

to polarization fluctuations connected with the ferroelectric phase transition at 100°C on heating. The dielectric resonance shows pronounced dispersion, likely due to a distribution of relaxation times.

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Study of organogenesis *in vitro* from callus tissue of *Flacourtia jangomas* (Lour.) Raeusch through scanning electron microscopy

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***In vitro* regeneration of shoot bud was obtained from nodal segment-derived callus tissue of *Flacourtia jangomas* (Lour.) Raeusch, a woody medicinal plant of dioecious habit. Induction of callus was obtained in MS basal medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l BAP. Highest number of shoot bud (7.4 ± 0.20) was noted in 2.0 mg/l BAP. Rhizogenesis was achieved in 1.0 mg/l NAA from both internode and leaf explant. Shoot bud organogenesis was observed through histological and SEM study. Under SEM, several clusters of shoot primordia together with trichomes of characteristic morphology as well as their structural details were noted.**

FLACOURTIA jangomas (Lour.) Raeusch (family Flacourtiaceae) is a woody, dioecious plant. It is found frequently in semi-wild conditions in the Brahmaputra valley and adjoining areas in the northeastern parts of India, and had probably migrated from Bangladesh and upper Myanmar. The plant is maintained in forests or orchards elsewhere. This plant has some medicinal as well as economic values. It is mainly cultivated for its edible fruit and hard wood. The fruits are either eaten raw or used for making jams and preserves^{1,2}. Different plant parts are also pharmaceutically used for the treatment of asthma, pre- and post-natal blood purification³, etc. The fruits are used in bilious conditions and in diarrhoea⁴. The plant contains tannin and a fixed oil⁵. Two limnoids, i.e. limolin and jangomolide have been obtained from its stem and bark⁶. However, no report is available on its *in vitro* organogenesis from callus tissue and its micromorphogenetic responses. Attempts have been made in this paper to investigate the organogenesis from callus tissue and the micromorphogenetic details of the differentiating structures through histological and scanning electron microscope (SEM) studies.

Young internodes (1.0 cm long; 0.1–0.15 cm in diameter; between 3rd and 4th node), nodal segments (0.5 cm long; 0.2 cm in diameter; 4th–5th node) and leaf segments (0.5–0.7 cm²; 3rd–4th young unfolded leaves) were taken as explants for callus induction. All these explants were collected from actively growing branches of 10–15-year-old *F. jangomas* trees in Ramana Forest, Golapbag, Burdwan. They were surface sterilized with HgCl₂ (0.1% w/v aqueous) for 5–10 min and washed

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thoroughly with sterilized distilled water. Then they were excised and transferred into callus induction medium. The basal medium was Murashige and Skoog's⁷ (MS) supplemented with 3% sucrose and 0.8% agar. The different plant growth regulators (PGRs) used were 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5–5.0 mg/l), naphthaleneacetic acid (NAA; 0.5–5.0 mg/l), benzylaminopurine (BAP; 0.5–2.0 mg/l) and 6-furfurylaminopurine (kinetin; 0.5–2.0 mg/l). The cultures were incubated at a temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $70 \pm 10\%$ under a 16 h photoperiod of about $40 \mu\text{E m}^{-2}\text{S}^{-2}$.

The calluses were subcultured into regeneration medium. NAA (0.1–5.0 mg/l), either singly or in combination with kinetin/BAP (0.2–5.0 mg/l), was used for caulogenesis. For rhizogenesis, different concentrations of NAA (0.1–3.0 mg/l) were used.

For SEM study, callus pieces with differentiated shoot buds and roots were collected at various developmental stages and first rinsed in 0.25 M phosphate buffer (pH 7.2), and fixed in 2% glutaraldehyde in the same buffer for 2 h at 4°C . After dehydration in a graded series of ethanol and finally with amyl acetate, 'critical point drying' was done and the callus pieces were coated with gold sputtering (in IB₂ ion-sputter-coated chamber) and observed under SEM (Hitachi, S-530).

For histological analysis, callus pieces with regenerated shoot buds were fixed in FAA solution (formalin:glacialacetic acid:70% alcohol in the ratio of 5:5:90). Standard methods of paraffin embedding and staining were followed for histological studies⁸. Sections ($10 \mu\text{m}$) were cut by a rotary microtome and were observed under both bright-field and fluorescence microscope by using conventional fast Green technique and coriophosphine O respectively.

Callus induction was optimum in MS medium with 2.0 mg/l 2,4-D and 0.5 mg/l BAP. Leaf segment showed the best response for callus induction. Callus induction was obtained from all of the explants used, but caulogenesis was achieved only from nodal segment-derived calluses; whereas rhizogenesis was found only from internode and leaf-derived calluses.

Highest number of shoot buds was developed (Figure 1a) in 2.0 mg/l BAP; it was 7.4 ± 0.20 (Table 1). For rhizogenesis (Figure 1b), 1.0 mg/l NAA was found to be effective (Table 2), although regeneration was achieved up to a concentration of 2.0 mg/l.

Under SEM, several groups of structures representing clusters of potential shoot primordia growing over the callus surface were observed. Each of these shoot primordia have a compact epidermal covering composed of a layer of more or less equal-sized cells. These shoot buds appear as finger-like protuberances (Figure 2a). Although the general callus appeared to have developed a cuticle layer over its surface, the shoot primordia appeared uncovered at this stage of their growth (after about four weeks of culturing).

Samples collected after about seven weeks of growth in the regeneration medium showed few of the shoot primordia developing into shoot buds with leaf primordia. Such buds, when observed under the SEM, revealed that

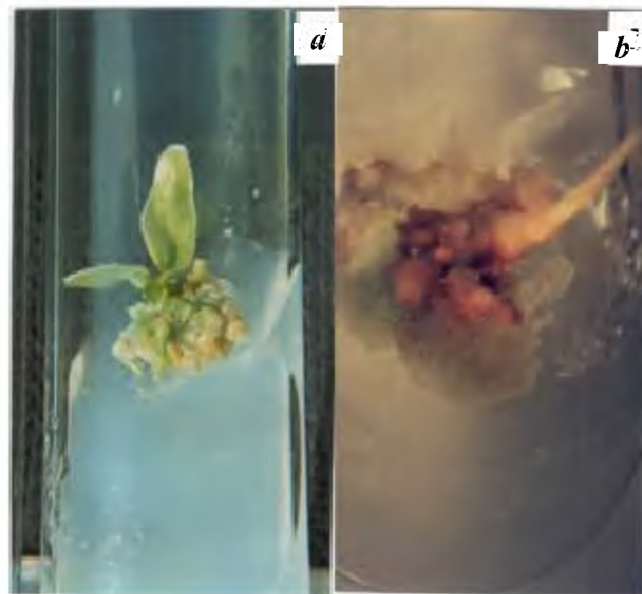


Figure 1. Organogenesis of nodal segment and leaf explant-derived callus tissue of *F. jangamas* in MS basal medium with different PGRs. **a**, Regeneration of shoot buds with one of them growing into a shootlet as seen after 28 days of subcultured callus tissue in MS medium with 2.0 mg/l BAP ($\times 2$); **b**, Regeneration of roots from explant-derived callus after 28 days of subculture on MS medium containing 1.0 mg/l NAA ($\times 4.5$).

Table 1. Shoot bud organogenesis in nodal segment-derived callus tissues of *F. jangomas*

Concentration of PGRs in MS medium (mg/l)			No. of shoot buds regenerated (mean \pm SE)
BAP	Kinetin	NAA	
–	–	–	–
0.1	–	–	–
0.5	–	–	3.4 ± 0.22
1.0	–	–	4.2 ± 0.18
2.0	–	–	7.4 ± 0.02
5.0	–	–	4.6 ± 0.18
–	0.1	–	–
–	0.5	–	1.3 ± 0.08
–	1.0	–	1.5 ± 0.09
–	2.0	–	1.9 ± 0.15
–	5.0	–	2.1 ± 0.13
0.2	–	0.1	–
0.5	–	0.1	2.3 ± 0.14
1.0	–	0.1	3.5 ± 0.22
2.0	–	0.1	2.7 ± 0.20
–	0.2	0.1	–
–	0.5	0.1	1.8 ± 0.13
–	1.0	0.1	3.1 ± 0.17
–	2.0	0.1	2.2 ± 0.16

Values are mean number of shoot buds after four weeks of culture involving altogether 30 samples in three experiments each having ten replicates.

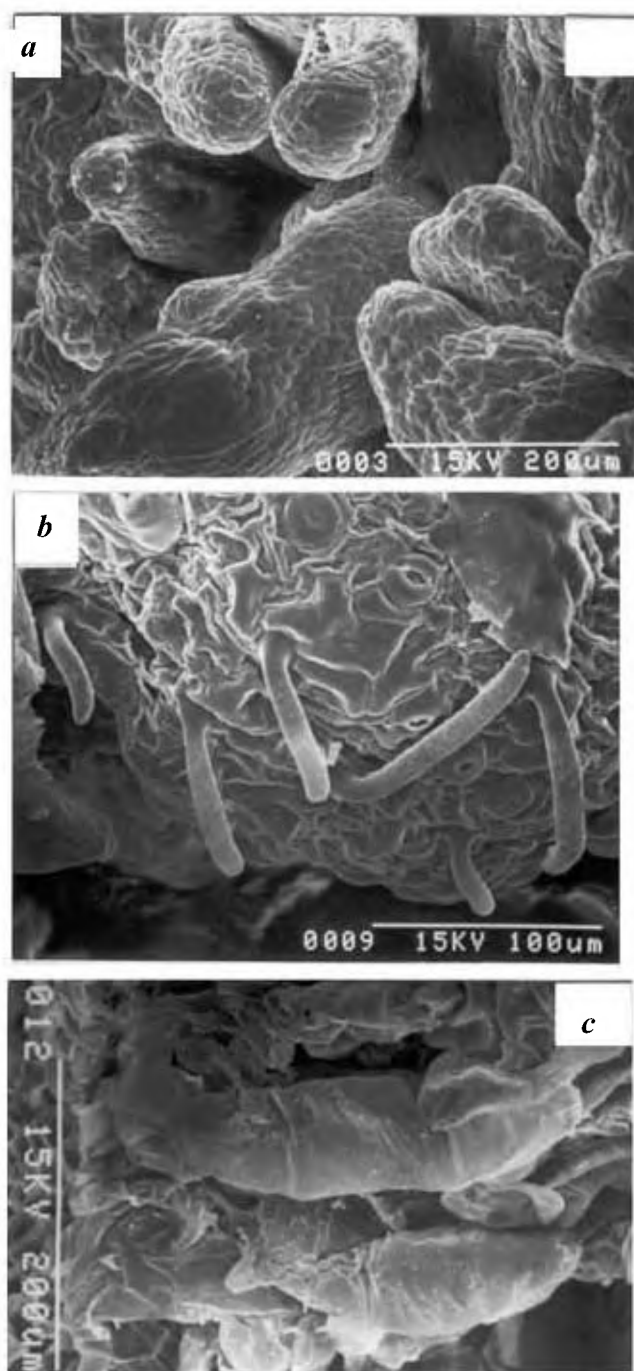


Figure 2. Scanning electron micrographs of regenerating shoot buds, their detailed structure and roots from *F. jangomas* callus tissue. **a**, A bunch of regenerating shoot buds from callus showing distinct epidermal layer; **b**, Emergence of apparently unicellular trichomes with characteristic elongated lenticular structures dotted on their surface as well as fully developed stomata on epidermal wall of shoot bud obtained after 60 days indicating further development of shoot; **c**, Two young roots regenerated from callus tissue.

the epidermal layer has cuticularized and distinct stomata had developed on it (Figure 2b). A number of unicellular trichomes with characteristic appearance of numerous,

Table 2. Rhizogenesis in callus tissue derived from internode and leaf segment explants of *F. jangomas*

Concentration of PGR in MS medium (mg/l)	No. of roots regenerated (mean \pm SE)	
	Internode	Leaf
NAA		
–	–	–
0.1	–	–
0.5	1.9 \pm 0.13	2.2 \pm 0.16
1.0	4.9 \pm 0.28	5.5 \pm 0.22
2.0	2.3 \pm 0.16	2.8 \pm 0.20

Values are mean number of roots after four weeks of culture involving altogether 30 samples in three experiments each having ten replicates.



Figure 3. Histological section of a shoot bud regenerating from callus tissue showing distinct shoot apex with two leaf primordia, one folded and the other straight, while observed under the fluorescence microscope after staining with coriphosphine O in M/15 Sørensen's phosphate buffer at pH 6.0 (\times 80).

regularly-spaced, eye-shaped markings on the surface (Figure 2b) were noticed. The regenerated roots (Figure 2c) that were observed under the SEM were sturdier, sparse and devoid of any root hair.

Histological section of differentiated shoot from callus tissue showed a shoot apex partially covered with two leaf primordia. A compact zone of meristematic tissue was observed near the apex (Figure 3).

In the present study, it was observed that all explants do not have the equal potential to regenerate shoot buds. Similarly, differential impacts of different explant types have also been reported earlier^{9,10}. Rhizogenesis was obtained from internodes and leaf segment-derived callus, but these two explants could not initiate shoot development. Only nodal segment-derived callus showed the potentiality of caulogenesis.

For triggering shoot bud regeneration in *F. jangomas*, BAP (2.0 mg/l) is an essential hormonal constituent of the medium. BAP without any auxin supplementation induces shoot bud regeneration in *Aegle marmelos*¹¹ and *Houttunia cordata*¹². In *Citrus aurantifolia*¹³, cytokinin alone was found to be effective for maximum shoot multiplication. It was previously noticed that in blueberry¹⁴ and garlic¹⁵ BAP gave the best response for multiplication of shoot buds, than other cytokinins.

The SEM study of shoot bud differentiation from callus showed that cells were aggregated into groups; these cells were also morphologically distinguished from other cells by having a cuticle-like covering. The trichomes were not associated with the shoot primordia at the initial stage, but they appear at a later stage. The presence of trichomes associated with shoot buds was reported earlier in several species¹⁶⁻¹⁸, but the characteristic eye-shaped marking was not found in those cases. Cuticularized epidermis was reported earlier in regenerated shoot buds of *Rosmarinus officinalis*¹⁶.

The presence of stomata on the epidermal layer is a striking feature which may indicate further development of shoot primordia into shoot buds with leaf primordia. Histological section of callus tissue with differentiated shoot bud also revealed that there was only shoot apex with two leaf primordia instead of any bipolar structure.

From these investigations, it has been observed that both caulogenesis as well as rhizogenesis from callus tissue are possible in *F. jangomas*, and BAP is effective to induce shoot bud organogenesis without any auxin supplementation. However, differential impacts of differ-

ent explant types in regeneration have been noticed here. In the course of regeneration, it has been observed from histological and SEM study that only caulogenesis has taken place instead of somatic embryogenesis.

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