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Short Communication

Plant regeneration from internode explants of *Euphorbia tirucalli*

Hidenobu Uchida[1], Osamu Nakayachi[1], Motoyasu Otani[1], Masataka Kajikawa[2], Yoshihito Kohzu[2], Katsuyuki T. Yamato[2], Hideya Fukuzawa[2], Takiko Shimada[1], Kanji Ohyama[1]*

[1] Research Institute of Agricultural Resources, Ishikawa Agricultural College, Nonoichi, Ishikawa 921-8836, Japan
[2] Laboratory of Plant Molecular Biology, Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa Oiwake-cho, Kyoto 606-8502, Japan
*E-mail: kohyama@ishikawa-c.ac.jp Tel: +81-76-248-3137 ext. 5139 Fax: +81-76-248-4191

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Abstract *Euphorbia tirucalli* is a potential source of commercially important chemicals such as sterols. Here we report the first successful plant regeneration from internode explants of *E. tirucalli*. Adventitious buds were efficiently induced on LS medium supplemented with 0.02 mg l^{-1} thidiazuron. On average of four experiments, 17.3 adventitious buds were induced from 12 explants on this medium. The adventitious buds grew into shoots during subsequent cultures on a hormone-free LS medium. For rooting treatment, we cultured these shoots on the LS medium containing 0.02 mg l^{-1} naphthalenacetic acid, followed by on the half-strength LS medium without vitamins, and were successful to obtain whole plantlets.

Key words: *Euphorbia tirucalli*, adventitious bud induction.

Members of genus *Euphorbia* are succulent shrubs distributed in subtropical regions in East Africa, South America, East Asia and India. These plants increase their biomass promptly in semi-deserts (Maugh 1976). *Euphorbia* plant cells accumulate sterols, triterpenoids, and diterpenoids (Ohyama et al. 1984a). These plants exude yellowish white latex when shoots are scratched (Saigo and Saigo 1983). The major components of *Euphorbia* latex are triterpenes (Biesboer and Mahlberg 1979; Yamamoto et al. 1981), and cracked or fermented latex can be used as fuel (Nielsen et al. 1977; Calvin 1980; Depeyre et al. 1994). Terpenoids and sterols in plants are industrially important chemical sources as vitamins, steroid compounds, insecticides, and anti-cancer drugs (Stohs and Rosenberg 1975; Heftmann 1975; Wu et al. 1991; Itokawa et al. 1989).

Recently, various sterol biosynthesis genes in plants have been isolated and characterized. Application of these information to plant gene modification would be useful for the production of transgenic plants with improved phytosterol production. Regeneration is indispensable to introduce foreign genes to the plants which contain important chemical substances. However, reports on regeneration of *Euphorbia* plants have been limited to *E. pulcherrima*, poinsettia, an ornamental species (Narayanaswami 1977; de Langhe et al. 1974; Nataraja et al. 1973; Nataraja 1975). In this study, we established method to regenerate a sterol-abundant plant, *E. tirucalli*. Our protocol is expected to contribute to genetic modification of given species.

The strain of *E. tirucalli* used in this study was maintained vegetatively and used in previous reports (Yamamoto et al. 1981; Ohyama et al. 1984b). Potted plants were grown in a greenhouse. Sterile explants were obtained by a method reported previously (Shimada et al. 1997) with several modifications. Shoots 5-cm long were excised from the potted plants, brushed with detergent, and washed with running tap water for at least 20 min. The rinsed explants were submerged in water with detergent *in vacuo* for five min. The explants were surface-sterilized with 70% (v/v) ethanol with 0.01% (v/v) Tween® 80 for 30 s, and with 3% (v/v) sodium hypochlorite solution containing 0.01% (v/v) Tween® 80 for five min *in vacuo*. They were further sterilized with 3% (v/v) sodium hypochlorite solution containing 0.01% (v/v) Tween® 80 three times for 10 min each, and then, washed with sterile distilled water three times for 10 min each.

The explants were planted onto solidified LS medium in 9-cm long glass tubes (Linsmaier and Skoog 1965; Shimada et al. 1997). The *in vitro* endophyte-free cultures were obtained after three subsequent 3-week cultures on the same medium. These *in vitro* plants grew to be more than 15 cm tall after subsequent culture on the

Abbreviations: BA, benzyladenine; NAA, naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; TDZ, thidiazuron.

LS medium in 20-cm long glass tubes, or in 500-ml plant culture vessels. Plants were grown at 27°C under approximately 40 μmol m^{-2} s^{-1} fluorescent illumination with a 16-h photoperiod.

For adventitious bud formation, we used the internodes generated *in vitro* as described above. These internodes were cut into segments 4 mm long each. Twelve internode explants were placed apolarly on solidified LS media supplemented with the phytohormones described below in a Petri dish (90×15 mm). The cultures were incubated under the same condition as described above. The internode explants were cultured on solidified LS medium containing benzyladenine (BA) at 0, 0.2 or 2.0 mg l^{-1} and/or 2,4-dichlorophenoxyacetic acid (2,4-D) at 0, 0.2 or 2.0 mg l^{-1}. After 5 weeks of culture, abundant callus formation was observed equally on the media containing both BA (0.2 or 2.0 mg l^{-1}) and 2,4-D (0.2 or 2.0 mg l^{-1}) (data not shown). Under these conditions, neither adventitious buds nor roots were observed (data not shown). Since efficient plant regeneration has been reported to be induced by addition of thidiazuron (TDZ) and naphthaleneacetic acid (NAA) (Shimada et al. 1997), this combination of phytohormones was tested (Table 1). After a 4-week culture on the media containing TDZ at 0, 0.02 or 0.2 mg l^{-1} and NAA at 0, 0.2 or 2.0 mg l^{-1}, the number of adventitious buds induced was the highest on the medium containing 0.02 mg l^{-1} TDZ without NAA (Table 1). Three subsequent experiments showed similar results. On the average of these four experiments, 17.3 adventitious buds were formed on 12 explants on the medium containing 0.02 mg l^{-1} TDZ without NAA (Table 1).

After a 4- to 6-week culture on the medium containing 0.02 mg l^{-1} TDZ, several adventitious buds appeared on an explant segment (arrows in Figure 1A). Most of these adventitious buds were formed on the periphery of segment adjacent to solidified medium. This is in contrast to the previous report that adventitious buds were generated in *E. pulcherrima* directly from callus subcultured in a shoot-inducting medium (de Langhe et al. 1974).

Explants with adventitious buds were subcultured on a hormone-free LS medium at two-week intervals. One

Table 1. Effect of phytohormones on internode explants of *E. tirucalli*.

Phytohorome (mg l^{-1})		Numbers of adventitious buds formed
TDZ	NAA	
0	0	1.3±1.3
0	0.2	3.3±1.9
0	2.0	0
0.02	0	17.3±8.6
0.02	0.2	0
0.02	2.0	0.3±0.3
0.2	0	0
0.2	0.2	0
0.2	2.0	0

Figures represent mean values ± standard errors in four different experiments. Twelve explants were used for each experiment.

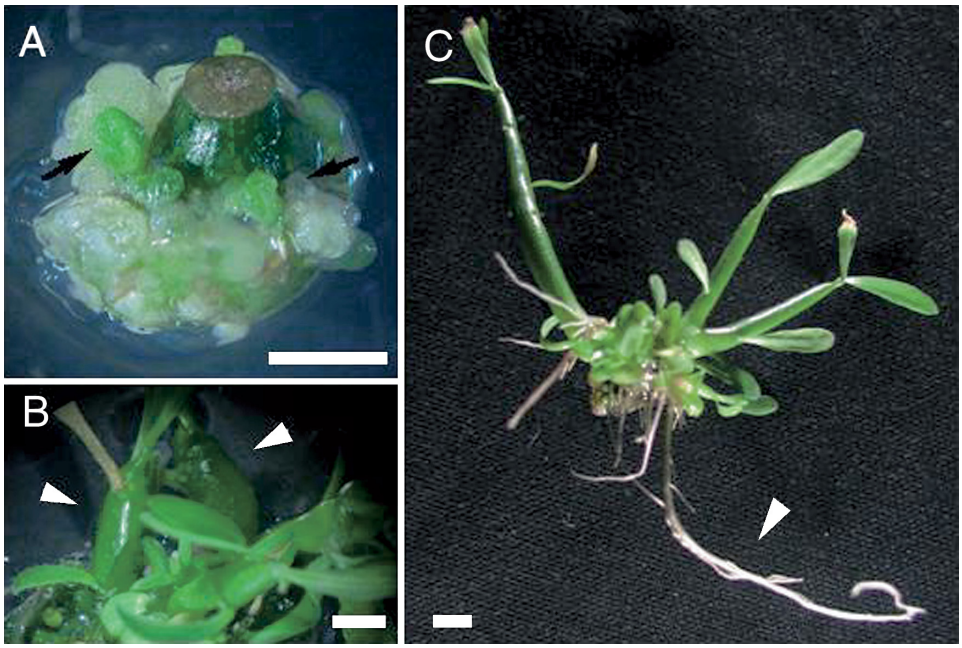

Figure 1. Plant regeneration from internode segments of *E. tirucalli*.

A, Adventitious buds (indicated by arrows) developed after a 5-week culture on LS medium supplemented with 0.02 mg l^{-1} TDZ; B, Trunk-shaped shoots (indicated by arrowheads) with expanded leaves developed after 4-week culture of the explants with adventitious buds on hormone-free LS medium; C, A whole plantlet developed from trunk-shaped shoots after a 3-week culture on LS medium supplemented with 0.2 mg l^{-1} NAA, followed by a one-week culture on the half-strength of hormone-free LS medium without vitamins. Arrowhead indicates a root. Bar=3 mm.

week after the initiation of culture on fresh hormone-free LS medium, the adventitious buds developed into mound-like shoots with curly leaves having a wavy edge. Four weeks after the start of culture on hormone-free LS medium, some of these shoots developed into trunk-shaped shoots (arrowheads in Figure 1B) with flat and knife-shaped leaves, as were normally observed in the potted plants. Subsequent culture on fresh hormone-free LS medium resulted in further growth of shoots.

For rooting, the trunk-shaped shoots were transferred to LS medium containing 0.2 mg l^{-1} NAA, which was most effective for root formation from internode explants. Rooting was confirmed one week after transfer onto this medium. These shoots thus obtained were subsequently transferred to the half-strength of hormone-free LS medium without vitamins for further development of the roots (arrowhead in Figure 1C).

This is the first report showing whole plantlet regeneration from explants of *E. tirucalli*. This method of plant regeneration of *E. tirucalli*, which accumulates important chemicals, should be an indispensable technique for the production of transgenic plants yielding larger amounts of phytosterols.

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