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SHORT COMMUNICATION

Direct Adventitious Shoot Formation from Apical Shoot Explants of *Euphorbia tirucalli*

Guohua Ma · Jaime A. Teixeira da Silva · Guojiang Wu

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Abstract The induction of adventitious buds from apical shoot explants of *Euphorbia tirucalli* was studied. On average, 10.5 adventitious buds were efficiently induced in a ring on the segment from one apical explant on MS (Murashige and Skoog) medium supplemented with 0.5 mg l^{-1} thidiazuron and 0.5 mg l^{-1} benzylaminopurine. The adventitious buds could develop into adventitious shoots during subsequent cultures on hormone-free MS medium. For rooting, shoot clumps were cultured on half-strength MS medium containing 0.2 mg l^{-1} α-naphthaleneacetic acid or indole-3-butyric acid. All the rooted plants survived establishment in soil within 2 months.

Keywords *Euphorbia tirucalli* · Adventitious bud · Root formation · Regeneration

Introduction

The genus *Euphorbia* includes succulent shrubs distributed in tropical and subtropical regions of the world. These plants increase their biomass promptly in semideserts (Maugh 1976). *Euphorbia* plant cells accumulate sterols and di- and triterpenoids (Nemethy and others 1979; Saigo and Saigo 1983; Ohyama and others 1984a). The major components of *Euphorbia* latex are triterpenes (Biesboer and Mahlberg 1979; Yamamoto and others 1981); cracked or fermented latex can be used as fuel (Nielsen and others 1977; Calvin 1980; Moffat 1980; Depeyre and others 1994). Terpenoids and sterols in plants are also industrially important sources of vitamins, steroid compounds, insecticides, and anticancer drugs (Stohs and Rosenberg 1975; Heftmann 1975; Itokawa and others 1989; Wu and others 1991). Recently, some sterol biosynthesis genes in *E. tirucalli* were isolated and characterized (Kajikawa and others 2004; Uchida and others 2009). Application of information to plant gene modification would be useful for production of transgenic plants with improved phytosterol production. Regeneration is a precondition and is indispensable in the introduction of foreign genes to plants which contain important chemical substances. However, regeneration of *Euphorbia* plants has been reported only in an ornamental species *E. pulcherrima* (Nataraja and others 1973; de Langhe and others 1974; Nataraja 1975; Narayanaswamy 1977) and *E. lathyris*, a potential source of petroleum-like products (Sach and others 1981; Lee and others 1982; Tideman and Hawker 1982). In the species *E. tirucalli* cell suspension cultures have been reported (Yamamoto and others 1981; Ohyama and others 1984a, b), while plant regeneration from callus and then adventitious buds induced from internode explants has been achieved (Uchida and others 2004). In this study we established a method without callus via direct adventitious shoot formation and plant regeneration in *E. tirucalli*. We expect our protocol to be useful as the first step of a protocol for genetic modification.

E. tirucalli plants are found growing outside the South China Botanical Garden. Apical shoots 5.0 cm long and 4–5 mm in diameter were excised from adult plants (about 20 years old), brushed with detergent (major component:

G. Ma (✉) · G. Wu
Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, China
e-mail: magh@scib.ac.cn

J. A. Teixeira da Silva
Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken 761-0795, Japan

0.2% sodium alkyl benzene sulfonate), and washed with running tap water for at least 30 min. The rinsed explants were submerged in water with detergent for 5 min, surface-sterilized with 70% (v/v) ethanol for 10 s, with 0.01% (v/v) Tween 80 for 15 min, and then washed with sterile distilled water three times for 15 min each. The explants were cut into 0.8-1.0-cm-long sections and inoculated abaxial side down onto solidified MS medium (Murashige and Skoog 1962) in 9-cm-long jars. The media contained different concentrations of benzylaminopurine (BAP), kinetin (KT), thidiazuron (TDZ), or α-naphthaleneacetic acid (NAA) alone or in combinations (Table 1). The jars were transferred to a culture room at 27°C and put under approximately 40 mmol m^2 s^{-1} fluorescent illumination with a 16-h photoperiod. After 4–6 weeks of culture, callus or adventitious bud formation was investigated. The experiments were repeated four times and data from the experiments were pooled and statistically analyzed by one-way ANOVA with a *post-hoc* test (PLSD, $p \leq 0.05$) to separate treatment means.

To study the origin and development of adventitious shoots of *E. tirucalli*, the histology of adventitious shoots induced from apical shoots was studied when they were cultured on induction medium containing 0.5 mg l^{-1} TDZ and 0.5 mg l^{-1} BAP for 4 weeks. Explants were fixed in FAA (18:1:1, 70% ethanol: glacial acetic acid: formaldehyde), stained with hematoxylin, then embedded in paraffin; 8-μm transverse sections were cut with a microtome, mounted on slides, examined, and photographed.

After 6 weeks of culture on media containing 0.5 mg l^{-1} KT or 0.5 mg l^{-1} BAP alone, callus or adventitious shoots were not induced. On media containing 0.5 mg l^{-1} TDZ or 0.1 mg l^{-1} NAA alone, some callus was induced. However, no adventitious shoots were visible. When 0.5 mg l^{-1} BAP

Table 1 Effect of different phytohormone combinations in the media on induction of *E. tirucalli* adventitious buds from apical shoot explants

Phytohormones (mg l^{-1})	Observed result	Percent of explants with adventitious shoot	Mean number of shoots per explant
KT 0.5	No callus	0	0 a*
BAP 0.5	No callus	0	0 a
TDZ 0.5	Callus, no shoot buds	0	0 a
NAA 0.1	Callus, no shoot buds	0	0 a
KT 0.5 + NAA 0.1	No callus, no buds	0	0 a
BAP 0.5 + NAA 0.1	No callus, shoot buds	25.5	1.6 b
TDZ 0.5 + NAA 0.1	Callus, shoot buds	56.8	7.3 c
TDZ 0.5 + BAP 0.5	No callus, shoot buds	81.6	10.5 d

The number of explants per treatment was 40, cultured for 6 weeks

* Means followed by the same letter in a column are not significantly different by the PLSD test ($p \leq 0.05$)

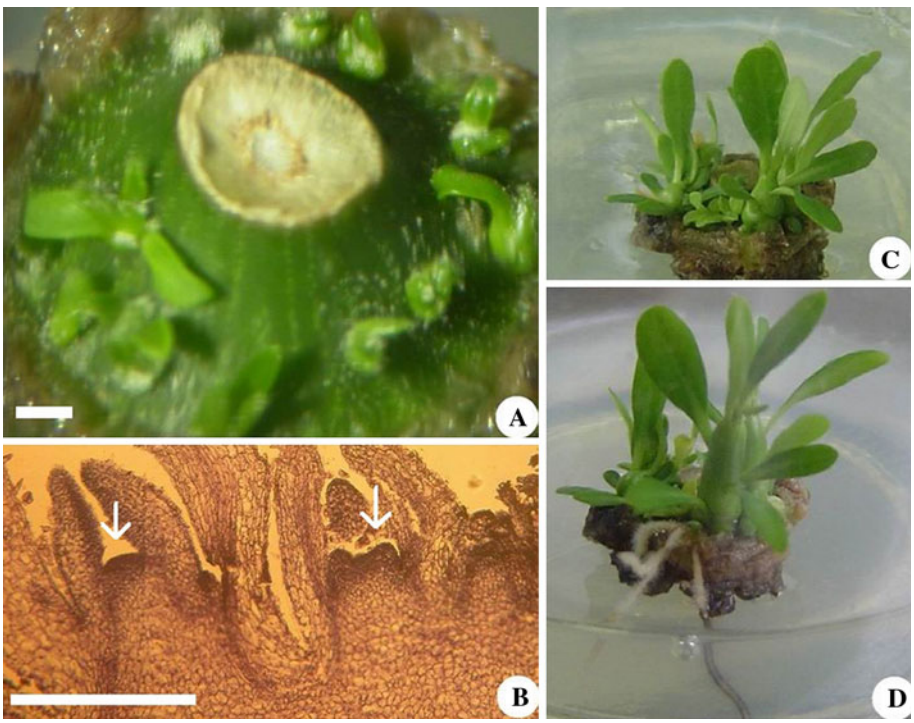

Fig. 1 Adventitious shoot formation induced from apical shoots and plant regeneration in *Euphorbia tirucalli* (scale bar = 2 mm). **a** Adventitious buds were induced from an apical shoot explant. **b** Histological section shows adventitious shoots (*white arrows*) that developed directly from inner tissues of the apical shoot explant after culturing for 4 weeks (scale bar = 1 mm). **c** An explant with adventitious buds was transferred to half-strength MS medium with no phytohormones and subsequently developed into an adventitious shoot cluster. **d** Root formation on rooting medium (1/2 MS) containing 0.2 mg l^{-1} NAA within 2 weeks

or 0.5 mg l^{-1} TDZ was combined with 0.1 mg l^{-1} NAA in the media, some adventitious shoots could be induced within 4 weeks (Table 1). However, no callus or adventitious shoots were induced on medium containing 0.5 mg l^{-1} KT and 0.1 mg l^{-1} NAA. This indicates that TDZ and BAP have a much greater effect on inducing adventitious buds than KT. Of the phytohormone combinations, a combination of BAP and TDZ in the induction medium induced the highest number of adventitious buds (15 at most). All of these adventitious buds formed in a ring on the segment without any callus being observed (Fig. 1a). Histological studies showed that adventitious shoots developed directly from the inner tissue of apical shoot explants (Fig. 1b). This result is different from what was previously reported in which adventitious buds were generated in *E. tirucalli* from callus when internode explants were induced on LS medium with 0.02 mg l^{-1} TDZ and 0.2 mg l^{-1} NAA (Uchida and others 2004). When explants with clumps of adventitious buds were transferred to MS regeneration medium containing no phytohormones, adventitious shoots and leaves grew within 1 week (Fig. 1c). When the clumps were transferred to half-strength MS medium with 0.2 mg L^{-1} NAA, adventitious roots were easily induced within 2 weeks (Fig. 1d). In a subsequent experiment, we carefully isolated adventitious shoots from adventitious shoot clumps into individual shoots and placed them on half-strength MS medium containing 0.2 mg l^{-1} NAA or 0.2 mg l^{-1} indole-3-butyric acid; greater than 90% of all shoots could root within 2 weeks (data not shown).

This is the first report showing whole plantlet regeneration directly from apical shoot explants of *E. tirucalli* in 2 months without an intermediate callus phase. Because this plant accumulates important chemicals, our protocol could be an indispensable technique for the production of transgenic plants yielding larger amounts of phytosterols.

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