_3 (1, 2.5, 5) + Ethylene (2.5,5)	,,
44.	Basal medium alone	,,
45.	**KNO_3 (50) ppm for 8 h treated *in vitro* ginger seeds cultured in half MS + 2, 4 – D (0.5) mg l^{-1} + BAP (6.0) mg l^{-1} + GA_3 (5.0) mg l^{-1} with 3 per cent sucrose**	**10.00**

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Above growth regulator treatment + Medium (Half MS, Full MS, Knudson C, Tomato embryo culture medium) + 3% sucrose
Explant – *In vitro* (80 DAP) produced seeds after treating with seed priming as given below
PEG 4000, PEG 6000 and Mannitol (3, 6, 9, 12, 15) % for 5 days
KCl, KH_2PO_4, Na_2HPO_4 and $CaCO_3$ (0.25, 0.5, 1.0, 2.0) % for 6 h
KNO_3 (1.0, 3.0, 5.0, 7.0, 10.0) % for 5 days
KNO_3 (25, 50, 100, 250, 500, 750, 1000) ppm for 8 h

regulators with different media as per Table 64. The treatments had no influence on seed germination.

Unsoaked seed of eighty days old after treating with 50 ppm KNO_3 for 8 h and cultured in half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose showed positive effect on germination with good root development after 2 weeks of inoculation. All other treatments failed to show positive response on germination

4.9.15 *In vivo* seed germination studies

After the various pre-sowing treatments as detailed from 4.9.4 to 4.9.14, the seeds were sown in sterile moist sand and moist filter paper. However, none of the treatments was effective for inducing seed germination.

4.9.16 Germinated seeds, their parentage and its media combinations

In all the above seed germination studies, the seeds selected were obtained through crosses between (autotetraploid and diploid), (diploid and autotetraploid), (diploid and diploid) and (autotetraploid and autotetraploid). However, the seed obtained through cross between diploid (Rio-de-Janeiro) and autotetraploid (Z – O – 78) germinated producing radicle after seed priming treatment (50 ppm KNO_3 for 8 h) and also the cross of autotetraploid (Z – O – 86) X diploid (Maran) after seed imbibing in water for one day when cultured in half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose.

Seed of cross between diploid (Rio-de-Janeiro) and diploid (Maran) and diploid (Rio-de-Janeiro) X autotetraploid (Z – O – 86) showed positive influence on germination showing radicle emergence after culturing in solid medium of half MS + 2, 4 – D (0.25) mg l^{-1} and BAP (9.0) mg l^{-1} along with 3 per cent sucrose.

4.9.17 Somatic embryoid formation

Somatic embryoids were developed from seeds of 80 DAP cultured in media combinations of half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose and also in half MS + 2, 4 – D (0.1) mg l^{-1}, BAP (8.0) mg l^{-1} mg l^{-1} along with 3 per cent sucrose after one month of inoculation. Maximum

somatic embryoids (4 - 5 per culture) were observed in media combination of half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose in the cross between diploid and autotetraploid while only (1 – 2 per culture) were obtained in cross between autotetraploid and diploid. No somatic embryoid formation was observed in crosses between diploid and diploid or autotetraploid and autotetraploid. One to two months old somatic embryoids were transferred to half MS and MS medium containing (2, 6, 8) ppm BAP and (0.1 to 1.0) ppm NAA + (1.0) ppm BAP and (0.2 to 0.5) ppm 2, 4 – D + (6.0 to 10.0) ppm BAP along with 3 per cent sucrose and incubated for 4 months (Table 64). There was no positive sign of plant regeneration.

Table 65. Effect of growth regulators on plant regeneration from somatic embryos of ginger.

Sl. No.	Growth regulators in mg l^{-1}	Plant regeneration
1.	BAP (2.0, 6.0, 8.0, 9.0)	Nil
2.	BAP (1.0) + NAA (0.1, 0.3, 0.5, 0.75, 1.0)	,,
3.	BAP (6.0, 7.5, 9.0, 10.0) + 2,4 – D (0.2, 0.25, 0.3, 0.5)	,,
4.	Basal medium alone	,,

Average of 10 observations - 15, 25 and 30 days after culturing
Media – Above treatments + Medium (Half MS, Full MS) + 3% sucrose
Explant – Somatic embryoids of ginger

4.9.18 Callus growth

Callus growth was observed after 25 days of seed germination in the germinated seed culture media containing half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose.

DISCUSSION

5. DISCUSSION

The investigations on "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during 2001 – 2003.

Ginger is propagated exclusively by vegetative means. Even then, the existing population shows variability for morphological, economic and qualitative traits including pest and disease resistance. Induction of variability through colchiploidy attempted at the Department of Plantation Crops and Spices during 1996 resulted in evolution of two autotetraploids and five variants. The available variability is not fully exploited for crop improvement, as hybridisation is not possible in this crop due to lack of seed set and fruit formation under natural conditions. Earlier attempts towards *in vivo* pollination by adopting different methods such as bud pollination, artificial cross pollination, chemical aided pollination, mentor pollination and pollination after decapitating stigma failed to induce seed set (Usha, 1984 and Sathiabhama, 1988).

The technique of *in vitro* pollination has been proved promising for overcoming pre-fertilization barriers due to incompatibility and for raising new genotypes. Valsala (1994), Renjith (1999) and Bhurke (2002) found that *in vitro* pollination technique involving application of pollen grains in a germinating medium in close vicinity of ovules effects fertilization in ginger, turmeric and kacholam respectively. Success was also obtained in seed set and seed development. However, the germination of *in vitro* produced seeds continues to be highly erratic and the conditions for successful regeneration are yet to be perfected.

The major aspects of the present investigations are

1. Evaluation of induced variants for variability in morphological characters, anatomical features, floral characters, economical attributes, quality attributes and incidence of pests and diseases.
2. Response to *in vivo* pollination.
3. Response to *in vitro* pollination.

4. Pollen pistil interaction.
5. Standardization of media and supplements for culture establishment.
6. Attempting controlled selfing and crossing among the selected cultivars.
7. Post pollination changes.
8. Seed viability test using tetrazolium staining.
9. Germination studies.

The results generated from the studies are discussed in this chapter.

5.1 EVALUATION STUDIES

5.1.1 Morphological characters

The variability in selected cultivars was examined for salient morphological characters and the results showed that the cultivars differed significantly with respect to various morphological characters except pseudostem circumference, leaf area and leaf area index (Tables 15, 16 and 17). Nair (1975) also reported that there was no significant difference in leaf area and leaf area index among the five types of ginger. V_1 recorded the maximum plant height and pseudostem height. V_{10} recorded maximum pseudostem thickness and circumference. Maximum number of tillers per plant and maximum number of leaves per plant were observed in V_2, whileV_9 had maximum number of leaves per tiller. Colour of the leaf varied from light green to dark green in the upper and lower surface with linear lanceolate shape common to all cultivars. Hairiness was absent on both surfaces of leaf and stipules were absent in all the selected cultivars. Maximum leaf length and breadth were observed in V_9 and V_3 respectively. Leaf was extremely thick in V_{10} when compared to lowest V_9. Autotetraploid (V_2) derived from Rio-de-Janeiro showed maximum leaf area with maximum leaf area index. . Number of roots was found high in V_8 whereas V_9 produced lengthy roots. V_4 was the most inferior cultivar with less plant height, pseudostem height, number of tillers per plant, number of leaves per tiller, number of leaves per plant, leaf area, leaf area index and root length, while V_5 recorded the lowest number of roots. Significant variation among ginger cultivars with regard to various morphological characters has been reported (Nybe, 1978; Mohanty *et al.*, 1981; Ravindran *et al.*, 1994; Korla and Tiwari,1999 and Singh 2001).

Maximum number of roots was observed in highest yielder (V_8), which was on par with V_{10}, V_3, V_2 and V_1. V_{10} had maximum pseudostem circumference and thickness and

leaf thickness while V_3 had maximum leaf breadth. The autotetraploid (V_2) derived from V_8 showed maximum number of tillers per plant, number of leaves per plant, root length, leaf area and leaf area index whereas autotetraploid (V_1) derived from V_{10} recorded maximum plant height and pseudostem height. The lowest yielder V_4 was found inferior with less plant height, pseudostem height, pseudostem circumference, lesser number of tillers per plant, number of leaves per tiller as well as per plant, leaf area and leaf area index.

5.1.2 Anatomical features

It is evident from the Table 18 that the ginger cultivars showed significant variation with respect to cuticle thickness, stomatal number, length and width and number as well as size of epidermal cells. Cuticle is a layer of wax like covering on the epidermis of leaves and it is meant to check transpiration. Upto 20 per cent of the total transpiration takes place through it and with the increase in the thickness of the cuticle the loss of water vapour is reduced. It also provides protection against some plant pathogens and against minor mechanical damage. It is of additional importance in agriculture, because it repels water used in various sprays containing fungicides, herbicides, insecticides or growth regulators. Because of the hydrophobic nature of the cuticle, most spray formulations contain a detergent to reduce the surface tension of water and allow it to spread on the foliage (Salisbury and Ross, 1999). Cuticle thickness of ginger cultivars ranged from 16.36 µm in V_5 to 24.54 µm in V_1. Maximum cuticle thickness was observed in autotetraploid V_1 followed by the corresponding diploid V_{10} which may have adaptation for water shortage areas due to reduced water loss through transpiration. All the variants recorded less stomatal number and increased size than diploids. Stomata are minute pores of elliptical shape surrounded by epidermal cells, mostly situated on leaves and maximum diffusion of water vapour and gaseous exchange take place through these pores. The loss of water vapour through stomata amount to about 80 – 90 per cent of the total loss (Pandey and Sinha, 1999). V_7, a variant had lesser number of stomata while V_8 had highest number. Both the autotetraploids (V_1 and V_2) recorded lesser stomatal number with increased size when compared to their corresponding diploids (V_{10} and V_8). Letchamo *et al.* (1994) also observed the lowest number of stomata with larger stomatal area in tetraploids of *Chamomilla recutita* when compared to diploids. The doubling of

chromosome number is associated with increase in cell size and such increase may be reflected in pronounced increase in the size of stomata (Sheeba, 1996). Stomatal length and width ranged among the cultivars from 147.24 to 212.68 μm and 81.00 to 130.88 μm respectively. Autotetraploid had lesser number of epidermal cells with maximum cell area. The above results are in accordance with the reports of Srivastava and Raina (1992) in *Clitoria ternatea* and Sheeba (1996) in ginger.

5.1.3 Floral characters

Among the ten cultivars studied, flowering was observed in V_1 (Z – O – 78), V_2 (Z – O – 86), V_8 (Rio-de-Janeiro), V_9 (Maran) and V_{10} (Himachal Pradesh). Cultivar difference with respect to floral characters was observed (Table 19). Nybe (1978) and Valsala (1994) also reported varietal difference in flowering and this variation in different types indicates that flowering is influenced by planting time, genotype and agroclimatic conditions.

5.1.3.1 Flowering season

The data on days to flowering revealed that flowering occurred within a period of 133.6 days (V_9) to 164.8 days (V_{10}) with a mean of 151.8 days after planting in the five cultivars. Cultivar difference was noted and V_9 (Maran) was the earliest flowering cultivar whereas V_{10} (Himachal Pradesh) and V_8 (Rio-de-Janeiro) were late flowering cultivars. V_1 (Z – O – 78) and V_2 (Z – O – 86) autotetraploids from Himachal Pradesh and Rio-de-Janeiro respectively flowered early compared to the diploids. V_8 (Rio-de-Janeiro) had maximum flowering duration (54 days) with maximum percentage of flowering (88.53 %) whereas V_2 (Z – O – 86) the corresponding autotetraploid had minimum flowering duration (25 days) and minimum percentage of flowering (34.72 %). But the cultivar V_1 (Z – O – 78), an autotetraploid from Himachal Pradesh showed enhanced flowering duration (40 days) when compared to V_{10} (32 days). This is in conformity with the finding of Valsala (1994) that early and late flowering cultivars were Maran and Rio-de-Janeiro respectively and that duration of flowering was maximum in Rio-de-Janeiro. The protracted flowering of Rio-de-Janeiro is advantageous for crossing between specified cultivars.

5.1.3.2 Inflorescence development and floral biology

Ginger inflorescence is a bracteate spike. In the present study, the spike required 28.33 to 30.33 days from initiation to first blooming and 13.66 to 22.33 days for completion of blooming which supports the observation of Usha (1984). Valsala (1994) also observed that ginger inflorescence took 30 to 32 days from initiation to first blooming and 9 to 18 days for completion of blooming which may be due to cultivar difference. Observations on inflorescence development revealed that the mean total length of the inflorescence was 18.00 cm with scape length of 12.91 cm and cone length of 5.09 cm, which was in accordance with the reports of Valsala (1994). In the flowered cultivars, the inflorescence emerged both from rhizome and shoot tip, maximum being from rhizomes. V_8 (Rio-de-Janeiro) had maximum number of inflorescence per plant (5.67) bearing maximum number of flowers per plant (117.14). The autotetraploids V_1 (Z – O – 78) and V_2 (Z – O – 86) recorded low flowering percentage and low number of flowers per scape compared with the corresponding diploids V_{10} (Himachal Pradesh) and V_8 (Rio-de-Janeiro). But the number of flowers per plant was higher in V_1 due to increased number of inflorescence per plant. Anthesis occurred between 3.00 and 4.00 p.m and anther dehiscence between 4.30 and 5.00 p.m (Table 20). According to Pillai *et al.* (1978) and Jayachandran *et al.* (1979), pollen shedding almost coincided with flower opening. On the contrary in the present study, a time lag of ½ to 2 h was observed between anthesis and anther dehiscence which supports the finding of Valsala (1994). Usha (1984) observed a gap of 10 to 25 min between anthesis to dehiscence.

5.1.3.3 Morphology of flowers

The flowers are small, borne in the axil of a bract. Flower length differed depending on the position of inflorescence of different cultivars (Table 21). The flowers attained maximum length at lower portion (3.72 cm) followed by middle (3.62 cm) and upper portion (3.43 cm) of inflorescence. V_1 (Z – O – 78), an autotetraploid recorded maximum flower length (3.69 cm) whereas minimum length (3.53 cm) was observed in diploid V_8 (Rio-de-Janeiro). Flowers and floral parts were bigger in tetraploids than diploids as already reported by Letchamo *et al.* (1994) in *Chamomilla recutita*, Ramachandran (1982) and Ramachandran and Nair (1992) in *Zingiber officinale*. The tubular calyx and

corolla were observed below with three yellowish lobes of which the dorsal lobe was curved over the anther. The posterior stamen of the inner whorl was the only fertile stamen and was enclosed by the deep purple coloured labellum. Stamen has short, broad filament and two prominent anther lobes. The pistil of the flower was characterized by a spiny stigma and a long style of mean length of 3.38 cm, which passes through the groove present between the two fertile anther lobes. Style length also differed with respect to flowers emerging at different positions of the inflorescence and it was maximum in the lower portion. V_1 with maximum flower length also recorded maximum style length (3.47 cm). The ovary which is trilocular recorded a mean length of 2.25 mm and diameter of 2.23 mm. The cultivar V_9 (Maran) produced the largest ovary with maximum length (3.00 mm) and breadth (3.02 mm) with the maximum number of ovules (23.8). The mean number of ovules per ovary was 21.56. The ovules measured a mean length of 549.02 µm and diameter of 338.98 µm. It was also observed that the cultivars V_1(autotetraploid derived from Himachal Pradesh), V_2 (autotetraploid derived from Rio-de-janeiro) and V_9 (Maran) produced bold ovules than the other diploids. Since the ovary contains 21.56 ovules, in successful seed set and development, 21 seeds per ovary can be expected. The observations on ovary and ovule size will be helpful monitoring their growth *in vitro*. The above observations almost corresponded with those made by Valsala (1994) in ginger cultivars.

5.1.3.4 Pollen morphology

Ginger flowers carry enough pollen grains in their single bilobed anthers. The pollen grains are round, highly heterogenous in size and filling and are limited by a thick exine. A well defined pore is absent in the pollen grains. This is in agreement with the observations made earlier by Sathiabhama (1988) and Valsala (1994).

5.1.3.5 Influence of season on size of pollen grains of ginger cultivars

The size of the pollen grains was found influenced by the season and it was observed that the pollen size was high during early season (95.43 µm) compared to mid (92.71 µm) and late season (88.33 µm) (Table 22). The larger sized pollen grains of diameter 70.89 µm during late season to 109.07 µm during early season were well stained

while smaller sized grains of 32.72 µm during mid season to 65.45 µm during early season were not stained. The size of the pollen grains varied from 38.18 µm to 103.62 µm among cultivars. These observations are also in agreement with those of Valsala (1994) who observed a range of 53.32 µm to 94.64 µm for pollen size. Cultivar difference was observed in pollen size. The autotetraploids recorded increased pollen diameter compared to corresponding diploids.

5.1.3.6 Influence of season and position of flowers in the inflorescence on pollen fertility and viability of ginger cultivars

The fertility of the pollen grains was found influenced by the season and position of flowers in the inflorescence (Table 23 and 24). The fertility was high during mid season (46.01 %) compared to late (36.62 %) and early season (21.38 %). The flowers emerging from upper (36.74 %) and lower (36.03 %) portions of the inflorescence recorded high fertility compared to middle portion (31.24 %). These observations are also in agreement with the reports of Pillai *et al.* (1978), who observed 35 per cent fertility while Sathiabhama (1988) recorded 15.58 to 39.67 per cent pollen fertility. But Usha (1984) reported only 12 to 16 per cent fertility. Valsala (1994) reported a pollen fertility ranging from 14.82 to 35.28 per cent. Bindu (1997) reported a mean pollen fertility of 12.05 per cent in early, 29.10 per cent in mid and 14.40 per cent in late season while 18.55 per cent in lower, 20.66 per cent in middle and 19.7 per cent in upper position of flowers in the inflorescence.

The influence of season and position of flowers in the inflorescence on viability of pollen grains did not follow the same trend as that of fertility and it was high in early (8.36 %) followed by mid (7.17 %) and late season (6.52 %) (Table 23 and 24). The results also indicated maximum viability from lower (9.18 %) when compared to middle (7.48 %) and upper (6.02%) portions of the inflorescence. The mean pollen germination reported in ginger by various workers in artificial medium was in the range of 2 to 15 per cent (Nair *et al.*, 1975; Pillai *et al.*, 1978; Jayachandran *et al.*, 1979 and Sathiabhama, 1988). But the ME_3 medium had registered a mean pollen germination of 15.06 per cent as reported by Valsala (1994). Bindu (1997) reported a mean pollen viability of 5.89 per cent in early, 5.06 per cent in mid and 2.84 per cent in late season

while 4.58 per cent in lower, 5.91 per cent in middle and 3.78 per cent in upper position of flowers in the inflorescence.

Even though pollen fertility was fairly high especially in autotetraploids, the viability in ME_3 medium was not correspondingly high. This may be due to the fact that acetocarmine test and ME_3 medium may not be fully efficient in bringing about full fertility and viability respectively. Therefore, tests other than acetocarmine method may be thought off for assessment of pollen fertility.

The aforesaid observations suggest that pollination during early and mid flowering season and taking pollen from flowers on lower and middle portions of inflorescence can result in better seed set in ginger.

5.1.3.7 Influence of season and position of flowers in the inflorescence on pollen tube length of ginger cultivars

The observations indicated that the season and position of flowers in the inflorescence influenced pollen tube length of ginger cultivars (Table 25). Maximum tube length was observed in mid season (240.68 μm) followed by late (184.96 μm) and early season (155.38 μm). It also showed maximum tube length from middle (226.05 μm) than lower (220.06 μm) and upper portion (134.90 μm) of the inflorescence. Bindu (1997) reported the same fact as maximum tube length in mid season (68.07 μm) followed by early (63.57 μm) and late (48.2 μm) and the same was maximum in flowers from lower position of the inflorescence (64.90 μm) followed by middle (62.50 μm) and upper positions (47.5 μm).

5.1.3.8 Influence of cultivars on fertility, viability and tube length of pollen grains

The genotype of the cultivar was found to influence the pollen fertility, viability and pollen tube length. The size of pollen grains ranged from 38.18 μm in V_9 to 103.62 μm in V_2 (Table 22). The pollen fertility and viability in five cultivars ranged from 20.68 per cent in V_{10} to 56.91 per cent in V_2 (Table 23) and from 3.80 per cent in V_{10} to 12.83 per cent in V_2 (Table 24) respectively. The pollen tube length ranged from 53.08 μm in V_{10} to 384.98 μm in V_2 (Table 25). The autotetraploids showed more fertility percentage (49.96 % in V_1 and 56.91 % in V_2) and more viability percentage (10.66 % in V_1 and

12.83 % in V_2) when compared to the diploids (V_8, V_9 and V_{10}). Pollen fertility was 85 per cent in tetraploids as against 13 per cent in diploid as reported by Ramachandran and Nair (1992). Sheeba (1996) also found that autotetraploids had high pollen fertility (64 %) than diploids (6%). The increased pollen fertility in the autotetraploid may be a consequence of the high frequency of quadrivalent formation and the high sterility in the diploid is probably due to heterozygosity for gross structural changes of chromosomes (Ramachandran and Nair, 1992). These observations give the indication that the chances of seed set will be more in crosses involving V_2 and V_1 as male parents which are autotetraploids and these cultivars will be a better choice in future generative breeding programmes.

The cultivars of ginger are reported to show varying degrees of meiotic abnormalities like the formation of univalents and multivalents at metaphase – I, presence of bridges and laggards at anaphase – I and micronuclei formation at telophase – I (Ramachandran, 1969; Pillai *et al.*, 1978; Ratnambal and Nair, 1983 and Sathiabhama, 1988). Hence it can be assumed that meiotic abnormalities during microsporogenesis contribute much towards pollen sterility in ginger. Excepting the autotetraploids, pollen fertility and viability were very low in the ginger cultivars necessitating large quantities of pollen grains to be used for pollination. It was easy to get large quantities of pollen for pollination after natural anther dehiscence. Anther dehiscence under *in vitro* condition was observed only after 5.00 p.m and pollination after 5.00 p.m facilitated use of large quantity of pollen grains.

Cultivar V_3, a variant evolved through colchicine treatment produced inflorescence in the first year but it failed to flower and in the succeeding year even the inflorescence was not produced. Variants such as V_4, V_5, V_6, V_7 also failed to produce inflorescence. This may be due to the physiological disturbances in the plant leading to no inflorescence production or production of rudimentary florets, which remain concealed inside the bract. Alternatively, it might be due to temperature sensitive mutant nature (similar to closed anther mutant in tomato), which requires low temperature for inflorescence production and flowering because colchicine treatment sometimes may act as mutagen.

5.1.4 Economic attributes

5.1.4.1 Rhizome characters

Rhizome characters like the number, length and girth of fingers are reported to influence yield, which may be more of a genetical character rather than environmental because all the cultivars were grown under identical conditions (Nybe, 1978). Significant variation was observed among the treatments for all primary, secondary and tertiary finger characters except length and breadth of secondary fingers and number and weight of tertiary fingers (Table 26, 27 and 28). But the year wise variation for primary finger was significant only with respect to length, breadth and internodal distance while variation between the treatments within year showed variation only in breadth and girth. The cultivar V_1 ranked top with maximum number of primary fingers but V_7 and V_4 recorded maximum length and breadth respectively. V6 registered maximum weight while V_1 had minimum weight. It is clear from Table 27 that the year wise variation of secondary finger was significant only for girth, weight, number of nodes and internodal distance, while variation between the treatments within year was significant only for weight and number of nodes. V_7 and V_3 recorded maximum number of secondary fingers and maximum weight respectively. In relation to tertiary finger, only number of nodes showed significant variation between years as well as variation between treatments within year. V_7 had maximum number of tertiary fingers with maximum weight. Nybe (1978) investigated variations in twenty five ginger types and reported significant variation for number of primary and secondary fingers. Girth and total number of fingers were reported to vary significantly among the twenty eight cultivars studied by Mohanty *et al.* (1981). Chandra and Govind (1999) reported weight and internodal distance of rhizome to be the most variable rhizome characters. Significant genotypic difference for rhizome length and breadth was observed by Korla and Tiwari (1999).

Among the selected cultivars, V_1 had maximum number of primary fingers while V_7 had maximum number of both secondary and tertiary fingers with maximum length and maximum weight of primary and tertiary fingers respectively. Maximum weight of primary finger and maximum length of tertiary finger was observed in V_6. V_3 and V_8 were found to have maximum weight and maximum length of secondary finger respectively. Maximum girth of primary, secondary and tertiary finger was observed in V_{10}.

5.1.4.2 Yield attributes

It may be seen from the Table 29 that the cultivars differed significantly for both fresh yield and dry rhizome yield during the two years. The rhizome yield also exhibited significant variation among the treatments within year and between years of cultivation in both yield per plant and yield per unit area. Pooled analysis of the fresh yield per plant and per hectare indicated V_8 as superior cultivar (175.35 g per plant and 16.02 t ha^{-1}) which was on par with V_1 (120.88 g per plant and 11.05 t ha^{-1}), V_2 (144.17 g per plant and 12.68 t ha^{-1}), V_3 (150.89 g per plant and 13.79 t ha^{-1}), V_9 (113.17 g per plant and 10.34 t ha^{-1}) and V_{10} (156.73 g per plant and 14.32 t ha^{-1}) while V_4 as inferior cultivar (50 g per plant and 4.57 t ha^{-1}). The pooled analysis of the dry rhizome yield indicated V_{10} as the superior cultivar with maximum dry rhizome yield (3.08 t ha^{-1}) and showing stable performance over the years. The cultivars V_1 (2.49 t ha^{-1}), V_2 (2.45 t ha^{-1}), V_3 (2.76 t ha^{-1}), V_8 (2.82 t ha^{-1}) and V_9 (2.2 t ha-1) were on par with the superior cultivar V_{10}. V_4 remained inferior in dry rhizome yield also (0.82 t ha^{-1}).

The highest dry rhizome yield was recorded by V_{10} considering the pooled data while V_4 remained inferior cultivar. Nair (1975) reported that the type Rio-de-Janeiro recorded the maximum yield followed by China, Maran, Sieraleon and Thinladium. Nybe (1978) reported fresh rhizome yield in the range of 2.74 (Arippa) to 28.54 t ha^{-1} (Nadia) and dry rhizome yield ranged from 1.13 (Uttar Pradesh) to 6.45 t ha^{-1} (Nadia). Thirty ginger cultivars studied by Sreekumar *et al* (1980) also indicated Rio-de-Janeiro to be suitable for fresh ginger production. Mohanty *et al* (1981) also made similar observations. Ravindran *et al.* (1994) evaluated ginger accessions for yield character and revealed considerable variability for this trait among the accessions. Significant genotype difference for yield per plant was observed by Korla and Tiwari (1999).

Positive correlation existed between rhizome weight and plant height, number of tillers, leaf number, leaf length and width (Sreekumar *et al.*, 1980; Saikia and Shadeque, 1992 and Singh, 2001). Nybe *et al.* (1980) reported that morphological characters such as length and girth of primary and secondary fingers to be positively correlated with yield.

Path coefficient analysis for rhizome yield and its related traits revealed very high positive direct effects of stomatal number as already reported by Das (1999) in ginger. Significant positive association of oil content with rhizome yield per plant and oleoresin content was observed by Singh *et al.* (2000).

V_8, which recorded maximum fresh rhizome yield exhibited maximum number of roots, lesser stomatal size and less susceptibility to shoot borer. The cultivars V_{10}, V_3, V_2 and V_1 were on par with the top yielder V_8. V_{10} which recorded highest dry rhizome yield had maximum pseudostem thickness and circumference with maximum leaf thickness while V_3 had maximum leaf breadth which were correlated with yield attribute. The autotetraploid V_2 derived from V_8 had maximum number of tillers per plant, number of leaves per plant, root length, leaf area, leaf area index while maximum plant height, pseudostem height, cuticle thickness and less susceptibility to leaf spot was observed in autotetraploid V_1 derived from V_{10} which might have contributed to increased productivity. Maximum number of inferior characters were observed in V_4 such as minimum plant height, pseudostem height, pseudostem circumference, number of tillers per plant, number of leaves per plant, leaf area, leaf area index and high susceptibility to soft rot as well as bacterial wilt which might have made it an inferior cultivar.

5.1.5 Quality attributes

There existed significant variation in quality attributes among the selected cultivars. The flesh and powder colour of rhizome showed variation from yellow to bright yellow. The U.K Standards prescribes light yellow colour for the powdered ginger as reported by Purseglove *et al* (1981).

Driage in the selected ginger cultivars ranged from 17.33 per cent in V_8 to 22.56 per cent in V_1 (Table 30). The autotetraploids V_1 and V_2 showed enhanced driage when compared to corresponding diploids while the other variants recorded less driage when compared to the check varieties. The variation in drying percentage under uniform conditions may be attributed to the difference in size of the rhizome as reported by Nybe (1978) and observed in this study.

Fibre content in ginger cultivars varied from 2.70 per cent in V_2 to 4.98 per cent in V_5 (Table 30). All the variants except V_5 recorded low fibre content compared to the check varieties. Purseglove *et al.* (1981) reported that the rhizomes which remain too long in the field become more fibrous, which reduces their market value. Kerala ginger is considered to be one of the best in the country, due to its lower fibre content, boldness and characteristic aroma and pungency. The 'Chernad' quality is best and the rhizomes are bigger and less fibrous and the 'Wynad' quality is considered to be inferior owing to

its still greater fibre content (Purseglove *et al.*, 1981). Gupta (1974) stated that among the exotics, 'Ta-kuang' and 'Chu-chiang' are popular cultivars from Taiwan, known for their soft, almost fibreless ginger and are in large demand for the production of ginger preserve. Sreekumar *et al.* (1980) reported fibre content ranging from 3.5 per cent in Tura to 6.0 per cent in Jorhat.

The cultivar V_2 had the maximum oil content (2.07 %) with the maximum oil yield per hectare, which was on par with V_{10}. Both oil content and oil yield per hectare were minimum in V_4 (Table 31). Purseglove *et al.* (1981) stated that in case of the spice intended for industrial extraction purposes, i.e., distillation of the essential oil or preparation of the oleoresin, the appearance is unimportant and in these cases, the aroma and flavour character, the content of volatile oil and the pungency level are the principal evaluation factors. The standard for "Oil ginger" from the Essential Oil Association of America specifies light yellow to yellow colour for the oil with the aromatic, persistent odour of ginger as reported by Purseglove *et al* (1981). In the present study, colour of oil among the selected cultivars varied from light yellow to dark yellow.

It was again observed that V_2 had high oleoresin content with maximum oleoresin yield per hectare extracted using acetone and ethyl acetate as solvents (Table 32 and 33). V_4 was found to be the most inferior cultivar with respect to oleoresin yield per hectare also. The oleoresin colour varied from pale brown to dark brown. The standard for "Oleoresin ginger" from the Essential Oil Association of America specifies dark brown viscous to highly viscous liquid with the characteristic odour and flavour of ginger as reported by Purseglove *et al* (1981). The maximum sensory score (++++) was given to V_8 for both oil and oleoresin. Purseglove *et al.* (1981) reported notable difference in the yield, aroma, flavour and pungency of the oleoresin due to variation in the geographical type of ginger employed, the age at which it is harvested, the choice of solvents and the method of extraction followed. The differential oleoresin yield obtained in the selected cultivars by the use of different solvents can be substantiated by the above reasons.

In conformity with the present findings, Nybe (1978) reported significant variation among the types with respect to quality attributes like crude fibre ranging from 3.47 per cent (China) to 6.47 per cent (Kuruppampady), oleoresin content ranging from 10.53 per cent (Rio-de-Janeiro) to 4.91 per cent (Wynad Mananody), oleoresin yield ranging from 504 kg ha^{-1} (Maran) to 69 kg ha^{-1} (Uttar Pradesh), oil content ranging from 2.40 per cent

(Karakal) to 0.57 per cent (Himachal Pradesh) and oil yield ranging from 97.82 kg ha^{-1} (Maran) to 11.11 kg ha^{-1} (Tafingiva). The differential behaviour of cultivars in various quality attributes under uniform conditions can be explained by the difference in genetic make up. The variations in quality attributes under different years of cultivation may be due to influence of seasonal variations and environmental conditions which was in accordance with the reports of Nybe (1978).

Among the different quality characters studied, autotetraploids V_1 and V_2 derived from Himachal Pradesh and Rio-de-Janeiro respectively recorded high driage, less fibre content and high oleoresin content when compared to the corresponding diploids. Volatile oil content was also highest in V_2. However, the corresponding diploids recorded maximum oil and oleoresin yield per hectare than its autotetraploid derivatives since oil and oleoresin yield per hectare is a function of both content and dry rhizome yield.

5.1.6 Incidence of pests and diseases

The incidence of pests and diseases also showed significant variation except leaf spot incidence among the selected cultivars (Table 34). With respect to shoot borer incidence per tiller and per plant, V_8 and V_7 were the least affected cultivars respectively while V_4 was the maximum affected cultivar. Soft rot incidence was minimum in V_{10} and maximum in V_4. Field screening for bacterial wilt showed that V_{10} had the least incidence while V_9 was highly susceptible to bacterial wilt. Ooze test carried out on the freshly harvested rhizome also indicated complete absence of oozing in V_{10} while the same was maximum in V_1, V_2, V_7 and V_8. Infection by the wilt pathogen and the incidence of disease can vary depending on the stage of growth, environmental conditions and part of the plant which may be the probable reason for the difference in severity in incidence at stand and harvested rhizomes. V_1 and V_6 recorded the least incidence of leaf spot whereas V_8 was the most susceptible cultivar. All the twenty five types in ginger studied by Nybe (1978) were susceptible to shoot borer, soft rot and leaf spot incidence. None of the accessions screened by Ravindran *et al.* (1994) showed tolerance or resistance to rhizome rot, bacterial wilt and leaf spot disease. He reported significant variation in incidence within cultivated ginger, which is in conformity with the present findings. The variation in incidence among the selected cultivars observed in this study may be due to genetical factors.

Among the selected cultivars, V_8 and V_7 were least susceptible to shoot borer incidence considering the percentage of attacked tillers and plant respectively. V_{10} was found least susceptible to both soft rot and bacterial wilt. Very light infection of leaf spot was observed in V_1 and V_6.

5.2 INVESTIGATIONS ON CONTROLLED *IN VIVO* POLLINATION IN GINGER

Different *in vivo* pollination techniques were employed earlier to overcome the barriers in fruit set and seed set. Bud pollination, artificial (self, sibling and cross) pollination and pollination using mixed pollen from other genera like *Kaempferia* and *Costus* failed to give positive results on fruit set. Removal of stigma as well as partial and complete removal of style and artificial pollination also failed to induce fruit set. Vijayasree (2001) observed failure of fruit set in turmeric after intra ovarian pollination. Bud stage was unsatisfactory as stigma was not receptive at this stage, which is in accordance with Sathiabhama (1988). The spiny stigma which prevents adherence of pollen grains and the lengthy style may be contributing factors for lack of seed set in ginger under *in vivo* conditions as observed by Sathiabhama (1988) and confirmed through this study.

High pollen sterility, low as well as slow germination of pollen grains and coiling of pollen tube are suggested reasons for failure of fruit set (Jayachandran and Vijayagopal, 1979 and Sathiabhama, 1988). Even in the autotetraploid cultivar V_2 (Z – O – 86) having the highest pollen fertility (56.91 %), the mean pollen germination noticed was only 12.83 per cent. The process of pollen germination has been observed to be very slow, with the pollen tube taking 24 h to reach its maximum length. By this time, abscission layer is formed in the stylar region and flowers are shed. Incompatibility is the failure of plants with viable pollen and ovule to set seed due to some physiological hindrance which prevents fertilization as reported by Crane and Lawrence (1952) and this might be reason for failure of fruit set in ginger.

5.3 INVESTIGATIONS ON *IN VITRO* POLLINATION IN GINGER

Ovule development was found in placental, modified placental and ovular pollination. Among the different methods tried, placental pollination showed maximum ovule swelling and maximum percentage of cultures with developed ovules (Table 35).

Valsala *et al.* (1996) and Bindu (1997) also reported seed set and seed development through placental, modified placental and ovular pollination technique in ginger, of which placental pollination was found to be the best.

5.4 POLLEN PISTIL INTERACTION AFTER *IN VITRO* AND *IN VIVO* POLLINATION

In higher plants, proper interaction between pollen and pistil is quite significant for the normal functioning of microspores and seed production. The fluorescence microscopic procedure of Kho and Baer (1968) has been employed in this investigation for studying pollen pistil interaction.

Pollen pistil interaction studies after *in vitro* placental pollination revealed that pollen tube growth is sufficient to cover the ovule length and to effect fertilization. Hence, there is chance of fertilization in case of *in vitro* placental pollination.

Pollen pistil interaction studies after *in vivo* stigmatic, stylar and intra ovarian pollination showed that pollen grains failed to germinate under *in vivo* condition. This failure probably due to inherent inability, was in accordance with the reports of Sathiabhama (1988). She also reported that none of the pollen grains germinated up to 2 h of pollination and only two germinated after 4 h of pollination and pollen tube failed to penetrate the stigma. Closely arranged spines in the stigma of ginger may be a hindrance for the pollen grains to gain contact with the stigmatic surface. Togari and Kawahara (1942) also observed complete suppression of pollen germination after selfing in sweet potato. The pollen is inhibited in the stigma for the simple reason that some kind of stimulus from the stigma which is essential for pollen germination is blocked by the incompatibility reaction as reported by Harrison *et al.* (1975). Martin and Cabanillas (1966) in their observation, besides the incompatibility barrier inhibiting the pollen germination, a physiological barrier between stigma and style were also hypothesized. Valsala (1994) also reported that there was no ovule development in ginger after intra ovarian pollination even under *in vitro* conditions.

According to Sears (1937), there are three sites of incompatibility viz., stigma, style and ovary. Inhibitory substances located within the stigma (Kumar, 1983 in sweet potato), style (Miller, 1938 in sweet potato and Han, 1994 in *Hibiscus syriacus*) and ovary (Sears, 1937 in *Gasteria verrucosa*; Moncur *et al.*, 1991 in *Acacia mearnsii*;

Sheeba, 2001 in adapathiyan) are suggested reasons for the incompatibility. Some inhibitory substances may be present in placental fluid produced by placental cells in the ovary which might have prevented the entry of pollen tube to the embryosac as reported by Willemse and Wittich (1996). The failure of pollen germination after *in vivo* stigmatic, stylar, intra ovarian pollination and *in vitro* intra ovarian pollination suggest that incompatibility at three sites, stigma, style and ovary are operating in ginger leading to failure of seed set. Pollen germination and successful seed set under *in vitro* placental pollination indicate that removal of the sites of incompatibility (stigma, style and ovary) would help to free the ovules from the inhibitory substances, promoting pollination and fertilization.

5.5 STANDARDIZATION OF MEDIA AND SUPPLEMENTS FOR CULTURE ESTABLISHMENT

5.5.1 Basal medium for culture establishment

Murashige and Skoog (1962) basal medium at half strength on supplementation with NAA, BAP and CW supported the maximum development of ovary (Table 36). The medium of SH (1972) and Nitsch (1951) with supplements were also found useful. However, the above media devoid of plant growth substances did not support ovary development, which was in accordance with the report of Valsala (1994).

The percentage of cultures showing maximum ovule development was more in half MS. Visual assessment of the cultures also revealed that half MS was superior to that of SH and Nitsch for increased size of ovules.

The suitability of MS medium with plant growth substances for *in vitro* culture of ginger has been reported earlier (Hosoki and Sagawa, 1977; Choi, 1991, Kackar *et al.*, 1993 and Nazeem *et al.*, 1996). Ilahi and Jabeen (1987) found half strength of MS inorganic to be more favourable than full strength.

Half MS medium was found best for good ovule development after *in vitro* placental pollination in Zingiberaceous crops as reported by Valsala (1994) and Bindu (1997) in ginger, Renjith (1999) and Vijayasree (2001) in turmeric and Bhurke (2002) in kacholam.

5.5.2 Influence of growth regulators on ovule development in ginger after *in vitro* placental pollination

The influence of auxins and cytokinins alone and in combinations on ovule development was studied to fix the optimum level. Auxins alone, NAA (0.5-1.0 mg l^{-1}), IAA (0.5 mg l^{-1}) and 2,4-D (0.5 mg l^{-1}) had favourable effect on ovule swelling and ovule development. NAA (0.5 mg l^{-1}) and (1.0 mg l^{-1}) showed moderate ovule swelling in 100 per cent of the cultures (Table 37). Cytokinin alone, BAP (2.5 mg l^{-1}) and kinetin (2.5 mg l^{-1}) induced ovule development and it was observed that BAP (2.5 mg l^{-1}) showed moderate ovule development in 83.33 per cent of cultures (Table 38).

Similarly, the combinations of auxins and cytokinins induced ovule development. The combination BAP (2.5 mg l^{-1}) + NAA (0.5 mg l^{-1}) was found better and the effect was manifested in 94.44 per cent of the cultures (Table 39).

The role of auxins in supporting ovary development into fruit is well documented. The developing seeds are a rich source of auxin (Nitsch, 1952) that is utilized for normal fruit growth in apple, pear and strawberry. Synthetic auxins such as NOA and 2,4-D can replace the stimulus provided by pollination.

The cytokinin activity at the early stage of embryogenesis is responsible for enhanced seed size by increasing cell number (Michael and Kelbitsch, 1972). Studies with isogenic mutants of barley, which vary in grain weight, demonstrated that large grain lines contain higher amounts of cytokinin at the very early stage of seed development than small grain lines (Kelbitsch *et al.*, 1975). According to Tollennar (1977), cytokinins are found in relatively high concentration in the liquid endosperm stage of early seed growth and their presence coincides with the higher rate of mitosis.

Raghavan and Torrey (1963) demonstrated that low concentration of IAA and kinetin aided growth of globular *Capsella* embryos. Neal and Topolesky (1983) found combinations of kinetin and GA_3 or kinetin and IAA to be most beneficial for the growth of tomato embryos excised 12 days after pollination. Philips *et al.* (1982) found that moderate levels of auxin with low levels of cytokinin aided the growth and survival of heart shaped interspecific hybrid embryos in *Trifolium*.

GA_3 (1 to 10 mg l^{-1}) and GA_3 (5 mg l^{-1}) + NAA (0.5 mg l^{-1}) or BAP (2.5 mg l^{-1}) in half MS + 3 per cent sucrose did not support ovule development following placental pollination which was in concurrence with the report of Valsala (1994). But GA_3 (1 mg l^{-1}) + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) had low ovule swelling and low percentage of cultures showing response (Table 43).

Euewens and Schwale (1975) have reported that in general, high auxins (IAA) and GA levels have been associated with active seed growth and fruit growth by cell expansion. In a number of plants, both GA and auxins are highest during early to mid embryo development, at a stage when cytokinins decreased rapidly.

Higher concentration of GA was found in the suspensor compared to the embryo and GA can be substituted for the suspensor in supporting the embryo growth in culture as reported by Yeung and Sussex (1999). However, in the present study GA did not support ovule development.

In the present investigation, tryptophan (0.25 to 1 mg l^{-1}) alone and also with BAP (2.5 mg l^{-1}) in half MS + 3 per cent sucrose did not support ovule development (Table 44). However, Guha and Johri (1966) have reported that Tryptophan favours ovary development and seed set in *Allium cepa* under *in vitro* condition.

Quartrano (1987) reported that hormones play an important role in different stages of seed development and germination. The concentration of cytokinins, auxins, gibberellins and abscisic acid were found relatively high in extracts from seeds of different developmental stages.

The aforesaid reports explain the requirement of growth regulators for proper development of ovules.

5.5.3 Influence of carbon source on ovule development

Both ovary and ovule developed at all levels of sucrose tried (3.0, 6.0, 9.0 and 12.0 %) along with hormones but the maximum development was observed at 3 per cent and 6 per cent level above which there was a gradual reduction in the ovule development. Maximum percentage of cultures showing ovule development (85%) was observed at 3 and 6 per cent sucrose level (Table 45).

The sucrose concentration has been reported to influence ovary development, ovule development and germination of seeds following *in vitro* pollination. In most of the

studies, sucrose has been used at a concentration of 4.0 to 5.0 per cent (Kanta and Maheshwari, 1963; Usha, 1965 and Rangaswamy and Shivanna, 1967). In *lilium*, swelling of the capsule was highest at sucrose concentration of 10.0 per cent but embryos were mostly found in ovaries cultured at 6.0 to 8.0 per cent sucrose (Tuyl *et al.*, 1991).

Since endosperm filling was obtained at 3.0 per cent sucrose level itself, along with BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} + CH (200 mg l^{-1}), 3 per cent sucrose as observed by Bhurke (2002) in kacholam can be considered as optimum for *in vitro* pollination of ginger.

5.5.4 Influence of organic media supplements on ovule development

Addition of coconut water to the media favoured high ovule development at 15 to 25 per cent v/v concentrations which was similar to the observations made by Valsala (1994). Coconut water contains a number of factors promoting cell division and a large number of free amino acids (Shantz and Steward, 1952, and Letham, 1974).

Casein hydrolysate (200-500 mg l^{-1}) which contains more than twenty amino acids was useful in promoting ovule development which was in accordance with the reports of Valsala (1994). Lower concentrations of 200 mg l^{-1} and 500 mg l^{-1} showed more favourable influence than higher concentrations. The favourable effect of CH on ovule development has been well documented (Kanta and Maheshwari, 1963 and Bindu, 1997).

L-glutamine (25 to 500 mg l^{-1}) showed moderate ovule swelling and the effect was not at all superior to control (Table 46). Collins and Grosser (1984) while reviewing the work on embryo culture opined that glutamine is a superior source of nitrogen for embryo culture. Emershad and Ramming (1984) observed a positive effect of L-cysteine and L-glutamine on the survival and increase in the size of grape embryos by *in ovulo* embryo culture.

Thus the basal media MS at half strength supplemented with NAA (0.5 mg l^{-1}), BAP (2.5 mg l^{-1}) and CW (15 %) v/v or CH (200 mg l^{-1}) was found most suitable for *in vitro* pollination studies in ginger (Plate 15).

5.5.5 Influence of vitamin supplementation on ovule development

The addition of double the quantity of the vitamin stock of MS medium in the medium of half MS + 3 per cent sucrose + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} enhanced high ovule development (Plate 16). Such a favourable effect of vitamin supplementation

was not seen in case of other media combinations tried as in Table 47. Vitamins such as B_1 and B_6 or even a mixture of vitamins (Maheshwari and Lal, 1961) stimulated ovary growth of *Iberis amara*. Vitamin E increased seed fertility in *Dendrobium nobile* (Ito, 1966). Castano and Proft (2000) reported that vitamin stock of MS medium supported the ovule development in chicory.

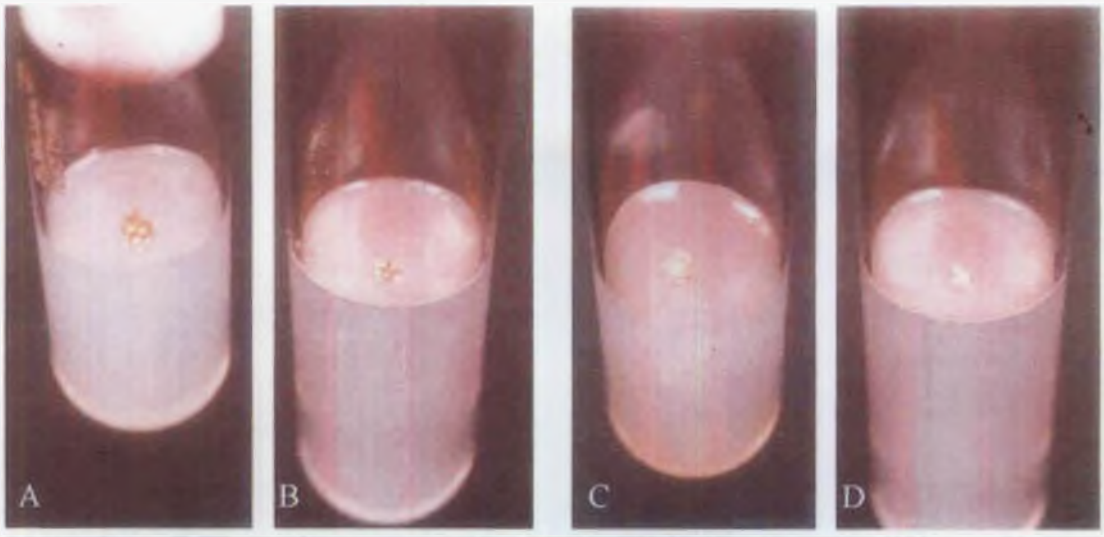
5.5.6 Influence of physical conditions on ovule development

Incubating the cultures in different physical conditions revealed that semi solid and solid media favoured high ovule swelling compared to liquid medium when the cultures were kept in continuous dark conditions (Table 48). Semisolid and solid media also induced ovule development in maximum percentage of cultures (95%) while in liquid medium, it was only 85 per cent (Plate 17). Keeping the cultures in continuous light, favoured moderate ovule swelling in semi solid medium and low ovule swelling in both solid and liquid media.

5.6 ATTEMPTING CONTROLLED SELFING AND CROSSING AMONG THE SELECTED CULTIVARS

5.6.1 Influence of selfing in ginger cultivars

The autotetraploids, Z-0-78 and Z-0-86 and the diploids, Rio-de-Janeiro, Maran and Himachal Pradesh could be selfed using *in vitro* placental pollination in different seasons and the mean percentage of cultures showing response and the mean number of ovules developed per culture were 77.77 per cent and 14.68 per cent respectively (Table 49). Maximum mean percentage of cultures showing well developed ovules and maximum number of ovules developed per culture was observed in mid season. This may be due to the higher pollen fertility and maximum pollen tube length in mid season when compared to early and late season. Z-0-86 recorded maximum percentage of cultures showing well developed ovules and maximum number of ovules developed per culture in both mid and late season while the same was maximum in Z-0-78 and Rio-de-Janeiro in early season. This might be due to the higher pollen size, fertility, viability and tube length observed in Z-0-86 in both mid and late season and maximum tube length observed in Z-0-78 in early season when compared to other cultivars. Z-0-86, an autotetraploid (V_2) has recorded the maximum pollen size, fertility, viability as well as tube length and the increased ovule size when compared to corresponding diploid V_8 (Plate 18). This may be the reason for

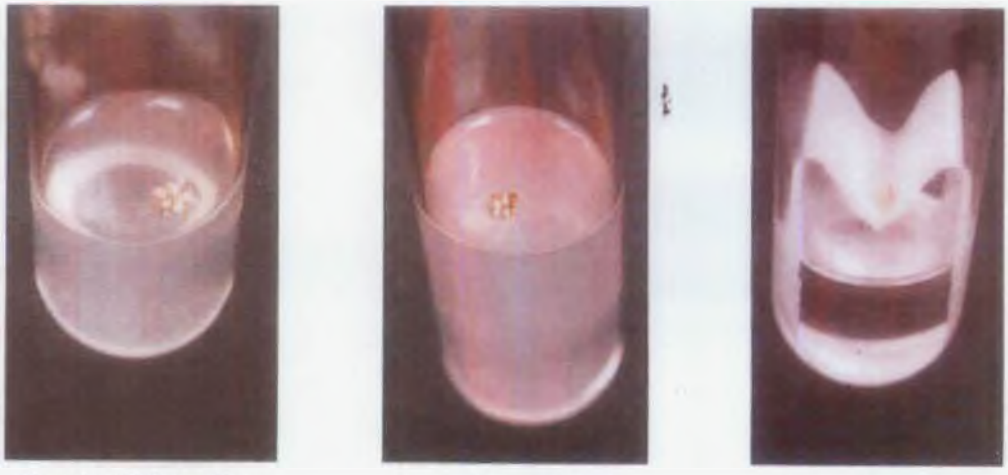


A. Half MS + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH 200 mg l^{-1}
B. Half MS + sucrose 3 % + 2,4 - D (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH 200 mg l^{-1}
C. Half MS + sucrose 3 % + Dicamba (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH 200 mg l^{-1}
D. Half MS + sucrose 3 % + IAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH 200 mg l^{-1}

Plate 15. Ovule development in different media combinations

Half MS + sucrose 3 % + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) + CH 200 mg l^{-1}

Plate 16. Ovule development in the best medium combination with doubled vitamin stock of MS medium



Semi solid medium Solid medium Liquid medium

Plate 17. Ovule development in different physical conditions of media

the maximum percentage of cultures with maximum number of well developed ovules per culture in Z-0-86. The low pollen fertility, viability, tube length and ovule size observed in Himachal Pradesh may be a reason for the poorest performance on selfing. Because of exclusive vegetative propagation, cultivars may possibly be homozygous. Hence the need for selfing does not usually arise. The results on *in vitro* selfing give an indication that self incompatibility is not present in ginger cultivars under *in vitro* conditions as reported by Bindu (1997).

5.6.2 Influence of crossing between ginger cultivars

The autotetraploids (Z-O-78) and Z-O-86) were treated both as female and male parent and were crossed with diploids (Rio-de-Janeiro, Maran and Himachal Pradesh). The results (Table 50) showed that the mean percentage of cultures showing response was 87. 80 per cent and the mean number of ovules developed per culture were 14.75, which differed, in early, mid and late season. The results on *in vitro* crossing give an indication that cross incompatibility is not a problem for seed set in ginger cultivars with *in vitro* pollination as reported by Bindu (1997). When compared to early and late season, maximum mean percentage of cultures showing well developed ovules and maximum number of ovules developed per culture was observed in mid season. Higher pollen fertility and maximum pollen tube length in mid season might be the probable reason. Autotetraploids (Z-O-78 and Z-O-86) as pollen parent crossed with diploids (Rio-de-Janeiro and Maran) showed maximum percentage of cultures showing well developed ovules and maximum number of ovules developed per culture in all the seasons (Plate 19). This might be due to the fact that these crosses had the favourable influence of higher pollen size, fertility, viability and tube length of autotetraploids and maximum number of ovules of diploids. The crosses involving Himchal Pradesh either as female or pollen parent recorded low percentage of cultures showing well developed ovules and minimum number of ovules developed per culture in all the seasons which can be related to low pollen size, fertility, viability, tube length and ovule breadth when compared to other parents. The crossing study suggests the possibility of transferring particular desirable character from one variety to another.

5.7 POST POLLINATION CHANGES

5.7.1 Seed set and development after *in vitro* placental pollination

Theoretically, each flower has the capacity of producing 21 seeds since the ginger flowers of the selected cultivars recorded a mean ovule number of 21.56.

The seed set per culture (66.66%) and number of well developed ovules per culture (7.82) at 80 DAP was observed to be more in semi solid medium than in solid medium (50 per cent and 5.93 respectively). Valsala (1994) observed 61.56 per cent mean seed set and 6.87 mean number of well developed seeds per culture at 80 DAP under *in vitro* conditions following placental pollination in ginger.

The ovules on the day of anthesis measured a length of 0.55 mm and breadth of 0.34 mm. The growth of seed was found very rapid during the initial twenty days (Plate 20) and showing a linear trend recording a mean length of 1.84 mm and breadth of 0.92 mm in semisolid medium while it was only 1.79 mm length and 0.88 mm breadth in solid medium (Table 51). The length and breadth showed linear increase with advance in age (Plate 21a) irrespective of the medium upto 80 DAP. Seeds from semi solid medium recorded maximum length (2.54 mm) and breadth (1.63 mm) at 80 DAP than seeds from solid medium (2.30 mm length and 1.45 mm breadth) at 80 DAP.

The colour of the ovules during the initial stage was creamy white which changed to purple red in a time lapse of 30-35 DAP (Plate 21b) indicating the stage of ripeness and turned to black within period of 55-80 DAP indicating full maturity. Maturity of ginger seeds was found two to three months as observed by Valsala (1994).

The small black arillate seeds had two seed coats among which the outer one is thick and the inner is thin. The seed coat encloses a cavity, which is typical of monocots. In the cavity, endosperm with embedded embryo is seen.

5.8 SEED VIABILITY TEST USING TETRAZOLIUM STAINING

Seed viability test showed that seeds from 40 DAP to 80 DAP were viable. The embryo portion was stained red in seeds from 40 DAP to 80 DAP which is in concurrence with Bindu (1997) in ginger. This gives a hint that seeds of 40 DAP is mature enough with well developed embryo so that seeds from 40 DAP onwards can be subjected to germination.

Z – O – 86 (Autotetraploid)

Rio-de Janeiro (Diploid)

Plate 18. Ovule development after selfing of ginger cultivars *in vitro*

Z - O - 86 X Rio-de Janeiro

Rio-de Janeiro X Z - O - 86

Plate 19. Ovule development after crossing of ginger cultivars *in vitro*

Plate 20. Seed set and development after 20 days of culturing

The works done by Gbehounou *et al.* (1993) in seeds of *Striga hermonthica* revealed that the supposed viability with tetrazolium colour test and germination is closely linked giving 80 per cent germination as against 92 per cent viability with tetrazolium test.

5.9 GERMINATION STUDIES OF *IN VITRO* PRODUCED SEEDS

5.9.1 Investigations on embryo rescue studies

Pre-fertilization barriers can be overcome by excision methods or *in vitro* pollination and fertilization technique whereas post fertilization barriers can be overcome by "Embryo Rescue Technology" (Bhojwani and Razdan, 1983).

5.9.1.1 Embryo culture

Embryos along with endosperm were rescued from seeds on 20, 40, 60 and 80 DAP and were incubated in half MS medium, Knudson C medium, Taira and Larter's modified Norstog medium and Tomato embryo culture medium containing auxins and cytokinins (Plate 22). The growth regulator combinations of NAA (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.00 mg l^{-1}) or IAA (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.00 mg l^{-1}) or 2,4-D (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.00 mg l^{-1}) with BAP (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 10, 15, 20 mg l^{-1}) were used (Table 52). Rescuing embryo along with endosperm is expected to remove the seed coat barrier, which may hasten the absorption of nutrients by the embryo. But a positive influence of embryo rescue on germination was not observed in this study.

Knudson C medium (Knudson, 1951) is a standard medium for germination of orchid seeds that lack characteristic endosperm whereas Tomato embryo culture medium with high salts, high sucrose and low vitamins and amino acids was found superior to other treatments for the growth of immature tomato embryos (Neal and Topoleski, 1983). Lekshmy (1989) developed plantlets from excised embryos in banana when cultured on modified Knudson's C medium. Taira and Larter's modified Norstog medium was successfully utilized by Raina (1984) for germinating hybrid embryos isolated from partially to highly shriveled caryopses from crossing between *Triticum durum* and *Secale cereale*.

Zygotic embryos of *Coffea bengalensis* germinated into plantlets when cultured on half MS with BAP 0.1 mg l^{-1} as reported by Muniswamy and Sreenath (1996). Burun and Poyrazoghu (2002) observed plantlet development from barley embryos in Randolph and Cox medium whereas Qing *et al.* (2002) reported that the best medium for culturing young embryos of apricot was modified SH medium, which would develop into young plantlets.

5.9.1.2 Ovule culture

The pollinated ovules in half MS + BAP (2.5 mg l^{-1}) + NAA (0.5 mg l^{-1}) at different stages of development (5, 10, 15, 20, 25, 50, 80 DAP) were cultured in half strength MS, full strength MS, Knudson C, SH, B_5, Tomato embryo culture and Taira and Larter's modified Norstog medium along with different growth regulator combinations (Table 53) could not induce favourable response on seed germination (Plate 23). Spiegel-Roy *et al.* (1985) obtained seedlings by culturing abortive ovules of grape on Nitsch medium with IAA 10^{-5} M and GA_3 10^{-6} M. Adventitious embryos induced from the papaya ovules were transferred to hormone free MS medium regenerated plantlets as reported by Tokumoto *et al.* (2000). Ishikawa *et al.* (2001) produced interspecific hybrids by culturing ovules with placenta 7-14 days after pollination on 2 g l^{-1} Gelrite-solidified MS medium containing 3 per cent sucrose whereas Mischishita *et al.* (2001) obtained hybrid seedlings from *Rhododendron kaempferia* ovules by exposing ovules of 4 MAP to GA_3 50 mg l^{-1}.

In the present embryo rescue studies, the lack of response to germination may be due to underdeveloped and undifferentiated embryos, block to nucleic acid and protein synthesis, failure to mobilize food reserves of embryos, deficiency of plant growth substances and presence of inhibitors (Bradbeer, 1988).

5.9.2 Effect of primary treatments on seed germination

The various primary treatments like keeping on moist filter paper, moist sterile sand, moist sterile sand with vermiculite (1:1), imbibition in water and on different forms (solid, liquid and semisolid) of various basal medium such as full strength MS, half strength MS, Knudson C, SH, B_5, Taira and Larter's modified Norstog and Tomato embryo culture medium did not promote seed germination (Plate 24). **Only** ***in vitro***

produced seed of 80 DAP imbibed in water for one day when transferred to half MS + 2, 4 – D (0.5) mg l^{-1} + BAP (6.0) mg l^{-1} + GA_3 (5.0) mg l^{-1} with 3 per cent sucrose induced germination showing radicle emergence (Table 54). But the *in vivo* produced seeds of turmeric, another member of Zingiberaceae family germinated on moist filter paper with adequate moisture and air under *in vitro* implying that there is no necessity of any complex media requirements and growth regulators (Renjith, 1999). Roy and Banerjee (2001) observed *in vitro* seed germination of *Geodorum densifolium* in all basal media such as Knudson C, half MS and Vacin and Went. In the present study, as the seeds were developed under *in vitro* conditions, the growth substances required for proper development of the embryo and for inducing germination may be lacking in the medium.

5.9.3 Influence of physical conditions of medium on seed germination.

The seeds obtained on 80 DAP were cultured in half MS (solid, semi solid, liquid) medium and full MS (solid, semi solid, liquid) medium with different combinations of growth regulators but **half MS + BAP (9 mg l^{-1}) + 2,4-D (0.25 mg l^{-1}) + sucrose (3 %) in solid medium induced seed germination with radicle emergence** (Table 55).

In the preliminary studies on *in vitro* germination of ginger seed Valsala (1994) got one seed germinated out of 20 seeds in a medium of half MS with 2,4-D 8.0 mg l^{-1} incubated for two months. The seeds were then transferred to a plant growth regulator combination of BAP 9.0 mg l^{-1} and 2,4-D 0.1 mg l^{-1} in half MS. This reveals that plant growth substances are required for seed germination under *in vitro* conditions and the optimum combinations and concentrations are important to achieve good results.

5.9.4 Pulse treatment with growth regulators

Seed treatment with various growth regulators at different concentrations also did not influenced seed germination (Table 56). But Korikanthimath and Mulge (1998) found GA_3 100 mg l^{-1} and NAA 7.5 mg l^{-1} for 12 h increased *in vivo* germination of cardamom seeds.

5.9.5 Scarification

The seed coat of mature ginger seeds (80 DAP) were dark and hard. Chemical and mechanical scarification to soften the seed coat did not favour seed germination (Table 57

and 58). In cardamom another member of Zingiberaceae with hard seed coat, germination percentage when plating on filter paper improved significantly (25%) after nitric acid treatment for 10 min and continuous water washing for 24 days as observed by Chaudhury and Chandel (1995).

5.9.6 Stratification

Stratification of seeds at low temperature (2°C and 4°C) for 3 to 24 h had no effect on seed germination (Table 59). According to Outcalt (1991) stratification increases speed and percentage of germination of Ocala Pine seed in dry soil.

5.9.7 Washing the seeds in running water and sowing

Seeds did not respond to germination even after washing in running water, which helps to remove any inhibitors present in the seed (Table 60).

5.9.8 Hydration – dehydration – rehydration treatment

Subjecting seeds to stress condition also did not induce germination. Stress condition was provided by dehydrating hydrated seeds for 12 h (Table 61).

All the above treatments as detailed from 5.9.4 to 5.9.8 were done before culturing the seeds in half MS alone and also with GA (1.0, 5.0, 10.0 mg l^{-1}) + Ethylene (0.25, 0.50, 0.75, 1.00 mg l^{-1}) and BAP (2.5, 5, 10 mg l^{-1}) + GA (1, 2, 5 mg l^{-1}) + 2,4-D (0.1, 0.3, 0.5 mg l^{-1}) and GA (0.5, 5, 25, 50, 100) mg l^{-1}. These treatments failed to influence seed germination.

5.9.9 Treatment with solvents, NaCl, NaOH, HCN, thiourea and pectinase enzyme

Solvents such as acetone, methanol and ethanol of different concentrations used for seed treatment resulted in failure of seed germination (Table 62). Seed treatment with NaCl, NaOH and HCN at different concentrations (Table 63) had no positive effect on germination. Treatment with enzyme pectinase also failed to induce germination. Failure of germination was observed even after thiourea treatment for 10 minutes.

5.9.10 Influence of manual pricking and cut at chalazal as well as micropylar end of seeds

Manual pricking of seeds by pins and cutting at chalazal or micropylar and or at both ends did not promote seed germination.

In all the above treatments as detailed in 5.9.9 and 5.9.10, the seeds were then cultured in half MS, full MS, Knudson C, tomato embryo culture alone and also with GA_3 (5 mg l^{-1}), BAP (6 mg l^{-1}) and 2,4-D (0.5 mg l^{-1}) and seeds failed to germinate.

5.9.11 Priming of seeds

Stress condition was provided by soaking the seeds in different concentrations of PEG 4000, PEG 6000 and Mannitol for 5 days which could not favour seed germination. But in tomato, Cantliffe and Abebe (1996) observed that priming of seeds with PEG 8000 solutions for 6-8 days consistently improved germination.

Priming of ginger seeds with KCl, KH_2PO_4, Na_2HPO_4, $CaCo_3$ each at concentrations of 0.25, 0.5, 1 and 2 per cent for 6 h did not promote germination. Though KCl, KH_2PO_4, Na_2HPO_4, $CaCo_3$ could not induce germination of ginger seeds, Paul and Sarma (1996) have reported that toria seeds soaked in 2 per cent KCl, KH_2PO_4, Na_2HPO_4 for 10 h showed significantly higher germination.

Out of different KNO_3 treatments, seeds obtained from 80 DAP primed with KNO_3 50 ppm for 8 h induced germination in half MS supplemented with BAP (6 mg l^{-1}), GA_3 (5 mg l^{-1}) and 2,4-D (0.5 mg l^{-1}) along with 3 per cent sucrose (Table 64) similar to the germination of eragrostis seed soaked in 50 ppm KNO_3 for 24 h as reported by Khandelwal and Sen (1996).

But pierced seeds imbibed in (1, 3, 5, 7, 10%) KNO_3 for 5 days failed to germinate. Demir and Gunay (1996) observed germination of cucumber seed after priming with 3 per cent KNO_3 for 5 days.

In all the above treatments, the seeds after treatment were then cultured in various basal medium alone and also with combinations of different growth regulators at various concentrations (Table 64). **But only seed obtained from 80 DAP cultured in half MS supplemented with BAP (6 mg l^{-1}), GA_3 (5 mg l^{-1}) and 2,4-D (0.5 mg l^{-1}) along with sucrose 3 per cent showed positive response to germination after KNO_3 (50 ppm) treatment.**

5.9.12 *In vivo* seed germination studies

In vitro developed seeds were subjected to various pre sowing *in vivo* treatments such as scarification, stratification, hydration - dehydration - rehydration, treatments with NaCl, NaOH, HCN, thio-urea, pectinase and with various solvents, nicking and cutting the seeds at micropylar end or chalazal end or at both ends, various hormonal treatments and seed priming chemicals as detailed under *in vitro* treatments and sown on sterile moist sand and moist filter paper (Plate 25). All the treatments failed to induce seed germination. The reason for lack of *in vivo* seed germination may be the lack of satisfactory conditions required for germination as supported by the germination studies *in vitro* where in various plant growth regulators are required to induce germination. But Rangaswamy and Shivanna (1967) observed *in vivo* germination of petunia seeds on moist filter paper.

5.9.13 Germinated seeds, their parentage and media combinations

In all the above seed germination studies, the seeds selected were obtained through crosses between (autotetraploid and diploid), (diploid and autotetraploid), (diploid and diploid) and (autotetraploid and autotetraploid). However, the seed obtained through cross of diploid (Rio-de-Janeiro) and autotetraploid (Z – O – 78) germinated after seed priming treatment (50 ppm KNO_3 for 8 h) and also seed from cross of autotetraploid (Z – O – 86) X diploid (Maran) germinated showing radicle emergence after seed imbibing in water for one day when cultured in half MS + 2, 4 – D (0.5 mg l^{-1}), BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose (Plate 26). Valsala *et al.* (2001) observed radicle emergence from *in vitro* produced seeds in a slightly altered media combination of half MS + sucrose 6 per cent + NAA (0.2 mg l^{-1}) + BAP (1.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + CW 15 % v/v.

Seed of cross between diploid (Rio-de-Janeiro) X diploid (Maran) and diploid (Maran) X autotetraploid (Z – O – 86) showed positive influence on germination produced radicle emergence after culturing in solid medium of half MS + 2, 4 – D (0.25) mg l^{-1} and BAP (9.0) mg l^{-1} along with 3 per cent sucrose (Plate 26). Half MS medium supplemented with 2, 4 – D (0.2 mg l^{-1}), BAP (5.0) mg l^{-1} along with 3 per cent sucrose and CW 15 % v/v resulted in radicle emergence of seed as reported by Valsala *et al.* (2001).

Plate 21a. Seed set and development at 30 DAP in semi solid media

Plate 21b. Seeds at 30 DAP showing red colour indicating stage of ripeness

Plate 22. Embryo culture for germination

Plate 23. Ovule culture for germination

Plate 24. Seed kept for *in vitro* germination

Plate 25. Seeds kept for *in vivo* germination

Seeds having permeable seed coats usually go through three recognizable phases of germination : (1) imbibition; water potential of the seed environment is higher than that in the seed, causing water molecules to flow through the seed epidermis into the embryo, leading to (2) the activation phase; in which stored seed hormones and enzymes stimulate physiological development leading to (3) growth of the radicle; ending the germination phase. Imbibition results in the expansion of both embryo and endosperm. When a seed is provided with water, the water is taken up by imbibitional forces within the seed. Imbibition is also able to take place when dry seeds are placed in fairly concentrated solutions of impermeable solutes whose water potentials are too low to permit embryo growth. GA_3 and KNO_3 which penetrate to the embryo and stimulate metabolic activity are often effective in bringing about germination as reported by Bradbeer (1988).

In seed priming the water potential of seeds is controlled by the osmotic potential of the seed environment. Osmotic priming is accomplished using chemicals that lower osmotic potential in the seed environment and make germination occur over a short period of time. The purpose of priming is to reduce the germination time and improve stand and percentage germination. Seeds were found to germinate when water potential reaches a critical physiological level in the seed (Bradford, 1995).

Bradbeer (1988) reported that failure of germination might be due to the dormancy caused by the embryo coverings (endosperm) such as restriction of gaseous exchange, restriction of water uptake, mechanical restriction of embryo growth, water-soluble inhibitors in the embryo coverings, dormancy from the failure to mobilize extra-embryonic food reserves. He found that seed dormancy may be broken by chilling, light, scarification, leaching, exposure to chemicals and elevated temperature. However, the above said treatments also failed to induce germination in the present study.

5.9.14 Somatic embryoid formation

Somatic embryoids were formed when *in vitro* produced seeds from the cross (diploid x autotetraploid) and (autotetraploid x diploid) were kept in half MS along with BAP (6 mg l^{-1}) + GA_3 (5 mg l^{-1}) + 2,4-D (0.5 mg l^{-1}) and also in half MS along with BAP (6 mg l^{-1}) + 2,4-D (0.1 mg l^{-1}) (Plate 27). In monocots, immature inflorescences, immature embryos, or mature seeds are the choice explants for initiating embryogenic cultures as reported by Bhaskaran and Smith (1990) which is in accordance with the

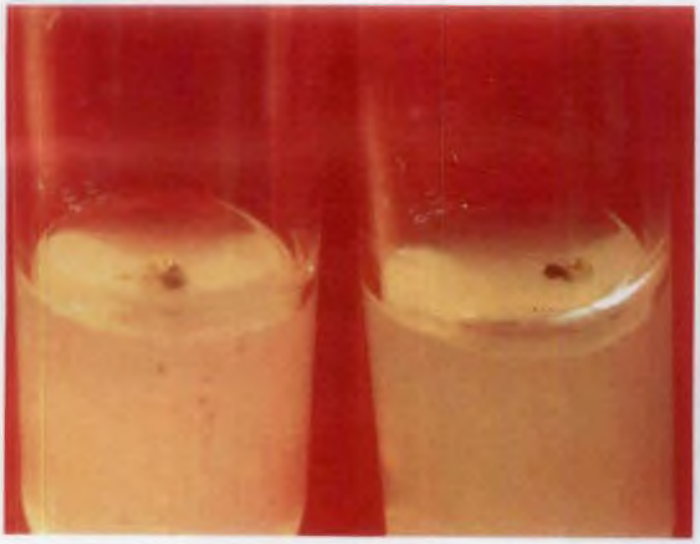
(Rio-de Janeiro) X (Z - O - 78)

Seed priming treatment (50 ppm KNO_3 for 8 h)

(Z - O - 86) X (Maran)

Seed imbibed in water for one day

Half MS + sucrose 3 % + 2,4-D (0.5 mg l^{-1}) + BAP (6.0 mg l^{-1}) + GA_3 (5.0 mg l^{-1})



(Rio-de Janeiro) X (Maran)

(Maran) X (Z - O - 86)

Half MS + sucrose 3 % + 2,4-D (0.25 mg l^{-1}) + BAP (9.0 mg l^{-1})

Plate 26. Germination of seeds *in vitro*, its parentage, media combinations and pre-treatments

present study. Plant regeneration from somatic embryoid was not observed even when it was transferred to MS containing BAP (2, 6, 8, 9 ppm), NAA (0.1 to 1 ppm) + BAP (1.00 ppm) and 2,4-D (0.2 to 0.5 ppm) + BAP (6 to 10 ppm) along with 6 per cent sucrose and incubated for 4 months (Table 65). Babu *et al.* (1992) reported plantlet formation from immature inflorescence in modified MS medium supplemented with BAP 10 ppm and 2,4-D 0.2 ppm. Kackar *et al.* (1993) observed somatic embryoid formation from leaf segments in MS medium + dicamba 2.7 µM and plant regeneration was achieved when it was transferred to MS containing BAP 8.9 µM.

5.9.15 Callus growth

Callus growth was observed in the germinated seed culture media containing half MS + 2, 4 – D (0.5 mg l^{-1}), BAP (6.0 mg l^{-1}) and GA_3 (5.0 mg l^{-1}) along with sucrose 3 per cent after 25 days of germination (Plate 28). Choi (1991) reported callusing was best and shoot and root formation were found on medium containing NAA 0.1 to 1 ppm and BAP 1 ppm. Joseph (1997) found that in kacholam a member of Zingiberaceae family half MS medium supplemented with BAP 0.5 mg l^{-1} and NAA 3.0 mg l^{-1} gave rise to somatic embryos from calli. Rout and Das (1997) reported efficient plant regeneration using callus derived from shoot primordia grown on MS media supplemented with BA (5 mg l^{-1}), IAA (1 mg l^{-1}) and shoots were rooted on half MS with IAA or IBA (1 mg l^{-1}). Palai *et al.* (2000) reported plant regeneration from callus cultures of ginger on MS with BAP 5 mg l^{-1} + IAA 1.5 mg l^{-1} and shoots were rooted on half MS with IAA 0.25-0.5 mg l^{-1}.

5.9.16 Future line of work

Evaluation of the induced variants indicated that the two autotetraploids are remarkable for their quality attributes but their yield potential is superseded by the incidence of diseases like soft rot and bacterial wilt. The increased pollen fertility of the autotetraploids is of importance from the breeding point of view. The successful seed set through *in vitro* placental pollination in crosses between autotetraploids and diploids can be taken to advantage in creating progenies with altered ploidy level that can be exploited for resistance to diseases along with economic attributes.

(Young stage) (Mature stage)

(Rio-de Janeiro) X (Z - O - 78)

Half MS + sucrose 3 % + 2,4-D (0.5 mg l^{-1}) + BAP (6.0 mg l^{-1}) + GA_3 (5.0 mg l^{-1})

(Young stage) (Mature stage)

(Z - O - 86) X (Maran)

Half MS + sucrose 3 % + 2,4-D (0.1 mg l^{-1}) + BAP (8.0 mg l^{-1})

Plate 27. Somatic embryoid formation in seed culture

(Rio-de Janeiro) X (Z - O - 78)

Half MS + sucrose 3 % + 2,4-D (0.5 mg l^{-1}) + BAP (6.0 mg l^{-1}) + GA_3 (5.0 mg l^{-1})

The studies on germination of *in vitro* produced seeds indicated that they were shy to germinate exhibiting only radicle growth even when subjected to different growth regulators in varying concentration. The results warrant the need to try the effect of different factors influencing germination singly and in combination with adequate number of replications to reach definite conclusions on seed germination.

The somatic embryoids and callus formed in seed culture create better opportunities for broadening the spectrum of variability, which necessitate attempts on proliferation of callus and somatic embryoids and their regeneration.

Successful seed germination or plant regeneration from somatic embryoids / callus formed in seed culture from crosses with different ploidy level can open up new vistas in crop improvement of ginger.


SUMMARY

6. SUMMARY

Investigations on "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)" were carried out at Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during 2001- 2003.

The salient findings of the study are summarized as follows:

1. Evaluation of seven induced variants along with three check varieties of ginger showed significant variation for all the morphological characters except pseudostem circumference, leaf area and leaf area index. Visual observations like leaf shape and pubescence and absence of stipules were consistent in all the cultivars. Maximum number of roots was observed in highest yielder (V_8), which was on par with V_{10}, V_3, V_2 and V_1. V_{10} had maximum pseudostem circumference, thickness and leaf thickness while V_3 had maximum leaf breadth. The autotetraploid Z – O - 86 (V_2) derived from V_8 showed maximum number of tillers per plant, number of leaves per plant, root length, leaf area and leaf area index where as autotetraploid Z – O – 78 (V_1) derived from V_{10} recorded maximum plant height and pseudostem height. The lower most yielder V_4 was found to be inferior with less plant height, pseudostem height, pseudostem circumference, lesser number of tillers per plant, number of leaves per tiller as well as plant, leaf area and leaf area index.
2. The autotetraploids V_1 and V_2 recorded increased cuticle thickness of 24.54 µm and 21.81 µm respectively compared to corresponding diploids [V_{10} (23.18 µm) and V_8 (20.45 µm)]. All the selected variants recorded lesser stomatal number with increased size compared to check varieties. The autotetraploids recorded maximum epidermal cell area [(51.57 mm^2 (V_1) and 53.99 mm^2 (V_2)] with less number of cells per mm^2 [(27.75 (V_1) and 26.50 (V_2)].

3. In ginger, flowering season extended from September to November. The cultivars flowered within a period of 133.6 to 164.8 days after planting. V_9 was the earliest flowering cultivar while V_{10} was the latest flowering cultivar. The duration of flowering was maximum in V_8 (54 days) and minimum in V_2 (25 days). The percentage of flowering ranged from 34.72 per cent in V_2 to 88.53 per cent in V_8.

4. Ginger inflorescence is a bracteate spike directly springing from the rhizome and rarely emerging terminally from the aerial shoot. Ginger inflorescence took 29.47 days form the visual initiation to first flower opening and the blooming completed within 16.53 days. The floral primordium recorded a mean length of 4.53 cm at the time of visual initiation and attained a mean length of 18.00 cm within one month. The maximum total number of inflorescence per plant was observed in V_8 (5.67) where as minimum in V_9 (3.00). The number of flowers contained per spike and per plant was maximum in V_8 (20.66 and 117.14 respectively). The anthesis in different cultivars started by 3 pm and continued upto 4 pm. Anther dehiscence took place between 4.30 pm and 5.00 pm. The variants except V_1 and V_2 failed to flower.

5. Cultivar difference was observed with respect to length of flowers emerging from different portions of the inflorescence recording a mean length of 3.59 cm. The flowers were characterized by a long style of mean length 3.38 cm ending in a spiny stigma. The ovary measured a mean length of 2.25 mm and diameter of 2.23 mm and recorded a mean ovule number of 21.56 per ovary. The ovary of V_9 was the largest followed by that of V_1. Microscopic measurement of ovule showed a mean length of 549.02 µm and breadth of 338.98 µm at the middle with maximum length in V_9 (575.87 µm) and maximum breadth in V_1 (363.19 µm).

6. The pollen grains were highly heterogeneous in size and round in shape. Seasonal influence was noticed on diameter of pollen grains which ranged from 43.08 µm to 95.43 µm in the different ginger cultivars. The fertility, viability and tube length of the pollen grains was found to be influenced by the season and position of flowers in the infloresence. The mean pollen fertility was highest (46.01 %)

during the mid period of the flowering season and the flowers emerging from the upper portions of the inflorescence (36.74 %). Pollen viability using ME_3 medium was highest in early season (8.36 %) and flowers from lower portion of the inflorescence (9.18 %). The mean tube length of the germinated pollen grains was maximum in mid season (240.68 µm) and for flowers from middle portion of inflorescence (226.05 µm).

7. The genotype of the cultivar influenced the pollen size, fertility, viability and tube length. Among the cultivars, V_2 recorded the maximum diameter of pollen grain (103.62 µm) with highest pollen fertility (56.91 %), viability (12.83 %) and tube length (384.98 µm). The autotetraploids showed more fertility percentage and viability percentage when compared to corresponding diploids (V_8 and V_{10}).

8. The cultivars differed significantly for all the primary finger characters but the year wise variation except for length, breadth, internodal distance and variation between treatments with in year except for breadth and girth was not significant. The treatments showed significant variation for all the secondary finger characters except length and breadth. Year wise variation was non significant for number, length and breadth while variation between the treatments with in year was significant only for weight and number of nodes. The treatments showed significant variation for all tertiary finger characters except number and weight of tertiary fingers. Year wise variation and variation between treatments with in year was significant only in case of number of nodes. Among the selected cultivars, V_1 had maximum number of primary fingers while V_7 had maximum number of both secondary and tertiary fingers with maximum length and maximum weight of primary and tertiary fingers respectively. Maximum weight of primary finger and maximum length of tertiary finger was observed in V_6. V_3 and V_8 were found to have maximum weight and maximum length of secondary finger respectively. Maximum girth of primary, secondary and tertiary finger was obtained in V_{10}.

9. The cultivars showed significant variation with regard to fresh rhizome yield and dry rhizome yield (t ha^{-1}) during the two years. Fresh rhizome yield ranged from

50.00 g per plant (V_4) to 175.35 g per plant (V_8) and 4.57 t ha^{-1}(V_4) to 16.02 t ha^{-1} (V_8). Dry rhizome yield ranged from 0.82 t ha^{-1}(V_4) to 3.08 t ha^{-1} (V_{10}). Among the variants, V_3, V_2 and V_1 recorded yield on par with highest yielder.

10. The cultivars exhibited variation in all quality attributes. Rhizome flesh and powder colour varied from yellow to bright yellow. The autotetraploids V_1 recorded the maximum driage (22.56 %) whereas V_2 recorded the lowest fibre content (2.70 %).

11. Highest oil content was observed in V_2 (2.07 %) and the lowest in V_7 (1.20 %). With respect to oil yield per hectare, V_8 registered the maximum value of 76.02 kg ha^{-1} followed by autotetraploid V_2 (55.50 kg ha^{-1}). The colour of oil varied from light yellow to dark yellow. Sensory evaluation indicated that V_8 had good sensory score ("++++") and the least preferred was V_5 and V_6 ("+").

12. With respect to oleoresin extracted with acetone and ethyl acetate, V_2 recorded maximum content (9.16 % and 7.74 % respectively), whereas V_8 gave the maximum oleoresin yield per hectare (280.15 kg ha^{-1} and 288.66 kg ha^{-1} respectively) followed by V_2 (246.28 kg ha^{-1} and 207.97 kg ha^{-1} respectively). The colour of oleoresin extracted using acetone and ethyl acetate varied from pale brown to dark brown. Sensory evaluation of oleoresin indicated that V_8 had the most pleasing aroma with acetone and ethyl acetate as solvents. When the extraction efficiency of the two solvents was compared it was found that the content was more with acetone (5.91 %) compared to ethyl acetate (3.86 %).

13. Significant variation was observed between cultivars for the incidence of shoot borer, soft rot, bacterial wilt while variation was non significant for leaf spot incidence. V_8 showed the least percentage of shoot borer attacked tillers (19.99 %) where as V_7 had the least percentage of attacked plants (29.37 %) while V_4 showed the maximum incidence of both attacked tillers (50.82 %) and plants attacked (67.32 %). V_{10} showed the least susceptibility to soft rot (20.28 %) and bacterial wilt (10.14 %). Ooze test from rhizomes confirmed the least incidence of bacterial wilt in V_{10}. V_1 and V_6 were found to be the least susceptible cultivars to

leaf spot, which is scored as very light (0.68) while V_8 showed the maximum incidence, which is scored as medium (2.00).

14. None of the various *in vivo* pollination techniques such as stylar, stigmatic, intra ovarian was effective in inducing fruit set in ginger.

15. Among the *in vitro* pollination techniques tried (placental pollination, modified placental pollination and ovular or test tube fertilization), placental pollination was found to be the best with maximum ovule swelling and maximum percentage of cultures with developed ovules.

16. The pollen pistil interaction studies after *in vitro* placental pollination showed that pollen grains germinated readily on the ovules under *in vitro* condition. It was observed that pollen germination starts with in 3 h of pollination and attained a length, which was sufficient to cover the total length of the ovule and to effect fertilization by 24 h. The pollen pistil interaction studies after different *in vivo* pollination methods showed that pollen grains failed to germinate under *in vivo* condition.

17. *In vitro* culture establishment studies revealed that ovary development and ovule swelling was good when it was incubated in half MS supplemented with BAP 2.5 mg l^{-1}, NAA 0.5 mg l^{-1}, CW 15 per cent v/v or CH (200 mg l^{-1}) and 3 per cent sucrose. Hence, this media can be taken as a template for mass production of seeds.

18. Vitamin supplementation by adding the vitamin stock of MS medium in double the normal quantity was beneficial for ovule development in the medium half MS + NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) with 3 per cent sucrose thus achieving the maximum percentage of ovule development (95 %).

19. Semisolid medium which favoured high ovule swelling with maximum percentage of cultures resulting in more seed set and number of well developed seeds per culture with maximum size was found preferable over solid and liquid

medium. Incubating the cultures under dark conditions was preferential than under light conditions.

20. Selfing of the five ginger cultivars *in vitro* by placental pollination was successful and showed ovule development. Maximum mean percentage of cultures showing well developed ovules and maximum number of ovules developed per culture was observed in the mid season. The mean percentage of cultures showing response was maximum in Z – O – 86 (90.74 %) with maximum mean number of ovules developed (16.01).

21. Crossing the autotetraploids with diploids and its reciprocal *in vitro* by placental pollination was successful and showed ovule development. Maximum mean percentage of cultures showing well developed ovules and maximum number of ovules developed per culture was observed in mid season. The mean percentage of culture showing response (100 %) was maximum in the cross of Maran X Z – O – 78 but maximum mean number of developed ovules (15.85) was observed in cross of Rio-de-Janeiro X Z – O – 78.

22. Ovules/seeds developed after placental pollination were creamy white during the initial stage of development which changed to purple red colour with in a period of 30 – 35 DAP and to black with in a period of 55 – 80 DAP.

23. The small arillate seeds had two seed coats, outer one being thick and inner one being thin. The seed coat encloses a cavity, which is typical of monocots. In the cavity, endosperm with embedded embryo is seen.

24. Tetrazolium staining of the well developed seeds revealed that the embryos were viable staining red which was more intense at 40 DAP than at 80 DAP.

25. The seeds after *in vitro* pollination were subjected to various *in vitro* and *in vivo* germination treatments. Though *in vivo* treatments failed to induce germination, 80 days old matured seed obtained from the cross diploid (Rio-de-Janeiro) X autotetraploid (Z – O – 78) germinated under *in vitro* conditions after seed priming treatment (50 ppm KNO_3 for 8 h) and also the cross of autotetraploid

(Z – O – 86) X diploid (Maran) after seed imbibition in water for one day when cultured in half MS + 2, 4 – D (0.5 mg l^{-1}), BAP (6.0 mg l^{-1}) and GA_3 (5.0 mg l^{-1}) along with 3 per cent sucrose showing radicle emergence. Seed of the cross between diploid (Rio-de-Janeiro) X diploid (Maran) and diploid (Rio-de-Janeiro) X autotetraploid (Z – O – 86) germinated after culturing in solid medium of half MS + 2, 4 – D (0.25 mg l^{-1}) and BAP (9.0 mg l^{-1}) along with 3 per cent sucrose producing radicle.

26. Embryo rescue studies (Embryo culture) could not induce germination.

27. Somatic embryoids were developed from seeds cultured in media combinations of half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose and also in half MS + 2, 4 – D (0.1) mg l^{-1}, BAP (8.0) mg l^{-1} mg l^{-1} along with 3 per cent sucrose. Maximum somatic embryoids (4 - 5 per culture) were observed in the cross between diploid and autotetraploid while 1 – 2 embryoid per culture was obtained in cross between autotetraploid and diploid. No somatic embryoid formation was observed in crosses between diploid X diploid or autotetraploid X autotetraploid. Plant regeneration from these somatic embryoids was not observed even when the cultures were transferred to half MS and MS media containing (2, 6, 8) ppm BAP and (0.1 to 1.0) ppm NAA + (1.0) ppm BAP and (0.2 to 0.5) ppm 2, 4 – D + (6.0 to 10.0) ppm BAP along with 3 per cent sucrose and incubated for 4 months.

28. Callus growth was also observed after 25 days of seed germination in the medium containing half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose.

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ABSTRACT

EXPLOITATION OF INDUCED VARIABILITY FOR CROP IMPROVEMENT IN GINGER (*Zingiber officinale* Rosc.)

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

Kerala Agricultural University

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KERALA, INDIA

2003

ABSTRACT

Investigations on "Exploitation of induced variability for crop improvement in ginger (*Zingiber officinale* Rosc.)" were carried out at Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara during 2001- 2003.

Evaluation of seven induced variants along with three check varieties indicated the superiority of the autotetraploids V_2 (Z – O – 86) and V_1 (Z – O – 78) in quality attributes. V_2, an autotetraploid from Rio-de-Janeiro was notable for lowest fibre content, highest oil and oleoresin content and increased driage and high cuticle thickness than its diploid counterpart. V_1, an autotetraploid from Himachal Pradesh exhibited highest driage, high oleoresin content and highest cuticle thickness. V_2 was more vigorous in growth exhibiting maximum yield contributing characters such as number of tillers per plant, number of leaves per plant, leaf area and also leaf area index and root length. V_1 showed maximum plant height and pseudostem height which are correlated with yield. The fresh and dry yield of rhizomes of V_1 and V_2 were found to be on par with V_8 the highest fresh rhizome yielder and V_{10} the highest dry rhizome yielder. V_3, a variant from Himachal Pradesh also produced yield on par with the highest yielder.

The yielding capacity of the autotetraploids especially V_2 was found to be superseded by the incidence of diseases like soft rot and bacterial wilt. V_1 was least susceptible to leaf spot. V_{10} (Himachal Pradesh) showing stable performance over the years had low incidence of soft rot and bacterial wilt.

The high pollen fertility and viability of the autotetraploids V_1 and V_2 and the highest pollen size and tube length observed in V_2 make them ideal as pollen parents in hybridization programmes.

None of the various *in vivo* pollination techniques such as stylar, stigmatic, intra ovarian was effective for inducing fruit set or seed set in ginger.

In vitro placental pollination with pollen grains suspended in ME_3 medium was found to the best with maximum ovule swelling and maximum percentage of cultures with developed ovules (90 %).

Pollen pistil interaction studies substantiated the lack of seed set under natural conditions due to failure of pollen germination owing to incompatibility barrieres operating in stigma, style and ovary. Elimination of these barriers in *in vitro* placental pollination enabled pollen germination and enough tube length to bring fertilization.

The experiments on culture establishment showed that MS semi solid medium at half strength supplemented with NAA (0.5 mg l^{-1}) + BAP (2.5 mg l^{-1}) along with 3 % sucrose + CH (200 mg l^{-1}) or CW (15 %) v/v supported maximum development of ovules. Addition of double the quantity of the vitamin stock of the MS medium to the culture establishment medium and incubation under dark conditions also favoured more ovule development. Semi solid medium was advantageous in obtaining maximum seed set and size at maturity.

Controlled selfing and crossing among the autotetraploids and diploid cultivars using *in vitro* placental pollination were successful and influenced ovule development. Mid season of flowering favoured maximum culture establishment and ovule development compared to early and late season in both selfing and crossing. Crossing enabled production of maximum number of developed ovules compared to selfing.

Ovules/seeds developed *in vitro* were creamy white during the initial stage of development which changed to purple red colour with in a period of 30 – 35 DAP and to black with in a period of 55 – 80 DAP. The small arillate seeds had two seed coats, outer one being thick and inner one being thin. The seed coat encloses a cavity, which is typical of monocots. In the cavity, endosperm with embedded embryo is seen.

Tetrazolium staining of well developed seeds revealed that the embryos were viable staining red which was more intense at 40 DAP than at 80 DAP seeds.

Seed germination studies indicated that subjecting the seeds to embryo rescue (embryo culture) and *in vivo* seed treatments failed to induce germination. Under *in vitro*

conditions, the seed of 80 DAP obtained from the cross diploid (Rio-de-Janeiro) X tetraploid (Z – O – 78) germinated after seed priming treatment (50 ppm KNO_3 for 8 h) and also the cross of tetraploid (Z – O – 86) X diploid (Maran) after seed imbibition in water for one day when cultured in half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose, showing good root development. Seed of the cross between diploid (Rio-de-Janeiro) X diploid (Maran) and diploid (Rio-de-Janeiro) X tetraploid (Z – O – 86) germinated showing radicle emergence after culturing in solid medium of half MS + 2, 4 – D (0.25) mg l^{-1} and BAP (9.0) mg l^{-1} along with 3 per cent sucrose.

Somatic embryoids were developed from seeds cultured in media combinations of half MS + 2, 4 – D (0.5) mg l^{-1}, BAP (6.0) mg l^{-1} and GA_3 (5.0) mg l^{-1} along with 3 per cent sucrose and also in half MS + 2, 4 – D (0.1) mg l^{-1}, BAP (8.0) mg l^{-1} along with 3 per cent sucrose in crosses between diploid and tetraploid. Plant regeneration from the somatic embryoids was not observed even after subjecting to media with different concentrations of various growth regulators.

Callus growth was also observed after 25 days of seed germination in the seed culture medium containing half MS + 2, 4 – D (0.5 mg l^{-1}), BAP (6.0 mg l^{-1}) and GA_3 (5.0 mg l^{-1}) along with 3 per cent sucrose.