CLONING OF GINGER (*Zigiber officinale*) THROUGH *IN VITRO* TECHNIQUE

By

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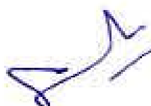

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*This is to certify that thesis entitled, **Cloning Of Ginger (Zingiber officinale) Through In VitroTechniques** Submitted to the **Department of Horticulture & Postharvest Technology**, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE in HORTICULTURE**, embodies the result of a piece of genuine research work carried out by IPSITA RAHMAN, Registration No. 03-01155 **under my** supervision and guidance. No part of the thesis has been submitted for any other degree in any other institutes.*

I further certify that any help or a source of information, received during the course of this investigation has been acknowledged.

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Place : Dhaka, Bangladesh

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DEDICATED
TO
MY
PARENTS

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June, 2008 *The Authoress*

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ABSTRACT

The experiment was conducted .at the Proshika plant tissue culture Laboratory,Manikganj,Dhaka during the month of September 2007 to May 2008 to study Cloning of ginger through *in vitro* Techniques. Sprouted buds from rhizome of ginger (variety Thai) were cultured in four different basal medium with different hormone (NAA, IAA, BA, KN, BAP, IBA & ADS) and their combination to find out suitable combination of media and also find out the best soil type for its successive growth and development. In case of single hormone NAA at (1mg/l) concentration with MS media showed highest shoot length (3.0cm), highest leaf number (2.2) and multiple tillers (2.2). In combined effect BA (2mg/l) and KN (1mg/l) with MS medium showed the best performance, with highest pseudostem length (1.4cm), highest leaf number(2.4) and highest multiple tiller (2.0). In case of LS medium with organic supplement and hormone BAP with KN with tomato juice gave highest shoot length (5.0cm) with 78% of rooting. In case of rooting hormone IBA at (1mg/l) with MS and White media achieved 82% root. For soil, garden soil: sand: soil (1:1:1) showed highest growth rate (plant height 11.8cm).It may be concluded that shoot induction was good in MS media and root induction was best in MS and White liquid rooting media.

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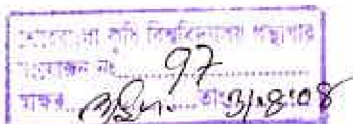
Abbreviation

Abbreviation	Full form
BA	Benzyl adenine
BAP	6- benzylaminopurine
Cg	Callogenesis
cm	Centimeter (s)
cb	Caltivar (s)
^{0}C	Degree Celsius
EDTA	Ethylene diamine tetra acetic acid
et al.	et alia = and other people
GA_3	Gibberellic acid
gm	Gram
g/l	Gram per liter
HCl	Hydrochloric acid
$Hgcl_2$	Mercuric chloride
IAA	Indole 3 acetic acid
IBA	Indole 3 butyric acid
Kn	Kinetin
mg	Milligram
mg/ l or mg l^{-1}	Milligram per liter
ml	Milliliter
mM	Millimole
min	Minutes
MS	Murashige and Skoog
N	Normal
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
No.	Number
PEG	Polyethylene glycol
PGR	Plant growth regulator
pH	Negative logarithm of H^+
w/v	Volume by volume
w/v	Weight by volume
2,4-D	2,4- dicloropphenoxy acetic acid
Zea	Zeatin
uM	Micromole
ul	Mircoliter

CHAPTER 1
INTRODUCTION



Introduction

Ginger (*Zingiber officinale*) is a perennial herb which extends underground in tuberous joints.

Ginger is an important spices crop of Bangladesh. It belongs to the family Zingiberaceae. It is an important tropical horticultural plant valued all over the world from ancient period for its aroma, flavor and medicinal properties. The important ginger producing countries are India, Jamaica, Sierra Leone, Nigeria, Southern China, Nepal, Taiwan, Thailand, Malaysia, Indonesia and Australia (Singh and Singh, 2000). India and China are the two major exporters of ginger in the world market. The major buyers of Indian ginger are the Middle East, USA and Western Europe. In Bangladesh, ginger grows well in Rangpur, Nilphamari, Dinazpur, Tangail, Rangamati, Khagrachari and Chittagong districts (Chowdhury *et al.*, 1998). Mainly ginger is cultivated in kharif season and seeds rhizome are planted in the month of March-April. Ginger requires a warm humid climate. The suitable temperature is 16-22^{0}c. Ginger cultivated either under heavy rainfall condition of 350-600mm or under irrigation. The crops thrive well in silt clay loam. The soil need to be deeply ploughed with fine-friable structure. Drainage system should be available there. The tree tolerates moderately acid to neutral soils (p^H 5.8-7.5). It prefers shade places than direct sunlight.

Ginger is used world wide as a cooking spice, Condiment and herbal remedy. Due to its pleas ant pungent and spicy aroma, ginger is used in the manufacture of a number of food products like ginger bread, confectionary, ginger-ate, curry powders, certain curried meats, table sauces, carbonated drinks, ginger brandy, wine and beer in many western countries Ginger has basic antiseptic properties and is used as carminative and stimulant (Singh and Singh 2000). Recently it is being used as chewing ginger.

In addition to its common usage as a spice, ginger rhizomes have a long history of use as a medicine throughout parts of Asia for its purport ant emetic and anti-inflammatory properties. It is also used in veterinary medicine and preparing Ayurvedic, Homeopathic and allopathic medicine (Pruthi, 1998). Chinas first great herbal, recommended ginger for cold fever, tetanus and leprosy. In ancient India ginger was called "Vishwabbesaj" the Universal medicine. It is commonly used to treat motion sickness and vertigo. The properties that ginger contain volatile oils 1-3%,protein 8.6%,resin,fiber 5.9%,ginger oil ,ash ,moisture 6.9%,fat 6.4%,carbohydrate 66.5% and little amount of calcium, potassium, vitamin-A, vitamin-B,Niacin.

BBS. (2005) reported that ginger was produced in only 6,826 hectare where the production was 38,265 tons. Per hectare production of ginger is only 5.6 tons. The present demand of ginger is 95,000 ton but the production is only 40,000 ton in 6000 hectare of land (BBS-2005). The need for herbal medicine manufacture is about 1, 20,000 kg (Islam, Shahbuddin *et a*l, 1997).

Micro propagation has many advantages over the conventional propagation for large scale production. Plant Tissue Culture Techniques have been useful in conservation of germ plasm of vegetative propagated crops and considered as an alternative to conventional field gene banks to safeguard against pests and environmental vagaries (Dodds, 1991). There are some early reports on *in vitro* culture of ginger (Balchandran *et al*., 1990; Rout and Dos, 1997). It is important to note that, is in the case with ginger (Babu *et al.*, 1992), the absence of seed mode conventional breeding methods are ineffective. Breeding of ginger is seriously handicappod by poor flowering and seed set. Clonal multiplication of ginger through shoot multiplication has been reported (Hosoki and Sagawa, 1977; Wang, 1989; Balachandran *et al*., 1990; Rout *et al*., 1997). Plantlets derived from in vitro culture might exhibit somaclonal variation (Larkin and Scowcroft, 1981) which is often heritable (Breiman et al., 1987). Traditionally, ginger was propagated by using rhizome. The rhizome can not be stored for long time as it is susceptible to fungal diseases, which affect the quality of the tubers to achieve high productivity, homogeneity and good quality tubers, pathogen-free planting material is crucial. Micro propagation of ginger is the best way to produce homogeneous plant lets from rhizome sprouts which is favorable for *in vitro* research activities and industrial scale use for spicy and medicines. There fore this experiment was carried out to investigate an efficient and systematic procedure for propagation with different combination of NAA, KN, IAA and BA of shoot-tip of *Zingiber officinale* through shoot apical meristem culture and the effect of IBA on root initiation.

Objectives:

1. To standardize the suitable media for *in vitro* shoot proliferation and root induction
2. To determine the suitable ratio of Auxin or Cytokinin for rapid shoot induction.
3. To find out the effective synergism of hormone
4. To find out the suitable organic supplements for shoot regenaration
5. To determine the suitable media for root induction
6. To find out the optimum soil conditions and fertilizer dose suitable for its *ex vitro* establishment.

CHAPTER 2

REVIEW OF LITERATURE

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REVIEW OF LITERATURE ON SHOOT REGENARATION

N.P.Anish *et al.*(2008) experimented that an *in vitro* method was developed for the rapid multiplication of Boysenberries Pulcherrima (Wall.) Kuntze, a threatened species of Zingiberaceae. Shoot tip explants developed from rhizome were transferred to different multiplication media containing various concentrations and combinations of BAP, KN and IAA. Though MS medium supplemented with single Cytokinin simulated shoot development, showed poor multiplication. Shoot tip explants achieved better response in BAP than in KN. Addition of KN (0.5 mg L^{-1}) to BAP (1.0 mg L^{-1}) containing medium promoted the rate of multiplication and obtained average 5.8 shoots in 5 weeks. Since rooting was also observed along with calogenesis, the plantlets were straight away transferred to greenhouse conditions. They were successfully established in the greenhouse condition with 85% survival. Hardened plantlets planted in the trial plot exhibited normal regeneration in the subsequent year and developed bulbils. In the following year, 65% of the tissue cultured plants exhibited regeneration. The vegetative and floral characters of the field grown plants were similar to their wild parents. Germination of bulbils produced from tissue cultured plants further confirm the suitability of in vitro plantlets for conservation purpose. The system suggests a feasible method for replenishing the wild population as the in vitro progenies accomplished their life cycle successfully in the field.

Sit,A.K. (2007) conducted that field evaluation on performance of different planting materials of ginger was made. Planting materials derived from rhizome of *in-vitro* regenerated plantlets harvested from field performed better than the planting materials like conventional planting materials and tissue cultured plantlets. Maximum yield/plant (356 g) was recorded from planting materials derived from in-vitro regenerated plantlets harvested from field. Maximum disease infestation was recorded on plants derived from conventional planting materials of ginger.

Saengnil and Sarathum. (2005) studied that the effects of some plant growth regulators and age of stem explants on callus induction of Curcuma sp. No. 50 *in vitro* were determined. Stem explants 1.0 cm in size measured from the base of Curcuma sp. plantlets could be induced to form calli when cultured on MS (Murashige and skoog, 1962) agar medium having 2,4-D. When combinations of 2,4-D (0-0.5 mg/l) and BAP (0-0.25 mg/l) were tested, it showed that 2,4-D singly added was superior to 2,4-D combined with BAP in promoting callus induction. The most effective 2,4-D level was 0.125 mg/l, providing the highest callus formation. The inclusion of 2,4-D and BAP or only BAP in the medium improved growth and development of shoot rather than callus growth. Explants from plantlets cultured for 2 months were more suitable for callus induction than those from 1- and 3month old plantlets. From histological study, it was found that calli may have originated from parenchyma of initial culture stem explants.

ChanLaiKeng and WengHing .(2004) experimented that `Zingiber officinale` buds from the rhizomes were used to produce in vitro shoots. These explants produced the largest number of multiple shoots (9.8 shoots per explant) when cultured on MS

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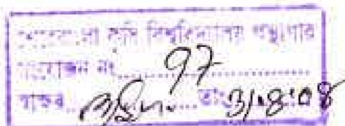

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medium supplemented with 2.0 mg benzyladenine/litre (BA) and 2.0 mg indole butyric acid (IBA)/litre. This medium was also suitable for in vitro propagation of other Zingiberaceae species: Alpinia conchigera, Alpinia galanga, Curcuma domestica [C. longa], C. zedoaria and Kaempferia galanga. Both C. domestica and C. zedoaria produced more multiple shoots when cultured in the liquid proliferation medium containing 2.0 mg BA/litre and 2.0 mg IBA/litre. To maintain the in vitro plantlets of Zingiberaceae species, they should be subcultured every 4 weeks. After executing proper acclimatization protocol, in vitro plantlets of A. galanga, A. conchigera, C. domestica, C. zedoaria, K. galanga and Z. officinale could be successfully planted in the field with high percentage of survival.

Xuan.Pu. *et al.* (2004) experimented that the optimum medium for the induction of ginger callus was MS medium supplemented with 2 mg 2,4-D/litre + 1 mg kinetin/liter. Addition of 2 mg kinetin/litre + 0.5 mg NAA/liter was suitable for the dedifferentiation of the plantlets.

Kirdmanee.C *et al.*(2004) studied that ginger buds were sterilized and cultured on Murashige and Skoog (MS) medium at 29+or-1 degrees C air temperature in the dark condition for one month. The meristems of developed shoots were cut into 0.15+or-0.05, 0.35+or-0.05, 0.55+or-0.05, and 0.75+or-0.05 mm in diameter and cultured on MS medium supplemented with 15% coconut water for rapid screening of bacterial infection. The bacteria-free shoots were multiplied on MS medium supplemented with 0, 2, 4, and 6 mg N6-benzyladenine (BA) litre-1 or 0, 2, 4, and 6 mg 6-(gamma - dimethylallylamino) litre-1 purine (2iP) [isopentenyladenine] at 25+or-1 degrees C air temperature for 5 weeks. The ginger plantlets were transferred to ex vitro and produced the bacteria-free rhizome. The bacteria-free rhizomes were compared with those of rhizome produced through conventional method in the commercial scale. The lowest bacterial infection of meristem was observed in the shoots cut into 0.15+or-0.05 mm in diameter. The shoots multiplied on the medium supplemented with 4 mg BA litre-1 were 1.9 times higher compared with those multiplied on the medium without BA. Vigorous growth, high survival percentage and high yield were observed in the ginger plants produced through bacteria-free rhizomes.

Him de Freite *et al.* (2003) conducted that the horticultural performance of ginger (Z. officinale) plants cultured *in vitro* and propagated from rhizome sections was evaluated under full sunlight and partial shade in Tarabana, Lara State, Venezuela. The results showed significant differences for the type of propagation and the condition of light, except for the variables number of leaves and root mass, respectively. The number of shoots, and fresh and dry mass of shoots was higher in *in vitro*-propagated plants, which were shorter, than those propagated by rhizome. Rhizome mass was greater in plants propagated conventionally, but root mass was smaller than in those propagated *in vitro*. The *in vitro* plants produced numerous small rhizomes, with a high number of fleshy roots and tuberous structures at the tips. All the evaluated variables were superior in partial shade, independently of the type of propagation, with the exception of roots mass in those plants produced from rhizome sections.

Sompop *et al.*(2003) experimented that *in vitro* propagation protocol for *Zingiber* cf. petiolatum I. Theilade, a wild species from Pattani province. Fruits were cleaned with 70 % ethanol and surface sterilised for 30 minutes with 1 % Clorox containing 10 micro liter of Twin 80, then rinsed 3 times in sterile distilled water. The fruits were cut and seeds germinated on Murashige and Skoog medium (MS) medium supplemented with 3 % sucrose. Three month-old seedlings were used as donor plant material. Terminal buds of the plants were inoculated on MS medium containing 6-benzylaminopurine (BA; 0.5-8 mg/l) alone or in combination with 1-naphthaleneacetic acid (NAA; 0.5 mg/l). Eight weeks after inoculation, the cultures were transferred to MS medium without plant growth regulator for four weeks. The cultures transferred from MS medium with 4 mg/l revealed the highest shoot induction rate of 6.75 (plus or minus) 1.51 shoots/response explant. Rooting was spontaneous and achieved in MS medium without plant growth regulator. Rooted plants were successfully transplanted to soil. The plants are being grown under greenhouse conditions for further taxonomic identification.

REVIEW OF LITERATURE ON ROOT INDUCTION

Malabadi, R.B. *et al.*(2002) conducted to develop a simple and efficient protocol for the rapid propagation of spiral ginger (C. specious) from *in vitro*-cultured rhizomes. The results showed that only rhizomes cultured on modified MS basal medium supplemented with 8.87 micro M BA+9.29 micro M kinetin (KN)+5.37 micro M NAA were able to form the maximum number of shoot buds with 10 to 11 roots. Luxuriant shoot elongation was observed when newly formed shoot buds with parental rhizome were cultured on modified MS basal medium containing 8.87 micro M BA+9.29 micro M KN+5.37 micro M NAA and further supplemented with 100 mg adenine sulfate/liter and 10% (v/v) coconut water. New rhizomes were able to produce plantlets with well-developed roots and these were successfully acclimatized and established under greenhouse conditions.

Arimura,C.T *etal.* (2002) studied that the effect of NAA (0.125, 0.25, and 0.5 mg/liter) and benzyl adenine (0.5, 1.0, and 2.0 mg/liter) on the development of ginger plantlets *in vitro* were evaluated. The highest shoot and root ratio, and fresh and dry weight were obtained from MS culture medium supplemented with 0.25 mg NAA/litre + 0.55 mg benzyl adenine/liter.

Nath,B. *et al.* (2001) experimented that the effect of plant growth regulators on the growth and yield of ginger (cv. Himgiri) rhizomes was studied in Solon, Himachal Pradesh, India, during 1997 and 1998. NAA, IAA, and IBA were applied to detached sprouts for 2 h only before planting in trays (1 ppm) or before transplanting in the field as well (0.5+0.5 ppm). The highest sprout survival rates (89.85 and 97.28%) were obtained with NAA and IAA at 0.5+0.5 ppm. IBA at 1 ppm gave the tallest plants (46.83 cm) with the highest number of leaves (31.45). All growth regulator treatments, except IBA at 0.5+0.5 ppm, were on a par with regard to the number of tillers. The heaviest rhizomes (49.62 g) and the highest yield (27.13 q/ha), net return (Rs. 15 333), and cost benefit ratio (1:0.039) were obtained with 1 ppm IBA.

Rout,G.R. *et al.* (2001) conducted of the induction of rooting in micro shoots of *Zingiber. officinale* cultivars Suprava, Turia local, Suruchi and V< sub>3</ sub>S< sub>18</ sub> was achieved on one-half-strength basal Murashige and Skoog medium supplemented with 0.5-1.0 mg/liter of either IAA or IBA and 2% (w/v) sucrose within 7-9 days of culture. Rooting was inhibited when the microshoots were cultured under higher concentrations of auxins. The micro shoots cultured on medium supplemented with NAA induced a large number of thin root hairs with friable calluses within 6-7 days. Peroxides activity was determined during root induction (0-day to the 10th day at every 2-day interval) from micro shoots derived in vitro. The activity was minimum in the inductive phase (primary) and at the maximum level during the root initiative phase. These findings may be useful in monitoring the rooting behavior in micro shoots derived from different subcultures and peroxides activity may be used as a marker for root initiation. at 2001 experimented that in the tissue culture of Faun ginger rhizome, a new method involving heat treatment of 0.2- to 0.5-mm-long shoot tip explants was shown to reduce the infection rate to <10%. The tissue culture results are as follows: on the optimum initial culture medium (MS medium) containing 2 mg benzyl adenine/liter and 0.2 mg IAA/liter for inducing calluses and buds, the bud induction rate was 233%. On the optimum subculture medium (MS medium) containing 0.1 mg kinetin/liter and 0.5 mg IAA/liter, the bud multiplication factor was 6.3 per month. Roots differentiated easily in unspecialized MS medium. When regenerated plantlets were grown indoors, the optimum light intensity was about 4000-6000 lx, and the optimum temperature was 25-26 degrees C. At the plantlet hardening stage, the optimum light intensity was about 2x104 to 3x104 lx. Under an air temperature of 24-28 degrees C, and 70-80% RH, the survival rate of plantlets after transplanting.

Rout *et al.* (2001) experimented that the induction of rooting in microshoots of Zingiber officinale cvs. Suprava, Turia local, Suruchi and V_3S_{18} was achieved on half-strength basal Murashige and Skoog's medium supplernented with 0.5-1.0 mg/l either indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) and 2 % (w/v) sucrose within 7-9 days of culture. Rooting was inhibited when the microshoots were cultured under higher concentration of auxins. The microshoots cultured on medium supplemented with NAA induced large number of thin root hairs with friable calluses within 6-7 days. Peroxides activity was deterrnined during root induction (0-day to the 10th day at every 2 day interval) from microshoots derived *in vitro*. The activity was minimum in the inductive phase (primary) and at the maximum level during the root initiative phase. These finding may be useful in monitoring the rooting behavior in micro shoots derived from different subculture and peroxides activity as a marker for root initiation.

Adaniya and Shirai. (2001) conducted that *in vitro* induction of tetraploid ginger (Zingiber officinale Roscoe) and its pollen fertility and germ inability were investigated. The growth of shoot tip cultures on agar MS medium containing 2.0 mg l^{-1} BA was greater than that of similar cultures in liquid MS medium with the same BA concentration. In liquid medium, the combinations of 0.5, 1.0, or 2.0 mg l^{-1} BA with 0.05 mg 1 1 NAA tended to enhance the growth of the cultures. The efficiency of tetraploid induction was assessed by treating shoot tip explants on agar or in liquid MS medium containing 2.0 mg l^{-} BA, 0.05 mg l^{-1} NAA, and 0.2% (w/v) colchicines for 4,

8, 12, and 14 days. The total number of tetraploids induced on solid medium was 18, but only five in liquid medium. On both media, the colchicines treatment for 8 days gave the maximum level of tetraploid induction. Therefore, it was found that the treatment of shoot tip explants on agar medium containing 2.0 mg l^{-1} BA. 0.05 mg l^{-1} NAA. and 0.2% (w/v) colchicines for 8 days was the most efficient way of inducing tetraploid ginger. Induced tetraploid strains,had higher pollen fertility and germ inability than the diploid counterparts, i.e., in the diploid strains, pollen fertility ranged from 0.3 to 6.2% and the germination rate from 0.0 to 0.1%, while in the tetraploid strains, pollen fertility ranged from 27.4 to 74.2% and the germination rate from 4.8 to 12.9%

Arimura,C.T. *et al*.(2000) studied that a micro propagation method using etiolated shoots in ginger cv. atibaia is presented. Basal portion of plantlets were grown at dark in Murashige and Skoog (MS) medium containing 0, 5, 10, 15 or 20 micro M NAA for 30 days to induce formation of etiolated shoots. Culture of shoots in MS supplemented with 25 micro M kinetin regenerated adventitious shoots along the nodes. These regenerated shoots were transferred to growth regulator-free MS for 30-35 days to induce rooting. This scheme makes it possible to regenerate 10-15 plantlets from each initial explant.

Devi,S *et al*.(2000) expwrimented that this work evaluated the *in vitro* growth and development of ginger explants submitted to different levels of NAA (0-0.25-0.5-0.75-1 mg/litter) or BAP (0-0.5-1.0-1.5-2 mg/litter), and NAA (0 and 0.5 mg/liter) + BAP (0 and 1 mg/litter), in solid and liquid media. NAA increased shoot length in both solid and liquid media. NAA at 0.5 mg/liter promoted the highest number of roots and the longest roots. BAP influenced the number of shoots, with maximum response at 1 mg/litter. In liquid media, the absence of BAP induced higher numbers of shoots. Shoot length was in regardless plant growth regulator treatment influenced by NAA and BAP. Root number and length were enhanced in liquid media regardless plant growth regulator treatment.

Jagadev *et al*. (2000) conducted that India has been known as the 'land of spices' from time immemorial. Among the spices, ginger contributes greatly towards human health and is regarded as a food medicine for several ailments. The major limitation in increasing production and productivity of ginger is lack of adequate disease.-free planting materials of high yielding varieties. As the major diseases are spread through contaminated seed-rhizomes, the possibility of producing pathogen-free planting materials using tissue culture is attractive. Therefore, the present investigation was undertaken to standardise a rapid, efficient, and reliable regeneration protocol for in vitro propagation of a high yielding ginger, cv. Suravi, collected from the high altitude research station at Pottangi (Koraput, a tribal district of Orissa), India. The axillary bud (0.2–0.5 mm size) from the sprouted rhizome was taken as the explants. The most ideal surface sterilant was found to be 0.1 $HgCl_2$ for 13 min, which reduced the total infection (fungal + bacterial) significantly to 3.3% and took shortest time for bud emergence (9.3 days) in standard Murashige and Skoog (MS) medium. The extent of survival (96.7%) and production of buds per explant (2.7) were maximum with this sterilant. MS medium supplemented with 3.0 mg/L benzyl amino purine (BAP) and

0.4 mg/L naphthalene acetic acid (NAA) was ideal for shoot proliferation and resulted in maximum number of total shoots from a single explants (36.0), maximum shoot length (6.1 cm) with 4.7 leaves after a second sub-culturing. For rooting, MS supplemented with NAA (0.5 mg/L) was found to be more effective and produced the maximum number of roots per shoot (13.3) and the maximum root length (2.0 cm) plus taking the least time for root initiation (10.3 days). The in vitro plantlets were prehardened in ½ MS liquid medium. The hardening and acclimatization media mixture of soil: sand: farm yard manure (1:1:1) was found to be best for survival of the plantlets in ginger

Smith and Hamill. (1980) studied that the growth and performance of micro propagated ginger was compared with 'seed'-derived plants in field trials conducted in south-eastern Queensland. In the first generation *ex vitro*, micro propagated plants had significantly ($P<0.01$) reduced rhizome yield with smaller knobs and more roots. Micro propagated plants had a greater ($P<0.01$) shoot: root (rhizome) ratio compared with seed-derived plants. Shoots from micro propagated plants were also significantly ($P<0.01$) smaller with a greater number of shoots per plant. The unusual shoot morphology of the micro propagated plants did not appear to be related to the presence of benzylaminopurine, a plant growth hormone added to the multiplication medium, as plants subculture for 3 cycles on a hormone-free medium also exhibited similar characteristics. Seed collected from the micro propagated plants and seed-derived plants was harvested and, despite the micro propagated seed being significantly ($P<0.01$) smaller, by the second generation *ex vitro* there were no significant differences between the treatments. Factors that can improve rhizome size, while reducing production costs, need to be identified before micro propagated plants can be recommended for routine use in the ginger industry as a source of disease and pest-free planting material.

Sharma,T.R. (1997) conducted that Explants from in vitro-cultured shoots of *Zingiber. officinale* cv. Himachal Local were cultured on MS medium with analytical-grade sucrose, ordinary sugar and raw sugar, or on potato extract medium with sucrose, dextrose or sugar. The effects of supplementing the medium with 2 mg benzyl adenine (BA)/liter, omitting agar and replacing distilled water with tap water in the preparation of the medium were also investigated. Shoot and root production were recorded after 30 days and well-rooted plantlets were transferred to pots for acclimatization. The highest number of shoots/explants was produced on MS medium + ordinary sugar and no growth regulators, followed by MS medium + sucrose. The longest roots were obtained on potato extract + dextrose. Use of tap water and omission of agar also gave good results. These techniques can be used to reduce the cost of micro propagation..

Sharma and Singh. (1997) studied that high-frequency in vitro multiplication of disease-free clones of ginger (*Zingiber officinale* Rosc.) was obtained by culturing small and active buds of ginger on MS medium supplemented with 2 mg/l Kin and 20 g/l sucrose. An average of 7.7 shoots per bud was obtained on this medium after 4 weeks of culture. A high multiplication rate of well-developed plantlets (7.0 shoots per bud) with a 6.8-cm shoot length and a 7.0-cm root length was also obtained on MS medium containing 2.0 mg Kin, 2.0 mg NAA and 20 g sucrose per liter. The

multiplication rate did not decrease even up to 28 months of subculture on the same medium. A simple method of successfully transferring more than 95% of tissue-cultured plants into pots was also standardized. In vitro-derived plants performed well under field conditions, were morphologically identical to the mother plants and were free from ginger yellows (*Fusarium oxysporum* f. sp. *zingiberi*). Well-developed rhizomes obtained from the tissue-cultured plants did not rot during storage of up to 6 months, thus indicating that the method is also effective in checking storage rot caused by *F. oxysporum* f. sp. *zingiberi.*

G.R Rout. (1997) conducted that efficient plant regeneration in *Zingiber officinale* Rose. was achieved using callus derived from shoot primordia and grown on MS media. Organogenesis was maximum on media supplemented with 5.0 mg/liter 6-benzyladenine, 1.0 mg/liter indole acetic acid, 100 mg/liter adenine sulfate and 3 percent (wtv) sucrose. D-glucose had internediary effect on rooting, but fructose, maltose, and mannitol had no effect. Of the conditions tested, shoot bud regeneration was highest at pH 5.7 or 5.8 and under 24 h illumination. The rate of shoot bud regeneration was positively correlated with the concentration of hormones in the nutrient media. Isolated shoots could be rooted on media containing half-strength MS mineral salts supplemented with 1.0 mg/liter indole-3-butyric acid or indole3-acetic acid and 2 percent (w/v) sucrose. Liquid media (agitated or static) were less effective than a solid (agar-gelled) media for root development. The *in vitro* derived plantlets could be hardened in the green house and successfully established in soil.

Rout G. R. *et al.*(1997) experimented that shoot multiplication of *Zingiber officinale* cv. V_3S_{18} was achieved by meristem culture on a Murashige and Skoog (MS) basal medium supplemented with 26.6 µM 6-benzylaminopurine (BA), 8.57 µM indole-3-acetic acid (IAA), and 1111.1 µM adenine sulfate and 3% (w/v) sucrose. In vitro rhizome formation from in vitro-raised shoots was achieved on MS medium supplemented with 4.44 µM BA, 5.71 µM IAA, and 3-8% (w/v) sucrose after 8 wk of culture. Cultural variations such as photoperiod, carbohydrate, nutrient composition, and growth regulators were tested for the maximum yield of rhizomes. Among the different photoperiods used, a 24-h photoperiod helped in the formation of more rhizomes as compared with other photoperiods. Of the different carbohydrates used, sucrose helped to achieve rhizome formation as compared to other carbohydrates. The micro rhizomes sprouted in a soil mixture within 2 wk of planting. The sprouted plantlets survived under field conditions with normal growth.

Kirdmanee. *et al.* (1996) studied that Ginger buds were sterilized and cultured on Murashige and Skoog (MS, 1962) medium at 29±1 °C air temperature in the dark condition for 1 month. The meristems of developed shoots were cut into 0.15±0.05, 0.35±0.05, 0.55±0.05, and 0.75±0.05 mm in diameter and cultured on MS medium supplemented with 15 % coconut water for rapid screening of bacterial infection. The bacteria-free shoots were multiplied on MS medium supplemented with 0, 2, 4, and 6 mg l^{-1} N-Banzylamine (BA) or 0, 2, 4, and 6 mg L^{-1} 6-(γ-dimethylallylamino) purine (2iP) at 25±1 °C air temperature for 5 weeks. The ginger plantlets were transferred to ex-vitro and produced the bacteria-free rhizome. The bacteria-free rhizomes were compared with those of rhizome produced through conventional method in the

commercial scale. The lowest bacterial infection of meristem was observed in the shoots cut into 0.15±0.05 mm in diameter. The shoots multiplied on the medium supplemented with 4 mg L-*1* BA were 1.9 times higher when compared with those multiplied on the medium supplemented without. The vigorous growth, high survival percentage and high yield were observed in the ginger plants produced through bacteria-free rhizome.

P.N. Jagadev *et al.* (1990) studied that India has been known as the 'land of spices' from time immemorial. Among the spices, ginger (Zingiber officinale Rosc.) contributes greatly towards human health and is regarded as a food medicine for several ailments. The major limitation in increasing production and productivity of ginger is lack of adequate disease.-free planting materials of high yielding varieties. As the major diseases are spread through contaminated seed-rhizomes, the possibility of producing pathogen-free planting materials using tissue culture is attractive. Therefore, the present investigation was undertaken to standardise a rapid, efficient, and reliable regeneration protocol for in vitro propagation of a high yielding ginger, cv. Suravi, collected from the high altitude research station at Pottangi (Koraput, a tribal district of Orissa), India. The axillary bud (0.2–0.5 mm size) from the sprouted rhizome was taken as the explant. The most ideal surface sterilant was found to be 0.1 $HgCl_2$ for 13 min, which reduced the total infection (fungal + bacterial) significantly to 3.3% and took shortest time for bud emergence (9.3 days) in standard Murashige and Skoog (MS) medium. The extent of survival (96.7%) and production of buds per explant (2.7) were maximum with this sterilant. MS medium supplemented with 3.0 mg/L benzyl amino purine (BAP) and 0.4 mg/L naphthalene acetic acid (NAA) was ideal for shoot proliferation and resulted in maximum number of total shoots from a single explant (36.0), maximum shoot length (6.1 cm) with 4.7 leaves after a second sub-culturing. For rooting, MS supplemented with NAA (0.5 mg/L) was found to be more effective and produced the maximum number of roots per shoot (13.3) and the maximum root length (2.0 cm) plus taking the least time for root initiation (10.3 days). The in vitro plantlets were prehardened in ½ MS liquid medium. The hardening and acclimatization media mixture of soil: sand: farm yard manure (1:1:1) was found to be best for survival of the plantlets in ginger.

Morón, A.(1987) experimented that the red ginger *(Alpinia purpurata K. Schum)* is a Zingiberaceae that is highly esteemed in international markets as an ornamental plant or as a cut flower. The species are originally from the South Pacific area and is adapted to the humid regions of Central America and the Caribbean. Since at the region it does not produce seeds, it has to be propagated vegetatively. The principal advantage of cultivating red ginger in vitro is its great capacity for multiplication beginning, with a fragment of tissue from a selected mother plant. The media used were based on the basal medium of Murashige and Skoog (MS). In the establishment phase, 4.33 g/l of commercial (MS) salts, 3% sucrose 100 g/l cysteine-HCl, ml/l of (MS) vitamins, 7 g/l of Difco Bacto-agar and 1, 2 or 3 mg/l of benzylaminopurine (BA) were used. The cysteine was eliminated in the multiplication phase, using the basic (MS) medium plus 3% sucrose, 1.5 g/l of Gelrite and 0, 2, 4 or 8 mg/l of BA and 0, 0.5 or 1 mg/l of naphthalene acetic acid (NAA). All the combinations of concentrations of both types

of growth regulators ere tried. For rooting in vitro, the concentration of (MS) salts was reduced to one half. Combinations of 0. 2 or 4 mg/l of NAA and 0, 0.5 or 1 mg/l of BA were tested. The survival percentage obtained for all explants in the establishment phase varied according to the type of disinfection. The best result was 73% survival and obtained by using sodium hypochlorite as the disinfectant and inoculating the same day. The tissue of red ginger did not cause any oxidation problems. Two mg/l of BA without NAA was the best treatment for shoot proliferation. It was found that NAA had a detrimental effect on shoot formation, as well as doses of BA exceeding 10 mg/l. Rooting in vivo in mist chambers resulted in the most rapid and economic method for preparing the cultures for natural conditions. The multiplication rate achieved in the laboratory was 5.47 in five months using 2 mg/l of BA, while that obtained in the greenhouse without treatments for shoot induction was 5 for the double perid of time.These rates show that the species studied responded properly to clonapropagation.

CHAPTER 3

Materials and method

Materials and method

The Experiment was conducted at Plant Tissue Culture Laboratory of Proshika Plant Tissue Culture Centre during 2007-2008.The materials and methods used in this investigation described below:

3.1. Glassware and Plastic Ware:

a. Glass vesicles (150 x 35 mm), conical flasks (125, 150, 500, and 1000) ml capacity, pipettes (1, 2, 5, and 10) ml capacity, measuring cylinders, funnels, etc. used in various experiments were of corning or Borosil make.

b. Locally manufactured glass jars with polypropylene screw caps were used as culture vessels. All glassware was cleaned using liquid soap and thoroughly washed in running tap water. Washed glassware was rinsed with distilled water and oven dried before use.

c. Culture vessels and flasks were plugged with cork prior to autoclaving. Flasks and bottles containing infected cultures were decontaminated by sterilization for hour, prior to washing.

d. Petri-plates (55 x 15 mm and 85 x 15 mm) were also used in the studies. Auto pipettes (0-20 ml, 20-200 ml and 200-10000 ml) and autoclave able micropipette tips (20, 200. and 100 ml capacities) were used for accurate addition of chemicals in the medium.

Glass ware sterilization:

All the empty glassware to be used for media preparation (Flasks, bottles, lest tubes, pipette tips) and instruments needed for sterile dissection and sub culturing (forceps, scalpel and filter paper pads) autoclaved at 121^{0}c and 1.1 kg/cm^2 pressure for 1 hour.

3.2. Plant Materials:

One of the Thai varieties of ginger (*Zingiber officinale* var. Thai) was collected from proshika laboratory field and used as experimental material. Active growing rhizomes from the mother trees of Thai variety were growing at Proshika PTC centers experimental fields. These rhizomes were cleaned in 5-10% liquid soap solution for 2-3 minutes and then rinsed by running tap water.

3.3. Explants preparation and sterilization:

Healthy rhizomes kept in moist chamber for proper sprouting. After 3-4 weeks of incubation juvenile buds were sprouted and these buds were considered as explants. Sprouted buds were excised from rhizomes and passed through a series of steps for sterilization as fallows:

1. Washed thoroughly under running tap water for 30 minutes to wash out the superficial contaminants and dusts.
2. Kept about 50 explants for 20 minutes in 500 ml beaker having 300 ml distilled water with 8-10 drops of Tween-80 and 5-7 drops of Savlon.
3. Washed thoroughly 5-7 timers with distilled water to remove Twin –80 and Savlon.
4. Immersed the explants in 0.1% $Hgcl_2$ (w/v) for 15 minutes. Then washed thoroughly with sterile distilled water 7-10 times to remove the traces of $Hgcl_2$. Trimmed further to get the optimum size of the explants for the inoculation. All these practices were performed under laminar airflow cabinet to ensure the preparation of contamination free explants as far as possible. Explants of approximately 1 to 2 cm in length were cut and with inoculated aseptically in to MS (Murashige and Skoog, 1962) as well as other media different concentrations and combinations of growth regulators.

3.4. Preparation of Stock Solution:

MS Media:

The first step in the preparation of culture media was the preparation of stock solution. . Various constituents of the medium were prepared into stock solutions for immediate use during the preparation of the medium. As different constituents were required in different concentration, separate stock solutions for macronutrients, Micronutrients, Vitamins, growth regulators etc were prepared separately and stored at refrigerator.

Stock solution I (macronutrients)

One liter stock solution-I was made up to 50times of the final concentration of the medium. At first 600ml of distilled water was taken in a 1500ml beaker and 82.5gmNHqNO_3,95gm KNO_3; 8.5gm KH_2PO_4 were added and dissolved to the last particle by random stirring with a magnetic stirrer. Then distilled water was added to make the volume 1000ml and stored in reagent bottle in refrigerator.

Stock solution II (macronutrients)

One liter stock solution II was made up to 100 times of the final concentration of the medium. At first 600ml of distilled water was taken in a 150ml beaker. Then 44gm $CaCl_2$ $2H_20$ weighted accurately and dissolved. Then distilled water was added to make the volume up to 1000ml and stored in reagent bottle in refrigerator.

Stock solution III (macronutrients)

One liter stock solution III was made up to 200 times of the final concentration of the medium. At first 600 ml of distilled water was taken in a 1500 ml beaker, and then appropriate amount of 74gm $MgSo_4.7H_20$ weighted accurately and dissolved. Then distilled water was added to make the volume up to 1000 ml and stored in reagent bottle in refrigerator.

Stock solution IV (micronutrients):

One liter (W/V) stock solution was made up 100 times of the final concentration of the medium. At first 600 ml of distilled water was taken in a 1500 ml beaker and then appropriate amount of 2.78gm $FeSo_4.7H_20$ and 3.73gm Na_2-EDTA.$2H_20$ were weighted accurately and dissolved. Then distilled water was added to make the volume up to 1000 ml and stored in refrigerator.

Stock solution V (micronutrients)

One liter stock solution V was made up to 200 times of the final concentration of the medium. At first 600ml of distilled water was taken in a 1500 ml beaker and then appropriate amount of 1.24gm H_3B0_3, 1.72 gm Zn So4.$7H_20$, 4.46gm $MnSo_4.4H_20$ were weighed accurately and dissolved. Then distilled water was added to make the volume up to 1000 ml and stored in refrigerator.

Stock solution VI (micronutrients)

One liter stock solution VI was made up to 100 times of the final concentration of the medium. At first 600 ml of distilled water was taken in a 1500 ml beaker and then appropriate amount of 83gm KI, 2.5gm CuSo4. $5H_20$, 25mg $NaMo0_4.2H_20$, 2.5mg $CoCl_2$ $6H_20$ were weighted accurately and dissolved. Then distilled water was added to make the volume up to 1000 ml and stored in refrigerator.

Stock solution VII(organic nutrients)

One liter stock solution VII was made up to 200times of the final concentration of the medium. At first 600 ml of distilled water was taken in a 1500 ml beaker and then appropriate amount of 20gm myo-inositol, 0.1gm Nicotinic acid, 0.1gm Pyridoxine Hcl, 0.02gm Thiamine, 0.4gm Glycine were weighted accurately and dissolved. Then the distilled water was added to make the volume up to 1000 ml and stored in refrigerator.

Other stock solution of White, PM and LS were followed by the methods of MS stock solution.

3.5. IN HCL / 1N KOH

a. For preparing 1 N HCL, 100 ml of liquid HCL was added to 900 ml distilled water.

b. In the same way 1N KOH, 40 gm of KOH was added to 1L of distilled water.

3.6. P^H: pH of all the media were maintained at a range about 5.5-5.8. The buffer solution was used to calibrate the p^H meter. Here $p^H_{-7}$and p^H_{-4} two buffer solutions were used. .

3.7. Growth regulators or Hormonal Stock

In addition to the inorganic nutrients, it is generally necessary to add ore or more phytohormones mainly auxins and cytokines to the media to support proper growth and development of tissues and organs.

To prepare any of hormonal stock solution, 10mg solid was dissolved in 1 ml of solved (0.1 N KOH). The mixture was then washed off with distilled water and collected in a 100 ml further addition of distilled water. So the strength of the hormonal stock solution becomes 1.0 mg /ml.

The treatment for this experiment is:

Four media

Five hormones

and three different combination of hormone were used in this experiment

Media and Growth regulators and used in these experiments were:

Murashige and Skoog

Whites

Phaytamax

Linsmaier

Growth regulators :

Auxins:

2, 4 dichlorophenoxy acetic acid (2, 4-D)

1H- In dole – 3 – butyric acid (IBA)

Naphthalene acetic acid (NAA)

Cytokines

6- benzylaminopurine (BDP)
N- (Turfurylamion) -1- H - purine - 6 amino (kinetin).
ADS(Adenosine sulphate)

Combination of hormone

BA and Kinetin
BA and ADS

3.8. Media Preparation

For the preparation of 200 ml of any media fallowing steps were fallowed:

i. The stock solution I, II, III, IV, V, VI, VII were taken in to a 500 ml conical flask in amounts of 4, 2, 1,2,1,0.1 and 1 ml respectively.

ii. Different required concentrations of hormonal supplements were added to this solution individually.

iii. 6 gm of sucrose for MS basal medium was dissolved in 200 ml mixed components.

iv. The whole mixture was then made up to 200 ml with further addition of distilled water.

v. P^H of the medium was adjusted to 5.8.

To prepare semi-solid medium, half amount of Agar, same amount of sugar of solid medium was used.

In the liquid medium all the ingredients were same but there was no agar. And to prepare rooting medium 1/2 MS was used and other ingredients were same. To prepare solid medium, 0.6% agar was added to the solution and melted at 110^0c for 30 mm in oven. Then the media was dispensed in to glass vessels in a volume of 20 ml. Other semi-solid, rooting medium were followed by the same method of preparing solid medium, except the liquid medium, its only needed autoclaved because there was no agar.

3.9. Media sterilization

All nutrient media were autoclaved at 1.1 kg/cm^2. Pressure at 121^{0}c for 20 minutes. Heat labile chemicals were added in the required quantity, after filter sterilization, in to the autoclaved media.

3.10. Culture condition

Cultures were incubated at 25$\pm$2^{0}c with a photoperiod of 16 has at 3000 lax light intensity by cool white fluorescent light and R.H. 65$\pm$5% Culture room was dust free and cleaned.

3.11. Experimental design

Experiment was designed as 2 x 3 factorials in a Completely Randomized Design with 5 replications and 5 plantlets per replication

3.12. Shoot regeneration and elongation

1 cm plant from the sprouts was inoculated to the regeneration, of shooting media. Different concentrations of hormone s NAA, BA, kinetin, ADS (0.5, 1.0, 1.5, 2.0, 2.5 ml/l) and also their synergism BA with kinetin, NAA and kinetin, BA and ADS etc along with MS and whites media were used for shoot generation and elongation.

3.13. Maintenance of subculture

Sprouts were cultured and after 15 days interval from the Sprout culture, two times data were collected on length of shoot, number of leaf, number of root and root length.

3.13. Root formation

Regenerated shoots of approximately 2-4 cm length were aseptically rescued from culture vessels and transferred to freshly prepared MS and white's rooting media supplemented with various concentrations of IBA (1mg /l) Hormone and type of media (solid, semi- solid and liquid).

3.14. Experimental design

Experiment was designed as 2 x 3 factorials in a Completely Randomized Design with 5 replications and 5 plantlets per replication.

3.15. Soil: The soils of the experimental site was clay loam and slightly acidic in nature. The pH soil 5.8-6.

Layout of the experiment

The experiment was conducted using the Randomized Complete Block Design (RCBD), 4 treatments with 10 replication and 1 plant per replication.

3.16. Pot preparation

Plants were planted in car then pot. The pots were prepared with friable garden soil with four different treatments as garden soil with poultry liter; garden soil with cow dung, garden soil with fertilizer (urea, TSP, MP) and normal garden soil as control. There were 10 pots for each treatment. 1-2 Plants were planted in one pot. The soil composition was sand: garden soil: (cow dung /fertilizer/ Poultry litter) (1:1:1).

1 Garden soil + Cow dung +Sand

2. Garden soil +Poultry liters +Sand

3. Garden soil+ Fertilizer + Sand

4. Garden soil + Sand

Manure and fertilizer application

The pots were prepared with different combinations of treatment as garden soil: cow dung: sand (1:1:1), garden soil: poultry litter: sand (1:1:1) garden soil: Chemical fertilizer: sand (1:1:1), here the chemical fertilizer urea, TSP, MP were used in the ration of N:P:K.the half amount of whale dose of chemical fertilizer were applied as basal dose and rest of that were top dressed after 120 days of transplanting.

Treatment of Plant

Before planting, the materials were treated with Ridomile-100 Ec @ 2 gm/l water for 15 minute to kill soil borne pathogens.

3.18. Transplantation

Rooted plants were kept in a room at normal temperature (30 ± 2^{0}c) in normal day light for 7 days for hardening. Plantlets were taken out from the culture vessels and washed carefully under running water for complete removal of media. Then the plantlets were transplanted to small earthen pot containing different composition and combination of garden soil with compost/ cow dung/ chemical fertilizer/ poultry litter and sand (1:1:1) ratio.

3.19. Intercultural operations

Necessary intercultural operation was taken as and when required for proper growth and development of the plants.

Weeding: Roughing weeding were done to keep the field free from weeds and to pulverize the soil.

Irrigation and drainage: Irrigation was applied as and when required. The proper drainage was also ensured.

Application of fungicides:

To control rhizome rot and other fungal disease, pot were sprayed with Metaryl 72 Wp (Mancozeb 64% + Metalaxyl 8%) in each 15 days interval.

3.20. Data Collection: Data were collected for the following plant character and as well as yield contributing characters:

Number of tiller per plant: Number of tillers per selected plant was recorded at the maximum plant growth.

Tiller length: Plant height from the ground level to the tip per selected plants was measured in centimeter (cm) with help of a meter scale at maximum plant growth.

Pseudo stem length: Pseudo stem length was measured from the ground level to the collar region of plant.

Number of leaves per plant:

Number of leaves per plant was conducted form ten selected plants at the maximum plant growth. Finally mean number of leaves per plant was calculated by dividing the total Number of leaves by number of plants.

Root length: Root length was measured from the growing point up to the tip of the root.

Number of root: Number of roots was counted per plant

3.21. Statistical analysis:

Weekly visual observation of culture was made and frequency of culture showing was recorded. Data were analyzed subject to standard deviation. Data presented in the tables were analyzed as mean $\pm$ SE.

a. Average length of pseudo stem, shoot and root:

Average pseudo stem, shoot and root length were calculated by this formula,

$$\bar{X} = \frac{\sum xi}{N}$$

Where $\bar{X}$ = Average length of pseudo stem.

$\sum$ = Summation

Xi = Length of shoot (from base to Collar)

N = Number of observation

b. Percentage of root induction:

$$\% \text{ of root induction} = \frac{\text{No of root induction}}{\text{Total no. of shoot inoculated}} \times 100$$

c. Leaf color :

DG = Dark green
LG =Light green
PG = Pale green

d. Growth vigor:

+++ = High vigor
++ = Medium vigor
+ = Poor vigor.

CHAPTER 4
Result & Discussion

RESULTS AND DISCUSSION

4.1. Comparison of Basal media:

In this experiment, the composition of basal media significantly influenced the numbers of shoots, leaves, shoot length, multiple shoot as well as growth vigor. There was significant difference between two media. At 15DAI as well s 30 DAI the MS Medium showed it good performance over the white medium. The highest Pseudo stem length (1.36 cm), shoot length (3.03cm), leaf number (2.25), multiple tillers (2.95) was found in MS medium, while non-significant effects were observed on the parameters in white medium. (Table1) Arimura.C.T (1999) showed that the best medium for plantlet Production from bud ex plant was MS supplemented with BAP and KN (both at 0.1 mg IL).

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Table 1. Effect of Basal Media^{x}:

SL. No	Media	Average						Growth Vigor	Tiller/ Explant
		pseudostem	length (cm)	Tiller length (cm)		Leaf number			
		At 15DAI	At 30DAI	At 15DAI	At 30DAI	At 15DAI	At 30DAI		
1.	White	0.88	1.31	2.37	3.67	2.25	4.35	++	0.65
2.	MS	1.36	2.21	3.03	4.76	2.25	4.90	+++	2.95

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test.

X, DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

Compositional difference is seen in between the two basal media (MS and White). All the macro and micro nutrients in MS and White media are not same and are not in equal amount. One of macro nutrients (NH_4NO_3) and organic nutrients (myo-inositol) are absent in White media.KNO_3 is also very little amount in White media. May be these reasons lower amount of nutrients or absence of nutrients causes weak functioning of White media.

4.2. Effect of plant growth regulators (NAA, BA, KN and ADS) on *in vitro* growth and development:

These hormones were used with MS and white media. The use of NAA, BA, KN and ADS exerted a significant effect on shoot, leaf number, and multiple shoot as well as growth vigor. Weekly observations were made and data were collected after two weeks for two times. The Pseudo stem length (PS Length), shoot length, leaf number and growth vigor stimulated by the single use of NAA, BA, KN & ADS. The highest

PS length (1.33 cm) and shoot length (3.01 cm) gained from the medium supplemented with NAA at 15 DAI. P.N. Jagadev et al. (1990) reported that MS medium supplemented with 3.0 mg/l BAP and 0.4 mg/l NAA ideal for shoot Proliferation and resulted in maximum number of total shoot(36.0) ; highest shoot length (6.1 cm) with 4.7 leaves after a second sub-culture. In this experiment leaves number were more (2.27) with KN at 15 DAI. The largest number of multiple shoots were found in BA (2.38) which significantly different with other hormone.

A1

A_2

Fig: A1-A2: *In vitro* shoot proliferation and elongation of ginger on (MS and White) media

B_1

B_2

Figures: B_1-B_2: Synergistic effects of BA & KN on shoot proliferation of ginger on (MS and white Media)

Laikeng and Thong- Weng (2004) found highest number multiple shoots (9.8 shoots / explants) when cultured on MS medium with 2.0 mg BA/L and 2.0 mg /l IBA- In this experiment growth vigor was highest '+++' In KN. But ADS had non significant effects over the parameters. NAA and BA is one kind of Auxin Hormone. The function of Auxin is to elongate stem and root promotion. NAA and BA has good effect on multiple shooting, shoot length & leaf number (Table 2).

Table 2. Effect of plant Growth regulators:

SL. No	Hormones	Average						Growth Vigor	Tiller/ Ex plant
		Pseudo stem length (cm)		Shoot length (cm)		Leaf number			
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI		
1	NAA	1.33 c	2.04 b	3.01 b	4.19 b	2.17 ab	3.97 a	++	2.08 b
2	BA	1.29 c	2.02 b	3.02b	4.05 ab	2.17 ab	3.95 a	++	2.38 c
3	Kinetin	1.19 b	1.99 b	2.96b	4.15 b	2.27 b	4.43 b	++	1.95 b
4	ADS	0.84 a	1.46 a	2.43a	3.92 a	2.12 a	4.02 a	+	1.27 a
	± SE (%)	0.032	0.037	0.046	0.062	0.045	0.071		0.093

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test

X, DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

Auxin act on elongating the growing point and in root promotion. KN act for cell division and help to initiate more leaf number. In this experiment NAA and BA has influenced on shooting and multiple tillering.

4.3. Effect of different PGR concentration on the development of ginger:-

In this experiment different hormone concentration (0.5, 1.0, 1.5, 2.0, and 2.5) mg/l were used with NAA, BA, KN and ADS growth regulators along with MS and white medium. Data were collected two times at 15 Days interval. In 1.0,1.5, 2.0 and 2.5 mg/l is equal but the highest length (1.29cm) for pseudo stem was observed in PGR free medium (control) which significantly similar to 1.0 mg/l concentration (length 1.26 cm) but differs with other concentration. P.N. Jagadev et al. (1990) found that MS medium supplemented with 3.0mg/l BAP and 0.4 mg /l NAA was ideal for shoot proliferation and resulted 36.0 shoots from a single explants, maximum shoot length (6.1 cm) with 4.7 leavers after second sub culture(followed by fig: 1 to 3).

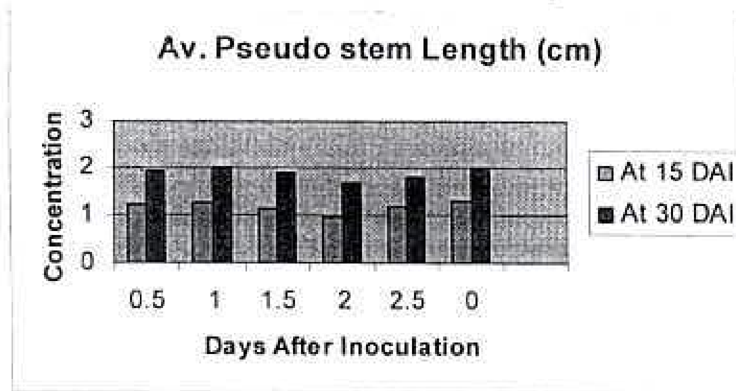


Fig 1: Pseudo stem length at different concentration

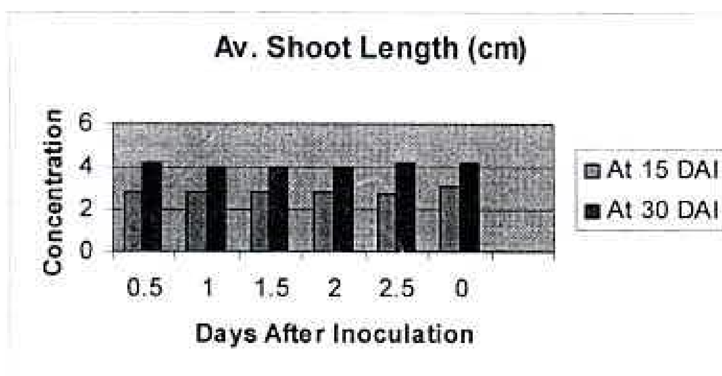


Fig 2: Shoot length at different concentration

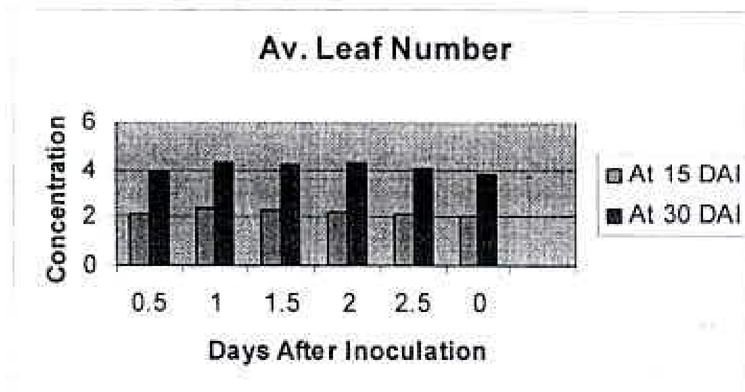


Fig 3: Number of leaf formation

In this experiment, leaf number (4.33) was more in 2.0mg/l concentration at 30 DAI. Nath.B (2001) found the tallest plant (46.83 cm) with highest number of leaves (31.45) at 1ppm IBA. Tiller number was highest in 0.5 mg/l but growth vigor (+++) was good in PGR free medium.

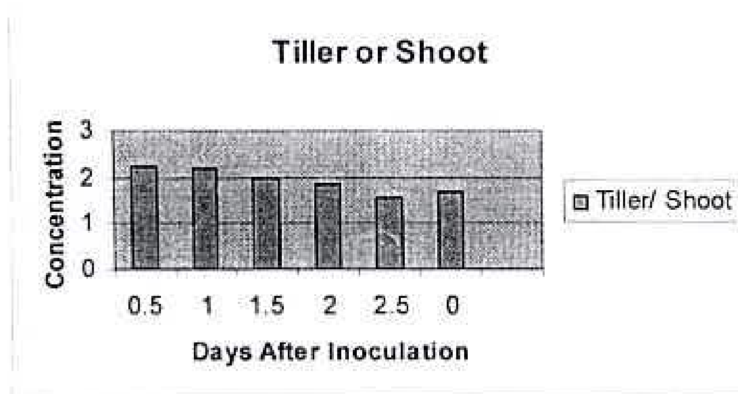


Fig 4: Number of multiple tillers per ex plant

Sometimes shoot proliferation or root induction is maximum at lower concentration or sometimes at higher concentration .The hormone concentration has a maximum limit. We have maintained this range to avoid unexpected result. Rout conducted that organogenesis was maximum on media supplemented with 5.0mg/l BA, 1.0mg/l IAA, 100mg/l ADS and 3 % (w/v) source.

4.4. Synergistic effects of plant growth regulators:

In this experiment different combination of hormone or synergistic effect of hormone were studied when supplemented with MS and with media. Data were collected interval for two times. In BA+KN(2=1)mg/l concentration the pseudostem length, tiller length, and leaf number is not equal to PGR free medium.But growth vigor and tiller/explant was highest in PGR free medium.But other combination were significantly different. The highest pseudo stem length (1.40 cm) , tiller length (3.15 cm) ,total leaf (2.4), growth vigor (+++), and multiple tiller (1.8) were obtained from the culture medium supplemented with BA (2mg/l) + KN (1mg/l), at 15 DAI Malabadi.R.B (2002) found that culturing of rhizomes of modified MS basal medium supplemented with 8.87 micro M BA+9.22 micro M 5.37 micro M NAA, Produced maximum shoot buds with 10 to 11 roots. The highest result of the parameters were also observed PGR (plant growth regulator) free medium (Table 4). Anish, M.Dan and M.Bejoy (2008) experiment and obtained that explants developed from rhizome through MS media supplemented KN (0.5mg/l) to BAP (1.0mg/l) and obtained average 5.8 shoots in 5 weeks (Table 4)

BA is effective for shoot elongation and KN is effective for cell division. So, the synergism of the two hormones with their optimum concentration showed best performance in this experiment

Table 4. Synergistic effects of plant growth regulators:

SL No	Media	Average						Growth Vigor	Tiller/ Ex plant
		Pseudo stem length (cm)		Tiller length (cm)		Leaf number			
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI		
1	BA(2mg/l) + KIN(1ng/l)	1.40 c	2.13 c	3.15 c	4.80 c	2.40 a	5.00 b	+++	1.8 a
2	BA(2mg/l) + KIN(2mg/l)	1.03 b	1.76 b	2.55 b	4.55 c	2.20 a	4.70 ab	++	1.7 a
3	BA(2mg/l) + ADS(2mg/l)	0.69 a	1.13 a	2.02 a	3.45 a	2.20 a	4.10 a	++	2.0 a
4	Control	1.34 c	2.01 c	3.07 c	4.04 b	2.20 a	4.70 ab	++	1.7 a
	± SE (%)	0.066	0.066	0.106	0.123	0.141	0.224		0.177

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test

X, DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

BA and KN are effective for shoot elongation and cell division. This two hormone with their optimum concentration showed good performance over the parameters, because BA and KN are different group of hormone and their function is different .so their combined action on the parameter in this experiment is good.

4.5. Effect of LS medium with different hormone and organic supplements:-

Different organic supplements (orange Juice, tomato Juice) with hormone were used in this experiment to find out the best performance of development. Weekly observations were made and data were collected two times.At 30 DAI the LS medium with no hormone showed the shoot length that is significantky differ with other combination but oter parameter like asleaf number 4.6 and percentage of root formation is significantly different.LS medium with BAP=KN=OJ showed the result(shoot length 3.41,leaf no 5.4,% of root formation 65) was more or less similar with other combination. At 30 DAI LS medium and BAP+KN+TJ, showed its best performance, in shoot length (4.99 cm), where leaf number (7.10) and percentage of root formation was (78%) in LS medium with BAP+NAA combination. Malabadi (2002) found luxuriant shoot elongation, when cultured on MS basal medium containing (8.3 micro M BA+ 9.29 micro M KN+ 5.37

micro M NAA) and 100 mg/l Adenine sulphate (ADS) and 10% (V/V) coconut water. In this experiment leaf color was dark in LS medium with no hormone combination (Table 5)

Table 5. Effect of LS medium with different hormones and organic supplements:

SL. No	Treatment	Average				% Root formation	Leaf Color
		Shoot Length (cm)		Leaf Number/plant			
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI		
1	LS+Agar	2.96 a	4.18 a	4.60 bc	6.40 c	46 b	DG
2	LS+BAP+KIN+Oj	3.41 b	4.74 bc	5.40 d	7.30 d	65 c	PG
3	LS+BAP+KIN+Tj	3.35 b	4.99 bc	5.00 cd	6.30 bc	77 d	LG
4	LS+BAP+NAA	3.45 b	4.57 ab	5.20 cd	7.10 d	78 d	PG
	± SE (%)	0.137	0.153	0.202	0.239	3.53	

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test

X, DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

Here, BAP, KN and NAA are different categories of growth regulators and their function is different to the parameters. BAP and NAA is kind of Auxin and their function is to elongate cell and promoted root. The other hand KN has functioned on cell division. Organic supplement has effect on shoot proliferation as Malabadi found. Here in this experiment organic supplement with hormone perform good result.

4.6. The effect of PM medium on the ginger plant:-

PM medium without hormone showed poor result in root formation that is difer with other combination but other parameter was more or less similar.Here PM medium were used with different hormone and organic supplements. At 30 DAI the highest shoot length was observed in PM+ NAA combination. Where leaf number (6.00) was in PM medium and BA+ TJ combination. But root number was highest in PM medium and KN+ OJ (orange Juice) combination (Table 6)

Table 6. Effect of PM medium with different hormones and organic supplements:

SL. No	Treatment	Average				% Root Formation	Leaf Color
		Shoot Length (cm)		Leaf Number/plant			
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI		
5	PM without Hormone	3.77 b	5.19 c	3.50 a	5.80 abc	27 a	DG
6	PM+NAA	4.53 c	5.70 d	3.30 a	5.20 a	23 a	LG
7	PM+KIN+Oj	3.42 b	4.73 bc	4.10 b	5.60 ab	60 c	DG
8	PM+BA+Tj	3.46 b	4.85 bc	4.80 cd	6.00 bc	31 a	LG
	% SE(±)	0.137	0.153	0.202	0.239	3.53	

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test

X, DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

Peptone is present in Phytamax (PM) medium, which is an organic nutrient. KN is functioned on root formation. Orange juice and tomato juice is kind of organic supplements and have influenced on shooting and rooting. The presence of peptone in PM media and combined action of KN, BA and organic nutrients may be the reasons for better growth and development.

C_1

C_2

Figures: C_1-C_2: Effects of LS and PM medium on shoot proliferation

4.7. Effect of IBA (1mg/l) on in vitro root induction of ginger plant:

IBA hormone was used with different condition of medium (solid, semi-solid and liquid). Here for root induction number of root, root length and percentage of root formation were studied. The maximum root number (11.1) was observed in with solid rooting medium which significantly indifferent to white liquid (11.1) and MS solid (10.2) rooting Medium. But the other combination were different with White liquid MS solid media. G.R. Rout et al. (2001) found highest number root, when micro shoots were culture on half strength basal MS medium supplemented with 0.5-1.0 mg/l either IAA or IBA and 2% (W/V) sucrose within 7-9 DAI. At higher concentration rooting was inhibited. In this experiment root length was highest in (7.3cm) white liquid medium where root formation percentage were (82%) higher in MS liquid medium (Table 7). Arimura. *et al.*(2002) found highest shoot and root and fresh, dry weight from MS media supplemented with 0.25mg/l NAA + 0.55mg/l BA.

Root formation percentage were higher in MS as well as with medium, in liquid condition the plant can easily absorb nutrient from liquid state, so the average result was good in this experiment.

Table 7. Effect of IBA (1 mg/l) on root induction:

SL. NO.	Media	Average				% Root formation
		No. of Root/Tiller		Root Length		
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI	
1	MS solid rooting media	8.0 b	10.2 ab	1.78 a	3.20 a	79 c
2	MS Semi-solid rooting media	6.8 ab	9.3 a	1.90 a	3.19 a	77 c
3	MS liquid rooting media	8.0 b	10.3 ab	1.78 a	3.14 a	82 c
4	White liquid rooting media	6.1 a	11.1 ab	4.00 b	7.35 c	60 b
5	White Semi-solid rooting media	5.6 a	11.5b	3.53 b	5.47 b	50 a
6	White solid rooting media	5.9 a	11.1 ab	3.91 b	6.11 b	80 c
	± SE (%)	0.288	0.437	0.045	0.086	3.47

Means followed by similar letters in each column are not significantly different at 5% level according to Duncan's Multiple Rang Test

X. DAI-Days after Inoculation, + = poor vigor, ++ = medium vigor, +++ = high vigor

4.8. Field performance evaluation:

When rooted plantlets reached at a desirable height, they were transferred for. Acclimatization in room temperature form a week. About 95% plantlets survived following the hardening and acclimatization process.

The earthen pot were prepared with sort of soil composition and transplanted with plants to select the best type of composition. They were-

1. Garden soil + Cow dung + Sand (1:1:1)
2. Garden Soil + Poultry liters + Sand (1:1:1)
3. Garden Soil + Fertilizer +Sand (1:1:1)
4. Garden Soil + Sand (1:1)

After 30 Days of observation from transplantation in soil, two times data were collected at two weeks intervals. Highest plant height at 15 DAI and 30 DAI (99 and 13.3 cm) were found in soil type-2, plant height were also highest (11.80 cm) in soil type 4. Cho-sangkyun et al. (1997) found significantly taller and had greater stem diameters in the carbonized rice husks : Peat medium (5:1 Proportion) than same medium (3:1 proportion) or perlite :vermiculite (1:2). The plant leaf number was highest in soil (type 2 and 4) respectively where leaves number were 8.10 at 30 DAT .The plantlets had poor growth in soil (type 2 and 3) but good performance in soil (type 1 and 4) where the growth vigor (+++) was high. The highest plant height, leaf number / plant, leaf color and growth vigor of *zingiber officinale* var. Thai. In the present study indicates that soil (type 1 and 4) could easily be adopted for large scale multiplication and cultivation. Cow dung is an organic fertilizer which has no residual effect and contains the nutrients that are needed by ginger plant (Table 8)

Table 8: Observation of plants performance in soil

SL. No	Treatment	Average				Leaf Color	Growth Vigor
		Plant Height (cm)		Leaf Number/plant			
		At 15 DAI	At 30 DAI	At 15 DAI	At 30 DAI		
1	Soil + Cowdung+sand	8.9 b	10.25 b	5.50 ab	7.20 a	DG	+++
2	Soil +Poultry litter+sand.	9.9 b	13.30 c	5.50 ab	7.60 ab	PG	++
3	Soil +Fertilizer+sand	3.6 a	6.70 a	5.10 a	8.10 b	DG	++
4	Soil +sand	8.1 b	11.80 bc	6.30 b	8.10 b	DG	+++
	± SE(%)	0.794	0.703	0.316	0.288		

D_1

Liquid media with (charcoal)

D_2

Solid media

Figures: D_1-D_2: Effects of IBA (1mg/l) on root induction (MS media)

]

E1

Solid media

E_2

Liquid media with
(Charcoal)

Figures: E_1-E_2: Effects of IBA (1mg/l) on tooting of Ginger (White media)

F1

F2

Figures: F_1, F_2, F_3, and F_4: Eight weeks old micro propagated plants establish on earthen pot in different soil composition

CHAPTER 5

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Ginger is an important tropical horticultural plant. It is an important spices crop of Bangladesh and valued all over the world from ancient period for its aroma, flavor and also medicinal properties. At present Bangladesh has been suffering for a big amount of spices and vegetables. There is an urgent need to develop and propagate superior and higher yielding varieties, which can fulfill the national market demand.

The conventional methods of vegetative propagation will be unable to meet the increasing demand for quality planting material. Developing a system of plant regeneration using in vitro techniques would be an effective and beneficial way of multiplication. The present work was there fore initiated with the objective of developing an in vitro method of plant multiplication. Important findings of the present study are summarized in the following paragraphs:

Find the suitable growing media for *in vitro* proliferation:

It is necessary to develop a best growing medium for in vitro propagation. Here, MS medium performed good result as maximum shoot length (4.7cm), more leaf (4.9cm) number and growth vigor (+++) over another medium (White medium).

Suitable method for *in vitro* proliferation of shoot:

Auxins and Cytokinins often used for shoot development in vitro. A favorable effect of Cytokinin (KN) and Auxin (BA) on in vitro shoot length and multiple shooting the present study was recommended that BA and KN at (singly) 1.0mg/l concentration performed the most favorable highest shoot length (4.1cm) and multiple tillering (2.3cm)

Best synergism for multiple shoot proliferation:

Auxins and cytokinins some times used as synergism. Here BA (2.0mg/l) + KN (1.0mg/l) has proved useful effect on vegetative development of ginger. Highest

growth vigor (+++); leaf number (5.0) and plant height (4.8cm) were observed in this experiment.

Selection of organic supplements for shoot proliferation:

In this experiment LS and PM medium were used with different organic supplements along with Auxin and cytokinin hormones. Here, LS+BAP+KN+OJ and LS+BAP+NAA combination showed the best result.

Induction of root:

Medium containing IBA 1.0mg/l was the most suitable for root induction with 80% occurrence in ginger shoots within four weeks of incubation. Average number of roots was (82%) in1/2 strength of MS basal liquid rooting medium, and average length of root (11.1cm) was obtained in white liquid rooting medium.

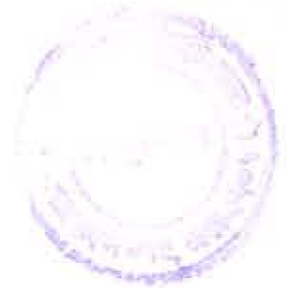
Growth observation of *In vitro* derived plant in earthen pot:

The hardened plantlets after acclimatization were established in earthen pots with over 90% successes. After one months observations period the plants were grown up to average length of (11.8cm) in Garden soil: sand.

Thus a protocol for the micro propagation of *Ζινγιβερ οφφιχιναλε* was established from sprout culture. This protocol developed through us could be used for clonal multiplication of ginger for high yield and genetic conservation of endogenous varieties. Farther researches and studies are needed to establish the convenient method of micro propagation.

CHAPTER 6

REFERENCES



REFERENCE

Arimura.1999.Micropropagation of ginger, PRAP Report Pacific Regional Agricultural Programme; **(7)** : 11-12.

Arimura, Finger and casali.2000.A first method for *in vitro* propagation of ginger. Tropical Science, **40 (2)** : 86-91

Arimura, Finger and Teixeira. 2002. NAAX BAP interaction on *in vitro* development of ginger. Acta Horiculturae, **(506)** : 289-291.

Balachandra,S.N., S.R.Bhat, and K.P.S. Chandel.1990. *In vitro* clonal multiplication of turmeric *(Curcuma spp.)* and ginger (*Zingiber officinales Rose.)*. Plant Cell Rep. **8:** 521-524.

Chan Laikeny and Thong Weng Hing. 2004. *In uitro* propagation of zingiberaceae sfecies with medicinal properties *Journal of plant Biotechnology*; **6 (3):** 181-188.

Cho-songkyum, Roh-kyunghee, Hyun-Dogn yun and Choi-inlok. 1997. RDA-*Journal of Industrial Crop Science*; **39 (2)** : 16-21.

Dodds,J.H.1991. *In vitro* Methods of conservation of plaint Genetic Resources. Chopman and Hall, London.

Islam,M.S., 2002. Policy Dialogue on Agriculture. Guide on Medicinal and Aromatic Plants of SAARC Countries.

Malbadi, 2002. *In vitro* propagation of spiral ginger. *Indian Journal of Genetics and Plant Breeding*, **62 (3)** : 277-278.

Pruthi, J.S. 1998. Spices and Condiments, Nationa Boo Trust, A-5 Green Park, New Delhi, pp, 147-154.

Rout, G.R., and P.Das, 1997. *In vitro* ogranogenesis in ginger (*Zingiber officinale Rose.) J. Herbs, Spices and Medicinal Plant*, 4: 41-51.

APPENDICES

Composition of MS (Murashige and Skoog, 1962) medium used in this study

CONSTITUENTS	CONCENTRATIONS (mg/1)
Macronutrients	
KNO_3	1900.00
NH_4NO_3	1650.00
$CaCl_2 2H_2O$	440.00
$MgSO_4$, $7H_2O$	370.00
KH_2PO_4	1700.00
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4$, $4H_2O$	22.30
$ZnSO_4$, $7H_2O$	8.60
Na_2MoO_4, $2H_2O$	0.25
CuSO4, $5H_2O$	0.025
$CoCl_2$, $6H_2O$	0.025
$FeSO_4$, $7H_2O$	27.80
Na_2 EDTA, $2H_2O$	37.30
Organic nutrients	
Myo-inositol	100.00
Nicotinic acid	0.50
Pyridoxinic HCI	0.50
Thiamine	0.10
Glycine	2.0
Sucrose	30,000
Agar Powder	6000
pH	5.8

PM Medium

Composition of PM Medium used in this study.

CONSTITUENTS	CONCENTRATIONS (mg/l)
Macro Nutrients	
NH_4No_3	825.00
$MgSo_4, 7H_2O$	90.45
KNO_3	950.00
$Cacl_2$	166.00
KH_2PO_4	85.00
Micro Nutrients	
$FeSO_4, 7H_2O$	27.85
Na_2 EDTA, $2H_2O$	37.24
$CUSO_4$	0.0125
$Cocl_2$	0.025
H_3PO_3	3.1
Na MnO_2	0.125
Mn $SO_4, 4G_2O$	8.45
Zn $SO_4, 7H_2O$	5.3
KI	0.415
Organic Nutrients	
Pepton	2000
Nicotinic acid	100.00
Pyridoxinic acid	0.5
Thiamine	1.00
Myo-insitol	200.00
Sucrose	20.000
Agar	6000
pH	5.4

Composition of White's (Am.J.Bot.1953) medium used in this study

CONSTITUENTS	CONCENTRATIONS (mg/l)
Macro Nutrients	
KNO3	80.00
Ca (NO3)2	288.00
Mg SO4, 7H2O	737.00
Na2 SO4	460.00
Na H2PO4	19.00
Micro Nutrients	
Kcl	65
Fe SO_4, $7H_2O$	27.80
Na_2 EDTA, $2H_2O$	33.6
$MnSO_4$, $4H_2O$	6.65
$2nSO_4$, $7H_2O$	2.67
H_3BO_3	1.5
KI	0.75
MoO_3	0.0001
$CuSO_4$	0.001
Organic Nutrients	
Nicotinic acid	0.5
Pyridoxinic acid	0.1
Thiamine	0.1
Glycine	3.0
Sucrose	20,000
Agar	6000
pH	5.5

Composition of Linsmaier and Skoog medium used in this study

Components	Concentration (mg/l)
Macronutients	
$NH_4 N0_3$	1650
$CaCl_2$	332.2
Na_2EDIA	37.26
$FeS0_4$	27.8
$MgS0_4$	180.7
$KP0_4$	170
Micro nutrients	
H_3BO_3	1900
$ZnS0_4$	8.6
$CoCl_2$	0.025
$Cus0_4$	0.025
Na_2EDIA	37.26
$FeS0_4$	27.8
$MgS0_4$	180.7
$MnS0_4$	16.9
Organic nutrients	
Molybdic Acid	0.25
Myo-inositul	100
Thiamine Hcl	0.4

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