MICROPROPAGATION OF GINGER (*Zingiber officinale* Rosc.)

Thesis submitted to the
University of Agricultural Sciences, Dharwad
In Partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

In

HORTICULTURE

By

RAJANI C. HIREMATH

DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD – 580 005

NOVEMBER, 2006

ADVISORY COMMITTEE

DHARWAD
NOVEMBER, 2006

(S.S. PATIL)
MAJOR ADVISOR

Approved by:

Chairman: _____
(S.S. PATIL)

Members: 1. _____
(A.A. PATIL)

2. _____
(P.R. DHARMATTI)

3. _____
(P.U. KRISHNARAJ)

4. _____
(SUMANGALA BHAT)

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

I. INTRODUCTION

India is called as the 'Spice Bowl of the World' for production of variety of spices with superior quality. Growing spices for various purpose has been famous since the ancient times. There are records about various spices and its properties in the 'Vedas' as early as 6000 BC. India is well known for the trade, since the period of exploration of sea routes, because of its varieties of spices and superior quality, which attracted foreigners to India.

According to the Bureau of Indian Standards (BIS), 63 spices are grown in India. India is the leading country in the world for production, consumption and export of spices.

Ginger is an important spice crop grown in India. It is herbaceous rhizomatous perennial plant belonging to the family Zingiberaceae, under the natural order scitaminae. It is a tropical plant believed to have originated in South East Asia probably India or China (Bailey, 1949 ; Parry, 1969). Ginger was introduced into Europe in the ninth century AD (Lawrence, 1984) and was brought to the Mediterranean region from India by traders during the 13th century.

Ginger is commonly used all over the world and especially in China, where it forms an essential ingredient in most of the dishes. It is used as spice and medicine. Apart from having tangy flavour, it has appreciable quantities of proteins (2.3%), carbohydrates (12%), fats (1%), minerals (1.2%), fibre (2.5%) and moisture (81%) of fresh rhizome (Swaminathan, 1974). It also contains appreciable amount of vitamin A and small amount of vitamin B. Hence, this crop finds a place in naturotherapy and herbal medicine prescription since vedic period.

Ginger is widely used in the preparation of soft drinks, beverages, such as ginger beer, ginger tea, ginger wine, cordials, liquors, gingerale and in candies, pickles preserves and baking products. Ginger forms a major ingredient in the traditional medicine of India. Ginger oil is used in pharmaceutical preparation as a carminative and stimulant for alcoholic gastritis etc.

Ginger is commercially cultivated in India, China, Taiwan, Philippines, Jamaica, Fiji, Africa, Mexico, Japan and Indonesia. India is the largest producer of ginger in the world accounting for 50 per cent of the world total production. In India, ginger is cultivated in an area of 83,940 ha with annual production of 3.06 lakh tonnes (Anon., 2002). India is also largest exporter of ginger, accounting to 6580 tonnes of dried ginger valued at Rs. 22.95 crores (Anon., 2002).

In Karnataka, it is grown in an area of about 8,421 ha with an annual production of 62610 tonnes (Anon., 2001).

The method of propagation of ginger is through pieces of underground rhizomes. But, this is slow process. Rhizome has dormancy period and only sproutes during the monsoon that to only 5 to 6 plants can be obtained from one single rhizome in a year. So, rapid method of multiplication is needed especially of newly developed high yielding varieties, which are available in small quantities. But, through tissue culture, dormancy problem could be overcome and it would be possible to cultivate the crop under favourable conditions.

Ginger cultivation is threatened by rhizome rot diseases caused by *Pseudomonas solanacearum* and *Phythium* sp. These are spread through infected seed rhizome. The major hindrance for crop improvement in ginger is due to lack of seed set. So, rapid multiplication of diseases free propagules on a large scale is needed. It is possible only through *in vitro* culture. It is estimated that 3 fold increase in the production of rhizomes could be possible by effective control of diseases and pests (Hosoki and Sagawa, 1977). Tissue culture can be used for embryo rescue and possible production of seeds in ginger.

Micropropagation provides a rapid, reliable system for the production of large numbers of genetically uniform plantlets. It offers a method to increase valuable genotypes rapidly and expedite the release of improved varieties. In addition, micropropagation ensures mass production of elite clones from hybrid or specific parental lines. Micropropogation ensures healthy seedlings with desirable characters.

Keeping these points in view, the present studies were conducted with the following specific objectives.

1. To standardize the source of explants for micropropagation.
2. To standardize the sterilization procedure for different explants.
3. To standardize the growth regulators for shoot growth.
4. To standardize the growth regulators for root growth.
5. Identification of suitable hardening medium for micropropagation.

II. REVIEW OF LITERATURE

Development of the plant tissue culture is historically linked to the discovery of the cell and subsequent propounding of the cell theory. The concept of 'Totipotency' which is an inherent part of the cell theory of Schleiden (1838) and Schwann (1839) is the basis for plant tissue culture. *In vitro* technique dates back to 1902, when Haberlandt predicted the totipotency of plant cells. Totipotency is the ability of a plant cell to develop into a complete plant. Major breakthrough in plant tissue culture were achieved with the discovery of auxins and cytokinins. The formulation of nutrient media *i.e.*, Murashige and Skoog (1962), medium is the most commonly used medium for culturing of large number of horticultural plants.

According to Murashige (1974) there are three possible methods available for micropropagation.

1. Enhanced release of axillary buds
2. Production of advantageous shoots through organogenesis.
3. Somatic embryogenesis.

Callus mediated organogenesis and somatic embryogenesis are not recommended for clonal propagation since there is a possibility of producing aberrants. In shoot tips and axillary bud cultures, genetic fidelity is maintained to a large extent. *In vitro* somatic embryogenesis is limited to a few species but still, acts as the most rapid method of plant regeneration (Evans *et al.*, 1981).

Currently, *In vitro* clonal propagation strategies have been developed for a number of economically important plant species. More and more species are becoming amenable for subject have been published by Murashige (1974, 1978), Hu and Wang (1983), Styler and Chin (1983), Sharp *et al.* (1984) and Litz (1985).

The present experiment was conducted to find out the best surface sterilizer, growth regulator, explant and hardening medium for *In vitro* multiplication of ginger. The relevant literature pertaining to these aspects has been reviewed and presented below.

2.1 ESTABLISHMENT OF CULTURE

The objective is to successfully place an explant into aseptic culture by avoiding contamination and then to provide an *In vitro* environments that promotes stable shoot production. The important aspects of this are explant disinfection, explant selection and culture medium (Hartmann *et al.*, 1997).

2.1.1 Explant types

The use of tissue culture as a tool for plant propagation could be particularly relevant for vegetatively propagated crop plants that resist conventional asexual propagation (Hackett, 1966) or when fast methods of mass propagation of single plant is required. The different explants such as axillary bud, shoot tips, meristem tips, root tips are commonly used. *In vitro* ginger multiplication, dormant buds on excised rhizomes can be forced to form shoots which can be rooted. This method is rather slow, particularly for plant breeders, as on an average only 20 plants can be produced per year from single, one year old plant (Leffring, 1971).

Illahi and Jabeen (1987) conducted an experiment in ginger using different explant materials *viz.*, young buds, stem cuttings taken from 3 month old plants, rhizome cutting with shoot bud primerdia and juvenile shoots and observed efficient plant regeneration.

Cronauer and Krikorian (1984) studied rapid multiplication of banana and plantain by *In vitro* shoot tip culture. Similarly, Swamy *et al.* (1983) reported that shoot tips isolated from rhizomes of the banana cv. Robusta were suitable material for *In vitro* plantlet production.

Haung (1995) observed that plants were regenerated from the shoot tips with 0.2 to 0.9 mm in length of ginger were best for *In vitro* propagation.

Rout and Das (1997) observed efficient plant regeneration in *Zingiber officinale* using callus derived from shoot primordia and grown on MS media. Similarly, Choi (1991) reported that callusing was best when base or middle portion, explants of ginger were cultured on medium.

According to Olivier (1996) axillary buds were the best source of explants for the successful clonal propagation of ginger. Clonal propagation of *Zingiber officinale* was made easy with explant taken from sprouting buds (Balachandran *et al*., 1990). Doraiswamy *et al.* (1983) reported that shoot tips isolated from the rhizomes of banana were found to be suitable material for plantlet formation *In vitro*.

Nadagouda *et al.* (1978) observed that plants were regenerated from the young vegetative buds excised from sprouting turmeric rhizomes. Similarly, Kuruvinshetti and Iyer (1981) reported that buds isolated from sprouting rhizomes of turmeric clone 15B were suitable material for plant production.

Shetty *et al.* (1982) observed that sprouting buds of turmeric clone 15 B can give good regeneration in *In vitro*. Kumar *et al.* (1985) the immature panicles of cardamom were best source of explants for clonal propagation without the intervention of callus of embroids. Leafy aerial Pseudostems and decapitated crown sections of ginger have been successfully cultured *In vitro* on MS medium (Ikeda and Tanabe, 1989).

2.1.2 Explant disinfection

In the process of sterilization living materials should not lose their biological activity, but only bacterial or fungal contaminants should be eliminated. The commonly used sterilants are bleach, ethanol, sodium hypochlorite, mercuric chloride. The type of sterilant used, concentration and time depends on the nature of explant and species (Razdan, 1993).

Berger *et al.* (1994) developed disinfection protocol for rhizomes by alcohol treatment of explants immediately after excision. The rhizomes were soaked in 1 per cent sodium hypochlorite or saturated Ca hypochlorite or soaking in $HgCl_2$ resulted in 62 to 90 per cent free from microorganisms.

Malmug *et al.* (1991) were able to establish contaminant free sprouting bud cultures from ginger by washing the explants, tween 80 followed by a 10 min treatment in a sodium hypochlorite solution (active chlorine – 0.5%).

Nadagouda *et al.* (1983) surface sterilized the sprouting buds of cardamom with 0.12 per cent (w/v) $HgCl_2$ solution for 15 min and then washed 4 to 5 times with sterile distilled water reduces the degree of contamination.

Pillai and Kumar (1982) surface sterilized the shoot apex of ginger with 0.1 per cent $HgCl_2$ solution and 90 per cent ethanol, washed with sterile distilled water for 3 to 4 times could get effective sterilization. Double sterilization with NaOCl (3.5%) and tween 80 for 15 minutes and again for 5 minutes resulted in significantly lower contamination of 10 days old suckers of banana variety 'Williams' (Hamill *et al*., 1993).

Vuylsteke and De Langhe (1985) surface sterilized 1 to 2 cm^3 of meristem tips of 11 banana cultivars by treating with ethanol (95%) for 15 seconds followed by a 15 minutes treatment in a hypochlorite solution (1.5%) to obtain successful results.

Gupta (1986) observed that banana and plantain tissues have phenolic compounds which oxidise rapidly and resulted in death of explants. To prevent phenolic oxidation he suggested the use of filter sterilized ascorbic acid 2.5 mg per ml. Maximum number of healthy and contaminant free cultures from buds of immature panicles of cardamom were obtained by cleaning with 70 per cent alcohol followed by 10-15 minutes treatment in a 0.12 per cent $HgCl_2$ solution (Kumar *et al*., 1985).

Sushma *et al.* (2005) developed disinfection protocol for *Hedychium spicatum*, a aromatic plant. The explants were treated with tween 20 for 10 min followed by the treatment with 1 per cent bavistin (BASF) for five minutes. Then surface sterilized with 0.1 per cent (w/w) $HgCl_2$ for 3 minutes and then washed with sterile distilled water for 5 minutes.

Kobza and Vachunova (1991) reported that chlorinated lime at 10 per cent concentration and $HgCl_2$ at 0.1 per cent for 10 minutes were the best sterilants for *Dracena* explants.

Rahaman *et al.* (2004) reported that rhizome buds were treated with solution of antiseptic [savlon 5% (v/v)] for 10 minutes. Then explants were washed with distilled water and finally treated with $HgCl_2$ (0.1%) for 14 minutes gave good results.

Wondyfraw and Surawit (2004) reported that buds of Korarima were rinsed with 70 per cent ethanol for 1 min followed by 2 step surface sterilization using 20 and 10 per cent hyter (6% v/v) sodium hypochlorite) mixed with 2 ml/l tween80 for 10 and 5 minutes, washed with sterile DW resulted in lowest degree of contamination.

2.2 SHOOT MULTIPLICATION *IN VITRO*

For obtaining desired responses in tissue culture, the role of growth regulators and their concentration will have to be carefully choosen. The most important development in the tissue culture of the planter were made with the discovery of growth redulators, auxins, gibberlins, cytokinins and abscicins and other organic compounds.

Wickson and Thimann (1958) discovered that cytokinins could release the lateral buds from apical dominance. In the presence of cytokinins, the dormant buds of vegetative apex are stimulated to grow and elongate. Skoog and Miller (1967) reported that the cell division or cell differentiation was also associated with auxins and cytokinins.

Nasirujjaman *et al.* (2005) grew turmeric rhizome bud on Murashige and Skoog medium containing different concentrations of BA and NAA. He observed the highest multiple shoots on the medium with 4 mg BA/l + 1 mg NAA/l. Ali *et al.* (2004) cultured turmeric emerging bud on Murashige and Skoog medium supplemented with BAP and kinetin. The highest multiple shoots were observed in the presence of 1 mg BAP + 0.25 mg kin/l.

Keshavachandran and Khader (1989) grew turmeric bud (Curcuma longa) cultivars CO-1 and BSR-1 on Murashige and Skoog medium supplemented with 1 mg kinetin/l, 1 mg BA/l. He found that the average number of shoots produced per bud was 2.11 in BSR-1 and 2.5 in CO-1.

Balchandran *et al.* (1990) reported that rhizome buds excised from *Curcuma longa* was inoculated on Murashige and Skoog medium with different combinations of BA and kinetin. For best shoot multiplication 3 mg BA/l was found to be good. Malmug *et al.* (1991) reported that shoot proliferation of the regenerated shoots was induced with the addition of 1 mg NAA + 5 mg BA/l.

Palai *et al.* (1997) observed that when *Zingiber officinale* cultivers cultured on Murashige and Skoog medium supplemented with increased concentration of BA from 6 to 8 mg/l, there was decreased multiplication of shoots.

Raju *et al.* (2005) reported that the best response of turmeric cultivars for shoot multiplication was obtained on Murashige and Skoog medium supplemented with 4 mg/l BAP and 1.5 mg/l NAA for Curcuma caesia (3.5 + 0.79 shoots/explant) and 1 mg/l BAP + 0.5 mg/l NAA for Curcuma zedoaria (4.5 + 0.15 shoots/explant).

Inden *et al.* (1988) observed that shoot tip of ginger cultured on Murashige and Skoog medium with 5 mg BA/l, 0.5 mg NAA/l. One shoot tip can produced more than 4 shoots within 9 weeks.

Faria and Illag (1995) studied the rapid propagation of *Zingiber* spectable *In vitro*. Multiple shoots were induced from axillary buds were transferred to half strength Murashige and Skoog medium containing 10 μMBA and 5 μM IAA. Shoot formation occurred when buds were transferred to half strength of MS medium containing only 10 μM BA.

Pandey *et al.* (1997) observed that explant Pseudo stems of ginger cultured on Murashige and Skoog medium with 5 mg/l BA + 0.5 mg/l NAA. The highest number of shoots produced with an average of 5.33 shoots after 5 weeks of culturing.

Doreswamy *et al.* (1991) cultured the banana apical and lateral meristems of cultivars Robusta and dwarf cavendish on Murashige and Skoog medium with adenine sulphate (2.05 μM), BA (22.2 μM) and IBA (29.6 μM) were found to be best for proliferation of multiple shoots (6-8 shoots).

Nadagouda *et al.* (1983) cultured young sprouted buds of cardamom on MS medium with kinetin. The highest number of shoots observed in presence of 2 mg/l BAP, 1 mg/l IAA, coconut water, biotin.

Dogra *et al.* (1994) achieved *In vitro* propagation of *Zingiber officinale* using rhizome buds. The buds produced multiple shoots when cultured on MS medium with 2.5 mg/l BA and 0.5 mg/l NAA.

Choi and Kim (1991a) showed that the addition of 0.5 mg per L NAA + 5.0 mg/l BA to the nutrient medium was best for regeneration.

Bhartendu *et al.* (1989) reported the multiple shoot formation on a identified MS medium with 2 mg/l BAP. But, the best results were obtained when bud cultured on liquid medium results 10 fold multiplication in 90 days.

Sanghamitra (2000) reported that plant regeneration was achieved from sprouted shoots of *Curcuma aromatica* on Murashige and Skoog's medium supplemented with BA alone (1-7 mg/l) or combination of BA (1-5 mg/l) and kinetin (0.5 – 1 mg/l). A concentration of 5 mg/l BA was best for shoot multiplication.

Hazare *et al.* (2005) studied the *In vitro* propagation of two turmeric cultivars by using rhizome bud as explants, with using different levels of BAP or in combination with kinetin. They found that 2 mg BAP + 2 mg kin per L was best for shoot multiplication.

2.3 *IN VITRO* ROOTING

Poonsapaya *et al.* (1993) observed that shoots rooted best when transferred to medium supplemented with 10 per cent activated charcoal with 0.5 mg/l NAA in *Zingiber cassumunar* Roxb.

Dogra *et al.* (1994) observed that the greatest number of roots were formed on MS medium supplemented with 1 mg/l NAA. Faria and Illag (1995) obtained optimum *In vitro* rooting from excised buds of ginger on MS medium fertified with 5 mg/l NAA or IAA.

Dipti *et al.* (2005) studied the *In vitro* multiplication and rooting of shoots in turmeric. She found the maximum rooting to multiple shoots were observed on half strength MS medium with 0.5 mg/l NAA.

Meenakshi *et al.* (2001) observed that the highest rooting was stimulated by subculturing the proliferated shoots on half strength MS media with 0.3 mg/l NAA during micropropagation of turmeric.

Sit and Tiwari (1998) reported that shootlets of turmeric were rooted on half strength MS medium with IBA at 0.0 to 0.5 mg/l and they were concluded that rooting did not occur in the absence of IBA and the number of roots per rootlet was proportional to IBA concentration.

Raju *et al.* (2005) studied the two species of curcuma (C. caesia and C. zedoaria) using rhizome bud explant. The best response for root multiplication was obtained on MS basal medium supplemented with 0.5 mg/l IAA. A maximum of 9.2 ± 0.15 and 8.9 ± 0.09 roots per explant were obtained for Curcuma caesia and Curcuma zedoraria respectively.

Mante and Tepper (1982) reported that shootlets of banana were rooted on MS medium with NAA (0.1 – 1.0 mg/l) or IBA (2 – 10 mg/l). Similarly, Rahaman *et al.* (2004) observed that rooting of shoots in turmeric was obtained on ½ MS medium with 0.1 – 1 mg/l IBA.

Choi (1991) reported that callusing was best when base or middle portion explants of ginger were cultured on medium containing 0.5 ppm NAA, while shoot and root formation were best on medium containing 0.1 to 1 ppm NAA + 1.0 ppm BA.

2.4 HARDENING

One of the major obstacles in the application of tissue culture methods for plant propagation has been the difficulty in successful transfer of plantlets from the laboratory to the field (Wardle *et al*., 1983). The reasons for such a difficulty appear to be related to the dramatic change in the environmental conditions. The environment of the culture vessel is one of low light intensity, with very high humidity (generally 100%) and poor root growth, while the greenhouse and/or field conditions are typified by very high light intensity, low humidity and microflora (Desjardins *et al*., 1987). Several workers have developed protocols to overcome some of these constraints. These reasons for such a difficulty appear to be related to the dramatic change in the environmental conditions.

Rooted plantlets of ginger were successfully transferred to a mixture of peat : sponge rock : vermiculite (2:1:1) in the greenhouse and eventually to full sun in the nursery (Hosoki and Sagawa, 1977).

Ali *et al.* (2004) successfully transferred turmeric plantlets to the greenhouse in pots containing soil with equal amount of sand + clay + compost. Plants were successfully established in field with 100 per cent survival rate.

Gonzalez and Mogollon (2004) tested sand, soil, saw dust, coconut and other components separately and in combinations for suitability as growth substrates and plants transplanted to media. The most suitable substrate found was coconut + saw dust (1.1) followed by sand + coconut + saw dust (1:1:1).

Salvi *et al.* (2000) observed complete plants of turmeric were transferred to sterilized soil in paper cups for 3 to 4 weeks and then to the field, where 95 per cent of plants survived to maturity. *In vitro* rooted ginger plants were transplanted into humus soil : kitchen garden soil (3:1) under 24 to 28, 70 to 80 per cent relative humidity in which more than 90 per cent survival rate was recorded (Congfa *et al*., 2001).

Samsudeen *et al.* (2004) were found that 85 per cent success when plant transplanted in potting mixture of garden soil, sand and vermiculite in equal proportions and kept in humid chamber initially for 22 to 30 days.

Martyr (1981) observed that cuttings uptake more water from peat : perlite (1:1, v/v) than from either peat : grit (1:1, v/v) or from peat alone. Water uptake by cuttings was not directly related to the water content of the medium per unit volume, which was greatest in the peat. This higher rate of uptake was reflected in the quicker rooting at the cuttings in the peat : perlite. Greenhouse acclimatization of plantlets was achieved in a 1:1 peat : perlite (volume basis) substrate by Conti *et al.* (1991).

III. MATERIAL AND METHODS

The present investigations on "Micropropagation of ginger (*Zingiber officinale* Rosc.)" was carried out in the tissue culture laboratory of the Department of Horticulture, University of Agricultural Sciences, Dharwad during the year 2004-06. The details of materials used and methods followed are presented below.

3.1 PLANT MATERIAL

Ginger (*Zingiber offinale* Rosc.) cultivar 'Bidar' local was taken for investigation. Rhizomes were kept in sand for sprouting. The stored sprouted rhizomes were used to get the explants.

3.2 EXPLANTS AND THEIR PREPARATION

3.2.1 Shoot tip

Shoot tip explants of 2-3 cm size were excised from the mother rhizome.

3.2.2 Axillary bud

Well developed axillary buds of size 1-2 cm were separated from the rhizome and their outer sheath were removed.

3.3.3 Root tips

Root tips of size 0.5-1 cm were separated from the mother rhizomes.

3.3 MEDIA

Murashige and Skoog (1962) basal medium was used for all the experiments. MS media were prepared from stocks solution (Appendix-I). Modification to the medium was done by adding growth regulators and other organic additives.

3.3.1 Preparation of stocks

Murashige and Skoog (MS) medium was commonly used for all the experiments. The stock solutions (10x) were prepared as given below with double distilled water, poured into well stoppered bottle and were stored in refrigerator at 4^0C.

Stock A: Macro nutrients – 1000 ml (10x)

Stock B: Micro nutrients – 1000 ml (10x)

Stock C: Vitamin – 100 ml (50x)

3.3.2 Preparation of growth regulator stocks

Stock solutions of kinetin (KIN) and 6-benzylamino purine (BAP) were prepared by dissolving them first in few drops of 1N NaOH and the volume was made upto the required concentration with double distilled water.

3.3.3 Preparation and sterilization of media

The stock solutions were mixed in required proportion along with growth regulators and sucrose. The volume was made up by adding double distilled water. The pH of the medium was adjusted between 5.6-5.8 by using either 0.1 N HCl or NaOH with the help of a digital pH meter. The volume was finally adjusted and required amount of agar and streptomycin was weighed and added into the medium. Agar in the medium was completely melted by gentle heating upto 90^0C and 15-20 ml of medium was poured into 25 x 150 mm pre sterilized glass culture tubes and plugged with non absorbent cotton wrapped in cheese cloth.

The media was autoclaved at 121^0C at 15 lbs/square inch pressure for 20 minutes and then allowed to cool to room temperature and stored in culture rooms until further use.

3.4 CULTURE ESTABLISHMENT

3.4.1 Surface sterilization of explants

Shoot tips, axillary buds, root tips were first washed in water with few drops of detergent (Teepol) and rinsed with distilled water 2 to 3 times and again they were immersed with Bavistin for 25 min and then rinsed with DW for 2-3 times. The final surface sterilization

Plate 1. Rhizomes kept in sand for sprouting

Plate 2. Sprouted rhizomes

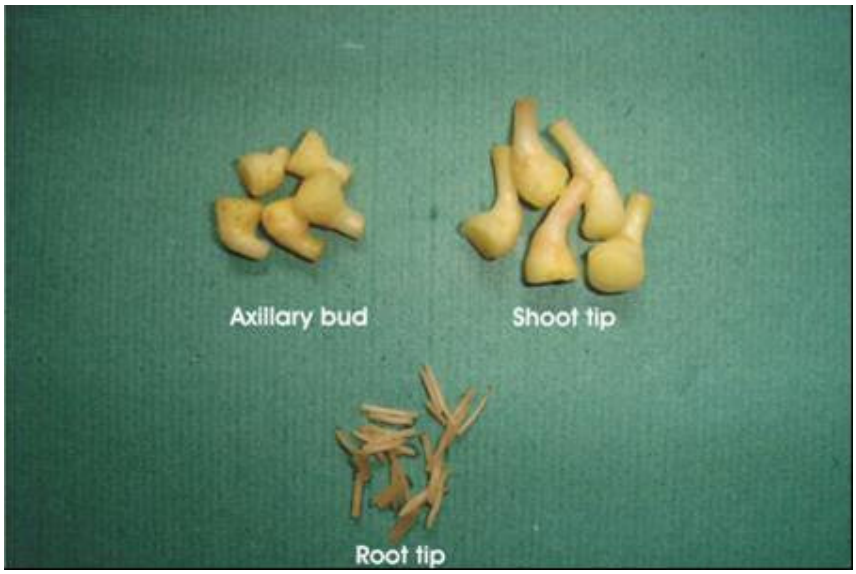

Plate 3. Explant preparation in ginger

was done with 0.1 per cent $HgCl_2$ for 12 min and then washed with distilled water for 3-4 times in the laminar air flow cabinet.

3.4.2 Inoculation

Sterilized explants were inoculated in test tubes containing the medial. The cut ends of explants were kept in such a way so as to have maximum contact with the medium.

3.4.3 Transfer area and maintenance of aseptic conditions

All the aseptic manipulations such as surface disinfection of explants, preparation and inoculation of explants and subsequent sub-culturing were carried out in the laminar air flow cabinet. The working table of laminar air flow cabinet and spirit lamp were sterilized by swabbing with absolute alcohol. All the required materials like media, spirit lamp, lighter, glass ware etc. were transferred on to the clean laminar air flow. The UV light was switched on for half on hour to achieve aseptic environment inside the cabinet where all manipulations were conducted.

3.4.4 Sub culture

Microshoots formed in the test tubes were taken out 5-6 weeks after inoculation. The shoots were separated by dissecting them in the sterile environment of laminar air flow cabinet with sterile dissecting needle and forceps. They were placed in the test tubes containing fresh media.

3.4.5 Rooting

The microshoots which were more than 2-3 cm in height were taken out and were placed in the tubes containing media with different concentrations of IBA and NAA for rooting.

3.5 HARDENING OF *IN VITRO* PLANTLETS

Young rooted plantlets were taken out of the test tubes, washed with distilled water and planted in net pots containing different hardening media. These plants were maintained in a prototype polytunnel. The plants were watered twice in a day initially, then once in a day after 8-10 days. Later they were transferred to green house after 15 days for further acclimatization.

3.5.1 Hardening media

1. Peat
2. Vermiculite
3. Sand

The media were first autoclaved at 121 ^{o}C for 20 minutes to make it sterile. They were filled into small plastic containers with holes at the bottom to ensure the drainage of excess water.

3.6 EXPREIMENTAL DETAILS

3.6.1 Experiment – I : Standardize the source of explants to micropropagation

Test : 't' test

Replications : 7

Number of explants used/treatment : 10

Treatment details

T_1 – Shoot tip

T_2 – Axillary bud

T_3 – Root tips

3.6.2 Experiment – II : Standardize the sterilization procedure for different explants

Design : CRD

Replications : 5

Number of explants used/treatment : 10

Treatment details

T_1 – Sodium hypochlorite (0.5%) 10 min

T_2 – Sodium hypochlorite (0.5%) 15 min

T_3 – $HgCl_2$ (0.1%) 10 min

T_4 – $HgCl_2$ (0.1%) 12 min

T5 – $HgCl_2$ (0.1%) 15 min

3.6.3 Experiment – III : Standardize the growth regulator for shoot (growth) multiplication

Design : CRD

Replications : 3

Number of explants used/treatment : 10

Treatment details

T_1 – MS

T_2 – MS + BAP 0.5 mg/l

T_3 – MS + BAP 1.0 mg/l

T_4 – MS + BAP 1.5 mg/l

T_5 – MS + BAP 2.0 mg/l

T_6 – MS + KIN 0.5 mg/l

T_7 – MS + KIN 1.0 mg/l

T_8 – MS + KIN 1.5 mg/l

T_9 – MS + KIN 2.0 mg/l

3.6.4 Experiment – IV : Standardize the growth regulator for root growth

Design : CRD

Replications : 3

Explants used/treatments : 10

Treatment details

T_1 – MS

T_2 – MS + IBA 0.5 mg/l

T_3 – MS + IBA 1.0 mg/l

T_4 – MS + IBA 1.5 mg/l

T_5 – MS + IBA 2.0 mg/l

T_6 – MS + NAA 0.5 mg/l

T_7 – MS + NAA 1.0 mg/l

T_8 – MS + NAA 1.5 mg/l

T_9 – MS + NAA 2.0 mg/l

3.6.5 Experiment – V : Identification of suitable hardening medium

Design : CRD

Replications : 6

Both explants used/treatment : 10

T_1 – Peat

T_2 – Vermiculite

T_3 – Sand

Shoot tip

Axillary bud

Root tip

Plate 4. Explants inoculated

3.7 COLLECTION OF DATA

3.7.1 Explants free from contamination

After inoculation of explants in test tubes, it was ensured to free from fungus, bacteria, browning etc.

3.7.2 Number of days taken for sprouting

The number of days taken to show initial differentiation of shoot from the date of inoculation of different explants was recorded and was expressed as mean number of days.

3.7.3 Per cent survival of explants

The number of explants survived and total number of explants inoculated were recorded and was converted into per cent.

3.7.4 Number of shoots produced per explants

While subculturing multiple shoots were separated, counted from explants and expressed as shoot per explant.

3.7.5 Number of days taken for initiation of shoots

Number of days taken to show initial differentiation of shoot after 45 days of inoculation was recorded.

3.7.6 Mean length of shoots

The shoot length was measured from base to the tip of the plantlet at the time of sub-culture and the average length was expressed in centimeters.

3.7.7 Number of days taken for initiation of roots

The number of days taken for initiation of roots, after inoculation was recorded.

3.7.8 Mean number of roots

The number of roots formed per microshoot were recorded and average was worked out.

3.7.9 Root length

From each shoot the length of longest roots was measured from the collar region to the highest root tip as a root length and expressed in cm

3.7.10 Per cent survival of plantlets

The number of plantlets survived out of total plantlets subjected to hardening was counted at different intervals and the percentage calculated.

3.8 STATISTICAL ANALYSIS OF DATA

The experimental data relating to contamination percentage, per cent survival of plantlets was transformed to arcsine values and analyzed under CRD. The data were subjected to analysis of variance test (ANOVA) as suggested by Panse and Sukhatmi (1967). Critical difference values were tabulated at one per cent probability where ever ‘F’ test found significant. The experimental data relating to explant type were analyzed under ‘t’ test.

IV. EXPERIMENTAL RESULTS

The results obtained in the present investigation on "micropropagation of *Zingiber officinale* Rosc. are presented under the following headings.

4.1 Standardization of the source of explants for micropropogation

4.2 Standardization of the sterilization procedure for different explants

4.3 Standardization of the growth regulators for shoot growth

4.4 Standardization of the growth regulators for root growth

4.5 Identification of suitable hardening medium for micropropagation

4.1 STANDARDIZATION OF THE SOURCE OF EXPLANTS FOR MICROPROPOGATION

4.1.1 Number of days taken for sprouting

The minimum time for sprouting was taken by shoot tip explant (5 days) to show primordial emergence followed by axillary bud (6 days) are presented in Table 1.

4.1.2 Per cent survival of explants

There was no significant difference between the treatments for per cent survival of explants.

4.1.3 Mean number of shoot produced per explants

Significant difference existed among the different types of explants for number of shoots formed.

The shoot tip explant produced the highest number of shoots (2), after the primordial emergence, followed by axillary bud explant which showed (1.5) number of shoots after sprouting.

4.2 STANDARDIZATION OF THE STERILIZATION PROCEDURE FOR DIFFERENT EXPLANTS

The explants of shoot tip and axillary bud treated with mercuric chloride and sodium hypochlorite for varying periods of time at different concentrations in order to establish maximum contaminant free cultures the results are presented in Table 2.

The highest percentage (90%) of healthy and contaminant free explants were established when they were exposed to 0.1 per cent mercuric chloride for a duration of twelve minutes. This was followed by establishment of 70 per cent of the explants when treated with the same surface sterilant for ten minutes. The least number of contaminant free cultures (37%) was obtained when 0.5 per cent sodium hypochlorite was used for ten minutes. When they were exposed to 0.1 per cent $HgCl_2$ for 15 minutes only 24 per cent contaminant free cultures obtained but, 33 per cent explants were died.

4.3 STANDARDIZATION OF THE GROWTH REGULATORS FOR SHOOT GROWTH

4.3.1 Number of days taken for initiation

There was significant difference with respect to time taken for initiation of shoots. The shoot initiation was early in BAP than KIN supplemented media.

Time taken for the shoot initiation was minimum (8.8 days) in 2 mg/l BAP, while it was maximum (11.1 days) in 0.5 mg/l KIN in shoot tip explant, followed by minimum (10 days) in 2 mg/l BAP, while maximum (11.6 days) was in 1.0 mg/l KIN axillary bud (Table 3 and 4).

4.3.2 Number of shoots produced per explant

There was significant difference between axillary bud and shoot tip explants with respect to the number of shoots produced. The maximum number of shoots were produced in shoot tip.

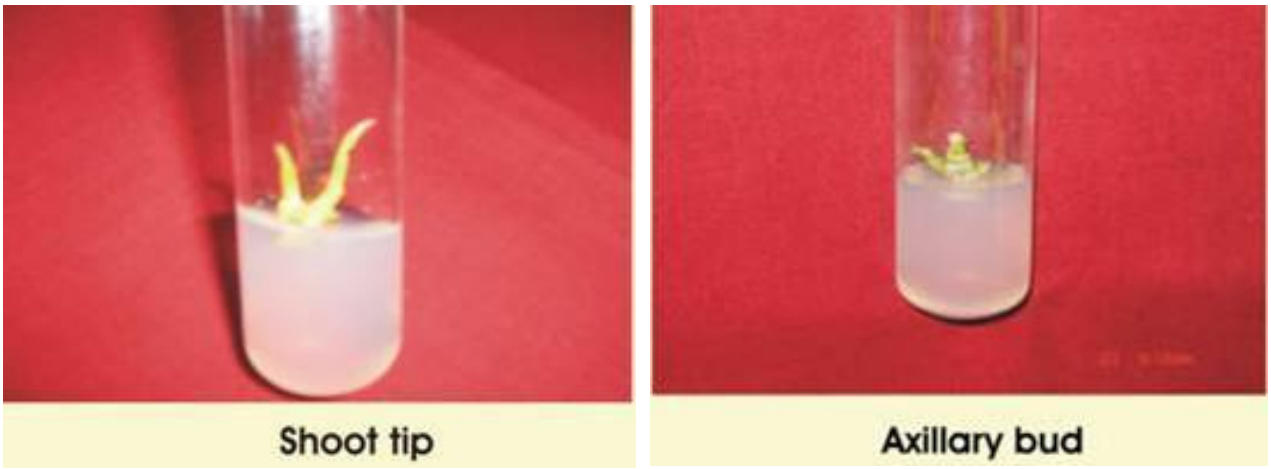

Plate 6. Hardening material

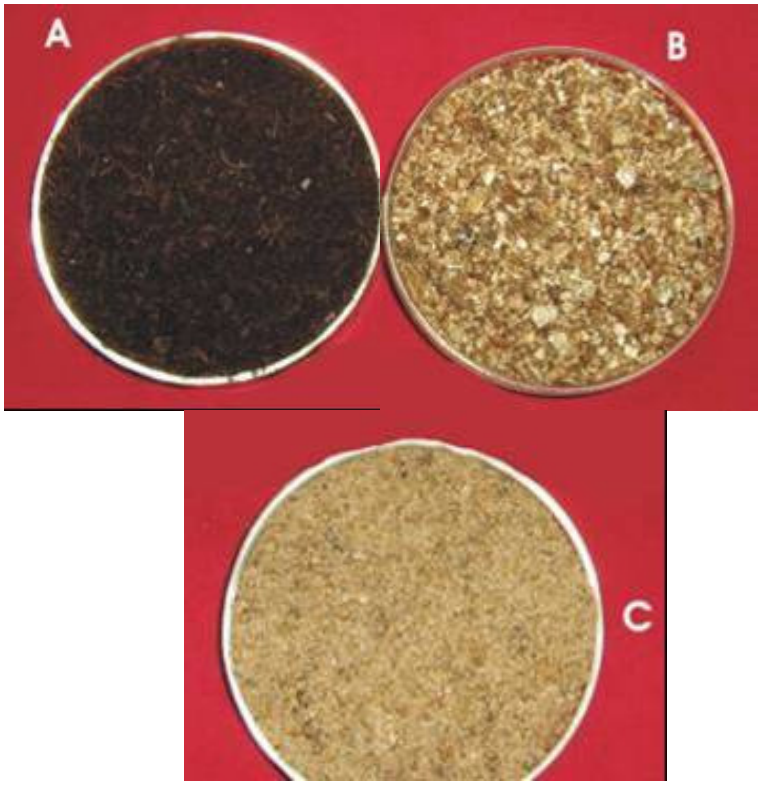

Plate 6. Hardening material

Table 1 : Influence of explant type on growth of *in vitro* shoots in ginger

Sl. No.	Explant type	No. of days taken for sprouting	Per cent survival of explants	No. of shoots produced/ explant
1	Shoot tip	5.0	81 (64.16)	2.0
2	Axillary bud	6.0	78 (62.03)	1.5
	T test	S	NS	S

Figures in parenthesis indicate arcsine transformed values
S – Significant NS – Non-significant

In shoot tip maximum number of shoots (5.1 shoots/explant) were produced in media supplemented with 2 mg/l BAP and minimum (1.1 shoots/explant) number of shoots were produced in 0.5 mg/l KIN supplemented medium (Table 3 and Plate 7).

In axillary bud explants maximum shorts (4 shoots/explant) were produced in media supplemented with 2 mg/l BAP and minimum (1.3 shoots/explant) number of shoots were produced in 1.5 mg/l KIN, supplemented medium (Table 4). Wherein single shoot production was observed in control (Plate 8).

4.3.3 Mean length of shoots

Shoots in media with BAP showed increased shoot length compared to KIN. The maximum (4.5 cm) and minimum (1.6 cm) shoot length were observed in control and 2.0 mg/l KIN supplemented media respectively. Increase in the cytokinin concentration in media decreased the shoot length.

4.4 STANDARDIZATION OF THE GROWTH REGULATORS FOR ROOT GROWTH

4.4.1 Number of days for initiation of roots

There was significant difference with respect to initiation of root primordia among the auxins used. Root initiation was early in IBA than NAA supplemented shoots. In shoot tip explant, time taken for root initiation was minimum (7 days) in 1 mg/l IBA treated shoots, while it was maximum (8.3 days) in 0.5 mg/l NAA (Table 5). In axillary bud explant, root initiation was minimum (7 days) in 0.5 mg/l IBA treated shoots, while it was maximum (8.5 days) in 2.0 mg/l NAA (Table 6). The shoots in control took 7/8 days for the emergence of root primordia.

4.4.2 Mean number of roots

Significant differences were noticed among the different treatments with respect to number of roots.

Among the treatments, the maximum number of roots were observed in shoot cultured on 1 mg/l NAA supplemented media (7) and it was minimum (3.0) in control in shoot tip. Results pertaining to this are presented in Table 5 and Plate 10. Followed by maximum number of roots were observed in 0.5 mg/l IBA (6.2) and minimum (2.5) in control in axillary bud explants. The number of roots per shoot were more in IBA than NAA (Table 5 and 6).

4.4.3 Mean length (cm)

Maximum root growth (3.3 cm) was recorded in control and minimum root growth (1.5 cm) was recorded in 0.5 mg/l NAA in shoot tip followed by maximum root growth (3.0 cm) was

Table 2 : Effect of surface disinfectants on per cent contamination and number of healthy cultured established in ginger explants

Sl. No.	Treatments	Exposure time (min)	No. of explants inoculated	No. of explants contaminated	No. of healthy cultures established
1	Sodium hypochlorite (0.5%)	10	30	19 (63)	11 (37)
2	Sodium hypochlorite (0.5%)	15	30	12 (40)	18 (60)
3	$HgCl_2$ (0.1%)	10	30	9 (30)	21 (70)
4	$HgCl_2$ (0.1%)	12	30	3 (10)	27 (90)
5	$HgCl_2$ (0.1%)	15	30	9 (24)	14 (43)
	S.Em$\pm$	-	-	0.4	0.7
	CD at 1%	-	-	1.4	2.5

Values in the parenthesis indicate percentage

recorded in control and minimum (1.3 cm) was recorded in 1.0 mg/l NAA in axillary bud explant.

4.5 IDENTIFICATION OF SUITABLE HARDENING MEDIUM FOR MICROPROPAGATED GINGER PLANTLETS

4.4.1 Survival percentage of plantlets

The data regarding the survival percentage of plantlets during hardening at different intervals are presented in the Table 7.

Significant differences were noticed for survival percentage of plantlets after 15 and 30 days after transferring to hardening media. The maximum survival was noticed on peat medium with 80 per cent at 15 and 30 days after transfer on to hardening medium. However, the lowest survival was on a sand showing 65 and 50 per cent at 15 and 30 days after transfer to hardening medium respectively.

4.5.2 Height of the plantlets

Significant differences were observed among the treatments. The maximum height was recorded in peat (5 cm) which was significantly superior to all other treatments. This was followed by vermiculite (4.1 cm) results are presented in Table 8.

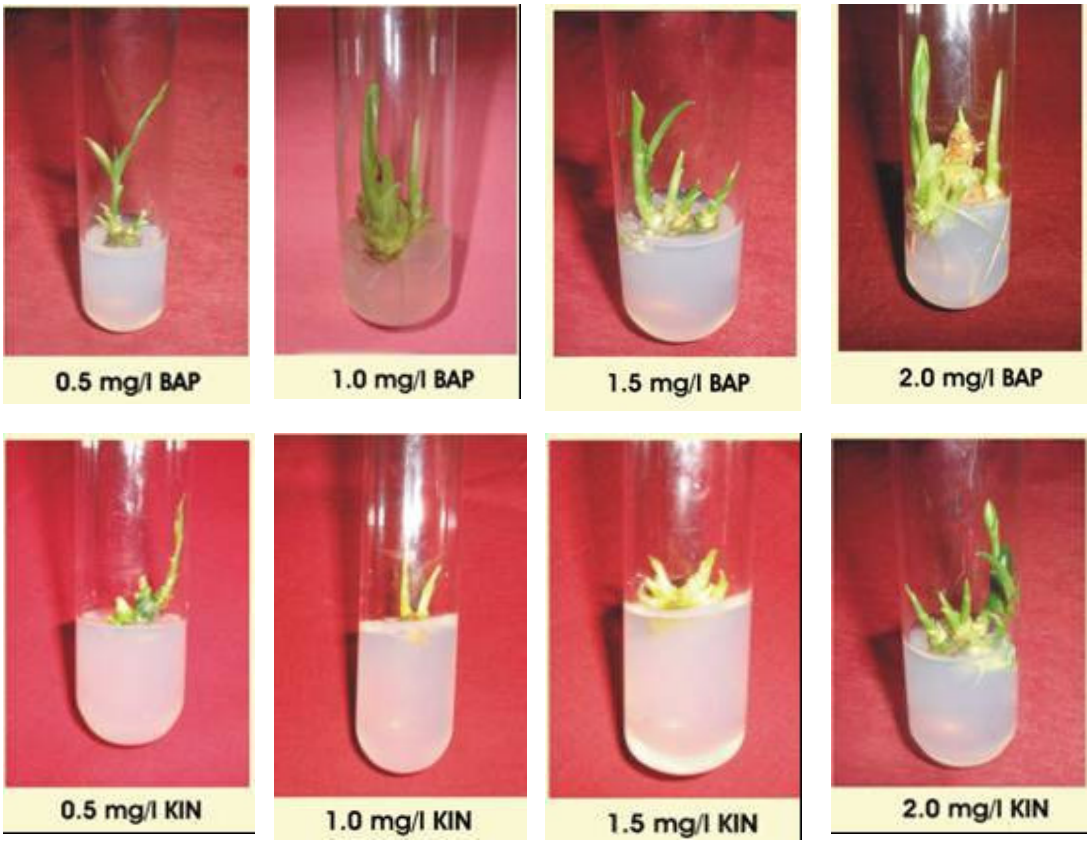

Plate 7. Multiple shoot production on MS medium with different levels of cytokinins

Plate 8. Single shoot formed on medium without growth regulators

Table 3 : Growth parameters of shoots as influenced by cytokinins in ginger (shoot tip)

Sl. No.	Treatment (mg/l)	No. of days taken for shoot initiation	No. of shoots	Mean length of shoots (cm)
1	MS	-	1.0	4.8
2	MS + 0.5 BAP	10.2	2.0	4.0
3	MS + 1.0 BAP	9.8	3.6	3.6
4	MS + 1.5 BAP	9.2	4.4	3.2
5	MS + 2.0 BAP	8.8	5.1	3.1
6	MS + 0.5 KIN	11.1	1.1	2.8
7	MS + 1.0 KIN	10.8	2.1	2.6
8	MS + 1.5 KIN	10.0	3.8	2.0
9	MS + 2.0 KIN	9.0	4.5	1.6
	S.Em±	0.02	0.01	0.18
	CD at 1%	0.08	0.04	0.72

4.5.3 Number of leaves per plant

Highest number of leaves was recorded in peat (4.0) and minimum (3.0) leaves were observed in sand.

Table 4 : Growth parameters of shoots as influenced by growth regulators in ginger (axillary bud)

Sl. No.	Treatment (mg/l)	No. of days taken for shoot initiation	No. of shoots	Mean length of shoots (cm)
1	MS	-	1.0	4.1
2	MS + 0.5 BAP	11.3	1.8	3.8
3	MS + 1.0 BAP	11.0	2.3	3.2
4	MS + 1.5 BAP	10.5	3.0	2.5
5	MS + 2.0 BAP	10.0	4.0	2.1
6	MS + 0.5 KIN	12.0	2.1	2.7
7	MS + 1.0 KIN	11.6	1.9	2.3
8	MS + 1.5 KIN	11.2	1.3	2.0
9	MS + 2.0 KIN	11.0	3.0	1.8
	S.Em$\pm$	0.03	0.17	0.15
	CD at 1%	0.09	0.68	0.60

Table 5 : Effect of auxins on *in vitro* production of roots of ginger (shoot tip explant)

Sl. No.	Treatment (mg/l)	No. of days taken for initiation	Average no. of roots	Root length (cm)
1	MS	7.5	3.0	3.3
2	MS + 0.5 IBA	7.6	6.0	1.3
3	MS + 1.0 IBA	7.0	7.0	1.5
4	MS + 1.5 IBA	7.9	6.5	1.8
5	MS + 2.0 IBA	8.0	5.8	2.3
6	MS + 0.5 NAA	8.3	5.0	1.8
7	MS + 1.0 NAA	7.5	4.3	2.0
8	MS + 1.5 NAA	7.4	3.8	2.5
9	MS + 2.0 NAA	8.5	3.2	2.8
	S.Em$\pm$	0.01	0.27	0.12
	CD at 1%	0.04	1.08	0.48

Table 6 : Effect of auxins on *in vitro* production of roots of ginger (axillary bud)

Sl. No.	Treatment (mg/l)	No. of days taken for initiation	Average no. of roots	Root length (cm)
1	MS	8.0	2.5	3.0
2	MS + 0.5 IBA	7.0	6.2	1.7
3	MS + 1.0 IBA	7.1	5.3	2.0
4	MS + 1.5 IBA	7.5	4.9	2.1
5	MS + 2.0 IBA	7.7	4.5	2.4
6	MS + 0.5 NAA	8.0	5.2	1.2
7	MS + 1.0 NAA	8.1	4.1	1.3
8	MS + 1.5 NAA	8.2	3.8	1.5
9	MS + 2.0 NAA	8.5	3.2	2.2
	S.Em$\pm$	0.10	0.21	0.11
	CD at 1%	0.40	0.84	0.44

1 mg/l IBA

1.5 mg/l IBA

2 mg/l IBA

Plate 9. Roots formed on medium Without growth regulators

1 mg/l NAA

2 mg/l NAA

1.5 mg/l NAA

Plate 10. Roots induced *in vitro* on MS medium with different quxins

Table 7: Effect of media on survival percentage of plantlets during hardening in ginger

Sl. No.	Medium	Survival	
		15 DAT	30 DAT
1	Peat	80 (63.4)	80 (63.4)
2	Vermiculite	75 (60.0)	75 (60.0)
3	Sand	65 (53.7)	50 (45.0)
	S.Em±	0.9	0.8
	CD at 1%	3.2	2.9

DAT – Days after transfer to hardening media
Figures in parenthesis indicate arcsine transformed values

Table 8: Effect of media on growth of ginger plantlets during hardening

Sl. No.	Medium	Plant height (cm)	No. of leaves/ plant
1	Peat	5.0	4.0
2	Vermiculite	4.1	3.5
3	Sand	3.5	3.0
	S.Em±	0.15	0.20
	CD at 1%	0.50	0.70

Plate 11. Establishment of plantlets on different hardening media

Plate 12. Secondary hardened plant

V. DISCUSSION

The present investigation was undertaken to standardize the protocols for culture establishment, multiple shoot production, *in vitro* rooting and also suitable hardening media for micropropagated plantlets of ginger were assessed.

5.1 CULTURE ESTABLISHMENT

The function of culture establishment is to disinfect the explant, establish of explant in culture media and stabilize the culture media and the explant for multiple shoot production (Mc Cown, 1986).

5.1.1 Standardization of the source of explants for micropropogation

The type of organs or explants chosen affect the successful establishment of the cultures and their subsequent growth. Not all the tissues or organs of a plant are equally capable of exhibiting morphogenesis (Hartmann *et al.,* 1997).

In the present study, to identify a suitable explant for *in vitro* propogation of ginger different explants were tried. Among the various explants, shoot tips gave the quickest response for initial growth and the highest number of multiple shoots. On the other hand, axillary bud took more time for the regeneration of shoots. This difference in response, among the different explants might be due to difference in physiological state of the explants (Sreelatha *et al.,* 1998). This may also be due to the fact that, the shoot tip has meristematic region where cell division and differentiation occurs (Hartmann *et al.,* 1997).

Murashige (1974) made a similar observation and reported that shoot tips are highly regenerative. The highest number of multiple shoots were produced by shoot tip explant. This may be due to excised apex when placed on the medium with high inorganic nutrient salt, further development of the terminal meristem is inhibited and this primordial axillary buds are forced into growth, resulting in the rapid proliferation into more number of short shoots (Hackett and Anderson, 1967).

In the present study, the shoot tip gave maximum multiple shoots and survival percentage which is in line with the findings of Malmug *et al.* (1991), Mukund (1998), Balakrishnamurthy and Rangaswamy (1992) in banana.

Root tip from rhizome was unable to establish *in vitro*.

5.1.2 Standardization of the sterilization procedure for different explants

In vitro propogation involves culturing explants under aseptic conditions in which surface sterilization or disinfection is one of the important prerequisites for successful micropropagation. Removing contaminants from the surface of the organ/explant is of prime concern (Hartmann *et al.,* 1997). The contamination of explants may be due to fungi, bacteria, moulds, yeasts etc., present on the surface or lodged in the cracks, scales etc. General disinfection procedures have been given by various workers for plant tissues (Doods and Robert, 1982). Disinfection requires the use of chemicals that are toxic to microorganisms but non-toxic to plant materials. Tissue culture became possible with the use of convenient and effective disinfectants such as ethanol, sodium hypochlorite, mercuric chloride, calcium hypochlorite and others (Krikorian, 1982).

The current investigation on effect of surface sterilants on reducing contamination rate and per cent of healthy cultured plants. It showed that $HgCl_2$ is better sterilant than NaOCl in reducing contamination rate. This is because the most useful radical in $HgCl_2$ is probably the chlorite, commonly present as bichloride of mercury. Mercuric chloride is extremely poisonous due to high bleaching action of two chloride atoms and also mercuric ions which combines strongly with protein causing death of organism (Pauling, 1955). Even though NaOCl consists of chlorine atom, its bleaching and disinfectant action is due to the slow decomposition of the salt to produce oxygen (Secrist and Powers, 1966).

The highest numbers of aseptic culture was obtained with $HgCl_2$ at 0.1 per cent for 12 minutes. Different authors have reported, differential response from rhizomatous crop, to get contaminant free cultures using different durations.

Raju *et al.* (2005) got the results using 0.1 per cent $HgCl_2$ for 15 minutes and Rahman *et al.* (2004) used 0.1 per cent $HgCl_2$ for 14 minutes to establish aseptic cultures in

turmeric. These findings are in conformity with the results obtained by Nadagouda *et al.* (1983) in cardamom and De Lange *et al.* (1987) obtained similar in ginger.

The higher concentration of $HgCl_2$ at 0.1 per cent for 15 minutes, observed more contamination and also death of the explants. This is due to the high bleaching activity of chlorine which killed the cells.

The lowest aseptic cultures were obtained with NaOCl 0.5 per cent. This is because of reduced effectiveness of chemicals at lower concentrations.

Optimal concentration of chemical and duration of chemical in the present investigation was $HgCl_2$ at 0.1 per cent for 12 minutes with respect to low contamination rate.

5.1.3 Standardization of the growth regulators for shoot growth

The results revealed that, the multiple shoot formation was more in BAP compared to KIN. This was in confirmation with the results of Wong (1986) and Zamora *et al.* (1986) who observed that BA is the cytokinin of choice for induction of shoot bud proliferation *in vitro* and BA has been found to be superior to kinetin in banana.

In the present study, the less number of days for initiation and the highest number of multiple shoots were observed in 2 mg/l BAP supplemented media. This was in confirmation with the results of Dipti *et al.* (2005), who reported that the highest number of multiple shoots in media supplemented with 2 mg/l BAP in shoot tip and 3 mg/l BAP in rhizome bud in turmeric, proved its superiority over KIN and NAA by producing more number of multiple shoots. BAP at 3 mg/l was most beneficial for proliferation in turmeric (Balachandran *et al.,* 1990). Similarly Winnar and Winnar (1981) reported that BAP 1 mg/l was most useful for development of multiple shoots. These findings are in conformity with the Keshavachandran and Khader (1989) and Shetty *et al.* (1982).

In control which had only a single shoot and mean length of shoot was maximum which may be because of apical dominance.

The results of the study revealed that the number of shoots increased and the mean length of shoots decreased as the concentration of cytokinins increased.

Media with the highest cytokinin concentration showed the maximum number of multiple shoots and lowest length of shoots. This may be due to the fact that suppression of apical dominance leads to the production of more number of multiple shoots and reduced shoot length.

5.1.4 Standardization of the growth regulators for root growth

Rooting of micro shoots require addition of auxins to the medium.

In the present investigation both NAA and IBA have been used for rooting. IBA induced early rooting compared to NAA.

The maximum number of roots were observed in media with 1 mg/l IBA. This was in confirmation with the results of Sit and Tiwari (1998) who found rooting of turmeric was best on 0.5 mg/l IBA. They concluded that rooting did not occur in the absence of IBA. Number of roots per shoot let was proportional to IBA concentration. Rahman (2004) also observed that IBA at 0.2 mg/l resulted in maximum number of roots. Further, the next best treatment in the present study was 1.5 mg/l IBA followed by 0.5 mg/l IBA.

But contradictory results were obtained by Meenakshi *et al.* (2001), who reported that maximum rooting observed in NAA 0.3 mg/l with maximum root length. These results were in accordance with the findings of Dogra *et al.* (1994) in ginger and Raju *et al.* (2005) in turmeric.

However, the maximum root length was observed in control.

5.1.5 Identification of suitable hardening medium for better establishment

The *in vitro* grown plantlets were used for hardening on three different media *viz.,* peat, vermiculite and sand. The highest survival percentage and better vigour of the plantlets was observed in peat medium followed by vermiculite and sand. This may be due to the optimum conditions like good aeration, higher water holding capacity and nutrients present in the medium which have boosted the growth of ginger plantlets. Hence, peat media was found to be most suitable for plant growth compared to vermiculite and sand. Similar results were also observed by Inden *et al.* (1988) in ginger.

It can be concluded that the peat is the best medium for production of *in vitro* plantlets of ginger.

Protocol for micropropagation of ginger

Based on the results, a protocol for micropropagation of ginger is given below.

1. Shoot tips of 2-3 cm should be isolated
2. The isolated explants are to be treated with a fungicide (Bavistin) for 25 minutes and then washed with distilled water. They are to be treated with 0.1 per cent $HgCl_2$ for 12 minutes and then washed 3-4 times with sterile distilled water in laminar air flow cabinet.
3. After sterilization, explants need to be cultured on MS medium for 1 month.
4. These shoots may be cultured on basal medium with 2 mg/l BAP for shoot multiplication.
5. After 2-3 subcultures micro shoots may be placed on MS medium containing 1 mg/l IBA for rooting.
6. These rooted plantlets can be hardened on peat media for 30 days in green house.

FUTURE LINE OF WORK

1. Induction of somoclonal variation for development of new varieties
2. *In vitro* microrhizome production
3. Somatic embryogenesis

VI. SUMMARY

The present study on micropropagation of *Zingiber officinale* Rosc. was conducted in the tissue culture laboratory in the Department of Horticulture, University of Agricultural Sciences, Dharwad during 2004-06.

Ginger is an important spice crop grown in India. It is herbaceous rhizomatous perennial plant. The method of propagation is through sections of underground rhizomes. However, it has a dormancy period and sproutes only during the monsoon that to only 5 to 6 plants can be obtained from one single rhizome per year. To overcome this, micropropagation may plays a important role in rapid mass propagation of ginger. Plants produced through micropropagation are true to type and are free from diseases. The present investigations were carried out to standardize surface sterilization of explants, suitable explant type for culture establishment, growth regulators for shoot multiplication and rooting and to evaluate suitable hardening media.

Of the various concentrations of $HgCl_2$ and NaOCl tried for surface disinfection. Among that the different concentration of $HgCl_2$ tried at 0.1 per cent emerged as the best treatment.

Different types of the explants *viz.,* shoot tip, axillary bud, root tip were tried. It was observed that shoot tip gave the best results and emerged as suitable explant for ginger culture establishment.

Among different concentrations of cytokinins *viz.,* BAP and kinetin. 2.0 mg/l BAP gave the highest number of multiple shoots.

Among different auxins tried at different concentrations for rooting of microshoots, MS medium with 1 mg/l IBA gave the highest number of roots.

Three different media *viz.,* peat, vermiculite and sand were tried for hardening of plantlets. Peat gave the maximum survival percentage with better plant growth resulting as a suitable medium for hardening.

VII. REFERENCES

ALI, A., MUNAWAR, A. AND SIDDIQUI, F. A., 2004, *In vitro* propagation of turmeric, *Curcuma longa* L. *International Journal of Biology and Biotechnology*, 1 : 511-518.

ANONYMOUS, 2001, Statistical data on horticultural crops in Karnataka state at a glance. Directorate of Horticulture, Lalbagh, Bangalore, pp. 66-67.

ANONYMOUS, 2002, Directorate of Economics and Statistics, New Delhi, p. 17.

BAILEY, L. H., 1949, Manual cultivated plants, 2nd Ed. McMilan Company, New York.

BALACHANDRAN, S. M., BHAT, S. R. AND CHANDEL, K. P. S., 1990, *In vitro* clonal multiplication of turmeric (*Curcuma* sp.) and ginger (*Zingiber officinale* Rosc.). *Plant Cell Reports*, 8 : 521-524.

BALAKRISHNAMURTHY, G. AND RANGASWAMY, S. R. S., 1992, Rapid propagation of banana through *in vitro* culture of shoot tips. *In : New Trends in Biotechnology* (Ed.) N. S. Subba Rao, C. Balagopalan and S. V. Ramakrishna, pp. 149-155.

BERGER, F., WAITES, W. M. AND LEIFERT, C., 1994, An improved surface disinfection method for shoot explants from Iris rhizomes infected with bacterial soft rot (*Erwinia carotovora* sp. Carotovora.). *Journal of Horticultural Sciences*, 69 : 491-494.

BHARTENDU, V., KUTMAR, D. AND KUNDAPURLAR, A. R., 1989, Large scale plantlet production of cardamom by shoot bud culture. *Plant Physiology and Biochemistry*, 14: 14-19.

CHOI, S. K. AND KIM, D. C., 1991, The study on the clonal multiplication of ginger through *in vitro* culture of shoot apex. *Biotechnology*, 33: 40-45.

CHOI, S. K., 1991, Studies on the clonal multiplication of ginger through the *in vitro* cuttings. *Research Reports of the Rural Development Administration*, 38 : 33-39.

CONGFA, L., ZHEPIN, W., YANGFENG, L., YONGJIA, Z. AND SHOUHUA, W., 2001, Study on shoot tip culture and rapid propagation techniques in tissue culture of Fuan ginger (*Zingiber officinale* Rosc.). *Acta Agriculturae Shanghai*, 17 : 21-24.

CONTI, L., FRANGI, P., TOSCA, A. AND YERGA, P., 1991, Breeding clones of *Gerber jamesonii* Hybr. suitable to micropropagation and pot cultivation. *Acta Horticulturae*, 300 : 103-105.

CRONAUER, S. S. AND KRIKORIAN, A. D., 1984, Multiplication of Musa from excised stem tips. *Annuals of Botany*, 53 : 321-328.

DESJARDINS, Y. A., GOSELIN AND YELLOW, S., 1987, Acclimatization of *In vitro* straw berry plantlets in Co_2 enriched environment and supplementary lighting. *Journal of American Society of Horticultural Science*, 112 : 846-852.

DIPTI, T., GHORADE, R. B., SWATI, M., PAWAR, B. V. AND EKTA, S., 2005, Rapid multiplication of turmeric by micropropagation. *Annual plant Physiology*, 19 : 35-37.

DOGRA, S. P., KORIA, B. N. NAD SHARMA, P. P., 1994, *In vitro* clonal propagation of ginger (*Zingiber officinale* Rosc.). *Horticultural Journal*, 7 : 45-50.

DOODS, J. H. AND ROBERT, L. W., 1982, *Experiments in Plant tissue Culture*, Cambridge University Press, London, p. 178.

DORAISWAMY, R., RAO, S. N. K. AND ELIAS, K. C., 1983, Tissue culture propagation of banana. *Scientia Horticulture*, 18 : 247-252.

DORESWAMY, R., SATIJRAM, L., PRAKASH, J. AND PIERIK, R. L. M., 1991, Tissue culture strategies for banana. *Proceedings of the International Seminar on New Frontiers in Horticulture*, Bangalore, November 15-28, pp. 219-223.

EVANS, D. A., SHARP, W. R. AND FLINCK, C. E., 1981, Growth and behaviour of cell cultures : Embryogensis and organogenesis. In Plant tissue culture : Methods and applications in agriculture (TA Thrope, Ed.). *Academic Press*, New York, pp. 45-114.

FARIA, R. T. AND ILLAG, R. D., 1995, Micropropagation of *Zingiber spectabile* Griff. *Scientia Horticulture*, 62 : 135-137.

GONZALEZ, M. T. AND MOGOLLON, N. J., 2004, Performance of red ginger (*Alpinia purporata vieilla*) in six substrates during the acclimatization phase. *Proceedings of the Interamericon Society for Tropical Horticulture*, 42: 77-81.

GUPTA, P. P., 1986, Eradication of Mosaic disease and rapid clonal multiplication of bananas and plantains through meristem tip cultures. *Plant Cell, Tissue and Organ Culture*, 6: 33-39.

HACKETT, W. P., 1966, Application of tissue culture to plant propagation. *Proceedings of International Plant Propagation Society*, pp. 88-92.

HACKETT, W. P. AND ANDERSON, J. M., 1967, Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices. *Proceedings of American Society for Horticultural Sciences*, 90: 365-369.

HAMILL, S. D., SHALLOCK, S. L. AND SMITH, M. K., 1993, Comparison of decontamination methods used in initiation of banana tissue cultures from field collected suckers. *Plant Cell Tissue and Organ Culture*, 33 : 343-346.

HANDLEY, L. W. AND CHAMBLISS, D. L., 1979, *In vitro* propagation of *Cucumis sativus* L. *Horticultural Science*, 14 : 22-23.

HARTAMANN, H. F., KESTER, D. E., DAUIES, F. D. Jr. AND GENEVE, R. L., 1997, *Plant Propagation – Principles and Practices*, 6th Ed. Prentice Hall of India Private Ltd., New Delhi, pp. 549-611.

HAUNG, J. H., 1995, *In vitro* propagation and preservation of ginger germplasm resources. *Scientia Agricultura Sinica*, 28 : 24-30.

HAZARE, S. T., KARNEWAR, S. D., KHEDKAD, C. D. AND PAWAR, V. N., 2005, *In vitro*, micropropagation and regeneration of turmeric. *Journal of Soils and Crops*, 15 : 304-307.

HOSOKI, T. AND SAGAWA, Y., 1977, Clonal propagation of ginger (*Zingiber officinale* Rosc.) through tissue culture. *Horticultural Science*, 12 : 451-452.

HU, C. Y. AND WANG, P. T., 1983, Meristem, shoot tip and bud cultures. In : Hand book of plant cell culture Vol. 1 techniques for propagation and breeding (D. A. Evans, W. R., Sharp, P. V. Ammirato and Y. Yamada, Eds.) MacMillan publishing Company, New York, pp. 177-227.

IKEDA, L. R. AND TANABE, M. J., 1989, *In vitro* subculture applications for ginger. *Horticultural Science*, 24 : 142-143.

ILAHI, I. AND JABEEN, M., 1987, Micropropagation of *Zingiber officinale* L. *Pakisthan Journal of Botany*, 19 : 61-65.

INDEN, H., ASAHIRA, T. AND HIRANO, A., 1988, Micropropagation of ginger. *Acta Horticulturae*, 230 ; 177-181.

KESHAVACHANDRAN, R. AND KHADER, M. A., 1989, Tissue culture propagation of turmeric. *South Indian Horticulture*, 37 : 101-102.

KOBZA, F. AND VACHUNOVA, J., 1991, Propagation of *Dracaena concina* Kunth. *Acta Universitatiq Agriculturae, Facultas, Horticulturae*, 6 : 51-55.

KRIKORIAN, A. D., 1982, Cloning higher plants from aseptically cultured tissues and cells. *Biological Reviews*, 57 : 151-181.

KUMAR, K. B., PRAKASH, K. P., BALACHANDRAN, S. M. AND IYER, R. D., 1985, Development of clonal plantlets from immature panicles of cardamom. *Journal of Plantation Crops*, 18 : 31-34.

KURUVINSHETTI, M. S. AND IYER, R. D., 1981, An evaluation of tissue culture technologies in coconut and turmeric. *Proceedings of the Fourth Annual Symposium on Plantation Crops*, 4 : 101-106.

LAWRENCE, B. M., 1984, Major tropics ginger (*Zingiber officinale* Rosc.). *Perfumer flavourist*, 9 : 16-20.

LEFFRING, L., 1971, Vegetative vermeerdering van gerbera vabel. *Bioemist*, 26 : 9.

LITZ, R. E., 1985, Somatic embryogenesis in tropical fruit trees, In : Tissue culture in Forestry and Agriculture. R. R. Henke, K. W. Hyghes, M. J. Constatin and A. Hollaender Eds., Plenum Press, New York, pp. 179-194.

MALMUG, J. J. F., INDEN, H. AND ASAHIVA, T., 1991, Plantlet regeneration and propagation from ginger callus. *Scientia Horticulture*, 48 : 89-97.

MANTE, S. AND TEPPER, H. B., 1982, Effect of hormones on multiple shoot initiation in banana. *Plant Cell and Tissue Culture*, 2 : 151-159.

MARTYR, R. F., 1981, New development in the uses of graded horticultural perlite. *Acta Horticulturae*, 126 : 143-146.

McCOWN, B. H., 1986, Woody ornamentals, shade trees and conifers. *In : Tissue Culture as a Plant Production System for the Horticultural Crops*. Eds, R. J. Zimmerman, R. J. Griesbach, F. A. Hammer Schlag and R. H. Lawson, Dordrecht : Martinus Nijhoft Publishers, Hoge, pp. 333-342.

MEENAKSHI, N., SULIKERI, G. S., KRISHNAMOORTHY, V. AND RAMAKRISHNA, V. H., 2001, Standardization of chemical environment for multiple shoot induction of turmeric (*Curcuma longa* L.) for *In vitro* clonal propagation. *Crop Research*, 22 : 449-453.

MUKUND, T., 1998, Standardization of micropropagation techniques banana cv. Kadali (*Musa acuminate* Colla). *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Bangalore, Karnataka.

MURASHIGE, T., 1974, Plant propagation through tissue culture. *Plant Physiology*, 22 : 135-165.

MURASHIGE, T., 1978, Principles of rapid propogation, In : Propagation of Higher Plants Through Tissue Culture a Bridge Between Research and Application (K. Huges, R. Henke and M. Constantin, Eds.) Technology Information Centre, USDE Oak, Ridge, pp. 14-24.

MURASHIGE, T. AND SKOOG, F., 1962, A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiology*, 15 : 473-497.

NADAGOUDA, R. S., MASCARENHAS, A. F., HENDRE, R. R. AND JAGANNATHAN, V., 1978, Rapid multiplication of turmeric (*C. longa* L.) plants by tissue culture. *Indian Journal of Experimental Biology*, 16 : 120-122.

NADAGOUDA, R. S., MASCARENHAS, A. F. AND MADHUSOODHANAN, K. J., 1983, Clonal multiplication of cardamom by tissue culture. *Journal of Plantation Crops,* 11 : 60-64.

NASIRUJJAMAN, K., UDDEN, M. S., ZAMAN, S. AND REZA, M. A., 2005, Micropropagation of turmeric (*Curcuma longa* L.) through *in vitro* rhizome bud culture. *Journal of Biological Sciences,* 5 : 490-492.

OLIVIER, J. J., 1996, The initiation and multiplication of ginger (*Zingiber officinale* Rosc.) in tissue culture. *In ligntings bulletin – Instituut Vin Tropiese en Subtropiese Gewasse*, 291 : 10-11.

PALAI, S. K., RAUT, G. R., DAS, P. AND EDISON, S., 1997, Micropropagation of ginger (*Zingiber officinale Rosc.*) Interaction of growth regulators and culture conditions. *Proceedings of the National Seminar on Biotechnology of Species and Aromatic Plants*, 8 : 20-24.

PANDEY, P. Y., SAGSWANSUPYAKOM, C., SAHAVACHADRIA, O., THAVEECHAE, N. AND PANDEY, Y., 1997, *In vitro* propagation of ginger (*Z. officinale*). *Kastetsaet Journal of Natural Science*, 3: 81-86.

PARRY, T. W., 1969, Spices Chemical Publishing Company, Inc., New York, 2 : 79-80.

PAULING, L., 1955, *College Chemistry*, W. H. Freeman and Company, San Francisco, p. 578.

PILLAI, S. K. AND KUMAR, R. B., 1982, Note on the clonal multiplication of ginger *In vitro*. *Indian Journal of Agricultural Sciences,* 52 : 397-399.

POONASAPAYA, P., PRAISINTU, K., PALEVITCH, D. AND PUTEUSKY, E., 1993, Micropropagation of *Zingiber cassumunar* Roxb. *Acta Horticulturae,* 344 : 557-564.

RAJU, B., ANITA, D. AND KALITA, M. C., 2005, *In vitro* clonal propagation of *Curcuma caesia* Roxb. and *Curcuma zedoaria* Rosc. from rhizome bud explants. *Journal of Plant Biochemistry and Biotechnology*, 14 : 61-63.

RAZDAN, M. K., 1993, An introduction to plant tissue culture, Oxford and IBH publishing company Pvt. Ltd., New Delhi, pp. 32-36.

REHMAN, M. M., AMIN, M. N., JAHAN, H. S. AND AHMED, R., 2004, *In vitro* regeneration of plantlets of *Curcuma longa* L. a volume spice plant of Bangladesh. *Asian Journal of Plant Sciences*, 3 : 306-309.

ROUT, G. R. AND DAS, P., 1997, *In vitro* organogenesis in ginger (*Zingiber officinale* Rosc.). *Journal of Herbs, Spices and Medicinal Plants*, 4 : 41-51.

SALVI, N. D., GOERGE, L. AND EPEN, S., 2000, Direct regeneration of shoots from immature inflorescence cultures of turmeric. *Plant Cell Tissue and Organ Culture*, 62 : 235-238.

SAMSUDEEN, K., BABU, K. N., MINOO, D. AND RAVINDRAN, P. N., 2004, Plant regeneration from anther derived callus cultures of ginger (*Zingiber officinale* Rosc.). *Journal of Horticultural Science and Biotechnology,* 75 : 447-450.

SANGHAMITRA, N., 2000, *In vitro* multiplication and microrhizome induction in *Cucurma aromatica* Sallsb. *Plant Growth Regulation*, 32 : 41-42.

SCHLEIDEN, M. J., 1938, Deitrage Zur phytogenesis, muller, Arch, Ant, Eiss, Med, pp. 137-176.

SCHWANN, T., 1939, Mikraskapische untersuch ugen uber die ubereinstimmung in der struktur and dem wach stume der tiere and pfanzen (Ostwards kallsiker) de exakten Wissenschaften, No. 176, Engelmann – Leipzig, p. 910.

SECRIST, J. H. AND POWERS, W. H., 1966, *General Chemistry*, Nostrand Company, Canada, p. 605.

SHARP, W. R., EVANS, D. A., AMMIORATO, P. V. AND YAMADA, Y., 1984, Hand book of plant cell culture, Volume 2, Crop Species MacMillan publishing Co., New York.

SHETTY, M. S. K., HARIDASAN, P. AND IYER, R. D., 1982, Tissue culture studies in turmeric. *Proceedings of the National Seminar on Ginger and Turmeric*, Central Plantation Crops Research Institute, Kasargod, pp. 39-41.

SIT, A. K. AND TIWARI, R. S., 1998, Micropropagation of turmeric (*Curcuma longa* L.). *Recent Horticulture*, 4 : 145-148.

SKOOG, F. AND MILLER, C. C., 1967, Chemical regulation of growth and organ formation in plant tissue cultivated *in vitro.* In : *Biological Action of Growth Substances, 11th Symposium Society of Experimental Biology*, 11 : 118-131.

SREELATHA, U., NAIR, R. S. AND RAJMOHAN, K., 1998, Factors affecting somatic organogenesis from leaf explants of Anthurium species. *Journal of Ornamental Horticulture*, 1 : 48-54.

STYLER, D. T. AND CHIN, C. K., 1983, Meristem and shoot tip culture for propagation, pathogen elimination and germplasm preservation. *Horticulture Review*, 5 : 221-227.

SUSHMA, K., VISHAL, R. AND SHAM, K. S., 2005, Conservation and propagation of high altitude medicinal and aromatic plant : Hedychium Spicatum. *Journal of Plant Biochemistry and Biotechnology*, 14 : 57-59.

SWAMINATHAN, M., 1974, Essentials of food and nutrition, volume II, Mysore Printing and Publishing House, Mysore, pp. 484-485.

SWAMY, R. D., RAO, N. K. S. AND CHACKO, E. K., 1983, Tissue culture propagation of banana. *Scientia Horticulture*, 18 : 247-252.

VUYLSTEKE, D. AND DE, LANGHE, E., 1985, Feasibility of *In vitro* propagation of bananas and plantains. *Tropical Agriculture*, 62 : 14-16.

WARDLE, K., DOBBS, K. B. AND SHORT, K. C., 1983, *In vitro* acclimatization of asceptically cultured plantlets to humidity. *Journal of American Society of Horticultural Science*, 108: 386-389.

WICKSON, M. E. AND THIMMANN, K. V., 1958, The antagonism of auxin and kinetin in apical dominance. *Physiologia Plantarum*, 11: 62-74.

WINNAR, W. D. AND WINNAR, D., 1989, Turmeric successfully established in tissue culture. *Information Bulletin Research Institute*, 193 : 1-2.

WONDYFRAW, T. AND SURAWIT, W., 2004, A micropropagation method for korarima (*Aframomum corrorima* (Braun) Tonsen). *Science Asia*, 30 : 1-7.

WONG, W. C., 1986, *In vitro* Propagation of banana (Musa spp.) : Initiation Proliferation and development of shoot tip cultures on defined media. *Plant Cell Tissue and Organ Culture*,

ZAMORA, A. B., BARBA, R. C. AND DAMASCO, O. P., 1986, Status and prospectus of tissue culture Research on banana. *Proceedings of International Workshop,* Davao, Philippines 25-27, February, pp. 78-88.

Appendix – I : Composition of Media

Constituents (mg/l)	MS
Macro nutrients	
KNO_3	1900
NH_4NO_3	1650
Ca (NO_3) $4H_2O$	-
$CaCl_2.4H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
$(NH)_2SO_4$	-
KCl	-
$NaH_2PO_4.H_2O$	-
Na_2SO_4	-
Micro nutrients	
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$CuSO_4.5H_2O$	0.025
$Na_2NO_4.2H_2O$	0.25
$CoCl_2$	0.025
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3

Murashige and Skoog (1962)

Appendix – II : Composition of Media

Constituents (mg/l)	MS
Vitamins	
Inositol	100
Nicotinic acid	0.5
Pyridoxine Hcl	0.5
Thiamine Hcl	0.1
Glycine	2.0
Carbon source	
Sucrose	2%
Agar	0.8%

Appendix – III : Abbreviations used in the text end their expansion

BAP	6-benzyl amino purine
NAA	Naphthalene acetic acid
IBA	Indole-3-butyric acid
KIN	Kinetin
^{o}C	Degree Celsius
cm	Centimeter
g	Gram
mg	Milligram (s)
mg/l	Milligrams per liter
MS	Murashige and Skoog
%	Per cent
pH	Hydrogen ion concentration
DW	Distilled water

MICROPROPAGATION OF GINGER (*Zingiber officinale* Rosc.)

RAJANI C. HIREMATH 2006 Dr. S. S. PATIL
Major Advisor

ABSTRACT

An investigation on micropropagation of zinger (*Zingiber officinale* Rosc.) was carried out during 2004-06 at the Tissue Culture Laboratory of Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad.

In the present investigation five sub experiments were carried out by following CRD design and 't' test in order to find out best surface sterilizer, explant, shooting media, rooting media and hardening material.

Regarding the suitability of explants, shoot tip was the best for culture establishment by producing more number of adventitious shoots in a shorter period of time i.e., early emergence of primordial than the axillary bud.

The study on surface sterilization revealed that explants treated with 0.1 per cent mercuric chloride for 12 minutes, showed the highest aseptic culture establishment. Among the two different explants *viz*., shoot tips axillary buds treated, shoot tips gave maximum survival percentage and healthy culture establishment.

Early response for sprouting and better culture establishment of shoot tip were observed on Murashige and Skoog (MS medium). Among the cytokinins, BAP and kinetin at different concentrations, 2.0 mg/l BAP. Produced more number of multiple shoots. Media with the highest cytokinin concentration showed the maximum number of multiple shoots and lowest length of shoots. On cytokinin free medium single shoot with maximum length were produced.

Among the auxins used in the rooting experiment the maximum number of roots, with less number of day taken for initiation were observed on 1 mg/l IBA supplemented medium. NAA was found less effective than IBA. On auxin free medium maximum root length was produced. Peat medium gave highest survival percentage at 15 and 30 days after transfer to hardening media and better vigour of the plantlets were observed in peat media, followed by vermiculture and sand media.