Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences 3 (3): 306-309, 2004
ISSN 1682-3974

In vitro Regeneration of Plantlets of *Curcuma longa* Linn. A Valuable Spice Plant in Bangladesh

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Abstract: Sprouted rhizome buds were collected from the field grown one year old rhizomes and surface sterilized by treating them with 0.1% $HgCl_2$ for 14 min. The dissected rhizome bud explants (ca.1 cm) of turmeric were cultured in MS medium supplemented with different concentrations and combinations of cytokinin and auxin. The best result towards development of multiple shoot from the cultured explants was obtained when MS medium was supplemented with 2.0 mg l^{-1} of BA. Rooting experiments with half strength of MS medium and various concentrations of NAA, IBA and IAA revealed that 0.1-1.0 mg l^{-1} of any auxins was found to be effective for rooting. But root forming performance of IBA was proved to be the best among the three auxins tested. Rooted shoots (plantlets) were gradually acclimatized and successfully established in plastic pot containing garden soil under natural condition. About 70% of the transplanted plantlets survived and were eventually established in the field.

Key words: *In vitro*, regeneration, plantlets, *Curcuma longa*

INTRODUCTION

Turmeric (*Curcuma longa* Linn.) is a perennial rhizomatous herb. It is believed to be originated in India as wild turmeric and is found growing in the hilly regions of Western and Eastern Ghats. It is now cultivated throughout the Indo-Malayan realm as well as other tropical and subtropical parts of the world[1].

Turmeric is traditionally used mainly as spices and pigments but it is also an important medicinal plant whose fresh rhizome and dried powder are popular remedies for disorders of blood and various skin diseases. It is also externally used for pains and bruises and for beautification of fairsex. The rhizome juice is used as anthelmintic as well as in asthma, gonorrhea and urinary diseases. The essential oil of the plant is used as antacid, carminative, stomachic and tonic[1,2].

In conventional method, turmeric is propagated vegetatively by perennial rhizomes. Low productivity, disease susceptibility and higher cost of seed rhizome production are major constraints faced by the growers. In the recent decades micropropagation techniques are being profitably used to overcome such constraints in various vegetatively propagated crops as well as ornamental and horticultural plants[3]. Considering high demand and greeter economic and medicinal value of turmeric, it is necessary to develop a suitable protocol for mass production of disease free stocks through tissue culture technique. There are many reports on *in vitro* propagation of some rhizomatous plants like ginger[4-6] cardamom[7] and *Alphinia calcarata*[8]. The tissue culture protocols for turmeric have also been reported by very few workers[9,10]. However, in Bangladesh no previous work was done on the *in vitro* propagation of this plant.

In the present study an effort was made to establish a protocol for the *in vitro* propagation of turmeric from rhizome bud. This work is obviously a first step in the advancement of turmeric tissue culture in Bangladesh.

MATERIALS AND METHODS

Rhizome buds were collected from the sprouted rhizomes as well as field grown plants of turmeric, they were brought to the laboratory and processed for aseptic culture. For surface sterilization the rhizome buds were excised, cleaned thoroughly under continuous flushing of running tap water for 20 min and then washed with a solution of antiseptic [Savlon 5% (v/v)] for 10 min. The explants (rhizome buds) were then washed repeatedly with distilled water and finally treated with $HgCl_2$ (0.1%) for 14 min in a laminar flow cabinet and washed three times with autoclaved distilled water to remove any trace of $HgCl_2$.

After surface sterilization, rhizome buds were trimed to appropriate sizes and cultured on the MS medium for shoot multiplication. The basal medium used for all

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the experiments were Murashige and Skoog mineral formulation (MS) containing standard salts and vitamins, 30 g ml^{-1} sucrose and 7 g ml^{-1} agar. The media were variously supplemented with 6-benzyl adenine (BA) and Kinetin (Kn) either individually or in different combinations with auxins, α-naphthaleneacetic acid (NAA). The pH was adjusted to 5.7±0.1 before adding agar and the media were autoclaved at 1.1 kg cm^{-2} for 20 min at 121°C. Cultures were incubated at 25±1°C with a photoperiod of 16 h at 2000-3000 lux light intensity of cool white fluorescent light.

RESULTS AND DISCUSSION

Establishment of aseptic culture: The rhizome buds from the fresh sprouts and young plants of turmeric were delicate in nature and found to be very vulnerable to $HgCl_2$ treatment. However, surface sterilization with 0.1% $HgCl_2$ solution for 5 min resulted in contamination of about 50% explants. The cultured explants showed fungal contamination within five days of incubation. About 90% contamination free cultures were obtained when the explants were treated for 14 min with 0.1% $HgCl_2$. These explants remained green and also showed healthy growth and proliferation of axillary shoots from the basal nodes. The primary establishment of the aseptic cultures *in vitro* needed surface sterilization of the explants from the *ex vitro* grown plants. Among several chemicals, $HgCl_2$ is considered as a potent surface sterilizing agent; however, its residual inhibitory effect is also greater than the other sterilizing agents commonly used in plant tissue culture[3,11,12]. As rhizome surface of turmeric is rugged and covered with scaly leaves so use of a strong sterilant like $HgCl_2$ was essential. Many other reports on using $HgCl_2$ for surface sterilization of difficult explants from *ex vitro* grown plants support present study[13,14].

Table 1: Effects of different concentrations of BA alone and in combination with NAA on shoot proliferation from rhizome bud explants. There were 15 explants in each treatment and data (x̄±SE) were recorded after 6 weeks of culture

Growth regulators (mg l^{-1})	% of explant showing proliferation	No. of per shoot culture	Average length of shoot per culture
BA3			
0.5	65.0	7.8±0.92	4.0±0.38
1.0	75.0	8.2±0.42	5.5±0.98
2.0	100.0	14.5±1.82	6.2±0.04
3.0	75.0	12.0±1.22	4.0±0.95
4.0	70.0	11.2±1.10	3.3±0.62
5.0	60.0	10.3±1.01	3.8±0.62
Kn			
0.5	45.0	4.4±0.91	3.0±0.48
1.0	50.0	5.0±0.25	3.9±0.41
2.0	55.0	7.5±0.82	5.0±0.52
3.0	60.0	7.0±0.51	5.0±0.32
4.0	40.0	6.2±0.42	4.5±0.25
5.0	30.0	5.5±0.35	3.2±0.15
BA + NAA			
1.0 + 0.1	60.0	5.5±0.52	3.7±0.33
+ 0.2	67.0	6.3±0.32	4.7±0.42
+ 0.5	67.0	6.3±0.27	4.3±0.33
+ 1.0	73.0	6.3±0.42	5.3±0.52
2.0 + 0.1	80.0	6.6±0.33	5.2±0.42
+ 0.2	87.0	7.1±0.21	6.3±0.33
+ 0.5	73.0	5.3±0.34	5.3±0.25
+ 1.0	67.0	5.5±0.44	4.4±0.35
3.0 + 0.1	53.0	4.3±0.21	4.5±0.44
+ 0.2	60.0	4.2±0.25	4.5±0.35
+ 0.5	47.0	3.3±0.32	3.5±0.52
+ 1.0	40.0	2.5±0.33	3.0±0.22
Kn + NAA			
1.0 + 0.1	33.0	1.5±0.31	2.3±0.23
+ 0.2	40.0	3.4±0.15	2.8±0.22
+ 0.5	53.0	3.2±0.25	3.1±0.15
+ 1.0	47.0	2.3±0.23	2.5±0.16
2.0 + 0.1	53.0	3.2±0.21	3.5±0.22
+ 0.2	67.0	5.2±0.31	5.0±0.41
+ 0.5	60.0	4.2±0.41	4.0±0.42
+ 1.0	47.0	3.3±0.25	2.1±0.02
3.0 + 0.1	47.0	2.8±0.19	1.5±0.21
+ 0.2	53.0	3.4±0.25	2.1±0.12
+ 0.5	47.0	2.6±0.33	1.8±0.10
+ 1.0	40.0	4.1±0.15	2.7±0.22

Table 2: Effects of different concentrations of auxins on adventitious root formation from *in vitro* shoots. There were 18 shoots in each treatment and data (x̄±SE) were recorded after 6 weeks of culture

Conc. of auxin (mg l^{-1})	% of shoots rooted	No. of roots per rooted shoots	Average length of roots (cm)
NAA			
0.1	100.0	7.5±0.62	6.2±0.21
0.2	100.0	10.5±1.81	6.3±0.31
0.5	94.0	10.8±1.02	6.1±0.45
1.0	82.0	14.4±1.23	4.3±0.31
IBA			
0.1	100.0	5.6±0.31	5.3±0.28
0.2	100.0	15.4±1.23	6.3±0.92
0.5	100.0	10.5±0.99	5.2±0.51
1.0	82.0	7.8±0.25	4.5±0.61
IAA			
0.1	61.0	2.3±0.21	3.2±0.25
0.2	56.0	3.5±0.32	4.1±0.21
0.5	44.0	5.8±0.22	3.2±0.22
1.0	50.0	4.2±0.31	2.9±0.23

Proliferation of shoots: Different experiments were conducted with a view to finding out optimum culture condition for maximum shoot multiplication from cultured explants. Multiple shoots were found to develop from rhizome bud of both the sources when cultured on MS medium supplemented with BA alone or in combination with NAA. However, initiation of multiple shoots in most of treatments was observed within three weeks of culture (Fig. 1A). The best shoot proliferation from rhizome bud explants was observed in MS+2.0 mg l^{-1} BA (Fig. 1B-F). In this treatment, 100% of the cultured explants produced shoot where number of shoots per culture was 14.5±1.82 and average length of shoots per culture was 6.2±0.04 cm (Table 1). Almost similar result was obtained

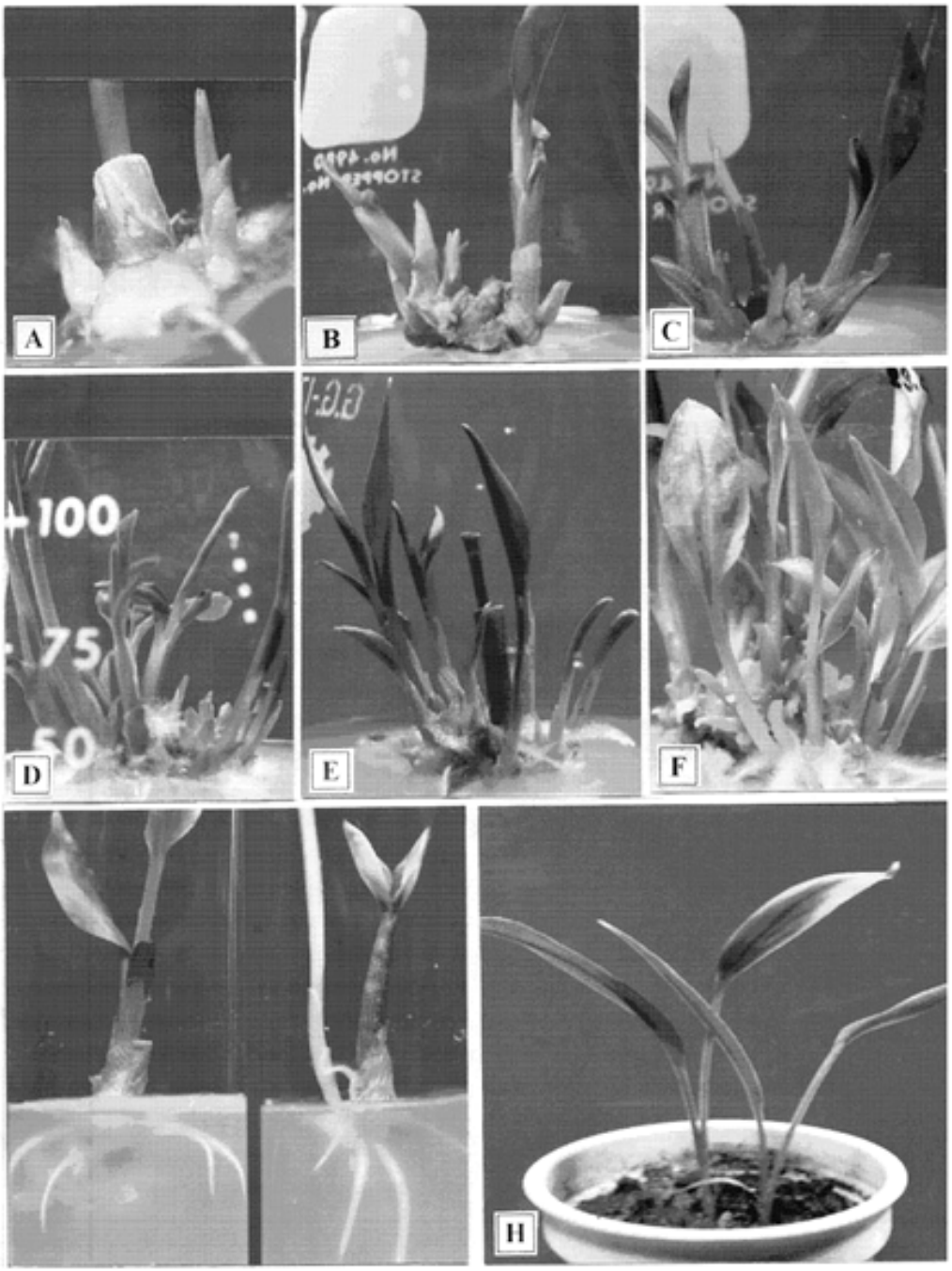

Fig. 1: A-H: Multiple shoot regeneration from rhizome bud explant of turmeric through tissue culture technique
A: Shoot buds initiation from rhizome bud explant on MS containing 2.0 mg l^{-1} BA after two weeks in culture
B and C: Development of multiple shoot buds on same medium after four and five weeks in culture, respectively
D: Development and elongation of multiple shoots on same medium after seven weeks in culture, respectively
E: Elongation of shoots on same medium after eight weeks in culture
F: Elongated multiple shoots on same medium after ten weeks in culture
G: Roots development in turmeric on ½ MS medium 0.2 mg l^{-1} IBA after six weeks in culture
H: Regenerated plantlet of turmeric transplanted in plastic pot under *ex vitro* condition

by Balachandran *et al.*[9], Hoque *et al.*[6] and Keshavachandaran and Khader[15].

The effect of BA and NAA on shoot initiation and multiplication from the cultured rhizome bud explants on MS medium was also studied in the present investigation. Different combinations of BA (1.0-3.0 mg l^{-1}) and NAA (0.1-1.0 mg l^{-1}) were used for this experiment. Best response was observed in 2.0 BA+0.2 mg l^{-1} NAA supplemented MS medium and almost 87% of the incubated explants showed shoot initiation. The present finding is in agreement with that of Inden *et al.*[16] who obtained good response for multiple shoot regeneration of ginger on MS medium containing BA with NAA.

Rooting of the proliferated shoots: Root formation was induced in the *in vitro* proliferated shoots by culturing them on half strength of MS medium with 0.1-1.0 mg l^{-1} either of NAA, IBA or IAA. Among the three types of auxin used in the present experiment, IBA was found to be most effective at different concentrations tested for producing roots on base of microshoots (Fig. 1G). Among the various concentration of IBA, 0.2 mg l^{-1} IBA was found to be the best concentration of auxin for proper rooting in which 100% shoots rooted within six weeks of culture (Table 2). The findings are in agreement with those observed in other rhizomatous plant species such as ginger[6], *Alpinia calcarata* [8]. *In vitro* regenerated plantlets those were transferred onto the soil, about 70% of them could tolerate transplantation shock and survived under *ex vitro* environment (Fig. 1H). Rest of the transplants could not survive either due to desiccation or microbial over growth that caused necrosis.

REFERENCES

1. Ghani, A., 1998. Medicinal Plants of Bangladesh: Chemical Constituents and uses. Principal Sources of Information. In: Medicinal Plants of Bangladesh. General Secretary, Asiatic Society of Bangladesh, Ramna Dhaka, Bangladesh, pp: 150.
2. Kirtikar, K.R. and B.D. Basu, 1996. *Curcuma* L. In: Indian Madicinal Plants. E. Blatter, J.P. Caius and K.S. Mhaskar (Eds.). Lalit Mohan Basu, Allahabad, India, 4: 2417-2426.
3. Torres, K.C., 1988. Tissue techniques for Horticulture Crops. Van Nostrand, Reinhold, New York.
4. Kackar, A., S.R. Bhat, K.P.S. Chandel and S.K. Malik, 1993. Plant regeneration via somaic embryogenesis in ginger. Plant Cell Tissue and Organ Cult., 32: 289-292.
5. Malamung, J.J.F., H. Inden and T. Asahira, 1991. Plantlet regeneration and propagation from ginger callus. Scientia. Hort., 48: 89-97.
6. Haque, M.I., S. Perveen and R.H. Sarker, 1999. *In vitro* propagation of ginger (*Zingiber officinale* Rosc.). Plant Tissue Cult., 9: 44-51.
7. Parvin, S., M. Hossain, M.A. Bari, S. Huda and M.S. Islam, 1999. *In vitro* Plant regeneration in cardamom (*Elettaria cardimomum* M.). 3rd Intl. Plant Tissue Cult. Conf. Dhaka (Mar. 8-10), Bangladesh, pp: 47.
8. Amin, M.N., M.A. Islam and M.A.K. Azad, 2001. Micropropagation and conservation of a threatened aromatic medicinal plant-*Alphinia calcarata* Rosc. 4 th Intl. Plant Tissue Cult conf. Dhaka (Nov. 1-3), Bangladesh, pp: 55.
9. Balachandran, S.M., S.R. Bhat and K.P.S. Chandel, 1990. *In vitro* clonal multiplication of turmeric (*Curcuma* sp.) and ginger (*Zingiber officinale* Rose.). Plant Cell Rep., 8: 521-524.
10. Nadgauda, R.S., A.F. Mascarenhas, R.R. Hendre and V. Jagannathan, 1978. Rapid multiplication of turmeric (*Curcuma longa* Linn.) plants by tissue culture. Indian J. Expt. Biol., 16: 120-122.
11. Bhojawani, S.S. and M.K. Razdan, 1983. Plant Tissue Culture: Theory and Practice. Developments in crop Sci. 5. Elsevier Sci. Publ., Amsterdam, The Netherlands.
12. Razdan, M.K., 1993. An Introduction to Plant Tissue Culture. Oxford and IBH Publ. Co. Pvt. Ltd., New Delhi, India.
13. Bennett, I.J. and McComb, 1982. Propagation of Jarrah (*Eucalyptus marginata*) by organ and tissue culture. Aust. For. Res., 12: 121-127.
14. Roy, S.K., L. Rahman and P.C. Datta, 1987. Propagation of Mitragyna parviflora Korth. Plant, Cell Tissue and Organ Cult., 12: 75-78.
15. Keshavachandaran, R. and M.A. Khader, 1989. Tissue culture propagation of turmeric. South Indian Hort., 37: 101-102.
16. Inden, H., A. Hirano and T. Asahira, 1988. Micropropagation of ginger. Acta Hort., 230: 177-184.