J. Product. & Dev., 12(2): 437– 452(2007)

IN VITRO PROPAGATION AND *IN VIVO* PRODUCTION OF *Zingiber officinale*,ROSC.PLANTS

Azza M.S. Arafa
Ornamental Horticulture Department, Faculty of Agric. , Cairo Univeristy, Egypt.

ABSTRACT

Series of experiments were undertaken at Plant Tissue Culture Laboratory, Agricultural Development System Project (ADSP), Ministry of Agriculture, during the two consecutive seasons of 2005 and 2006, to determine the influence of different sterilization materials and time, types of media, different levels of BA and NAA and sucrose concentrations on some growth and rhizome characters of common (ginger), (Zingiber officinale, Rosc.). The obtained results could be briefed in the following points: - Increasing the level of $HgCl_2$ on NaOCl and time of immersing shoot tips, led to gradual inhibition of survival and contamination (%).- Sterilization with mercuric chloride at 0.3% level for 5 minutes followed by 1.0% sodium hypochlorite for 5 minutes also, inhibited contamination completely and 100% survival. - The best shoot number and root length were resulted by liquid media supplemented with BA at 3.0 and 1.0 mg/l, respectively. While, solid medium free from BA gave the best shoot length and number of roots.- Control treatment produced the highest length of shoot and root as well as number of roots, but number of shoots was the largest due to 0.5 mg BA + 0.5 mg NAA/l. treatment. - Raising concentrations of sucrose, reduced survival (%) gradually. The 70 and 90g/l sucrose resulted the highest formation (%) and number of rhizomes.

Under greenhouse conditions, the favourable size of rhizome, as well as the levels of NPK fertilization were evaluated for growth and development of common ginger, in the two seasons. Plant height, number of leaves and fresh weights of vegetative growth and rhizomes, were increased progressively by increasing the size of the planted rhizomes (cuttings) in the greenhouse. The size of rhizomes (40 – 50g) gave growth values more than double the values of 20g rhizome. - In the greenhouse, raising N or K ratios increased gradually number of branches, leaves and roots, plant height ,root length and fresh weight of vegetative growth and rhizomes.

Key words: *In vitro, Zingiber officinale, Zingiberaceae,* Sterilization materials, NAA, BA, rhizome formation, *In vivo* , size of rhizomes, NPK, fertilization.

INTRODUCTION

Zingiber officinale ,Rosc., common ginger or canton ginger, a member of *Zingiberaceae* family is a moderately size group of monocolydenous plants, produce

culinary root and stem growth, with tuberous, aromatic rhizomes. It is perennial herb native to tropical and subtropical Asia (Huxley, 1992).Ginger is currently the tenth most important spices in world terms of its economic point of view. Ginger rhizomes are famous spice used in herbal medicine and as raw material in food beverage and pharmaceutical industries. It is rich in secondary metabolites as oleoresin (Bhagyal-akshmi and Singh, 1988). Ginger should have fertile soil and partial shade and equire warm temperature. Propagation by division, of rhizomes in spring, (Bailey and Bailey, 1976). However, such practice of propagation tends to spread diseases. Most improvement programs are confined to evaluation and selection of naturally occurring clonal variation means of plant propagation and a tool for crop improvement (Vasil, 1988).

Clonal multiplication of, *Zingiber officinale*, Rosc. through multiplication has been reported by many researchers as Rout *et al* (1997) and Khatun *et al* (2003).Using the best material and time for sterilization of culture to obtain clean and actively growing in it was suggested. Rice *et al.* (1992) concluded that hypochlorite were powerful bactericidal, fungicidal and sporicidal. On *Zingiber officinale*, Rout *et al.* (2001) and Khatun *et al.*(2003) used mercuric chloride as a bactericidal and fungicidal disinfectant, disinfectant agents. Double sterilization (with two disinfectants) was recorded by Arafa *et al.* (1999) on *Diffenbachia exotica* and Zayed (2000)on *Spathiphyllum* as well as Nower (2002)on some ornamental bulbs and Hussein (2004) on three *Aglaonema* species.

Several factors were found to affect the regeneration potential of plant tissue cultured *in vitro*. Of these factors, plant growth regulators. Flick *et al.* (1983) and Thorpe(1994)pointed out that the cytokinins /auxin balance was more important for regeneration. Many researchers suggested the use of BA (benzyl-adenine) and NAA (naphthalene acetic acid) for good morphogenesis, such as Arimura *et al.* (2000) and Rout *et al* (2001) on ginger (*Zingiber afficinale* Rose).However, Palai *et al.* (1997) found that shoots were reduced as BA concentration was raised from 6-8 mg /L .Sucrose is important in tissue culture, as a source of carbon and energy, with bulbs and rhizomes were important for bulbs formation *in vitro* culture by high concentration (Niimi *et al.*,2000). In a trial to induce ginger rhizome formation, *in vitro*, Bhat *et al* (1994) concluded that 9 or 12 % sucrose was the more effective.

For *in vivo*, size and weight of ginger rhizome used in planting greatly affect the subsequent growth; Malty *et al.* (1988) obtained promising results when used rhizome weight of 40 g as planting material. The levels of N: P: K fertilization had a great impact on growth, development and rhizome yield, (Pradeepkumar *et al.*, 2001 and Majumdar *et al.,* 2003).

The present investigation aimed to evaluate sterilization treatments as well as the effect of plant growth substances, sucrose concentrations on morphogenesis of ginger (*Zingiber afficinale* ,Rosc.) *in vitro*. Also, the effect of size of rhizome and different N: P: K levels on the growth, development and rhizome characters under greenhouse conditions.

MATERIALS AND METHODS

This investigation was carried out during the two consecutive seasons of 2005 and 2006 at the Plant Tissue Culture Laboratory, Agricultural Development System Project (ADSP), and Ministry of Agriculture to find out the suitable methodology for propagation of common ginger (*Zingiber officinale*, Rosc.) using tissue culture technique. This work included the following investigations:

***First part:* (*In vitro* propagation)**: clean rhizome pieces with shoot tips 2.0 cm long were thoroughly washed well in sterile distilled water .Then, the shoot tips were excised with the sharp blade and collected from rhizome, and well washed for two hours under current water, and taken to test the suitable sterilization method, including the use of sodium hypochlorite solution (NaOCl) at the levels of 1.0, 2.0, 3.0 and 4.0 % or mercuric chloride solution ($HgCl_2$) at 0.1, 0.2, 0.3 and 0.4 %, for different times of sterilization: 5, 10, 15, 20 and 25 minutes. The sterilized shoots tips were cultured in 250 ml jars containing MS (Murashige and Skoog, 1962) incubated for 15 days in growth room at ambient temperature under 16 hrs illumination of 2000 lux. Survival and contamination percentages were estimated. The investigation included 40 treatments (8 sterilization levels x 5 sterilizing time). Each treatment contained 9 jars in 3 replicates, *in vitro.* Further investigation was done to detect the effect of sterilization at 0.1, 0.2 and 0.3 % mercuric chloride for 5, 10 and 15 minutes, followed by immersion in 1.0 % sodium hypochlorite for 5 minutes, on survival and contamination percentages of common ginger shoot tips cultured on MS medium. This investigation contained nine treatments (3 sterilization treatments x3 times of sterilization). Each treatment included 9 jars in three replicates, *in vitro*.
Excised shoot tips, sterilized with 0.3 % $HgCl_2$ for 5 minutes followed with 1.0 % NaOCl for 5 minutes (the best treatment for sterilization in the 2nd investigation), were cultured on solid or liquid media, supplemented with the five levels of BA (benzyl-adenine): 0.0, 0.5, 1.0, 2.0 and 3.0 mg /L, *in vitro*. The investigation included 10 treatments (5 BA levels x 2 media). After 16 weeks, number and length (cm) of shoots and root were recorded.

The experimental design of the three foregoing investigation was factorial in completely randomized design with three replicates. Shoot tips were cultured *in vitro* in 750 ml jars containing 35ml of MS medium. This investigation aimed to detect the effect of supplementation of 0.5, 1.0 and 2.0 mg BA /L combined with NAA (Naphthalene acetic acid) at 0.25 or 0.5mg/l, on number and length (cm) of shoots and roots. The investigation consisted of 6 treatments replicated thrice in completely randomized design. In order to find out the best sucrose concentration as a source of energy for MS medium, *in vitro* on survival and rhizome formation percentage and number of rhizomes/ shoot tip of common ginger. Six sucrose concentrations were: 30, 50, 70, 90, 110 and 130 g /L, replicated three times.

***Second part:* (*In vivo* production):** this investigation was consummated to evaluate the influence of rhizome cutting size of common ginger on its growth and

development. Weight of rhizome cutting were 20, 20- 30, 30- 40 and 40-50 g /rhizome. They were planted in 35 cm clay pots containing medium of 2:1:1 (by volume) peatmoss: sand: clay, the physical and chemical properties of the medium are shown in Table A.

The analysis of the medium was conducted using the method described by Jakson (1967), thoroughly mixed with 250 g /pot decomposed organic fertilizer, under greenhouse conditions. Routine agricultural practices as watering, weeding …etc was done whenever required .Planting occurred on March 15th, 2005 and 2006. On 15th, November, such data were obtained: plant height (cm), number of leaves and fresh weight of vegetative growth and rhizomes (g). The layout of this investigation was four treatments in three replicates, each included 10 pots. In two seasons (2005 and 2006) investigation, about 50g rhizomes of common ginger were planted under greenhouse conditions, on March, 15th, in clay pots containing 2: 1: 1 (v/v/v) peatmoss: sand: clay, mixed with 250 g /pot decomposed organic fertilizer, to evaluate different NPK fertilization ratios. NPK treatments were the following ratios: 0, 120: 60: 120, 120: 60: 180, 240: 60: 120, 240: 60: 180, 360: 60: 120 and 360: 60: 180. Fertilization using, ammonium sulphate (20.5 %N), calcium super-phosphate (15.5 % P_2O_5), and potassium sulphate (48% K_20). The fertilizers added at two equal occurred after 3 and 5 months from planting as recommended by Wiroatmodjo (1990). Each treatment was replicated three times, each having 10 pots. On November, 15th, 2005 and 2006, the following data were recorded: number of branches, leaves and roots, length (cm) of branches and roots and fresh weight (g) of vegetative growth and roots.

The experimental design for the investigations *in vivo* was randomized complete block design. Data analysis of variance was carried out according to Steel and Torrie (1980),using Duncan's Multiple Rang Test $P \leq 0.05$.

Table A: Physical and chemical properties of medium used for growing *Zingiber officinale* plants.

Physical properties										
Soil particle size distribution (%)							**Organic Matter (%)**	**Electrical Conductivity (dS/m)**		
Coarse sand	**Fine sand**	**Silt**	**Clay**							
11.3	71.2	6.5	11.0				7.3	1.3		
Chemical properties										
Soluble anions (meq./ L)			**Soluble Cations (meq./L)**				**Available elements (ppm)**			**pH**
HCO_3	**Cl^-**	**SO_4^{--}**	**Ca^{++}**	**Mg^{++}**	**Na^+**	**K^+**	**N**	**P_2O_5**	**K_2O**	
2.13	7.8	6.4	4.48	2.84	7.69	1.18	523	85.7	318	6.4

RESULTS AND DISCUSSION

First part : In vitro propagation of Zingiber officinale, Rosc.

The 1st investigation: Influence of some sterilization materials for different times on survival and contamination percentages, *in vitro*. Data in Table 1 revealed that increasing NaOCL or $HgCl_2$ levels individually led to significantly progressive depression on survival and contamination percentages. It is clear that mercuric chloride had more effect on sterilization but less reductive on survival percentage. The largest and lowest survival (%) occurred due to the 0.1% $HgCl_2$ and the 4.0 % NaOCl, respectively. Increasing time of sterilization caused significant gradual suppression on both survival and contamination percentages. The 100 % survival was produced by a variety of combined treatments between some levels of materials and time used, namely 1.0 % NaOCl for 5 minutes; 0.1% $HgCl_2$ for 5, 10 and 15 minutes; 0.2% $HgCl_2$ for 5 and 10 minutes and 0.3% $HgCl_2$ for 5 minutes, whereas, the 4.0 % NaOCl for 25 minutes, depressed completely survival (%). But, the 0.4 % $HgCl_2$ for 15, 20 and 25 minutes were the best treatments that suppressed completely contamination in *in vitro* culture of ginger shoot tips.

The 2nd investigation: Influence of some double sterilization treatments for different times on survival and contamination percentages, *in vitro*. Raising $HgCl_2$ levels gradually reduced significantly survival and contamination percentages. Also increasing time of sterilization significantly and progressively improved both characters in most cases. Sterilization with 0.3 $HgCl_2$ for any time from 5 to 15 minutes followed by 1.0 % NaOCl for 5 minutes suppressed contamination completely, however, sterilization for the less time (5 minutes) resulted 100% survival. Russel and Chopra (1990) suggested that the action of mercuric chloride might be due to lyses of microbial cells through its effects on their cell walls or reaction with thio groups in the microbial enzymes. However, Rice *et al* (1992) mentioned that hypochlorite is powerful antimicrobial agents. The present results are in a parallel line with the findings of Arafa *et al* (1999) on *Dieffenbachia extoica* and Hussein (2004) on three *Aglaonema* species, that $HgCl_2$ at 0.1 for 5 minutes followed by commercial Clorox (5.25 % NaOCl) diluted to 50% (2.63% NaOCl) for 20 min. was the most effective sterilization treatment give the highest survival percentage with no contamination.

The 3rd investigation: Influence of different levels of BA (benzyl-adenine) supplemented to solid or liquid media on shoots and roots growth, *in vitro*. Data in Table 3 and Figure 1 indicated that raising BA levels increased gradually and significantly the number of shoots /shoot tip, but reduced progressively and significantly both shoot length and number of roots. Roots were the longest by 1.0 mg BA/l, whereas 2.0 mg/l BA. Shoot number significantly favoured liquid medium,

Tab1

Table 2: Effect of some doula- sterilization treatments on the percentage of survival and contamination of *Zingiber officinale*, Rosc., shoot tips grown *in vitro*.

Treatments		Survival (%)				Contamination (%)			
		Time of sterilization /minutes			Mean	Time of sterilization /minutes			Mean
		5	10	15		5	10	15	
NaOCl (5min) 1%	$HgCl_2$% 0.1	100 a	100 a	100 a	100.0 a	40 a	40 a	30 b	36.67 a
	0.2	100 a	100 a	90 b	96.67 b	20 c	20 c	10 d	16.67 b
	0.3	100 a	90 b	90 b	93.33 c	00 e	00 e	00 e	0.00 c
Mean		100.0a	96.67b	93.33c		20.0a	20.0a	13.33 b	

Means with different letters, in the same column are significantly different ($P<0.05$) using Duncan's multiple range test

while solid medium significantly increased shoot and root length (cm) and number of roots. As for combined treatments, the 3.0mg BA/l supplemented to liquid medium produced significantly the largest number of shoots (16.0), whereas, the longest shoots (5.1 cm) and number of roots (3.5) were significantly resulted by solid medium free from BA. But the longest roots (3.95 cm) occurred significantly due to liquid medium supplied with 1.0mg BA/l. the lowest figures of the foregoing characters resulted by solid or liquid media free from BA, 3.0 mg BA /L supplied to liquid medium, 3.0mg/l BA supplemented to solid or liquid media and 0.5 mg BA /L added to liquid medium, respectively. In this respect, Agretious *et al* (1996) pointed out that the maximum number of *Aplinia calcarata* shoots was obtained from rhizome bud explants on MS medium supplemented with BA (1.5 mg /L) and IAA (0.5 mg /L). Palai *et al* (1997) noted that ginger shoot bud multiplication decreased as BA concentration increased from 6 to 8 mg /L. Arimura *et al* (2000) observed that root number and length of *Zingiber officinale* were enhanced in liquid media regardless plant growth regulator treatment.

The 4th investigation: Influence of different levels of BA (benzyl-adenine) and NAA (naphthalene acetic acid) on some shoot and root characters. It is evident from Table 4 that the 0.5 mg /L BA + 0.5 mg /L NAA resulted the larger number of shoots (8.50),while the lowest shoots number (1.00) occurred by the control (0.0 BA + NAA). On the other hand, the longest shoots and roots as well as the highest number of roots (5.2 cm, 4.0 cm and 3.25, respectively) were produced significantly by the control treatment, as compared to other treatments, whereas, the least number of shoots (1.0) resulted significantly from the control. The shortest shoots (1.60 cm) occurred due to 0.5 mg /L BA + 0.5 mg/L NAA treatment, but 2.0 mg /L BA + 0.5 mg /L NAA produced the least number and length of roots (1.75 root and 1.20 cm, consecutively). Such results are in a parallel line, for shoots number with those of

Table3 : Effect of different levels of BA with solid and liquid media on number of shoots, shoot length (cm), number of roots and root length (cm) of *Zingiber officinale*, Rosc. Shoot tips after 16 weeks

Treatments (mg /L)		Shoots		Roots	
		Number	Length (cm)	Number	Length (cm)
Control		1.00 e	5.20 a	3.25 a	4.00 a
0.25 NAA +	0.5BA	7.50 b	2.30 b	2.50 a-c	2.10 c
	1.0BA	4.50 d	1.75 c	2.25 bc	1.90 c
	2.0BA	4.25 d	2.50 b	2.50 a-c	2.80 b
0.5 NAA +	0.5BA	8.50 a	1.60 c	2.25 bc	2.90 b
	1.0BA	6.50 c	2.20 b	3.00 ab	3.00 b
	2.0BA	4.25 d	1.65 c	1.75 c	1.20 d

Means with different letters, in the same columu are significantly different (P<0.05) using Duncan's multiple range test.
**S.M: solid medium **L.M: liquid medium

Arimura *et al* (2002) on ginger that NAA + BA at low levels improved shoot multiplication and shoot /root ratio, while, on *Zingiber cassumunar* (Roxb.)
Chirangini and Sharma (2005)found that, the most effective media for multiplication of microshoots were MS media with NAA and BA producing at the rate of 8 microshoots per explants.

The 5th investigation: Influence of some sucrose concentrations (g/l) on *in vitro* rhizome formation .It appears from data in Table 5 and Figure 1 that the highest percentage of survival (100%) resulted significantly by the lowest concentrations (30 and 50g/l) sucrose. Survival (%) was reduced significantly and progressively as sucrose concentrations were decreased. But, percentage of rhizome formation increased gradually up to 70g/l sucrose which resulted significantly the highest (85.0 %), and then it decreased progressively and significantly. The least percentages of survival and rhizome formation (50.0 and 35.0 %, successively) were produced by the highest sucrose concentration (130 g /L).Whereas, the largest and the lowest number of rhizomes were 6.25 and 2.50 occurred due the 90 and 30 g/L sucrose, consecutively. Such results are in agreement with those of Bhat *et al* (1994) who mentioned that for ginger rhizome induction, only sucrose (at 9 % or 12 %) was found to be effective. Jeong (1996) mentioned that as sucrose concentration was increased from1% to 9% the number of bulblets was increased. Niimi *et al*, (2000) found that the bulblet growth of *Lilium rubellum* was more stimulated in the medium containing 250 mM sucrose than in the medium 150 mM sucrose.

Tab4

Table 5: Effect of sucrose concentration on *in vitro* survival (%), rhizomes [formation (%), number and fresh weight (g)] of *Zingiber officinale*, Rosc. after 16 weeks.

Sucrose conc.(g /L)	Survival (%)	Rhizomes		
		Formation (%)	Number	Fresh weight (g)
30	100 a	25 e	2.50 d	0.56 b
50	100 a	65 b	4.50 b	0.91 a
70	90 ab	85 a	3.50 c	0.47 c
90	80 bc	70 b	6.25 a	0.35 d
110	70 c	45 c	5.25b	0.28 d
130	50 d	35 d	5.00 b	0.29 d

Means with different letters, in the same column are significantly different ($P<0.05$) using Duncan's multiple range tests.

Second part: In vivo production of Zingiber officinale, Rosc.

The 6th investigation: Influence of size of rhizome on some growth and development parameters under greenhouse conditions**.** Data in Table 6 revealed that the bigger the size of rhizome was, the greater the plant height (cm), number of leaves and fresh weight of vegetative growth and rhizomes formed in both seasons of the investigation. The longest plant height and number of leaves were 69.0 cm and 12.0 in the first season and 72.67 cm and 12.33 in the second one, due to the biggest rhizome (40-50 g /rhizome), amounting to more than double the values resulting from the least size of rhizome (20 g). The 40-50 g size resulted 33.39 and 34.67 g fresh weight of vegetative growth and 74.09 and 76.24 g., fresh weight of rhizomes, during the first and second seasons, respectively. Such figures were about 5 and 3.5 times the values produced by the 20g rhizome. These results are in coincidence with those obtained by Malty *et al.* (1988) who postulated that the best weight of ginger (*Zingiber officinale* Rose) rhizome as planting material was 40g.

The 7th investigation: Influence of NPK fertilizers on growth and development of ginger propagated by 50 g. /rhizome under greenhouse conditions: The lowest N: P: K (120: 60: 120) level of fertilization produced significantly the highest records of number of branches , leaves and roots, length of branches and roots and fresh weights of vegetative growth and rhizomes, during in the both seasons, whereas the lowest values resulted by the highest N: P: K ratio (360: 60: 180), in comparison to most fertilization mixtures, as shown in Table 7 and Figure 1. It is evident that raising nitrogen and potash ratio in fertilization mixture caused inhibition in all the studied parameters. Such results might be explained by the findings of Dayankatti and Sulikeri (2000) observed that growth characters were highest at 125 Kg N /ha. While,

Fig 1

Table 6: Effect of size of rhizome on growth and development of *Zingiber offcinale* Rosc., grown in greenhouse during two seasons.

Size of rhizome (g)	Plant height (cm)	Number of leaves	Fresh weight of vegetative growth	Fresh weight of rhizomes
		First season (2005)		
20	31.67 c	5.67 b	6.47 c	21.10 d
20- 30	50.33 b	7.00 b	16.10 b	35.65 c
30- 40	57.00 b	10.67 a	22.67 b	47.47 b
40- 50	69.00 a	12.00 a	33.39 a	74.09 a
		Second season (2006)		
20	32.33 d	6.33 b	7.12 d	22.07 d
20- 30	50.00 c	7.67 b	16.52 c	36.83 c
30- 40	58.67 b	11.67 a	22.00 b	49.09 b
40- 50	72.67 a	12.33 a	34.67 a	76.24 a

Means with different letters ,in the same column are significantly different (P<0.05) using Duncan's multiple range test.

Table 7: Effect of different concentration of NPK fertilization on growth and development of *Zingiber offcinale* Rosc., grown in greenhouse during two seasons.

NPK	Number of branches	Plant height (cm)	Number of leaves	Fresh weight of vegetative growth (g)	Number of roots	Root length (cm)	Fresh weight of rhizomes (g)
				First season : 2005			
Control	1.33 cd	71.53 c	13.00 b	62.28 bc	6.33d	8.67 d	85.50 d
120:60:120	3.67 a	105.5 a	17.00 a	115.40 a	10.00 a	22.00 a	116.0 a
120:60:180	1.67 cd	88.67 b	14.67 b	66.31 bc	7.33 cd	13.00 c	88.05 cd
240:60:120	2.00 bc	93.17 b	13.33 b	55.47 cd	7.67 bc	15.33 bc	90.67 c
240:60:180	2.67 b	101.50 a	16.00 a	73.39 b	8.67 b	18.33 b	105.60 b
360:60:120	1.00 d	44.17 d	8.00 c	45.83 d	4.33 e	6.33 de	56.29 e
360:60:180	1.00 d	37.77 d	6.00 d	25.61 e	1.67 f	5.00 e	44.70 f
				Second season : 2006			
Control	1.33 c	70.73 c	12.67 b	64.83 d	5.67 cd	8.33 d	85.87 d
120:60:120	3.33 a	110.0 a	17.33 a	119.40 a	9.33 a	21.00 a	117.30 a
120:60:180	1.67 bc	89.73 b	14.00 b	72.96 c	6.67 bc	12.33 c	89.36 cd
240:60:120	2.00 bc	91.50 b	14.33 b	58.73 d	7.00 bc	14.67bc	95.45 c
240:60:180	2.67 ab	104.40 a	16.33 a	87.71 b	8.00 ab	17.67 b	108.00 b
360:60:120	1.33 c	45.37 d	7.67 c	48.33 e	4.00 d	6.00 de	60.66 e
360:60:180	1.33 c	36.77 d	5.33 d	27.66 f	1.33 e	4.33 e	46.67 f

Means with different letters ,in the same column are significantly different (P<0.05) using Duncan's multiple range test

Pradeepkumar *et al* (2001) found that combinations of 150 kg N with 50 or 100 kg K and 75kg N with 150 kg k, were significantly superior to other combinations with respect to yield. They added that N fertilizer significantly affected plant height up to 75 kg N /ha. Whereas, Ajithkumar and Jayachandran (2001) pointed out that 50kg N + 100 kg KO_2 produced the highest yields.

CONCLUSION

From the previous results concluded that:

(1) *In vitro* propagation of ginger, immersing ginger shoot tips in mercuric chloride at 0.4 % for 5 minutes followed by immersion for 5 minutes in 1.6 % sodium hypochlorite resulted 100% survival with no contamination. Solid medium free from plant growth substances give the best number of roots and shoot length**.** Addition of 70-90 g sucrose /L *in vitro* was favourable for rooting characters.

(2) *In vivo* production, planting 40 – 50 g rhizome (weight)in greenhouse produce the best growth and rhizome characters. In order to obtain the highest growth, root and rhizome parameter under greenhouse conditions, plants would be fertilized with N: P: K at 120: 60: 120.

REFERENCES

Ajithkumar, K. and Jayachandran, B.K. (2001). Effect of major nutrients on yield of ginger (*Zingiber officinale,* Rosc) intercopped in cocorut garde. *J. Spices and Arom. Crops*, **10** (1): 17-23.

Arafa, A.M.S.; Ebrahim, M.K.H. and Ibrahim, I.A. (1999). Role of benzyl-adenine and activated charcoal in optimizing the culture media of *in vitro* cultured *Dieffenbachia extoica* cv. Tropic Snow. *Bull. Fac. Sci., Assuit Univ.*, **28** (2D): 187 – 198.

Arimura, C.T.; Finger, F.L.and Casali,V.W.D.(2000). Effect of NAA and BAP on ginger (*Zingiber officinale* ,Rosc.) sprouting in solid and liquid medium. *Rev. Brasil. Plantas Medic***., 2** (2): 23-26.

Arimura, C.T.; Finger, F.L.;Teixeira, J.B.; Ming, L.C. (ed); Craker, L.E. (ed.); Scheffer, M.C. (ed.) and Chaves, F.C.M. (2002). NAA x BAP interaction on *in vitro* development of ginger. *Acta Horticulturae*, **569**: 289 – 291.

Bailey L.H and Bailey, E. (1976). *Hortus Third*. MacMillan Publ. Co., Inc., N.Y., USA.

Bhagyal-akshmi , S.M. and Singh, N.S. (1988). Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale*,Rosc.) with a high yield of oleoresin. *J. Hort. Sci***., 63** (2): 321 – 327.

Bhat, S.R.; Chandel, K.P.S. and Kackar,A. (1994). *In vitro* induction of rhizomes in ginger (*Zingiber officinale*, Rosc.) .Ind. J. Exp. Biology, **32** (5): 340-344.

Chirangini and Sharma(2005).*In vitro* propagation and microrhizome induction in *Zingiber cassumunar* (Roxb.)an antioxidant –rich medicinal plant. *Journal of Food, Agriculture &* Environment,**3** (1):139-142.

Dayankatti, B.S. and Sulikeri, G.S. (2000). Effect of plant population and nitrogen levels on growth, yield and quality of giger growth altributes. *Karnataka J. Agric. Sci*., **13** (4): 1047 – 1048. (Cf. CAB Abst. 2000/08 – 2002/2007, record 205 of 278).

Hussein, M.M.M. (2004). *In vitro* propagation of three species of Aglaonema plants., *Arab. Univ. J. Agric. Sci*, Ain Shams Univ., Cairo, **12** (1): 405 – 423.

Huxley, A. (1992). Dictionary of Gardening. Stockton Press, N.Y., U.S.A., **4**, 518-519.

Jakson,M.L.(1967). *Soil Chemical Analysis Prentice-Hall*. Inc. England. Cliffs, U.S.A., 219-221.

Jeong,J.H.(1996):*In vitro* propagation of bulb scale section of several Korean native lilies. *Acta Hort*.,**414:**269-276.

Khatun, A.; Nasrin, S. and Hossain, M.T (2003). Large scale multiplication of ginger (*Zingiber officinale*, Rosc.) from shoot - tip culture. *J. Biol. Sci.,* **3** (1): 59-64.

Majumdar,B.; Venkatesh, ,M.S.; Kailash-Kumar ;Patiram and Kumar, K.(2003). Effect of N levels,FYM and mother rhizome removal on yield, nutrient up take and quality of ginger (*Zingiber officinale*, Rosc.) and different forms of N build up in an acidic Alfisol of Meghalaya.*Crop Research Hisar,* **25** :3,478-483.

Malty, I.K.; Sengupta, D.; som, M.G. ; Jana ,P.K. and Bose, I.K. (1988). Growth and yield of *Zingiber officinale* ,Rosc. As influence by some agronomic practices in the plains of West Bengal. *Acta Horticulturac*, **188 A.:** 117-122.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue culture. *Physiol. Plant*., **15:** 473-497.

Nower,A.A.A. (2002). Studies on production of some ornamental bulbs and establishment of gene transfer system through tissue culture techniques . Ph.D. Thesis., Fac. Agric., Cairo Univ., Egypt.

Niimi,Y.;Misaki,Y. and Nakano,M.(2000): Production of commercial bulbs of *Lilium rabellum*, Baker:Changes in carbohydrates in bulblets and sugars of liquid medium during their culture. *J. of the Japanese Society for Horticultural Science*, **69** (2):161-165.

Palai, S.K.; Rout ,G.R. ; Das ,P.; Edison, S. (ed.); Ramana,K.V.(ed); Sasikumar,B. (ed.); Babu, K.N.(ed)and S.J. Eapen (1997). Micropropagation of ginger (*Zingiber officinale*, Rosc.) interaction of growth regulators and culture conditions. Biotechnology of spices, medicinal and aromatic plants. *Proc. Natl Seminar on Biotechnology of Spices and Aromatic Plants*, Calicut, India, 24-25 April, 1996. 1997; 20-24 (Cf. CAB Abst. 1996 – 1998/07, record 110 of 278).

Pradeepkumar, T.; Mayadevi ,P.; Aipe, K.C. ; Manomohandas, T.P.; M.P. Giridharan, M.P. ; Satheesan K.N. and Kumaran, K. (2001). Optimum dose of nitrogen and potassium for ginger in Wynad, *Kerala. J. Spices and Arom. Crops*, **10** (1): 7-11.

Rice, R.D.; Alderson, P.G.; Hall ,J. and Ranchhod, A. (1992). *Micropropagation: Principles and Commerical Practice.* (2nd Ed.), Pergamon Press, Oxford, U.K. 129-149.

Rout, G.R.; Palai, S.K. and Das, P. (1997). *In vitro* micropropagation of ginger (*Zingiber officinale*, Rosc.): interaction of growth regulators and culture conditions. *Ind. J. Herbs Spices*, **67**:437-441.

Rout, G.R.; Palai, S.K.;Samantaray,S. and Das, P. (2001). Effect of growth regulator and culture conditions on shoot multiplication and rhizome formation in ginger (*Zingiber officinale*, Rosc.)*in vitro*. *In Vitro* Cell. *Dev. Biol.-Plant,* **37**:814-819.

Russel, A.D. and Chopra, I. (1990). *Understanding Antibacterial Action and Resistance*. Ellis Horwood, N. Y., U.S.A., 54.

Steel ,R.G.D. and Torrie,J.H. (1980). *Principles of Statistics .A Biometrical Approach*.Second Ed.,Mc Graw-Hill Kogakusha,L.T.D.

Thorpe,T.A.(1994). *Morphgensis and Regeneration*. In: Vasil ,I.K. and Thorpe, T.A. (eds.),Plant Cell and Tissue Culture, 37-70.Kluwer Academic Publishers.

Vasil, I.K (1988) Progress in the regeneration and genetic manipulation of cereal crops. *Biotechnol*., **6**: 397 – 402.

Wiroatmodjo, J. (1990). Agronomic manipulation for exportable size of gingers (*Zingiber officinale*, Rosc.) cv.. *Badak. Indonesian J. Trop. Agric*., **1** (2): 80 – 82. (CAB Abst., 1993 – 1994, Record 86 of 278).

Zayed ,E.M.M.(2000).*In vitro* propagation of *Spathiphyllum*.M.Sc.Thesis, Fac. of Agric.,Cairo Univ., Egypt .

إكثار نباتات الزنجبيل فى مزارع الأنسجة وإنتاجها فى الصوبة.

عزة محمد سعيد عرفة
بساتين الزينة – كلية الزراعة – جامعه القاهرة

أجرى البحث فى معمل زراعة الأنسجة النباتية بمشروع تطوير النظم الزراعية، بوزارة الزراعة، خلال الموسمين المتعاقبين ٢٠٠٥ و٢٠٠٦ للتعرف على مدى تأثير مواد ومدة التعقيم المختلفة، أنواع البيئات، مستويات مختلفة من البنزيل أدنين ونفتالين حامض الخليك وتركيزات مختلفة من السكروز على بعض صفات النمو وتكوين الريزومات للزنجبيل العادي *Zingiber officinale* Rose, فى الزراعة المعملية وتحت ظروف الصوبة٠

تم تقدير أفضل حجم لأجزاء الريزومات من أجل الزراعة وكذا معدل التسميد ن: فو٢ا٥: بو٢ا على نمو وتطور الزنجبيل العادي لمدة موسمين. ويمكن إيجاز النتائج المتحصل عليها في الآتي:

- أدت زيادة تركيز كلوريد الزئبق أو هيبوكلوريت الصوديوم ومدة غمس قمم الأ فرع، إلى تثبيط تدريجي في نسبة البقاء حية وكذلك التلوث.
- التعقيم بكلوريد الزئبق ٠.٣% لمدة ٥ دقائق متبوعة بـ ١.٠% هيبوكلوريت الصوديوم لمدة ٥ دقائق أيضاً، ثبطت التلوث تماماً وبقيت كل البراعم حية.
- نتجت أحسن أعداد للأفرع وأطوال للجذور على البيئة السائلة المضاف إليها ٣,٠ ملليجرام لكل لتر بنزيل أدنين ويليها فى أفضل النتائج إضافة ١ملليجرام لكل لتر بنزيل أدنين.
- أعطت البيئة الصلبة الخالية من البنزيل أدنين أحسن طول للأفرع وعدد الجذور.
- أنتجت معاملة المقارنة أعلى طول للأفرع وللجذر وكذلك عدد الجذور، ولكن عدد الأفرع كانت أعلى نتيجة إضافة ٠,٥ ملليجرام لكل لتر بنزيل أدنين + ٠,٥ملليجرام لكل لتر نفثالين حامض الخليك.
- زيادة تركيز السكروز فى البيئة أدى لنقص نسبة بقاء القمم النامية حية تدريجياً ولكنها أنتجت أعلا نسبة تكوين وعدد للريزومات مع إضافة ٧٠ أو٩٠ جرام سكروز٠
- زاد طول الأفرع، وعدد الأوراق والأوزان الطازجة للنمو الخضري والريزومات، تدريجياً، بزيادة حجم الأجزاء النباتية المزروعة، في الصوبة. أعطت الأجزاء النباتية بحجم ٤٠ – ٥٠ جرام قيم الصفات أكبر من ضعف قيم الأجزاء النباتية بحجم ٢٠ جرام.
- أدت زيادة معدلات التسميد بالنيتروجين، والبوتاسيوم إلى زيادة تدريجية في عدد الأفرع وعدد الأوراق والجذو، وزيادة طول كل من النبات والجذر، والوزن الطازج للنمو الخضري والريزومات وذلك عند زراعة نباتات الزنجبيل في الصوبة.