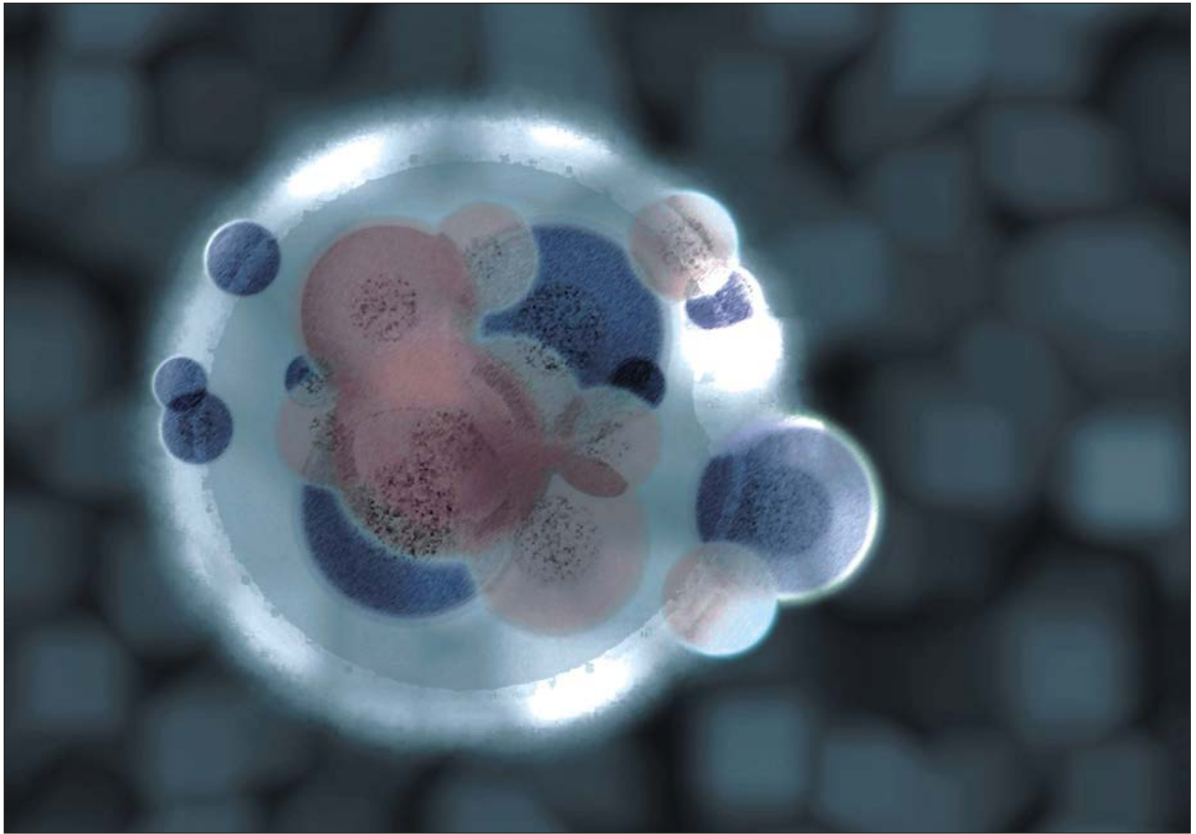

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Micropropagation of Turmeric (*Curcuma longa* Linn.) through *in vitro* Rhizome Bud Culture

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Abstract: An ideal micropropagation method of turmeric (*Curcuma longa* Linn.) has been developed using rhizome bud explants. Woody Plant Medium supplemented with different concentrations of BAP alone or in combination with different concentrations of NAA produced varying degree of multiple shoots. A supplementation of 4.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA gave the best result. In this case, 95% of the inoculated explants induced multiple shoots within 8-10 days of inoculation and the average number of shoots per explant was 6.70. Rooting was spontaneous in almost all the treatments. Most of the regenerated shoots were successfully transferred to soil under field conditions.

Key words: *Curcuma longa*, rhizome bud, micropropagation

INTRODUCTION

Turmeric (*Curcuma longa* Linn.) belongs to the monocot family Zingiberaceae and is an important medicinal plant as well as a spice of Bangladesh. It's pharmaceutical name is Rhizoma Curcumae. Turmeric is a necessary ingredient of curry powder. It is used extensively in Indian dishes and in Southeast Asian cooking.

Turmeric has distinctive medicinal properties and is found in most pharmacopoeias. Turmeric has been used for centuries in the Indian traditional system of medicine, the Ayurvedics. The rhizome is bitter; carminative, maturant, diuretic; useful in liver problem, urinary discharges, scabies, bruises[1]. It improves the complexion and is useful in diseases of blood, leucoderma, scabies, inflammations[2,3] ozoena, bad taste in the mouth, biliocusness, dyspepsia[4], ulcer[5,6] eliphantiasis, snake-bite, smallpox, swellings, boils, sprains[1].

Turmeric is exclusively vegetatively propagated crop using rhizomes. Flowering of turmeric is very rare. Even when it flowers, hardly any seed is produced. For this reason, turmeric cultivators are to use at least one healthy bud containing rhizome pieces as seed. Because of this, it is necessary to retain 20-25% of annual production for raising the following season crop. Moreover, it's rhizome multiplication is very low. In a growing season (8-10 months), only 10-15 lateral buds can be produced. Preservation of rhizome seeds is a hard job. It requires much attention, time and space. Besides these, they are prone to damages due to different factors such as adverse environment, insect and pathogen attack etc. Low productivity, disease susceptibility and higher cost of production are major problems faced by turmeric growers. In recent years, tissue culture techniques are being profitably used to overcome such problems in various crops as well as ornamental and horticultural plants. Rapid clonal multiplication of ginger, a related species, by shoot tip culture was reported by many workers[7-11]. The present study was undertaken to explore similar possibilities in turmeric.

Although there have been only a few reports on micropropagation of turmeric in the past, in recent years, tissue culture scientists around the globe[12,13] are using powerful *in vitro* techniques for large scale propagation of turmeric plant. But so far, to our knowledge, no such report on regeneration protocol of turmeric has been reported from Bangladesh.

MATERIALS AND METHODS

Explants were taken from unexpended leaves of sprouting rhizomes (rhizome bud) of an elite clone of turmeric grown in Rajshahi region of Bangladesh. At first, the rhizome buds were cut apart from rhizomes keeping the leaf sheath intact with a little portion of rhizome tissue. The unnecessary parts like roots and other residuals were removed from the rhizome buds. Then the explants were washed thoroughly under running tap water for 2 h to remove loose contaminants and taken in conical flask containing distilled water with few drops of Tween 80 (wetting agent) and washed further by vigorous shaking for about 7 min. This was followed by washing five times with distilled water. Finally, inside the laminar air-flow cabinet, surface disinfection was done by treating the explants with 0.2% aqueous solution of $HgCl_2$ for 10 min. Then the materials were washed 5 times with

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sterile distilled water immediately to remove all traces of $HgCl_2$.

After surface sterilization, the explants were prepared under laminar air-flow cabinet by removing the outer leaf sheathes. Then, small segments (1.0 cm) of rhizome buds with innermost leaf tissue were excised with a sterilized scalpel. Prepared explants were carefully inoculated in culture vessels containing sterilized agar gelled Woody Plant Medium[14] with different phytohormonal supplements. Data were taken after 30 days of culture.

Pyrex culture tubes (25×150 mm) having single explants were used for experimentation. The medium was solidified with TC agar from North Carolina Biological Supply Co., USA (5 g L^{-1}). pH of the medium was adjusted to 5.8 prior to autoclaving for 21 min at 120°C at 15 lbs psi pressure. Sugar was added at the concentration of 30 g L^{-1}. The cultures were kept in a growth chamber under 16 h light period at 27±1°C temperature.

RESULTS AND DISCUSSION

In the first set of experiments, rhizome buds were cultured on Woody Plant Media (WPM) supplemented with different concentrations of KIN alone or in combination with different concentrations of NAA. All these failed to produce multiple shoots. But when BAP was applied either singly or with NAA, different degrees of multiple shoot formation was noted.

Table 1: Effects of different concentrations of BAP and NAA in WPM on shoot multiplication from rhizome bud explants of turmeric. (Data recorded after 30 days of culture)

Hormonal concentrations (mg L^{-1}) BAP + NAA	No. of explants cultured	Mean No. of explants regenerated	Percentage of regeneration	No. of shoots/ explant	Days to shoot initiation	Root induction
0.5 + 0.0	20	11	55	1.000	20-25	+
1.0 + 0.0	20	14	70	1.000	20-25	+
1.5 + 0.0	20	15	75	1.000	15-20	+ +
2.0 + 0.0	20	15	75	1.500	10-15	+ +
2.5 + 0.0	20	1	80	1.375	10-15	+ +
3.0 + 0.0	20	17	85	3.420	10-15	+ +
3.5 + 0.0	20	18	90	4.230	8-12	+ +
4.0 + 0.0	20	19	95	6.250	8-12	+ +
5.0 + 0.0	20	17	85	4.150	10-15	+ +
6.0 + 0.0	20	16	80	3.570	10-15	+ +
2.0 + 0.1	20	14	70	1.143	9-12	+
2.0 + 0.2	20	15	75	1.145	12-15	+
2.0 + 0.3	20	15	75	1.200	12-15	++
2.0 + 1.0	20	16	80	1.250	8-12	+++
3.0 + 0.1	20	15	75	2.900	10-15	+
3.0 + 0.2	20	14	70	2.950	9-13	+
3.0 + 0.3	20	16	80	2.800	9-12	+
3.0 + 1.0	20	17	85	3.150	8-12	+++
4.0 + 0.1	20	16	80	5.500	10-15	++
4.0 + 0.2	20	18	90	5.550	9-13	++
4.0 + 0.3	20	17	85	5.900	9-12	++
4.0 + 1.0	20	19	95	6.700	8-10	+++

+ Few, ++Moderate, +++Profuse

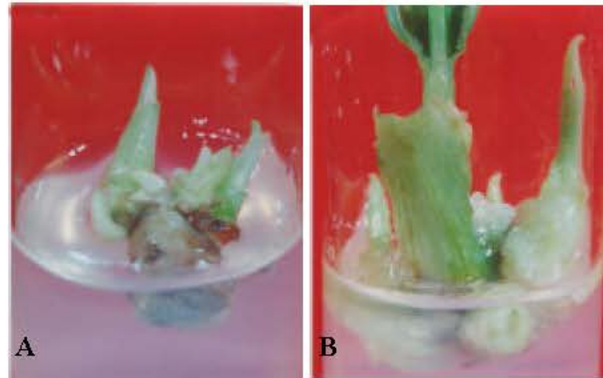

Fig. 1: A) Regeneration of multiple shoots in WPM + 4.0 mg L^{-1} BAP
B) Multiple shoot formation in WPM + 4.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA

It is evident from Table 1 that among all these treatments, best response was observed in 4.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA supplemented WPM (Fig. 1B). In this combination, almost 95% of the inoculated explants showed regeneration within 8-10 days of inoculation and the average number of shoots per explant was 6.70. The second highest response was observed in WPM + 4.0 mg L^{-1} BAP (Fig. 1A). In this supplement, 95% of the inoculated explants showed regeneration within 8-12 days of inoculation and in average, about 6.25 shoot buds were regenerated from each explant. Satisfactory root development was observed in all the hormonal supplements.

The ability of BAP to induce auxiliary branching is well documented[15]. In general, herbaceous plants are highly responsive to BAP treatments and most cultured herbaceous species produce robust, well-formed shoots suitable for further shoot proliferation[16]. Fanizza and Uicciardi[17] reported that high BAP (2.0 mg L^{-1}) produced multiple shoot from *Vitis vinifera*. Chowdhury[18] reported the effect of BAP on shoot proliferation of Indian rose and he noted that 2.5 mg L^{-1} BAP was optimum for shoot production. The positive effect of BAP on multiple shoot regeneration was also observed in some other fruit plants[19,20].

In ginger (*Zingiber officinale* Rosc.), a related species of turmeric, role of BAP in shoot multiplication was reported[10,12,21]. Malamug *et al.*[22] was able to regenerate multiple shoots from callus derived shoots of ginger using BAP and NAA in modified MS medium. Several reports are also available where MS medium with different concentrations and combination of BAP and NAA were used for multiple shoot regeneration from different explants of ginger[10,23]. Sunitibala *et al.*[24] reported optimum clonal propagation of turmeric by

rhizome bud culture in MS media containing 2.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA. In consistence with these results, our present study also revealed the role of BAP (singly and also in combination with little amount of NAA) in shoot multiplication of *Curcuma longa* Linn. in WPM.

The regenerated shoots with spontaneously formed roots were successfully transferred to soil.

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