

In vitro propagation of tikhur (*Curcuma angustifolia* Roxb.): A starch yielding plant

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In vitro regeneration of *Curcuma angustifolia* Roxb. was achieved through shoot meristem culture. The shoot buds (2-3 cm long) from rhizome were inoculated on MS medium supplemented with 3.0 mg/L BAP for initiation and elongation of shoots. As a result, 1.87 ± 0.28 shoots per explant were produced. These shoots were transferred on MS medium supplemented with 3.0 mg/L BAP and 25 mg/L adenine sulfate for further shoot multiplication. About 6.9 ± 0.69 micro-shoots per explant were produced within 6 wk. The roots appeared from shoots on shoot establishment as well as multiplication media. The rooted plants were transferred to pots and acclimatized, which showed 83% survival with normal growth.

Keywords: *Curcuma angustifolia*, meristem culture, micropropagation, shoot bud

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Curcuma angustifolia Roxb. (Family: Zingiberaceae) produces edible rhizome rich in starch content¹. The rhizome is processed to obtain *tikhur*, which is sold in the market. *Tikhur* flour is cooked and consumed in many parts of India². The plant grows wild in its natural habitat and usually perpetuates through vegetative reproduction.

Overexploitation has made *tikhur* scarce in natural habitat and costly in the market. Cultivation of superior clones would greatly enhance its production and quality. In nature, propagation of *tikhur* occurs through rhizome, which is a slow process. Tissue culture techniques offer opportunity for fast multiplication of superior clones in relatively small space and time. Tissue culture of some *Curcuma* spp. has been reported earlier. *In vitro* plantlet regeneration has been achieved in *C. amada*³⁻⁵, *C. aromatica*⁶, *C. longa*⁷⁻¹¹, *C. zedoaria*¹² and *Curcuma* sp.^{13,14}. However, the reports are lacking on *in vitro*

propagation of *C. angustifolia*. Here, we report a protocol of this important species.

The rhizomes of *C. angustifolia* were collected from natural forests of Pithora, about 100 km from Raipur, India. After washing, the rhizomes were planted on sand beds of a greenhouse in the month of July. The shoot buds appeared within 15-20 d. The sprouted rhizomes collected from sand beds were washed thoroughly in running tap water to remove sand particles. The shoot buds, 2-3 cm long, were excised from rhizome under aseptic condition, sterilized in 0.2% mercuric chloride for 15 min and rinsed 3-4 times in sterilized water. The outer leaves were removed from shoot buds and shoot meristems with remaining inner leaves were used as explants. These shoot bud explants were inoculated on establishment medium of Murashige and Skoog¹⁵ (MS) medium supplemented with 0.0-5.0 mg/L BAP for initiation and elongation of shoots. After 6 wk, the elongated shoots along with original explants were placed on shoot multiplication medium of MS+3.0 mg/L BAP+0-100 mg/L adenine sulfate.

All the media adjusted at pH 5.7 were sterilized at pressure 1.05 kg/c m² for 25 min. After inoculation, the cultures were placed in tissue culture room at $25 \pm 2^\circ\text{C}$ under 16/8 h (light/dark) photoperiod with 1000 lux light intensity. Regenerated plants were acclimatized in the greenhouse at 12 h light/dark regime, 70% relative humidity and $25-30^\circ\text{C}$ temperature.

The optimum shoot bud initiation (80%), number of shoots (1.87 ± 0.28) and shoot elongation (48 ± 5.5) occurred in explants placed on MS medium supplemented with 3.0 mg/L BAP (Table 1). On this establishment medium, shoots also produced optimum number of leaves (2.7 ± 0.35 /shoot) having maximum width (18 ± 1.7 mm/leaf) and roots (5.5 ± 0.41 /shoot). However, further increase in BAP concentration of medium had adverse effects. Poor growth at higher concentration of BAP and Kn was also reported in *C. longa*⁷. The shoots that grew from explants on MS+3.0 mg/L BAP were further used for shoot multiplication.

The shoots placed on MS medium supplemented with 3.0 mg/L BAP and 25 mg/L adenine sulfate exhibited maximum shoot proliferation as well as root

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Table 1—Effect of BAP on explant establishment of *C. Augustifolia* Roxb.

MS medium +BAP (mg/L)	Shoot bud induction (%)	No. of leaf mean±SE	Max. leaf width (mm) mean±SE	No. of shoot mean ± SE	Shoot length (mm) mean ±SE	No. of root mean ± SE
0.0	60	1.73±0.31	12±1.4	0.82±0.18	30±3.7	6.3±0.52
3.0	80	2.75±0.35	18±1.7	1.87±0.28	48±5.5	5.5±0.41
5.0	50	2.3±0.4	13±1.8	1.27±0.21	35±4.0	4.1±0.38

Table 2—Effect of adenine sulfate on shoot multiplication of *C. Augustifolia* Roxb.

MS+ BAP (3.0 mg/L)+adenine sulphate (mg/L)	No. of leaf mean±SE	Leaf width mean±SE	No. of shoot mean±SE	Shoot length (mm) mean±SE	No. of root mean ± SE
0	3.78±0.32	14.1±0.26	1.98±0.26	37.67±3.23	9.11±0.98
25	16.5±2.13	14.7±1.29	6.9±0.69	32.8±1.25	16.10±1.50
50	6.2±0.77	11.7±0.80	2.6±0.36	29.8±2.08	6.8±0.99
100	5.89±0.42	11.78±1.26	2.44±0.24	26.11±1.48	4.67±0.55

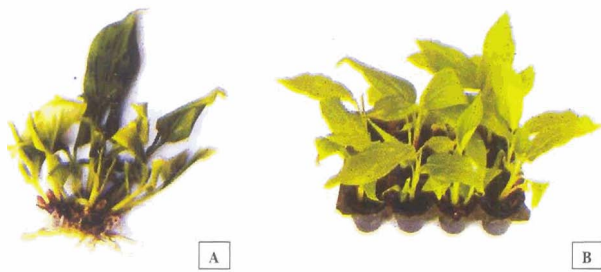


Fig. 1—Micropropagation of *C. angustifolia* through shoot tip culture: A. Shoot proliferation on MS medium containing 3 mg/L BAP and 25 mg/L adenine sulfate; B. Micropropagated plants in net pot placed in the greenhouse for acclimatization.

formation (Fig. 1A). On this medium, a single shoot produced 6.9 ± 0.69 micro-shoots of 32.8 mm length, having 16.5 ± 2.13 leaves/shoot and 16.10 ± 1.50 roots/shoot (Table 2). The shoot multiplication is considered as an important step for commercial exploitation of a micropropagation protocol. However, the optimum requirement at propagule proliferation stage differs from species to species. In *C. aromatica*, shoot multiplication was optimum at 5 mg/L BAP⁶; whereas for *C. zedoary*, 3 mg/L BAP was suitable for shoot multiplication¹². On the other hand, high multiplication in *C. longa* was observed when it was placed on liquid MS medium supplemented with $72.64 \mu\text{M}$ TDZ¹¹.

The root induction and elongation from shoots occurred on establishment as well as shoot multiplication media. Thus, only splitting of

individual shoot from the bunch produced complete plantlets. Earlier, spontaneous rooting was also reported in *C. longa*^{7,11} and *C. aromatica*⁶. The transfer of plantlets into liquid medium supported on filter paper platform was reported to ensure healthy root system⁷; however, this step was not included for *C. angustifolia*, as normal roots developed on solid medium. The *in vitro* regenerated plants were transferred to coco peat substrate in the net pots (M/s Swapnil Plastic, Nasik). The net pots were placed inside the greenhouse for acclimatization at 70% relative humidity and 25-30°C temperature. Total 80 plants were transferred to pots. After one month, 66 plants survived with normal growth and morphology (Fig. 1B). The results suggest that the present micropropagation protocol can be exploited for commercial production of *C. angustifolia* saplings.

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