Technology

Pak. J. Sci. Ind. Res. 2007 **50**(2) 145-148

Enhanced *Zingiber officinale* Shoot Multiplication in Liquid Culture

Tauqeer Ahmad, Nasreen Zaidi*, Nuzhat Habib Khan and Zia-ur-Rehman
Plant Biotechnology Laboratory, Food & Biotechnology Research Centre,
PCSIR Laboratories Complex, Ferozepure Road, Lahore-54600, Pakistan

(received December 10, 2005; revised December 9, 2006; accepted December 28, 2006)

Abstract. An efficient method of micropropagation of ginger (*Zingiber officinale*) that can produce 19-fold increase in plantlets after every 12 weeks from a single shoot tip has been developed. Among the various growth regulators used, a combination of 17.6 μM 6-benzylaminopurine (BA) with 2.46 μM indole butyric acid (IBA), 0.94 μM kinetin (Kin) and/or 11.42 μM indole-3-acetic acid (IAA), with half and three quarter strength of Murashige and Skoog (MS) medium proved as the best for morphogenesis. An appropriate combination of cytokinin and auxin gives better results as compared to cytokinin or auxin alone. In addition to the chemical composition of the medium, its physical form can influence the growth pattern and the multiplication rate of cultures. The liquid phase enhanced effectivity of media in case of shoot multiplication. It was also noted that shoot number increased due to "agitation" factor. All the regenerated shoots had functional roots in the same media of shoot multiplication.

Keywords: ginger micropropagation, shoot multiplication, *in vitro,* growth regulators

Introduction

Ginger (*Zingiber officinale*) is one of the oldest and widely used spices. It is esteemed for its aroma, flavour and pungency. It is an indispensable ingredient of continental dishes and is used in food processing, confectionary and beverage industries.

It is a vegetatively propagated crop. The multiplication rate is very slow. In a growing season (8-10 months), only 10-15 lateral buds are produced (Bhagyalakshmi and Singh, 1988). The ginger crop is also susceptible to many diseases i.e. Rhizome-rot disease caused by many species of *Pythium* and the Ginger Yellows caused by *Fusarium oxysporum* f. sp. zingiberi, which results in heavy losses. Conventional method of propagation (by rhizome sections) favours the spread of diseases. Nearly 87% of the field infection of ginger is transmitted through infected rhizomes (Dohroo, 1989). Because of it, selection and rapid multiplication of high yielding and locally adapted varieties, having preferred rhizome characters, can solve many of the problems faced by the ginger growers.

The clonal propagation of ginger was successfully undertaken by inducing growth in axillary and apical buds as reported by earliers (Nadgauda *et al.*, 1980; Hosoki and Sagawa, 1977), shoot tips (Hoque *et al.*, 1999; Inden *et al.*, 1988; Sato *et al.*, 1987; Sakamura *et al.*, 1986; Pillai and Kumar, 1982), meristem (Bhagyalakshmi and Singh, 1988), leafy aerial pseudostem and decapitated crown sections (Ikeda and Tanabe, 1989), stem discs and axillary buds (Noguchi and Yamakawa, 1988a; 1988b), active buds (Sharma and Singh, 1997) and terminal buds (Prathanturarug *et al.*, 2004) *in vitro*.

It is highly desirable to develop a method for the production of healthy clones with high multiplication rates. Plant regeneration via organogenesis was reported by Malamug *et al.* (1991), and rapid clonal multiplication of ginger through shoot induction had been worked out by Hosoki and Sagawa (1977). A maximum of six shoots per culture were obtained in 4 weeks, Nadgauda *et al.* (1980), also obtained similar results by culturing large bud explants of local variety. The objective of this study was to investigate rapid *in vitro* propagation technique.

Materials and Methods

The rhizomes of *Zingiber officinale* Rosc. were procured from local market and thoroughly washed with ample running tap water, wrapped in newspaper sheets and kept them in a dry closet. Within a week the quiescent buds sprouted. Bud stubs (7-8 mm) were sliced, collected and dipped for 5 min in diluted 1:1 commercial bleach, and washed thoroughly with sterile distilled water. Further sterilization was carried out in laminar airflow cabinet. Explants were treated with 0.1% mercuric chloride for 15 min and continuously swirled. Then explants were washed three times with sterile double distilled water. Using a sterilized sharp scalpel the explants were retrimmed and transferred to MS culture medium (Murashige and Skoog, 1962), gelled with 0.8% agar, and no growth regulator. When the buds grew into 10-15 mm shoots, they were aseptically dissected under dissecting microscope to cut away 0.1-0.5 mm size apical meristematic dome with one or two

*Author for correspondence; E-mail: drnasreenz@yahoo.com

leaf primordia. Eventually, they were placed on the meristesm culture media described by Bhagyalakshmi and Singh (1988). For multiple shoot formation the shoots obtained from meristems were subcultured on the media mentioned in Table 1. In this experiment effect of mode of culturing was also determined. So, the explants were shaken in liquid state on gyratory shaker at 80 rpm or kept static, on the media gelled with 0.8% agar. In all the cases the pH of the media was adjusted 5.8 prior to autoclaving for 15 min at 15 lb psi. The cultures were maintained at 25±2 °C under 16 h photoperiod maintained with cool white fluorescent light of 2000-4000 Lux intensity. The shoots obtained from meristems were subcultured once again on meristem culture media (Bhagyalakshmi and Singh, 1988), to allow further development and then transferred to shoot multiplication media (Table 1). The first subculture was after five weeks and subsequent ones at three weeks interval. The number and lengths of shoots were recorded. Plantlets with well-developed roots were thoroughly washed in running tap water to remove adhering medium and transplanted to the pots containing mixture of sand and soil in equal proportions. The potted plants were covered with polyethylene bags to maintain humidity. The plantlets were regularly irrigated with ½ MS and/or Hoagland solution (Hoagland and Arnon, 1950). After hardening plants were shifted to the green house.

Results and Discussion

A combination of growth regulators 17.6 μM benzylaminopurine (BA) with 2.46 μM indol butyric acid (IBA), 0.94 μM kinetin (Kin) and or 11.42 μM indole-3-acetic acid (IAA) proved to be the best for morphogenesis. The present study utilized tissue culture techniques with a possibility of obtaining more than 19-fold increase in shoot production every 8-12 weeks. A combination of BA and Kin has been recommended for ginger bud cultures (Nadgauda *et al.*, 1980; Hosoki and Sagawa, 1977). Different chemical addenda such as activated charcoal, ascorbic acid, adenine sulphate, and MS salts reduced to ½ or ¾ level and a defined ratio of cytokinin and auxin were required for the present study. The combination of 17.6 μM BA, 2.46 μM IBA and 0.94 μM Kin with ½ MS medium in M_4 and M_5 media (Table 1), was found optimum for sustained multiplication of ginger cultures. Rout *et al.* (1995) achieved *in vitro* shoot multiplication of turmeric with 4-fold increase with a combination of 17.75 μM BA with 5.70 μM IAA. In this study, 17.6 μM BA with 11.42 μM IAA also proved better and yielded up to 17-fold increase in shoot multiplication (Fig. 1). Presence of BA in the medium was pivotal for enhancing the number of shoots per culture. As the concentration of BA increased from 8.8 μM to 17.6 μM, it also doubled (Table 1), irrespective of the physical condition of the medium. In contrary, when the concentration of BA increased to 22.9 μM in the absence of other growth regulators, the shoot number reduced to half as compared to those in M_3 to M_6 media, where the concentration of BA was 17.6 μM (Table 1). Bhagyalakshmi and Singh (1988) also reported about their study on ginger where average shoot count was reduced by lowering the Kin concentration.

The present study also showed that 2.46 μM IBA 17.6 μM BA + 0.94 μM Kin in M_3, M_4 and M_5 media (Table 1), found to be an effective auxin and cytokinin combination for multiplication of shoots (Table 2). When BA was reduced to 8.8 μM (Table 1) and used alone or with 2.46 μM IBA, the number of shoots was less (Table 2). This shows an appropriate combination of cytokinin and auxin gives better results as compared to auxin or cytokinin alone. Sharma and Singh (1995) also obtained a good combination of 4.44 μM BA and 0.27 μM IAA for shoot proliferation in ginger.

In addition to the chemical composition of the medium, its physical form can influence the growth pattern and the multiplication rate of cultures (Table 2). As far as physical condition of the media is concerned, liquid phase enhanced effec-

Table 1. Modified MS media showing details of growth regulators, organic adjuvant and antioxidant

Media code	MS strength	Growth regulators (μM)				Adenine sulfate (μM)	Ascorbic acid (g/l)	Activated charcoal (g/l)
		BA	IBA	IAA	Kin			
M_1	3/4	8.8	2.46	-	0.47	205	0.1	2.0
M_2	3/4	8.8	2.46	-	0.94	205	0.1	0.1
M_3	3/4	17.6	2.46	-	0.94	205	0.1	0.2
M_4	1/2	17.6	2.46	-	0.94	205	0.1	0.2
M_5	1/2	17.6	2.46	-	0.94	205	0.1	-
M_6	3/4	17.6	-	11.42	-	205	-	-
M_7	3/4	22.9	-	-	-	-	-	-

BA = benzylaminiopurine; IBA = indole butyric acid; IAA = indole-3-acetic acid; Kin = kinetin

Fig. 1. Multiple shoot formation on medium supplemented with 11.42 µM IAA and 17.6 µM BA after 8 weeks.

tivity of the media in case of shoot multiplication. Sharma and Singh (1995), reported that liquid medium being more effective than solid medium for *Zingiber officinale* and produced highest number of shoots. The data recorded during the present study for shoot multiplication in liquid phase was also promising as compared to that in solid phase (Table 2). In case of media M_1 and M_2, there was no significant shoot multiplication on solid (S) media. Whereas, significant difference of shoot number was observed in static liquid (SL) media (Table 2). In M_1 medium shoot number was more i.e. 8±1.08 as compared to that in M_2 i.e. 4±0.70. It was also noted that shoot number increased due to agitation factor. Average shoot count in M_1 and M_7 was more in SL phase. Thus, it was concluded that besides physical condition, influence of chemical constituents remains significant as ever. However, in all the media employed, the average shoot count was less in solid culture media. The findings of Bhagyalakshami and Singh (1988) were otherwise i.e. solid media being suitable for micropropagation of ginger. There was no significant difference in composition of M_4 and M_5 media. M_5 lacked activated charcoal too. It was observed that the shoots produced in M_4 were more shiny and glossy as compared to that in M_5 medium. This may be due to antioxidant activity of activated charcoal that adsorbed the phenolic discharge of the culture. Adenine sulphate, a vitamin known

Table 2. Effect of growth regulators, other addenda and physical condition of the media on shoot multiplication and shoot length of the *in vitro* ginger

Media code	Physical condition	Number of shoots per culture	Shoot length cm ± S.E
	SL	8.33 ± 1.08	13.00 ± 0.07
M_1	AL	6.66 ± 2.26	10.66 ± 0.81
	S	3.00 ± 1.41	9.33 ± 1.08
	SL	4.00 ± 0.70	8.66 ± 1.08
M_2	AL	5.00 ± 1.41	9.66 ± 1.47
	S	3.33 ± 0.40	6.33 ± 1.08
	SL	12.66 ± 1.47	12 ± 0.40
M_3	AL	14.00 ± 0.70	12.33 ± 0.40
	S	9.00 ± 1.22	11.66 ± 2.16
	SL	15.66 ± 1.08	10.33 ± 0.40
M_4	AL	18.00 ± 0.70	12.33 ± 1.77
	S	14.00 ± 2.54	10.00 ± 1.41
	SL	14.00 ± 4.94	11.33 ± 0.40
M_5	AL	19.66 ± 4.49	11.66 ± 0.40
	S	15.66 ± 1.08	12.33 ± 1.77
	SL	14.00 ± 4.35	12.00 ± 0.70
M_6	AL	17.66 ± 6.01	11.33 ± 1.08
	S	7.00 ± 0.70	9.66 ± 0.40
	SL	9.33 ± 0.40	12.66 ± 0.40
M_7	AL	8.33 ± 1.63	13.00 ± 0.70
	S	7.66 ± 1.47	11.00 ± 1.41

SL = static liquid; AL = agitated liquid; S = solid

to act as a cytokinin, was added to promote shoot formation. It is also reported to enhance embryogenesis (Halperin and Wetherell, 1964). Ascorbic acid was added as antioxidant.

There is slight variation in shoot length (Table 2). In M_1, M_2, M_6 and M_7, solid media shoot lengths are short, may be due to composition of media, as in M_5 medium the case is otherwise and in M_4, shoot length in solid medium is same as in agitated liquid (AL) medium. In some cases, the shoots produced on AL and SL media were vitrified and did not withstand survival on transference to soil. All the regenerated shoots had functional roots. But in M_4 and M_5 media, root growth was vigorous. This may be due to low salt concentration. In M_7 media, roots were less as compared to those in the rest of the media. The previous studies conveyed that roots were produced irrespective of the presence of BA in culture media but roots were of the root hair type (Malamug *et al.*,

1991). The meristem-derived ginger plants showed no variation in phenotype and productivity an essential requirement in micropropagation.

The present work confirmed that *in vitro* propagation of ginger, can be effectively geared to multiple folds in AL media containing an appropriate combination of cytokinin and auxin; a reduced level of sucrose as a carbon source and an adequate application of antioxidants. This efficient method of micropropagation may provide opportunities for the production of rhizomes as vegetative seed source under controlled conditions.

References

Bhagyalakshmi, Singh, N.S. 1988. Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. *J. Hortic. Sci.* **63:** 321-327.

Dohroo, N.P. 1989. Seed transmission of pre-emergence rot and yellows in ginger. *Plant Dis. Res.* **4:** 73-74.

Halperin, W., Wetherell, D.F. 1964. Adventive embryony in tissue culture of wild carrot, *Daucus carota*. *Am. J. Bot.* **51:** 274-283.

Hoagland, D.R., Arnon, D.I. 1950. The water-culture method for growing plants without soil. *Coulombia Agriculture Experimental Station Circular* **347:** 1-32.

Hoque, M.I., Perveen, S., Sarker, R.H. 1999. *In vitro* propagation of ginger (*Zingiber officinale* Rosc.). *Plant Tissue Cult.* **9:** 45-51.

Hosoki, T., Sagawa, Y. 1977. Clonal propagation of ginger (*Zingiber officinale* Rosc.) through tissue culture. *Hortic. Sci.* **12:** 451-452.

Ikeda, L.R., Tanabe, M.J. 1989. *In vitro* subculture applications for ginger. *HortScience* **24:** 142-143.

Inden, H., Asahira, T., Hirano, A., 1988. Micropropagation of ginger. *Acta Hortic.* **230:** 177-184.

Malamug, J.J.F., Inden, H., Asahira, T. 1991. Plantlet regeneration and propagation from ginger callus. *Sci. Hort.* **48:** 89-97.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* **15:** 473-497.

Nadgauda, R.S., Kulkarni, D.D., Mascarenhas, A.F., Jagannathan, V. 1980. Development of plantlets from cultured tissues of ginger (*Zingiber officinale* Rosc.). In: *Proc. Natl. Sym. Plant Tiss. Cult., Genetic Manipulation and Somatic Hybridization of Plant Cells*, P. S. Rao, M. R. Heble, M.S. Chadha (eds.), pp. 358-368, BARC, Bombay, India.

Noguchi, Y., Yamakawa, O. 1988a. Rapid clonal propagation of ginger (*Zingiber officinale* Rosc.) by roller tube culture. *Jpn. J. Breed.* **38:** 437-442.

Noguchi, Y., Yamakawa, O. 1988b. Formation of multiple bud clump and somatic embryo of ginger (*Zingiber officinale* Rosc.). *Jpn. J. Breed.* **38:** 80-81.

Pillai, S.K., Kumar, K.B. 1982. Note on the clonal multiplication of ginger *in vitro*. *Indian J. Agric. Sci.* **52:** 397-399.

Prathanturarug, S., Angusmalec, D., Pongsiri, N., Suwacharangoon, S., JenjiHikul, T. 2004. *In vitro* propagation of *Zingiber petiolatum* (Holttum) I. Theilade, a rare Zingiberceous plant from Thailand. *In vitro Cell. Dev. Biol. Plant* **40:** 317-320.

Rout, G.R., Palai, S.K., Samantaray, S., Das, P. 1995. Metabolic changes during *in vtiro* multiplication of *Curcuma longa* L. *Acta Bot. Hung.* **39:** 383-392.

Sakamura, F., Ogihara, K., Suga, T., Taniguchi, K., Tanaka, R. 1986. Volatile constituents of *Zingiber officinale* rhizomes produced by *in vitro* shoot tip culture. *Phytochemistry* **25:** 1333-1335.

Sato, M., Kuroyanagi, M., Ueno, M., Shimomura, K., Satake, M. 1987. Plant tissue culture of Zingiberaceae (I) *In vitro* propagation of ginger (*Zingiber officinale* Rosc.). *Plant Tiss. Cult. Letters* **4:** 82-85.

Sharma, T.R., Singh, B.M. 1997. High-frequency *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. *Plant Cell Reports* **17:** 68-72.

Sharma, T.R., Singh, B.M. 1995. *In vitro* microrhizome production in *Zingiber officinale* Rosc. *Plant Cell Reports* **15:** 274-277.