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- **Short communication**

In vitro clonal propagation of BARI Ada-1 (*Zingiber officinale* Rosc.)

S. Yesmin*, A. Hashem, M. M. Khatun, S. Nasrin, T. Tanny and M. S. Islam[1]
National Institute of Biotechnology, Ganakbari, Savar, Dhaka-1349, Bangladesh

Ginger (*Zingiber officinale* Rosc.), a herbaceous rhizomatous perennial herb belonging to the family Zingiberaceae is commercially grown in many tropical regions and is native to tropical South East Asia (Sathyagowri & Seran, 2011). It is commonly used all over the world as spice and medicine and is a common ingredient for various food and beverages. It has a long history of being used as medicine in Asian, Indian and Arabic herbal traditions. Ginger has been used to help treatment of arthritis, colic, diarrhea and heart conditions. It has been used to help treatment of the common cold, flu-like symptoms, headaches and painful menstrual periods (http://www.whfoods.com). Ginger is one of the most important spice crops in Bangladesh. Bangladesh is now seventh largest producer and third largest consumer of ginger. Bangladesh produces about 72084 metric tons of ginger in an area of 8407 hectare of total land in the year 2012 (FAO, 2012). In Bangladesh the productivity and quality of this crop are low mainly as it suffers from a large number of bacterial, viral, fungal and nematode allied diseases.

Breeding of ginger is hampered due to poor flowering and seed setting, therefore it is propagated vegetatively through rhizome (Kambaska & Santilata, 2009). Vegetative propagation of ginger has the high risk of spreading systemic infections. Ginger cultivation is threatened by rhizome rot diseases caused by *Pseudomonas solanacearum* and *Phythium* sp. These are spread through infected seed rhizome.

So, rapid multiplication of diseases free large scale propagules are needed. It is possible mainly through *in vitro* culture. It is estimated that 3 fold increases in the production of rhizomes could be possible by effective control of diseases and pests, (Hosoki & Sagawa, 1977). Therefore, an efficient *in vitro* regeneration protocol considers the best alternative (Hamirah *et al*., 2010).

The present investigations were carried out using rhizome bud with a view to develop a suitable protocol for micropropagation and acclimatization in order to aid large scale multiplication with better survival rate of locally grown BARI ada-1 variety.

Local variety of ginger namely, BARI ada-1 was used in this study. Healthy rhizomes were collected from field grown plant and kept in sands for sprouting. After 45 days these

[1] Bangladesh Atomic Energy Commission, E-12/A, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
*Corresponding author: sabinanib79@gmail.com

stored rhizomes were sprouted and rhizome buds were used as explants (Fig. 1). Rhizome buds about 1 to 2 cm long were collected and washed thoroughly under running tap water for 30 minutes to remove dust particles and rinsed with mild detergent. Then the buds were immersed in 0.5% bavistin solution for 10 minutes and washed four to five times with distilled water. The explants were then transferred to the laminar air flow cabinet and disinfected with 0.1% mercuric chloride (HgCl2) for 20 minutes and washed four times with sterilized distilled water. The sterilized rhizome buds were then aseptically trimmed to about 1.0 cm long and were inoculated on MS (Murashige & Skoog, 1962) medium supplemented with different concentrations and combinations of BAP, Kn and NAA. For root induction, well developed shoots were separated and transferred to half and full strength MS media supplemented with different concentrations (0.1, 0.2 and 0.3 mg/l) of IBA and NAA. All the media contain 3% sucrose and 0.8% agar as solidifying agent. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 16 hrs light/8 hrs dark photoperiod (cool, white florescent light) and 25±2º C temperature. The cultures were maintained by the regular subculture on fresh medium after two-three weeks interval.

Table 1. Effects of different concentrations and combinations of BAP, Kn and NAA on shoot induction and multiplication from rhizome buds of *Gingiber officinale* Rosc. (Data were recorded after eight weeks of culture)

Growth regulators (mg/l)		Explants inducing shoots (%)	No. of shoot bud induced after 4 weeks	No. of shoots/explants after 8 weeks	shoot length (cm)
		Mean	Mean ± SE	Mean ± SE	Mean ± SE
Basal MS (Control)		0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
BAP	NAA				
0.5		46.66	1.47 ± 0.19	3.47 ± 0.35	3.0 ± 0.30
1.0		56.66	2.0 ± 0.19	5.6 ± 0.42	3.61 ± 0.27
2.0		75.00	2.8 ± 0.26	7.93 ± 0.58	4.31 ± 0.29
3.0		**83.33**	**4.07 ± 0.30**	**14.53 ± 0.79**	**5.24± 0.31**
4.0		60.00	2.40 ± 0.25	6.73 ± 0.45	4.18 ± 0.26
5.0		43.33	1.87 ± 0.21	4.53 ± 0.30	3.63 ± 0.23
2.0	0.3	66.66	3.4 ± 0.28	7.67 ± 0.58	4.48 ± 0.20
3.0	0.3	76.66	4.12 ±0.29	12.6 ± 0.72	5.02 ± 0.18
4.0	0.3	56.66	2.5 ± 0.32	6.55 ± 0.33	3.34 ± 0.13
2.0	0.5	70.00	3.45 ± 0.31	9.36 ± 0.91	4.72 ± 0.20
3.0	**0.5**	**90.00**	**5.85 ± 0.27**	**25.78 ± 0.73**	**6.19 ± 0.30**
4.0	0.5	60.00	3.11 ± 0.26	7.37 ± 0.41	4.20 ± 0.18

Results are mean ± SE of 30 replications.

Different experiments were carried out to find out optimum culture conditions for maximum shoot induction and multiplication from rhizome bud explants. When explant were cultured on MS medium without any growth regulators, they are failed to produce

shoots and became brown in color. Highest percentage (83.33%) of explants were responsive and maximum number (4.07 ± 0.30) of shoot bud initiation was recorded on MS medium supplemented with 3.0 mg/l BAP (Table 1, Fig. 2) alone. The explants with small shoots and shoot buds were subcultured to fresh media of same composition for shoot multiplication. The maximum number (14.53 ± 0.79) of shoots per explants and highest shoot length (5.24± 0.31 cm) were found in MS medium supplemented with 3.0 mg/l BAP after 8 weeks of culture initiation (Fig. 3).

Table 2. Effects of IBA and NAA on *in vitro* root induction in regenerated shoots of *Gingiber officinale* Rosc. on half and full strength MS. (Data were documented after four weeks of culture)

MS strength	Growth regulators (mg/l)		Rooted shoots (%)	Days required for rooting	No. of roots/ shoot	Length of roots (cm)
Basal MS ½ (Control)			0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
½ MS	IBA	NAA				
½ MS	0.1		93	20 -22	6.60 ± 0.48	4.25 ± 0.22
½ MS	**0.2**		**98**	**18-20**	**7.40 ± 0.50**	**5.36 ± 0.30**
½ MS	0.3		92	20-22	5.50 ± 0.40	3.60 ± 0.21
½ MS		0.1	94	20	4.70 ± 0.42	2.92 ± 0.11
½ MS		0.2	97	17-18	5.50 ± 0.34	3.96 ± 0.17
½ MS		0.3	91	21-22	4.40 ± 0.31	3.13 ± 0.15
Basal MS (Control)			0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00

Results are mean ± SE of 30 replications.

Among the different concentrations and combinations of cytokinins and auxin, MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA was found to be most suitable for clonal multiplication of ginger variety bari ada-1(Table 1). The highest percentage of responsive explants (90.00%) the maximum number (5.85 ± 0.27) of shoot bud induction/explants, maximum number of shoot multiplication (25.78 ± 0.73) and the highest shoot length (6.19 ± 0.30 cm) were found in MS + 3.0 mg/l BAP + 0.5 mg/l NAA. Sathyagowri & Seran (2011) and Inden *et al*. (1988) reported 5.0 mg/l BAP and 0.5 mg/l NAA was the best for shoot multiplication in other ginger variety.

In most of the cases, regenerated plantlets developed roots spontaneously in same regeneration medium from shoots cluster. But they were found to be inadequate for transplantation and therefore, adequate root induction is necessary. The best root induction and development was found in half strength MS medium containing 0.2 mg/l IBA. The highest percentage (98%) of root formation, maximum number of roots (7.40 ± 0.50) and highest length (5.36 ± 0.30) of roots were recorded in this medium within three weeks of inoculation (Table 2, Fig. 5). Similar result was also obtained by Hoque *et al*. (1999). After sufficient development of roots, plantlets were successfully transplanted into small plastic pots containing soil (Fig. 6). Following proper acclimatization plantlets were transferred to field and 85% plants survived. This regeneration protocol can be applicable for rapid and large scale micropropagation of ginger in the days to come.

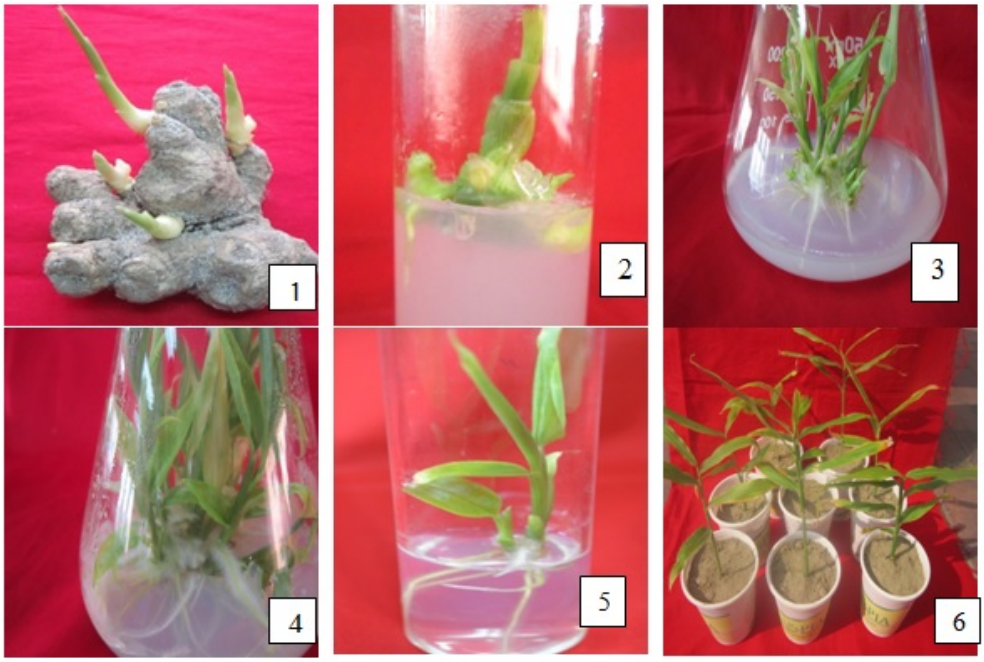

Figure 1-6. Different stages of plantlets development from rhizome buds of *Gingiber officinale* Rosc. 1. Sprouted rhizomes; 2. Initiation of shoot buds in MS medium supplemented with 3.0 mg/l BAP; 3. Shoot multiplication in MS + 3.0 mg/l BAP; 4. Multiplication of shoots in MS + 3.0 mg/l BAP + 0.5 NAA 5. *In vitro* rooting in ½ MS medium supplemented 0.2 mg/l IBA; 6. Acclimatization of *in vitro* plantlets in plastic pots

REFERENCES

Food and Agriculture Organization of the United Nations (FAO). 2012. FAOSTAT. http://faostat3.fao.org/home/index.html.

Hamirah, M. N., Sani, H. B., Boyce, P. C. and Sim, S. L. 2010. Micropropagation of red ginger (*Zingiber montanum* Koenig), a medicinal plant. *AsPac J. Mol. Biol. Biotechnol.* **18** (1): 127-130.

Hoque, M. I., Perveen, S. and Sarker, H. R. 1999. *In vitro* propagation of Ginger (*Zingiber officinale* Rosc.) *Plant tissue cult.* **9**: 45-51.

Hosoki, T. and Sagawa, Y. 1977, Clonal propagation of ginger (*Z. officianale* Rosc.) through tissue culture. *Hort. Sci.* **12**: 451-452.

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

Kambaska, K. B. and Santilata, S. 2009. Effect of plant growth regulator on micropropagation of ginger (*Zingiber officinale* Rosc.) cv-Suprava and Suruchi. *Journal of Agriculture Technology*. **5** (2): 271-280.

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

Sathyagowri, S. and Seran, T. H. 2011. *In vitro* plant regeneration of ginger (*Zingiber officinale* Rosc.) with emphasis on initial culture establishment. *Int. J. Med. Arom. Plants*, **1**(3): 195-202.

(http://www.whfoods.com). The World Healthy Foods.