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In vitro multiplication of *Curcuma longa* Linn.–an important medicinal zingiber

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Abstract
Curcuma longa Linn. is a herbaceous perennial plant belonging to the family Zingiberaceae. *In vitro* protocol for the regeneration of plantlets from the sprouted rhizomes of turmeric was optimized. Murashige and Skoog media supplemented with different concentrations and combinations of cytokinins and varied percentage of sucrose were experimented. Murashige and Skoog media supplemented with benzyl amino benzene (BAP) at the concentration of 2mg/l and sucrose 3% showed the best regeneration in comparison to Kinetin when used singly. Combination of 2mg/l BAP and 3mg/l Kinetin resulted in highest number of shoots. The same media showed spontaneous rooting. Healthy regenerated plantlets were selected for hardening in sterile mixture of garden soil and sand in the ratio of 1:1. Ninety three percent of the micropropagated plantlets survived to maturity when transferred to soil.

Keywords: Benzyl amino purine, Cytokinin, *Curcuma longa*, Kinetin, micropropagated plantlets.

Turmeric (*Curcuma longa* Linn.) belongs to the family Zingiberaceae. It is a perennial rhizomatous herb supposed to have Indian origin (Ghani, 1998) and is used both as food and medicine. Also known as the golden spice as well as the spice of life, it is found growing well in the hilly areas of both the Western and Eastern Ghats. Currently it is cultivated in both the tropical and subtropical parts of the world (Ghani, 1998).

Turmeric is traditionally used mainly as spices, pigments and medicine. Turmeric has efficacy in both pharmacological as well as clinical studies used to cure different diseases like dyspepsia (Thamlikitkul *et al.* 1989), peptic ulcers (Prucksunand *et al.* 2001), and gastric ulcers (Muderji *et al.* 1981; Sakai *et al.* 1989; Rafatullah *et al.* 1990; Kositchaiwat *et al.* 1993; Masuda *et al.* 1993). The essential oil obtained from the plant can be used as antacid, carminative, stomachic and tonic (Ghani, 1998; Kirtikar *et al.*, 1996). The pharmacology of curcumin contained in turmeric was investigated and it proved that curcumin has anti-inflammatory activity, which returns the levels of serum glutamic oxaloacetic transaminase and serum glutamic transaminase to normal after its administration to inflamed rats (Srimal and Dhawan, 1973).

In recent years, tissue culture technique has been used for the large scale propagation of many plants. Micropropagation of turmeric is done conventionally by using recurrent rhizomes. Flowering of turmeric is very rare. Even when it flowers, hardly any seed is produced. So, at least one healthy bud containing rhizome pieces are used by turmeric cultivors as seed. Because of this reasons it is necessary to retain 20-30% of annual production for raising the following season crop. Moreover, its rhizome multiplication is very low. Besides these, they are prone to damages due to different factors such as adverse environment, insect and pathogen attack etc. Due to the low yield, vulnerability and higher price of seed, there are major constraints faced by the growers for the production of rhizomes. Now a days, micropropagation techniques have been used advantageously to conquer such problems in many vegetatively propagated crops as well as decorative and horticultural plants.

Because of high demand and greater economic importance along with its medicinal importance, it is essential to develop a suitable protocol for mass production of disease resistant plants through tissue culture technique. The suitable protocols for in vitro regeneration of plantlets of turmeric have also been reported (Balachandran *et al.*, 1990; Nadagauda *et al.*, 1978).

In the present paper an attempt was made to set up a protocol for the *in vitro* propagation of *Curcuma longa* to get disease free plantlets.

Materials and Methods

Sprouted rhizomes were collected from the field grown plants of turmeric (*Curcuma longa* Linn.). They were then brought to the laboratory and processed for aseptic culture. They were washed carefully under running tap water to remove the soil. Buds which emerged on rhizomes were excised with sharp blade and were surface sterilized by immersing in 1% extran for 10 min and then washed 5 times with double distilled water for 5 min each. After that, these were dipped in 0.1% mercuric chloride for 10 min, then again washed 5 times with sterile double distilled water to remove the traces of mercuric chloride under a laminar flow cabinet. Next, they were treated with 70% ethanol for 1 min and

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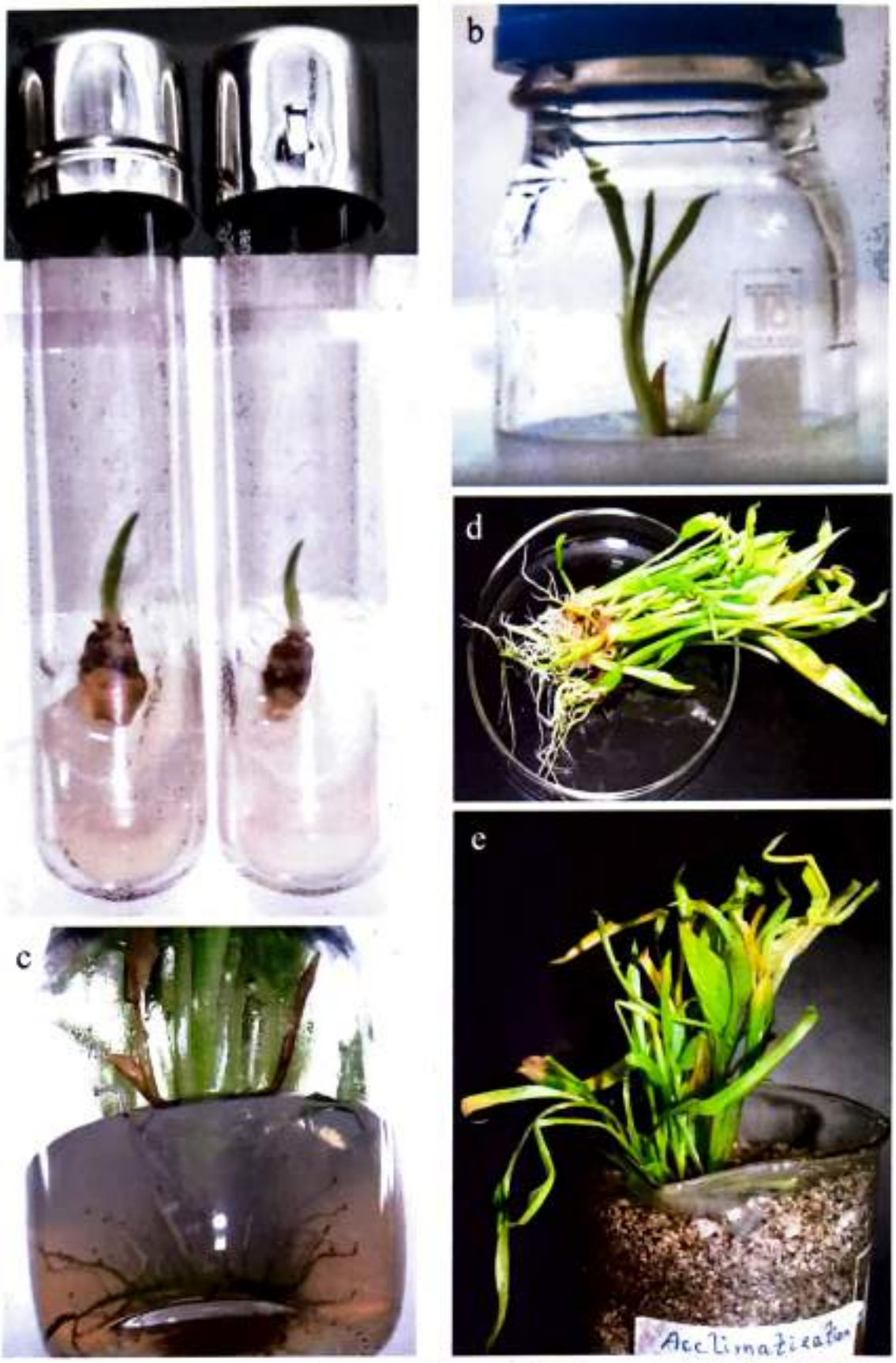

Fig1: Stages of micropropagation of *Curcuma longa* Linn. (a) Rhizomatous bud of *Curcuma* inoculated on MS media. (b) Formation of plantlets. (c) and (d) rooting from *in vitro* regenerated plantlets. (e) Transfer and hardening of *in vitro* grown plantlets in beaker containing soil and sand.

Table1: Effect of BAP and Kinetin on the regeneration of *Curcuma longa* Linn. in MS media with 3% sucrose when used alone.

Hormones	M S Medium with 3% sucrose	
(mg/l)	No. of Shoots	Height of Shoots (cm)
BAP		
1	1.93±0.34	1.10 ± 0.10
2	8.41 ± 0.33	5.82 ± 0.13
3	8.12 ± 0.41	6.10 ± 0.60
4	6.34 ± 0.49	6.63 ± 0.21
5	4.80 ± 0.10	4.93 ± 0.20
Kinetin		
1	1.93 ± 0.34	1.12 ± 0.11
2	2.97 ± 0.23	3.30 ± 0.20
3	3.87 ± 0.49	4.38 ± 0.50
4	5.47 ± 0.29	6.27 ± 0.42
5	4.67 ± 0.06	4.89 ± 0.14

finally, washed several times with sterile double distilled water for 10 min each.

After surface sterilization, rhizome buds were trimmed in 2-3 mm height and blotted dried on sterile blotting paper and inoculated on the Murashige and Skoog medium (Murashige and Skoog, 1962) for shoot and root development. Different concentrations and combinations of cytokinins like BAP (benzyl amino purine) and Kinetin were experimented in this study. To access the effect of different phytohormones sucrose was used as a carbon source at the rate of 30mg/l in the present experiment. Agar at the rate of 8mg/l was used as a solidifying agent in the culture media. The pH of the media was adjusted to 5.7 ± 0.1 with 0.1N NaOH or 1N HCl prior to the addition of agar, followed by autoclaving at 121 ° c for 20 min at 15 psi. The growth regulators were filtered sterilized and poured to the culture media. Then slants were prepared in a laminar flow cabinet. After 20 min the media became solidified in the test-tubes and inoculations of explants were done carefully. Cultures were incubated at 25 ° c with a photoperiod of 16 hrs at 2000-3000 lux light intensity of cool white fluorescent light.

From the *in vitro* regenerated plantlets, the healthy plantlets having good numbers of root were selected for hardening. The plantlets were removed from the media and were washed with double distilled water properly to get rid of the traces of agar sticking to the roots. The plantlets were then transplanted into beaker containing autoclaved mixture of soil and sand (1:1). Later they were transferred to the garden and planted in the field after a month.

Data related to height of the plants, number of shoots produced per plant were recorded.

Results and Discussions

The explants proliferated within 20-25 days after inoculation. But after few days of inoculation it was found that some of the explants were contaminated with fungi and bacteria. Such contaminated cultures were rejected and kept out from the culture room. Surface sterilization with 0.1% $HgCl_2$ solution for about 5 min resulted in fungal contamination of the cultured explants after few days of incubation. But when the explants were treated with 0.1% $HgCl_2$ for about 10 min more or less contamination free cultures were obtained. These explants remained green and healthy growth and proliferation of axillary shoots can be observed.

Explants grown on the basal medium did not produce shoots, roots or callus. To find out the optimum culture condition for maximum shoot and root proliferation from the cultured explants, different experiments were conducted with different hormonal concentrations.

Different stages of micropropagation of *Curcuma long* Linn (Fig. 1). MS media supplemented with 2mg/l BAP showed the maximum rate of shooting as well as rooting (Table 1).

Explants grown on MS media supplemented with different concentration of BAP showed some good results. It was found that sucrose concentration has a profound effect on the proliferation and growth of explants (Table 2). Maximum regeneration was noticed when explants were cultured on MS media supplemented with 2mg/l BAP and 3% sucrose. Extremely low regeneration was observed when the media was supplemented with 1% sucrose supplemented with 2mg/l BAP(2.1 shoot/ explant) and 3mg/l BAP (1.47 shoot/explant), where as MS media supplemented with 3% sucrose along with 2mg/l BAP(8.41 shoot/ explant) and 3mg/l(8.12 shoot/explant) had high regeneration rates. Thus it can be inferred from this that the concentration of sucrose in the media plays a vital role in regeneration of plantlets along with the phytohormones.

To observe the effects on regeneration different types of cytokinins like BAP and Kinetin were added to the media (Table 3). Media supplemented with BAP at a concentration of 2mg/l produced maximum number of plantlets (8.41), while maximum number of plantlets in case of Kinetin was observed at a concentration of 4mg/l (5.47). All the cultures resulted in moderate to spontaneous rooting. Rahman *et al.* 2004 also observed high effectiveness of BAP in turmeric tissue culture. Lower concentration of cytokinin in media led to less number of plantlets while it declined above a critical level. This may be inferred as high concentration of cytokinin in the media may produce some inhibitory effect and thus the rate of shooting is declined.

Different combinations and concentration of BAP and Kinetin were also tried to see the effect on regeneration. Media containing 2mg/l BAP and 3mg/l Kinetin showed the maximum number of plantlets/ explant (9.60) followed by 3mg/l BAP and 4mg/l Kinetin (8.53), where maximum shoot height (8.30) was obtained in media with 2mg/l BAP and 4mg/l Kinetin followed by 2mg/l BAP and 3mg/l Kinetin (7.60). Moderate to profuse rooting was observed in the most of the combinations. The number of plantlets regenerated and the rate of rooting was found to be proportional. Thus it can be concluded that combinations of BAP and Kinetin is more effective than when used singly.

In plant tissue culture, the ultimate success of *in vitro* propagated plantlets lies in its growth in the external

Table2: Effect of different concentration of sucrose (%) and BAP (mg/l) on the regeneration of *Curcuma longa* Linn. in MS media.

Concentration of Sucrose (%)	BAP (mg/l)	No. of Shoots
1	2	2.10
1	3	1.47
2	2	3.47
2	3	2.95
3	2	8.41
3	3	8.12

environmental conditions in pots. The in vitro propagated plantlets with well developed root and shoot system were successfully transferred to the beaker containing autoclaved mixture of soil and sand. After hardening the regenerated plantlets were transferred to the field where the plantlets showed 93% survival to maturity.

Thus based on these observations, we can conclude that this paper may prove useful to get huge number of desired cultivar within a very short span of time.

Table3: Effect of different concentrations of BAP and Kinetin on the regeneration of *Curcuma longa* Linn. in MS media with 3% sucrose.

BAP+Kinetin (mg/l)	MS Medium with 3% sucrose	
	No. of Shoots	Shoot Height (cm)
1+1	1.99±0.34	1.70±0.61
1+2	3.32±0.41	2.30±0.20
1+3	5.40±0.25	3.34±0.32
1+4	5.61±0.32	6.17±0.27
2+1	5.73±0.59	2.60±0.80
2+2	7.42±0.18	2.99±0.22
2+3	9.60±0.59	7.60±0.29
2+4	7.40±0.47	8.30±0.62
3+1	5.84±0.13	2.71±0.12
3+2	7.08±0.20	2.93±0.03
3+3	7.23±0.29	4.59±0.50
3+4	8.53±0.30	7.59±0.19

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