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Shoot bud regeneration from leaf explants of a medicinal plant: *Enicostemma axillare*

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Adventitious shoot bud regeneration was achieved from leaf explants excised from a medicinal plant *Enicostemma axillare* (Lam.) Raynal cultured on Murashige and Skoog (MS) medium. MS medium supplemented with 8.9 µM N6-benzyladenine (BA) was found to be more efficient for adventitious shoot bud regeneration than MS medium containing a combination of 8.9 µM BA and 0.54 µM NAA. Adventitious shoot buds were induced directly on the margin and mid vein of the leaf explant. The shoot buds multiplied in media containing 4.4 µM BA. Hormone-free MS medium was used for further growth and development. Roots were developed in media containing 0.054–0.54 µM NAA. Results suggest that *Enicostemma axillare* can be mass propagated within a short time for the production of microbe-free plants for the extraction of drugs.

TRADITIONAL medicine based on herbal remedies has always played a key role in the health system of many countries. An estimated three quarters of prescription drugs are derived from plants[1,2], and were discovered because of their prior use in indigenous medicine and related purposes. Several thousands of medicinal plants are disappearing from the earth due to neglect and human activities.

Enicostemma axillare (Lam.) Raynal belonging to the family Gentianaceae is a species known for its medicinal properties. It is used as a bitter tonic, stomachic, laxative, blood purifier and as a drug for curing dropsy and malaria[3]. It is also used in rheumatism, abdominal ulcers, hernia, swellings, itches and insect poisoning[4]. It contains ophelic acid. *In vitro* propagation of this useful medicinal plant species could provide a means of disease-free healthy clones for the extraction of pure drugs. The results pertaining to *in vitro* shoot regeneration for mass propagation of this species is presented in this paper.

Top shoot cuttings of *Enicostemma axillare* were collected from healthy disease-free plants growing in Nagamali Hills, Madurai, India, and washed in soap water prior to surface sterilization. The fully-expanded leaves were excised and surface-sterilized with 20% commercial chlorox solution containing 1.05% sodium hypochlorite and a drop of tween 20 for 15 min. After washing in sterile distilled water, the leaves were dipped in 0.1% mercuric chloride solution for 3 min followed by thorough rinsing in sterile distilled water three times. Finally the plant materials were dipped in 70% ethanol for one second and rinsed in sterile distilled water. Surface-sterilized leaves were cut into 5 mm pieces and used as explants. These explants were placed on the MS medium[5] containing different concentrations of BA in 25 × 150 mm culture tubes. The pH of the media was adjusted to 5.6 prior to autoclaving. All the cultures were incubated under 1000 lux light intensity provided by white fluorescent lamps for 16 h photo period at 25 ± 1°C. For each treatment, 40 replicates were made and the experiment was repeated twice. The explants were subcultured once in 15 days. After 45 days the shoot buds were transferred to hormone-free media for shoot growth and elongation. The isolated plantlets were planted on MS media containing 0.054–0.54 µM NAA for rooting. Rooted plantlets were washed and planted on autoclaved soil mix containing sand, peat moss and humus (1:1:1) for acclimatization.

Leaf explants enlarged after 2 weeks of incubation in media with 8.9 µM BA and 0.54 µM NAA, produced light green callus along the cut surfaces mainly along the midrib region and at the petiolar base. After 30 days in culture, several globular shoot bud initials were observed on the surface of the callus. This kind of globular shoot bud initials also developed directly on the midrib of the explants (Figure 1 *a*) in media containing 8.9 µM BA alone. After 20 days, the globular buds proliferated in the same media. These shoot buds developed into plantlets (Figure 1 *b*) when transferred to hormone-free media or media containing low concentration of BA (0.44 µM). Each one of the isolated shoot buds when

Figure 1. *a*, *Enicostemma axillare* (Lam.) Raynal leaf explant showing the shoot buds; *b*, Leaf explant with cluster of plantlets.

subcultured on the same media with BA produced 18–20 adventitious shoot buds. Roots were induced on the media containing 0.054–0.54 μM NAA after 30 days in culture.

This study provides a protocol for the adventitious shoot bud regeneration from leaf explants of *Enicostemma axillare* for mass propagation. MS media supplemented with 8.9 μM BA induced shoot bud regeneration on several plants[6,7]. In the present study, leaf explants showed higher morphogenetic potential in MS media containing BA than media containing BA in combination with low concentration (0.54 μM) of NAA. Frequent isolation and subculture of the shoot buds enhanced the multiplication rate of the shoot buds. The shoot buds maintained for a longer period in the media with BA showed vitrification[8]. This problem was solved by reducing the concentration of BA in the multiplication stage and frequent subculturing (once in 15 days) in fresh media. Plantlets acclimatized inside the growth chamber for 20–30 days showed 100% survival in the greenhouse. Thus, it is possible to develop an efficient *in vitro* propagation system of the medicinal plant *Enicostemma axillare* which can be successfully mass produced, thereby providing a source for the extraction of drugs, and also ensuring conservation of this species in nature.

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Electron-probe micro analysis study of the Pipliya meteorite

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A meteorite fell in an uncultivated field near Pipliya–Kalan, Rajasthan, at about 8.30 p.m. on 20 June 1996. The meteorite is an aggregate of welded angular fragments of basaltic rock of variable granularity. Its basaltic composition and genomictic welded brecciated structure implies eucritic association. We give here a concise geological information report (GIR) of the meteorite with its electron-probe data.

'SHOOTING' stars are commonly observed nearly every night. The sight of a noctilucent meteorite evokes awe and amazement amongst its viewers. To the scientists, especially meteoriticists, a meteorite provides an invaluable, rather a unique, sample of material of the earliest stages of formation of the solar system[1–3]. One of the

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